Cholesteryl ester transfer protein and high density lipoprotein responses to cholesterol feeding in men: relationship to apolipoprotein E genotype

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Abstract The apolipoprotein (apo) E isoform is an important determinant of the plasma lipoprotein distribution of apoE and of the metabolism of apoE-containing lipoproteins. We have determined the effects of apoE genotype on the plasma lipoprotein response to cholesterol feeding in 30 young normal male subjects (5 E3/2, 11 E3/3, 14 E4/3) under rigorously controlled dietary conditions. Two diets, differing only in cholesterol content (low cholesterol (LC): 80 mg cholesterol/1000 kcal and high cholesterol (HC): 320 mg cholesterol/1000 kcal), were compared using a random crossover design. At the end of the HC as compared to the LC period, total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and HDL_2-C increased by an average of 15%, 21%, 7%, and 23%, respectively, for the three genotype groups combined ($P < 0.001$ for each). The LDL-C response to dietary cholesterol did not differ among the apoE genotypes. However, the increase in HDL-C varied significantly according to the apoE genotype (E3/2: 0 change, E3/3: +4%, E4/3: +12%; $P < 0.05$). The plasma cholesteryl ester transfer protein (CETP) response to cholesterol feeding also differed amongst the three apoE genotype groups (E3/2: +37%, E3/3: +18%, E4/3: +9%) ($P < 0.05$). ApoE genotype has significant and opposite effects on plasma CETP and HDL-C responses to dietary cholesterol in men.—**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.** Cholesteryl ester transfer protein and high density lipoprotein responses to cholesterol feeding in men: relationship to apolipoprotein E genotype. *J. Lipid Res.* 1993. **34:** 437–446.

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The isoform of apolipoprotein E (apoE) secreted into plasma has significant effects on the lipoprotein distribution of apoE and on the hepatic clearance of VLDL and chylomicron remnants. Individual concentrations of plasma cholesterol within populations have been shown to differ dependent in part on apoE phenotype (1, 2), and this variable has also been proposed as a genetically based determinant of plasma lipoprotein responsivity to dietary fat and cholesterol (3).

Gregg and colleagues (4) and, more recently, Weisgraber (5) have demonstrated that apoE distribution amongst plasma lipoproteins differs according to apoE isoform. E4 has a lower affinity for HDL as compared to VLDL and this appears to be due to conformational changes in the lipoprotein binding (COOH terminal) domain of E4 induced by the Cys^{112} to Arg mutation in the amino terminal domain (6). The E2 isoform, on the other hand, distributes in both VLDL and HDL but demonstrates impaired binding to hepatic E receptors (7). The apoE content and isoform present on VLDL and chylomicron remnants would thus be expected to alter the clearance rate of these particles by the liver. Hence, one would anticipate more rapid hepatic clearance of triglyceride-rich remnant particles by E4/3 or E4/4 subjects and slower clearance of these particles by E3/2 or E2/2 individuals and, indeed, this appears to be the case for chylomicron remnants (8, 9). The metabolism of apoB-100-containing lipoproteins also differs according to apoE phenotype. It has recently been demonstrated that E4/4 subjects convert more $VLDL_2$ apoB to LDL apoB and exhibit decreased LDL fractional catabolic rates as compared to E3/3 subjects (10). In addition to effects on lipoprotein clearance, individuals with different apoE phenotypes have been shown to have variable efficiency of cholesterol absorption (11) and consequently might be expected

Abbreviations: VLDL, very low density lipoprotein; HDL, high density lipoprotein; HC, high cholesterol; LC, low cholesterol; TC, total cholesterol; TG, triglyceride; CETP, cholesteryl ester transfer protein.

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to display different effects of dietary cholesterol on plasma lipoprotein concentrations.

We have determined the effects of apoE genotype on the plasma lipoprotein response to cholesterol feeding in 30 young normal male subjects. These studies clearly demonstrate that the LDL cholesterol response to cholesterol feeding is not dependent on apoE genotype. However, we have determined that there is an important relationship between apoE genotype and the change in HDL cholesterol in response to cholesterol intake. Using plasma samples obtained in these studies, we have explored the mechanism of the effect of apoE genotype on the HDL response to dietary cholesterol. We have measured plasma CETP concentrations, as this plasma protein may influence HDL cholesterol concentration (12) and because in animal models, plasma CETP concentration is increased in response to dietary cholesterol (13-15). We show that the human plasma CETP response to increased dietary cholesterol varies with apoE isoform in a fashion that is opposite to HDL cholesterol responses.

TABLE 2. Sample menu

High Cholesterol Diet	Low Cholesterol Diet
2% Milk (200 ml)	2% Milk (200 ml)
Orange juice (125 ml)	Orange juice (125 ml)
Oat bran muffin (200 g)[a]	Oat bran muffin (200 g)[b]
Butter (5 g)	Butter (5 g)
Yogurt 1.4% m.f. (125 g)	Yogurt 1.4% m.f. (125 g)
Apple juice (200 ml)	Apple juice (200 ml)
Egg salad/pita roll[a]	Egg substitute salad/pita[b]
Potato salad (150 g)	Potato salad (150 g)
Chocolate chip cookies[a]	Chocolate chip cookies[b]
Apple (200 g)	Apple (200 g)
Fish in wine sauce	Fish in wine sauce
Spaghetti carbonara[a]	Spaghetti carbonara[b]
Whole wheat roll (150 g)	Whole wheat roll (150 g)
Butter (5 g)	Butter (5 g)
Carrots (125 g)	Carrots (125 g)
Strawberry crepe[a]	Strawberry crepe[b]

[a]Items made with whole eggs.
[b]Items made with egg substitute mixture.

METHODS

Subjects

Normal male volunteers from the student population of McGill University were screened for plasma lipids and apoE phenotype. Subjects were selected on the basis of normal serum lipids, absence of underlying disease, and willingness to comply with a strict dietary regimen. The study was given IRB approval by the Royal Victoria Hospital Research Institute and written informed consent was obtained from all subjects.

