Association between serum lipids and apolipoprotein E phenotype is influenced by diet in a population-based sample of free-living children and young adults: The Cardiovascular Risk in Young Finns Study

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Abstract Apolipoprotein E (apoE) is a genetic determinant of coronary heart disease and lipid levels in several populations. We studied whether the association of apoE alleles with serum lipids varies with diet in a population of free-living young Finns. One thousand twelve subjects, aged 9–24 years, were studied as a part of the Cardiovascular Risk in Young Finns Study in 1986. Serum lipid concentrations and apoE phenotypes were determined, and the composition of the diet was assessed by the 48-h recall method. The subjects were divided into three groups according to the intake of dietary saturated fatty acids (SAFA, g/1000 kcal) and cholesterol (mg/1000 kcal). Group one (high SAFA-cholesterol group) was formed from subjects belonging to the highest tertiles of both cholesterol and SAFA intakes (n = 175); group two (middle SAFA-cholesterol group) consisted of subjects belonging to the middle respective tertiles (n = 119); and group three (low SAFA-cholesterol group) consisted of subjects belonging to the lowest respective tertiles (n = 192). The statistical significance of the association of serum total cholesterol and low density lipoprotein (LDL) concentration with apoE phenotype increased from the low SAFA-cholesterol group (P = 0.024 for total cholesterol and P = 0.015 for LDL-cholesterol, respectively) to the high SAFA-cholesterol group (P = 0.0022 and P = 0.00073, respectively). The middle SAFA-cholesterol group fell between these two groups. The average serum cholesterol lowering effect of the $\epsilon 2$ allele in the low, middle, and high SAFA-cholesterol groups was; −0.22 mmol/l, −0.33 mmol/l, and −0.52 mmol/l, respectively, while the $\epsilon 4$ allele raised these levels by +0.27, +0.36, +0.52 mmol/l, respectively. The results for serum LDL-cholesterol were parallel to those of serum total cholesterol. The average effect of the $\epsilon 2$ allele on serum triglyceride was to raise its level in the subject belonging to low SAFA-cholesterol group only (P = 0.0008). The average effects of the $\epsilon 2$ and $\epsilon 4$ alleles on serum high density lipoprotein (HDL) cholesterol were to raise its concentration in the high SAFA-cholesterol group only (P = 0.013). Our results suggest that the effect of the apoE allele on serum total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglyceride is influenced by diet and provides further evidence that this is also true among free-living subjects consuming their ordinary diet.—**Lehtimäki, T., T. Moilanen, K. Porkka, H. K.** Åkerblom, T. Rönnemaa, L. Räsänen, J. Viikari, C. Ehnholm, and T. Nikkari. Association between serum lipids and apolipoprotein E phenotype is influenced by diet in a population-based sample of free-living children and young adults: The Cardiovascular Risk in Young Finns Study. *J. Lipid Res.* 1995. **36:** 653–661.

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Plasma lipoprotein concentrations are determined by genetic and environmental factors, such as diet (1–5). The apolipoprotein E (apoE) genetic polymorphism influences the coronary heart disease (CHD) risk factors, plasma total and low density lipoprotein (LDL) cholesterol concentrations (6–8). ApoE is a ligand for lipoprotein recep-

Abbreviations: SAFA, saturated fatty acids; IDL, intermediate density lipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; CHD, coronary heart disease.

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tors (6, 9–11), and its most important physiological function is probably to mediate specific uptake of plasma very low density lipoproteins (VLDL), chylomicron remnants, and intermediate density lipoproteins (IDL) by the liver (6). ApoE appears in three major isoforms, E2, E3, and E4, coded by three codominant alleles, $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$, respectively (12–16). These lead to six major apoE phenotypes; E2/2, E3/2, E4/2, E3/3, E4/3, and E4/4 (12, 17).

Atherosclerosis has its basis in childhood and early adulthood (18–20). The genetic influence of apoE phenotype on atherosclerosis is evident already in 15- to 30-year-old subjects whose serum cholesterol and the degree of atherosclerotic lesions in thoracic and abdominal aorta increases according to phenotypes E3/2 < E3/3 < E4/3 (21). An increased frequency of the E4 isoform has been reported in subjects with angiographically documented coronary artery disease or myocardial infarction (8, 22, 23). In most populations (7) the E4 isoform is associated with high serum total and LDL-cholesterol concentrations (13–16, 24, 25). Utermann (7) observed that the effect of the $\epsilon 4$ allele on serum total cholesterol differs among populations and hypothesized that these differences may be due to the different dietary habits (e.g., fat intake) of these populations. On the other hand, several studies have shown that dietary cholesterol is significantly associated with serum cholesterol concentrations in humans (5), although to a lesser degree than dietary saturated fatty acids (SAFA).

The associations of apolipoprotein E (apoE) phenotype and serum lipids during different dietary interventions have been intensively studied (26–35). Despite several intervention studies, there is still controversy as to whether the association of apoE phenotypes with serum lipids depends on the intake of dietary fats (26–35). This question has not been studied in representative population samples of free-living people consuming their ordinary diets.

The CHD mortality rate in Finnish middle-aged men is one of the highest in the world (36). It is also known that serum lipid levels measured in children and young adults predict CHD, total mortality, as well as mortality due to cardiovascular diseases in middle age (37). To investigate the CHD risk factor levels in Finnish children and adolescents, a comprehensive multicenter study was launched, with a base-line survey in 1980 and follow-ups in 1983 and 1986. The studies included a dietary interview, serum lipid determinations, and, in 1986, apoE phenotyping. The purpose of the present work was to test the association of apoE alleles with serum lipids in groups having different intakes of SAFA and dietary cholesterol in this population-based sample of free-living Finnish youths.

SUBJECTS AND METHODS

Subjects, sample selection, and ethics

The Cardiovascular Risk in Young Finns Study is a Finnish population-based follow-up survey to investigate atherosclerosis precursors in children