Diets

Low cholesterol (LC) (80 mg cholesterol per 1000 kcal) and high cholesterol (HC) (320 mg cholesterol per 1000 kcal) diets were designed which were identical in fatty acids, fiber, and all other nutrients (**Table 1**). The source of supplementary cholesterol in the HC diet was whole egg. In the LC diet, eggs were replaced by Fleischmann's Eggbeaters® plus a mixture of vegetable oil triglycerides (palm oil, hydrogenated palm oil, olive oil, and peanut oil) to make an egg substitute mixture identical to eggs in macronutrient and fatty acid composition. Batches of the individual vegetable oils were prepared at the beginning of each study and aliquots of each oil were analyzed in triplicate for fatty acid composition by gas-liquid chromatography on a Hewlett-Packard 5890 gas chromatograph using a Carbowax-30N fused silica capillary column. The oils were then combined in appropriate quantities to match the fatty acid composition of egg yolk. The fatty acid composition of egg yolk used for comparison was from the Canadian Nutrient File (16). The diets consisted of a 7-day rotating menu based on typical North American foods. The amount of dietary cholesterol was adjusted to meet the diet prescription exactly by supplementing meals with a milk-based drink (providing less than 6% of total daily calories), containing milk, fruit, syrup, and egg or egg substitute, if necessary. A typical day's menu is shown in **Table 2.** The basic menu was the same for each subject with minor adjustments based on individual food preferences (for example, spices, type of juice or cereal). All food items and recipe ingredients were weighed to 0.1 g with the exception of fruit and low fat items, such as juice, 2% milk, and 1.4% yogurt, purchased in preportioned containers. The latter were found to be accurate to within 2% of reported weight. Each diet was individually designed to meet estimated energy requirements based on 4-day weighed food records, diet history, Harrison-Benedict equation, and habitual activity patterns. During the study period, subjects were weighed daily and, if necessary, the energy level of the diet was adjusted to maintain constant body weight (±1 kg). Meat, poultry, and fish portions were standardized for fatty acid and cholesterol content by the choice of very low fat cuts and by purchasing these items from the same supplier for all

TABLE 1. Nutrient composition of study diets

Composition	Low Cholesterol	High Cholesterol
Cholesterol (mg/1000 kcal)	80	320
Fat (% energy intake)	34	34
Carbohydrate (% energy)	49	49
Protein (% energy)	16	16
Poly:mono:sat (% total fat)	18:36:46	18:36:46
Fiber (g/1000 kcal)	6.3	6.3

studies. Subjects ate their evening meal in the metabolic kitchen at the Royal Victoria Hospital and other meals and snacks were packed for home or school consumption. Each subject was supplied with a microwave oven for the duration of the study to facilitate reheating of meals consumed at home. A detailed record was kept of any deviations from the study diet.

Protocol

Each subject consumed both diets using a random crossover design. Each diet period lasted for 35 days with a 2-week interval between the two diets (**Fig. 1**). Blood was collected after a 14-h fast on day 34 and day 36 of each study period, under standardized conditions of posture with minimal tourniquet. Blood was collected on ice in tubes containing EDTA (1 mg/ml) and immediately centrifuged at 4°C. All lipoprotein separations and analyses were started within 48 h of sample collection.

Plasma lipoproteins and CETP

Total cholesterol, triglycerides, and HDL-C were measured by the Lipid Research Clinics protocol using automated methods, standardized with the Centers for Disease Control in Atlanta (17). VLDL ($d < 1.006$ g/ml), IDL ($1.006 < d < 1.019$ g/ml), LDL ($1.019 < d < 1.063$ g/ml), HDL_2 ($1.063 < d < 1.125$ g/ml), HDL_3 ($1.125 < d < 1.21$ g/ml) were isolated ultracentrifugally. Plasma apolipoproteins (apo) A-I and B were determined by an ELISA technique (18, 19) using a modified coating buffer and timing of the individual steps to allow each assay to be completed in a single day. The between-run coefficients of variation between replicate samples were 1.9% for total cholesterol, 3.5% for triglyceride, 3.5% for HDL cholesterol, 3.0% for apoA-I, and 3.2% for apoB. ApoE (20), apoA-II (21), and CETP (21) were determined by radioimmunoassay. We have previously demonstrated that, in normal subjects, CETP mass in plasma correlates well with cholesteryl ester transfer activity measured in vitro ($r = 0.86$, $P < 0.05$) (22). LpA-I was determined by differential immunoassay (23).

ApoE genotype

Subjects were initially screened by apoE phenotyping, using analytical isoelectric focusing of the apoVLDL with and without neuramidase and cysteamine treatment (24). ApoE phenotypes were confirmed by apoE genotyping using the polymerase chain reaction and allele-specific oligonucleotide primers (25).

Adipose tissue CETP mRNA levels

Adipose tissue biopsies (5 g) were obtained from three subjects at the end of each of the LC and HC diets from a periumbilical skinfold using a liposuction technique. This methodology was developed late in the study and only three of five available subjects were able to participate in this procedure. Total cellular RNA was extracted using the acidic guanidinium isocyanate technique as described by Chomczynski and Sacchi (26). Northern transfer of poly A+ mRNA was performed to verify the size of the CETP message (27). The abundance of CETP mRNA was determined by a solution hybridization ribonuclease protection assay (15). Using conditions previously described, 30 μg of test total RNA from human adipose tissue was hybridized to an anti-sense RNA probe prepared from the human cDNA (289 bp fragment, nucleotides 674–957) subcloned into pGEM4EZ. After 16–18 h of hybridization at 48°C, samples were digested by RNase T2 for 2 h at 30°C (15) and [^{32}P]RNA-RNA hybrids were analyzed on 6% polyacrylamide-urea sequencing gels. Protected fragments were visualized by autoradiography and quantitated by densitometry. RNA mass was determined by comparison with a standard curve of CETP cRNA hybridized simultaneously. For this purpose, sense strand RNA was synthesized by in vitro transcription and its mass was quantitated precisely by standard methods using [^{3}H]NTP incorporation. The intra-assay variation was 8% or less.

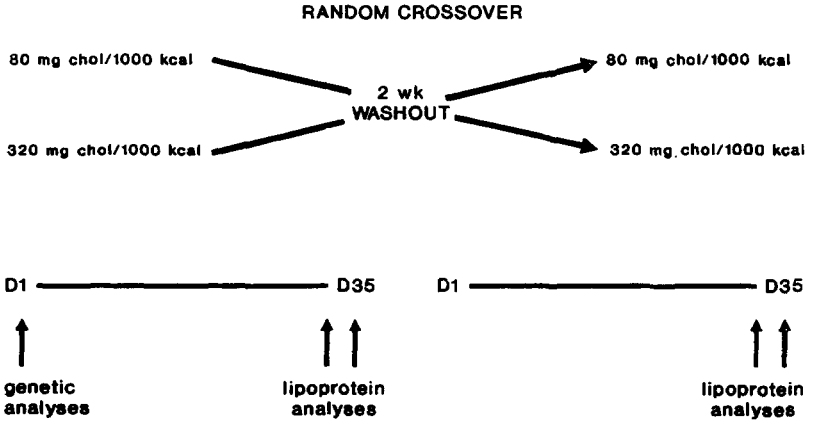

Fig. 1. Study protocol. Each subject ingested the two diets in random order. Each diet phase lasted 35 days with a 2-week washout period between diets. Blood samples were collected after an overnight fast on two occasions during the last 3 days of each study period for complete lipoprotein analyses.

Statistical analyses

Paired *t*-tests were used to compare differences in biochemical variables between the LC and HC diets for the entire population of 30 subjects. Analysis of variance was performed to compare responses to dietary cholesterol in subject groups differing in apoE genotype. Analysis of covariance was used to determine interactions between specific genetic and biochemical variables (28).

RESULTS

A total of 30 subjects participated in the study (5 E3/2, 11 E3/3, and 14 E4/3). The mean age was 23.0 (±2.6) years and mean body mass index (BMI) was 22.4 (±2.0). There were no differences in either age or BMI amongst apoE genotype groups.

Compliance

All subjects completed both phases of the study. Compliance was excellent as assessed by detailed reporting of protocol deviations and maintenance of body weight within 1.0 kg of initial weight in all subjects. The average cholesterol intake was 261 mg/d (range 208 to 352 mg/d) on the LC diet and 1044 mg/d (range 832 to 1408 mg/d) on the HC diet.

Plasma lipoproteins

Plasma lipoprotein concentrations by apoE genotype at the end of each of the LC and HC diets are shown in **Table 3.** There was a nonsignificant trend towards higher levels of TC and LDL-C for the E4/3 subjects on each of the LC and HC diets. E4/3 subjects had higher levels of HDL-TG and lower levels of HDL_3-C as compared to E3/2 subjects at the end of the LC diet but these differences were not significant at the end of the HC diet. VLDL-C and apoE concentrations were significantly higher for E4/3 subjects as compared to E3/3 subjects at the end of the HC but not the LC diet ($P < 0.05$ for VLDL-C and $P < 0.02$ for apoE by ANOVA). The plasma lipoprotein concentrations for the entire group of 30 subjects at the end of each of the high and low cholesterol diets are presented in **Table 4.** Mean increases occurred in TC, LDL-C, and HDL-C of 15.4%, 21.2%, and 7.0%, respectively, at the end of the HC as compared to the LC diet ($P < 0.001$ for each). Cholesterol enrichment of the LDL particle also occurred as assessed by an increase in the LDL cholesterol (mmol/l) to apoB (mg/dl) ratio (2.96 ± 0.35 {LC} vs. 3.07 ± 0.35 {HC}, $P < 0.05$). No significant changes occurred in total TG or VLDL-TG or VLDL-C. The HC diet resulted in a significant increase in HDL_2-C for subjects in each of the three apoE

TABLE 3. *Comparison of plasma lipoprotein levels at the end of each of two test diets* according to apoE genotype

Diet	E3/2 (n = 5)	E3/3 (n = 11)	E4/3 (n = 14)
Low cholesterol diet			
TC mmol/l	3.73 ± 0.45	3.62 ± 0.75	4.07 ± 0.68
TG mmol/l	0.64 ± 0.18	0.65 ± 0.19	0.85 ± 0.27
VLDL-C mmol/l	0.20 ± 0.09	0.24 ± 0.10	0.26 ± 0.05
VLDL-TG mmol/l	0.33 ± 0.14	0.35 ± 0.17	0.51 ± 0.25
LDL-C mmol/l	2.16 ± 0.37	2.12 ± 0.68	2.62 ± 0.58
HDL-C mmol/l	1.37 ± 0.11	1.26 ± 0.24	1.19 ± 0.28
HDL_2-C mmol/l	0.60 ± 0.19	0.59 ± 0.27	0.61 ± 0.21
HDL_3-C mmol/l	0.76 ± 0.21	0.67 ± 0.11	0.58 ± 0.11[a]
HDL-TG mmol/l	0.08 ± 0.04	0.11 ± 0.05	0.16 ± 0.06[a]
ApoE mg/dl	6.79 ± 2.44	3.59 ± 2.14	10.98 ± 8.09[b]
CETP mg/dl	0.143 ± 0.029	0.153 ± 0.027	0.155 ± 0.037
High cholesterol diet			
TC mmol/l	4.20 ± 0.59	4.17 ± 0.90	4.73 ± 0.79
TG mmol/l	0.73 ± 0.17	0.73 ± 0.17	0.74 ± 0.19
VLDL-C mmol/l	0.24 ± 0.06	0.19 ± 0.06	0.26 ± 0.05[b]
VLDL-TG mmol/l	0.42 ± 0.16	0.41 ± 0.17	0.39 ± 0.14
LDL-C mmol/l	2.58 ± 0.54	2.65 ± 0.87	3.13 ± 0.59
HDL-C mmol/l	1.37 ± 0.08	1.30 ± 0.25	1.35 ± 0.32
HDL_2-C mmol/l	0.71 ± 0.14	0.73 ± 0.26	0.74 ± 0.28
HDL_3-C mmol/l	0.64 ± 0.07	0.56 ± 0.08	0.60 ± 0.10
HDL-TG mmol/l	0.12 ± 0.04	0.15 ± 0.05	0.14 ± 0.05
ApoE mg/dl	7.85 ± 6.14	6.49 ± 3.50	11.49 ± 7.15
CETP mg/dl	0.196 ± 0.046	0.175 ± 0.039	0.172 ± 0.038

Mean ± SD for each group.
[a]ANOVA F-test, $P < 0.05$ for differences between genotype groups E3/2 and E4/3.
[b]ANOVA F-test, $P < 0.05$ for difference between apoE genotype groups E3/3 and E4/3.

TABLE 4. Lipoprotein levels on low and high cholesterol diets

Lipoprotein	Low Cholesterol Diet	High Cholesterol Diet
mmol/l		
TC	3.85 ± 0.69	4.44 ± 0.83[a]
TG	0.74 ± 0.24	0.73 ± 0.18
VLDL-C	0.24 ± 0.10	0.23 ± 0.07
VLDL-TG	0.42 ± 0.22	0.40 ± 0.15
LDL-C	2.36 ± 0.62	2.86 ± 0.72[a]
LDL-TG	0.16 ± 0.05	0.18 ± 0.08
HDL-C[b]	1.25 ± 0.25	1.34 ± 0.26[c]
HDL-TG	0.13 ± 0.06	0.14 ± 0.05

Values given as mean ± SD; n = 30.
[a]$P < 0.0001$ by paired *t*-test.
[b]Changes in HDL-C in response to cholesterol feeding differed depending on apoE genotype. Because there were proportionally more apoE4/3 and E3/2 subjects in the present study, the changes noted here in HDL-C should not be considered representative of average population responses.
[c]$P < 0.001$ by paired *t*-test.

genotype groups ($P < 0.0001$ for the 30 subjects combined) and a significant decrease in HDL_3-C for the E3/2 and E3/3 subjects but not for the E4/3 subjects ($P < 0.05$ by ANOVA for difference in HDL_3-C response of E4/3 vs. E3/2 and E3/3 groups) (**Fig. 2**).

Apolipoproteins and CETP

ApoB increased by 18% ($P < 0.0001$), apoA-I by 5% ($P < 0.05$), total plasma apoE by 23% ($P < 0.05$), and CETP by 16% ($P < 0.0001$) at the end of the HC as compared to the LC diet. No significant change occurred in apoA-II. LpA-I increased to an extent similar to apoA-I but this change was not significant (**Table 5**).

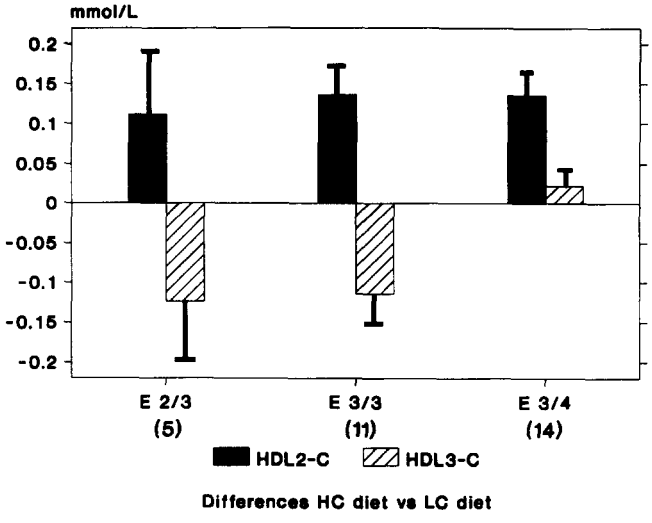

Fig. 2. Effects of apoE genotype on HDL subclass distributions on high as compared to low cholesterol diets. Data are expressed as mean and SEM. HDL_3-C was lower for E3/2 and E3/3 subjects and HDL_2-C was higher for all three genotype groups at the end of the high as compared to the low cholesterol diet. The HDL_3-C response differed significantly ($P < 0.05$ by ANOVA) for the E4/3 subjects as compared to the E3/2 and E3/3 subjects.

Adipose tissue levels of CETP mRNA

Levels of CETP mRNA as a percentage of total RNA increased in all three subjects studied by 30 to 89% ($P < 0.01$) (**Table 6**).

Variation in individual lipoprotein response

There was a wide range of lipoprotein response to dietary cholesterol. Individual increases in LDL-C ranged from 0 to +62%. Changes in HDL-C ranged from −14% to +58%. There was no effect of apoE genotype on the response of LDL-C, apoB, or apoE to dietary cholesterol (Table 3). However, the change in both total HDL-C and in HDL subclass distribution varied significantly according to the apoE genotype. HDL_2-C increased in all groups but to a slightly lesser extent in the E3/2 subjects. HDL_3-C decreased significantly in both E3/2 (−16%) and E3/3 (−17%) groups but did not change in the E4/3 group (+4%) ($P < 0.01$ vs. E3/2 and E3/3). Thus the net effect on total HDL-C was a large increase in the E4/3 subjects (+12%), a smaller increase in the E3/3 subjects (+4%), and no change in the E3/2 group because, for the latter subjects, the increase in HDL_2-C was balanced by a similar decrease in HDL_3-C ($P < 0.05$ for between-group differences by ANOVA). The changes noted in plasma CETP concentrations in response to cholesterol feeding also differed significantly amongst the three groups. In contrast to HDL-C, plasma CETP increased least in E4/3 subjects and most in E3/2 subjects (**Fig. 3**). Plasma CETP increased by a mean of 37% in the E3/2 individuals, by

TABLE 5. Plasma apolipoproteins and CETP concentrations on low and high cholesterol diets

Variable	Low Cholesterol	High Cholesterol
	mg/dl	
ApoB	79.3 ± 20.0	92.9 ± 23.5[a]
ApoE	7.52 ± 6.60	9.22 ± 6.26[b]
ApoA-I	126.2 ± 21.3	132.1 ± 23.7[b]
ApoA-II	19.4 ± 5.9	17.8 ± 5.1
LpA-I	100.7 ± 21.8	105.8 ± 24.9
CETP[c]	0.153 ± 0.032	0.177 ± 0.039[a]

Values are given as mean ± SD; n = 30.
[a]$P < 0.0001$ by paired *t*-test.
[b]$P < 0.05$ by paired *t*-test.
[c]Changes noted in plasma CETP concentrations in response to cholesterol feeding differed depending upon apoE genotype, and, as E3/2 and E4/3 genotypes were over-represented in the present study, these changes in CETP should not be considered representative of average population responses to cholesterol feeding. Changes in apolipoproteins did not vary by apoE genotype.

TABLE 6. Adipose tissue CETP mRNA levels of high and low cholesterol diets

Subject	ApoE Genotype	Plasma CETP		CETP mRNA[a]	
		LC	HC	LC	HC
		mg/dl		*pg/μg total RNA*	
GG	E3/2	0.110	0.161	0.212	0.275
DB	E3/2	0.126	0.145	0.073	0.130
PR	E4/3	0.140	0.125	0.044	0.083

Note that in the one apoE4/3 subject studied there was no increase in plasma CETP despite an increase in adipose tissue CETP mRNA level.
[a] $P < 0.009$ for changes in CETP mRNA levels between diets.

18% in the E3/3 subjects, and by only 9% in the E4/3 subjects ($P < 0.05$ for E3/2 vs. E4/3).

Relationship between changes in CETP and other lipoproteins

The relationship between changes in CETP and various lipoprotein and demographic variables was examined in the population of 30 subjects. There were no significant correlations between either BMI or caloric intake (and hence total cholesterol intake on each diet) and the increase in LDL cholesterol in response to cholesterol feeding. Nor were there any relationships between plasma lipoprotein concentrations at the end of the LC diet and the increase in LDL cholesterol on the HC diet. The change in LDL-C between the two diets correlated positively with the change in the plasma concentration of CETP ($r = 0.45$) as well as with the change in HDL_2-C ($r = 0.41$). Although across the apoE genotype groups the mean changes in CETP and the mean changes in HDL-C were inversely related (Fig. 3), the changes in CETP and the changes in HDL-C were not correlated as a whole ($r = 0.08$). Analysis of covariance demonstrated that the change in plasma concentration of HDL-C was significantly related to the change in CETP ($P = 0.025$), in a positive direction, when apoE genotype was controlled in the model. There was no interaction between apoE genotype and change in CETP. ApoE genotype and the change in plasma CETP level had independent effects on the change in HDL-C and together explained 40% of the variation in response of HDL-C to the high cholesterol diet (**Table 7**). A similar model was used for the analysis of changes in HDL_3-C which included apoE genotype, change in CETP level, and a term for the interaction of apoE genotype and change in CETP. This model explained 42% of the variation in the response of HDL_3-C. A significant interaction was found between the change in CETP and apoE genotype, suggesting that the effect of change in CETP on change in HDL_3-C is influenced by apoE genotype. The change in CETP had a positive effect on the change in HDL_3-C when considered alone (regression coefficient, 6.15) but when the apoE genotype was taken into account, it was apparent that E3/2 and E3/3 subjects experienced a fall in HDL_3-C in association with an increase in CETP (net decrease of magnitude, 7.68 and 12.26, respectively) while the E4/3 subjects experienced an increase in HDL_3-C in association with a small increase in plasma CETP concentrations (**Table 8**).

DISCUSSION

In the 30 subjects studied here, the average increase in LDL-C (+21%) in response to moderate change in cholesterol intake was substantial and certainly suggests that limitation of total cholesterol intake is a highly important recommendation for the public at large as well as

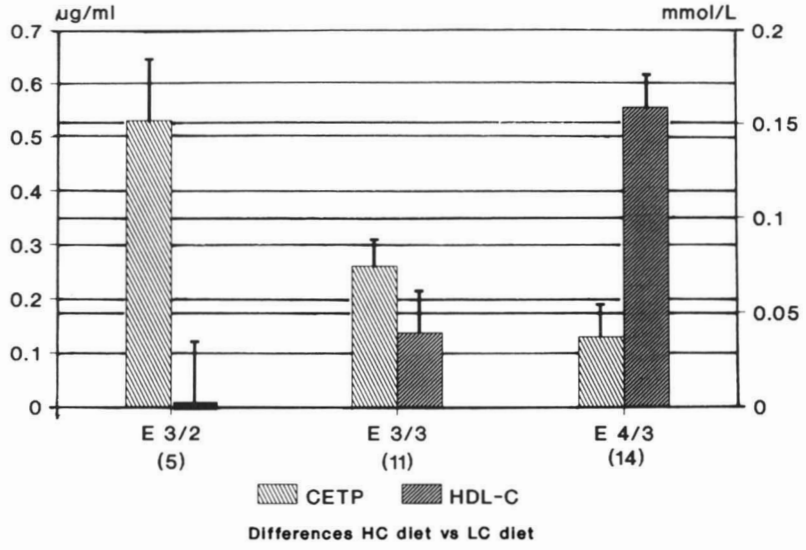

Fig. 3. Relationship of apoE genotype to CETP and HDL-C response to cholesterol feeding. The vertical scale represents changes in plasma concentrations of CETP and HDL-C upon changing from the low cholesterol diet to the high cholesterol diet. Data are expressed as mean and SEM. Between-group differences for both CETP and HDL-C are significant by ANOVA ($P < 0.05$). The number of subjects in each group is given in parentheses.

TABLE 7. Effects of apoE genotype and change in plasma CETP on changes in HDL-C for high versus low cholesterol diet

Parameter	Estimate	*P* Value
Intercept	5.18	0.0001
E3/2	−9.00	0.001
E3/3	−5.57	0.003
E4/3[a]	0	
Change in CETP	7.27	0.025

Table of coefficients for model predicting changes in HDL_3-C upon changing from low to high cholesterol diet.
[a]E4/3 is used as the reference group, therefore coefficient is zero.

for hypercholesterolemic patients. In accord with previous studies (29–34), the LDL response to cholesterol feeding was highly variable (0 to +62%) but contrary to our original hypothesis, individual variability was not a function of apoE genotype. In retrospective analyses of patients participating in the Helsinki Heart study (3) and the North Karelia Study (35), and in hypercholesterolemic patients treated with soya protein (36), subjects with one or more E4 alleles appeared to have exhibited greater response to dietary intervention, but none of these studies addressed the effects of E genotype on the response to dietary cholesterol per se. In addition, in the study of Tikkanen et al. (35), greater sensitivity to diet was evident only in E4/4 homozygotes, a group that was not included in the present investigation. Other well-controlled dietary experiments have also failed to detect an effect of apoE phenotype on the response to dietary fat and cholesterol (34, 37, 38). Katan and co-workers (39) have reported that inconsistencies exist in individual serum cholesterol response to dietary cholesterol. Although lack of reproducibility of responses to dietary change may often be related to inadequate dietary and environmental control, it remains possible that repeated testing of E genotype groups could reveal trends in responsivity not noted here. As part of this study, data have been collected on sterol balance, cholesterol absorption, postheparin lipolytic activities, and a number of other genetic variables. The effects of these variables on the LDL cholesterol response to cholesterol feeding will be the topic of a second communication.

While the subjects were consuming the low cholesterol diet, there was a trend towards higher total and LDL cholesterol levels for the E4/3 group which is in agreement with a number of previously published reports that apoE phenotype influences mean serum cholesterol levels within population groups (1, 2). There also were statistically significant differences in HDL_3-C and HDL-TG, which varied inversely across the three genotype groups. HDL_3-C was highest and HDL-TG lowest in the E3/2 group on the low cholesterol diet, while HDL_3-C was lowest and HDL-TG highest in the E4/3 subjects. The significance of these observations is not clear but may be related to the fact that VLDL triglyceride values were also higher (although not significantly so on the LC diet) in the E4/3 subjects. Triglyceride enrichment of HDL occurs in the presence of higher levels of VLDL triglyceride, making HDL a better substrate for hepatic lipase. The action of hepatic lipase could be expected to enhance the catabolism of HDL_3 as well as HDL_2 particles (40).

In this rigorously controlled, crossover dietary study, cholesterol feeding resulted in an increase in total HDL cholesterol. The E3/2 and E3/3 subjects, but not the E4/3 subjects demonstrated a reduction in the concentration of HDL_3-C, and all three genotype groups showed an increase in the concentration of HDL_2-C on the HC as compared to the LC diet. ApoA-I increased to a lesser extent than HDL-C, and apoA-II did not change. The increase in the concentration of the HDL population containing apoA-I only (LpA-I) was similar to that of total A-I but did not reach significance. Previous studies have also suggested that cholesterol feeding results in increases in total HDL-C and apoA-I with an increase in the HDL_2 subfraction (37, 41, 42).

Cholesterol feeding also resulted in significant increases in the plasma concentration of two components of the reverse cholesterol transport pathway, CETP and apoE. CETP mediates a major route for the return of HDL-derived cholesteryl ester to the liver by a process that involves cholesteryl ester/triglyceride exchange between apoA-I-containing lipoproteins and apoB-containing lipoproteins (43).

Plasma CETP concentration is strongly correlated with the abundance of CETP mRNA in both liver and adipose tissue in cynomolgous monkeys, suggesting that the variable synthesis in these organs accounts for a major part of the variation in plasma CETP concentration (13). In animals, an increase in dietary cholesterol leads to an increase in CETP mRNA abundance in both liver and adipose tissue (13–15) in association with increased plasma CETP concentrations. Although only three subjects were studied (Table 6), there appears to be a similar increase

TABLE 8. Effects of apoE genotype and change in plasma CETP on changes in HDL_3-C for high versus low cholesterol diet

Parameter	Estimate	*P* Value
Intercept	0.02	0.99
E3/2	−0.76	0.87
E3/3	−1.20	0.64
E4/3[a]	0	
Change in CETP	6.15	0.19
Interaction between CETP and apoE genotypes		
E3/2	−13.83	0.13
E3/3	−18.40	0.03

Table of coefficients for model predicting effects of changes in plasma CETP and apoE genotype on changes in HDL_3-C for high versus low cholesterol diet.
[a]E4/3 is used as the reference group, therefore coefficient is zero.

in CETP mRNA in adipose tissue in humans. Furthermore, recent studies in mice bearing the human CETP transgene, the presence of a 3.2 kb upstream and 2.0 kb downstream natural flanking region (NFR) sequence is associated with a fivefold increase in transcription rates for the CETP transgene in response to dietary cholesterol, an effect not seen in animals transfected with the CETP gene in association with a metallothionine promoter rather than its own flanking sequences (44). In addition, cultured macrophages secrete more transfer activity after cholesterol loading (45). Thus the CETP gene appears to be responsive to changes in cellular cholesterol and it is possible that the effect of dietary cholesterol on plasma levels of CETP is mediated by an effect on regulatory cholesterol pools in tissues such as liver and adipose. The teleological significance of increased CETP production in response to cholesterol delivery to peripheral cells is not yet completely clear. Certainly, CETP mediates a major route for reverse cholesterol transport and, provided that clearance of recipient particles for cholesterol transfer is not impaired, increased CETP may facilitate return of cholesterol from peripheral cells to the liver. CETP produced in peripheral adipose or other cells may also play a role in local, as well as centripetal, cholesterol transport.

In the present study, apoE genotype was a significant determinant of both the HDL and CETP responses to cholesterol feeding. The E3/2 subjects demonstrated large increases in CETP and had no net increase in HDL-C. E4/3 subjects had a small increase in CETP and a major increase in the plasma concentration of HDL-C, whereas E3/3 subjects showed an intermediate response in terms of both CETP and HDL changes.

The shift in the HDL subclass distribution in response to cholesterol feeding was also found to differ according to apoE genotype. HDL_2-C increased in all groups but to a slightly lesser extent in the E3/2 subjects. HDL_3-C decreased significantly in both E3/2 and E3/3 groups but did not change in the E4/3 group. Thus the net effect on total HDL-C was a large increase in the E4/3 subjects, a smaller increase in the E3/3 subjects, and no change in the E3/2 group. Covariant analysis demonstrated a significant interaction between apoE genotype and the change in plasma CETP in determining the HDL_3-C response to cholesterol feeding. One interpretation of these results is that, as there is greater distribution of apoE in HDL in the E3/2 and E3/3 groups, there is enhanced catabolism of HDL_3. Thuren et al. (46) have recently demonstrated that apoE enrichment of HDL makes it a better substrate for hepatic lipase. It is also possible that the increase in both subfractions of HDL in the E4/3 subjects reflects a direct effect of apoE genotype on cholesteryl ester transfer out of HDL. As the E4 isoform distributes preferentially in VLDL as compared to HDL particles, one would expect a lower content of apoE in HDL, and effects of apoE on the lipid transfer reaction have also been reported (47). Although across the apoE genotype groups the mean changes in CETP and the mean changes in HDL-C were inversely related, linear regression analysis of the individual values showed that the change in CETP and the change in HDL-C were not correlated ($r = 0.08$). Analysis of covariance, controlling for apoE genotype, demonstrated that the change in the plasma concentration of CETP was significantly and positively related to the change in HDL-C. The two variables, apoE genotype and the change in plasma CETP, explained 40% of the observed variation in HDL-C change in response to dietary cholesterol.

Given the small numbers, one must be cautious in the interpretation of these analyses but the results suggest that the mechanism by which apoE genotype regulates the HDL response to cholesterol feeding may be distinct from the effects on CETP mass and may be related to other factors such as effects on lipid transfer activity or on the hepatic clearance of chylomicron remnants. When the effect of apoE genotype on the HDL-C response is controlled, it would appear that the factors that increase plasma CETP in response to cholesterol feeding also enhance the HDL-C response. In previous studies, we have demonstrated that there is a small but positive correlation between plasma concentrations of CETP and HDL-C in normal subjects ($r = 0.41$, $P < 0.05$), suggesting that the concentration of plasma CETP is, in part, a function of apoA-I availability for CETP binding in agreement with the documented affinity of these two proteins for each other (21).

Further studies are required to define the mechanism by which different isoforms of apoE alter the plasma CETP response to cholesterol feeding. As E3/2 subjects exhibit delayed chylomicron remnant clearance and E4/3 individuals exhibit more rapid chylomicron clearance (8, 9), it is possible that the increased CETP response in E3/2 subjects may reflect greater delivery of chylomicron remnant cholesterol to peripheral cells, such as adipocytes, which are one source of plasma CETP.

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REFERENCES

1. Utermann, G., N. Pruin, and A. Steinmetz. 1979. Polymorphism of apoprotein E. III. Effect of a single polymorphic gene locus on plasma lipid levels in man. *Clin. Genet.* **15:** 63–72.
2. Robertson, F. W., and A. M. Cumming. 1985. Effects of apoprotein E polymorphism on serum lipoprotein concentrations. *Arteriosclerosis.* **5:** 283–292.
3. Manttari, M., P. Koskinen, C. Ehnholm, J. K. Huttunen,

and V. Manninem. 1991. Apolipoprotein E polymorphism influences the serum cholesterol response to dietary intervention. *Metabolism.* **40:** 271–221.
4. Gregg, R. E., L. A. Zech, E. J. Schaefer, D. Stark, D. Wilson, and H. B. Brewer. 1986. Abnormal in vivo metabolism of apolipoprotein E_4 in humans. *J. Clin. Invest.* **78:** 815–821.
5. Weisgraber, K. H. 1990. Apolipoprotein E distribution among human plasma lipoproteins: role of the cysteine–arginine interchange at residue 112. *J. Lipid Res.* **31:** 1503–1511.
6. Wilson, C., M. R. Wardell, K. H. Weisgraber, R. W. Mahley, and D. A. Agard. 1991. Three-dimensional structure of the LDL receptor-binding domain of human apolipoprotein E. *Science.* **252:** 1817–1822.
7. Havel, R. J., Y. Chao, E. Windler, L. Kotite, and L. Guo. 1980. Isoprotein specificity in the hepatic uptake of apolipoprotein E and the pathogenesis of familial dysbetalipoproteinemia. *Proc. Natl. Acad. Sci. USA.* **77:** 4349–4353.
8. Brenninkmeijer, B. J., M. J. Stuyt, P. N. M. Demacker, A. F. H. Stalenhoef, and A. van't Laar. 1987. Catabolism of chylomicron remnants in normolipidemic subjects in relation to the apoprotein E phenotype. *J. Lipid Res.* **28:** 361–370.
9. Weintraub, M. S., S. Eisenberg, and J. L. Breslow. 1987. Dietary fat clearance in normal subjects is regulated by genetic variation in apolipoprotein E. *J. Clin. Invest.* **80:** 1571–1577.
10. Demant, T., D. Bedford, C. Packard, and J. Shepherd. 1991. Influence of apolipoprotein E polymorphism on apolipoprotein B metabolism in normolipemic subjects. *J. Clin. Invest.* **88:** 1490–1501.
11. Kesaniemi, Y. A., C. Ehnholm, and T. A. Miettinen. 1987. Intestinal cholesterol absorption efficiency in man is related to apoprotein E phenotype. *J. Clin. Invest.* **80:** 578–581.
12. Barter, P. J., L. B. Chang, H. H. Newnham, K. A. Rye, and O. V. Rajaram. 1990. The interaction of cholesteryl ester transfer protein and unesterified fatty acids promotes a reduction in the particle size of high density lipoproteins. *Biochim. Biophys. Acta.* **1045:** 81–89.
13. Quinet, E., A. Tall, R. Ramakrishnan, and L. Rudel. 1991. Plasma lipid transfer protein as a determinant of the atherogenicity of monkey plasma lipoproteins. *J. Clin. Invest.* **87:** 1559–1566.
14. Jiang, X. C., P. Moulin, E. Quinet, I. J. Goldberg, L. K. Yacoub, L. B. Agellon, D. Compton, R. Schnitzer-Polokoff, and A. R. Tall. 1991. Mammalian adipose tissue and muscle are major sources of lipid transfer protein mRNA. *J. Biol. Chem.* **266:** 4631–4639.
15. Quinet, E. M., L. B. Agellon, P. A. Kroon, Y. L. Marcel, Y. C. Lee, M. E. Whitlock, and A. R. Tall. 1990. Atherogenic diet increases cholesteryl ester transfer protein (CETP) mRNA levels in rabbit liver. *J. Clin. Invest.* **85:** 357–363.
16. Health and Welfare Canada. 1988. Canadian Nutrient File. Bureau of Nutritional Sciences. Canadian Government Publishing Centre, Supply and Services Canada, Ottawa K1A 0S9.
17. Lipid Research Clinics Program. 1974. Manual of Laboratory Operation. Lipid and Lipoprotein Analysis. U.S. Government Printing Office, DHEW Publication (NIH) 75628. Bethesda, MD. 1–81.
18. Bury, J., and M. Rossenau. 1985. Quantification of human apolipoprotein A-I by enzyme immunoassay. *Clin. Chem.* **31:** 247–251.
19. Ordovas, J. M., J. P. Petersen, P. Santaniello, J. S. Cohn, P. W. F. Wilson, and E. J. Schaefer. 1987. Enzyme-linked immunosorbent assay for human plasma apolipoprotein B. *J. Lipid Res.* **28:** 1216–1224.
20. Milne, R. W., R. Theolis, R. Verdery, and Y. L. Marcel. 1983. Characterization of monoclonal antibodies against human low density lipoprotein. *Arteriosclerosis.* **3:** 23–30.
21. Marcel, Y. L., R. McPherson, M. Hogue, H. Czarnecka, Z. Zawadski, P. Weech, M. E. Whitlock, A. R. Tall, and R. W. Milne. 1990. Distribution and concentration of cholesteryl ester transfer protein in plasma of normolipemic subjects. *J. Clin. Invest.* **85:** 10–17.
22. McPherson, R., C. J. Mann, A. R. Tall, M. Hogue, L. Martin, R. W. Milne, and Y. L. Marcel. 1991. Plasma concentrations of cholesteryl ester transfer protein in hyperlipoproteinemia. Relationship to cholesteryl ester transfer protein activity and other lipoprotein variables. *Arteriosclerosis.* **11:** 797–804.
23. Parra, H. J., H. Mezdour, N. Ghalim, J. M. Bard, and J. C. Fruchart. 1990. Differential immunoassay of human LpA-I lipoprotein particles on ready-to-use plates. *Clin. Chem.* **36:** 1431–1435.
24. Ghiselli, G., R. E. Gregg, L. A. Zech, E. J. Schaefer, and H. B. Brewer. 1982. Phenotype study of apolipoprotein E isoforms in hyperlipoproteinemic patients. *Lancet.* **2:** 405–407.
25. Main, B. F., P. J. H. Jones, R. T. A. MacGillivray, and D. K. Banfield. 1991. Apolipoprotein E genotyping using the polymerase chain reaction and allele-specific oligonucleotide primers. *J. Lipid Res.* **32:** 183–187.
26. Chomzynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162:** 156–159.
27. Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, and M. R. Green. 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucleic Acids Res.* **12:** 7035–7056.
28. Snedecor, G. W., and W. G. Cochran. 1967. Statistical Methods. Iowa State University Press, Ames, IA.
29. 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.
30. McNamara, D. J., R. Kolb, T. S. Parker, H. Batwin, P. Samuel, C. D. Brown, and E. H. Ahrens. 1987. Heterogeneity of cholesterol homeostasis in man. Response to changes in dietary fat quality and cholesterol quantity. *J. Clin. Invest.* **79:** 1729–1739.
31. Mistry, P., N. Miller, M. Laker, W. Hazzard, and B. Lewis. 1981. Individual variation in the effects of dietary cholesterol on plasma lipoproteins and cellular cholesterol homeostasis in man. *J. Clin. Invest.* **67:** 493–502.
32. Packard, C. J., L. McKinney, K. Carr, and J. Shepherd. 1983. Cholesterol feeding increases low density lipoprotein synthesis. *J. Clin. Invest.* **72:** 45–51.
33. Ginsberg, H., N. A. Le, C. Mays, J. Gibson, and V. W. Brown. 1981. Lipoprotein metabolism in nonresponders to increased dietary cholesterol. *Arteriosclerosis.* **1:** 463–470.
34. Zanni, E. E., V. I. Zannis, C. B. Blum, P. N. Herbert, and J. L. Breslow. 1987. Effect of egg cholesterol and dietary fats on plasma lipids, lipoproteins, and apoproteins of normal women consuming natural diets. *J. Lipid Res.* **28:** 518–527.
35. Tikkanen, M. J., J. K. Huttunen, C. Ehnholm, and P. Pietinen. 1990. Apolipoprotein E4 homozygosity predisposes to serum cholesterol elevation during high fat diet. *Arteriosclerosis.* **10:** 285–288.

36. 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 apolipoprotein E phenotypes. *Am. J. Clin. Nutr.* **53:** 1191–1196.
37. 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.
38. Boerwinkle, E., S. A. Brown, K. Rohrback, A. M. Gotto, 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.
39. Katan, M. B., A. C. Beynen, J. H. M. DeVries, and A. Nobels. 1986. Existence of hypo- and hyperresponders to dietary cholesterol in man. *Am. J. Epidemiol.* **123:** 221–234.
40. Patsch, J. R., S. Prasad, A. M. Gotto, Jr., and G. Bengtsson-Olivecrona. 1984. Postprandial lipemia. A key for the conversion of high density lipoprotein$_2$ into high density lipoprotein$_3$ by hepatic lipase. *J. Clin. Invest.* **74:** 2017–2023.
41. Schonfeld, G., W. Patsch, L. L. Rudel, C. Nelson, M. Epstein, and R. Olson. 1982. Effects of dietary cholesterol and fatty acids on plasma lipoproteins. *J. Clin. Invest.* **69:** 1072–1080.
42. Cole, T. G., W. Patsch, I. Kuisk, B. Gonen, and G. Schonfeld. 1983. Increases in dietary cholesterol and fat raise levels of apoprotein E-containing lipoproteins in the plasma of man. *J. Clin. Endocrinol. Metab.* **56:** 1108–1115.
43. Tall, A. R. 1986. Plasma lipid transfer proteins. *J. Lipid Res.* **27:** 361–367.
44. Jiang, X. C., L. B. Agellon, A. M. Walsh, J. L. Breslow, and A. R. Tall. 1992. Dietary cholesterol increases transcription of the human cholesteryl ester transfer protein gene in transgeneic mice. *J. Clin. Invest.* **90:** 1290–1295.
45. Faust, R. A., J. H. Tollefson, A. Chait, and J. J. Albers. 1990. Regulation of LTP-I secretion from human monocyte-derived macrophages by differentiation and cholesterol accumulation in vitro. *Biochim. Biophys. Acta.* **1042:** 404–409.
46. Thuren, T., K. H. Weisgraber, P. Sisson, and M. Waite. 1992. Role of apolipoprotein E in hepatic lipase-catalyzed hydrolysis of phospholipid in high density lipoproteins. *Biochemistry.* **31:** 2332–2338.
47. Nishikawa, O., S. Yokoyama, H. Okabe, and A. Yamamoto. 1988. Enhancement of nonpolar lipid transfer reaction through stabilization of substrate particles with apolipoproteins. *J. Biochem.* **103:** 188–194.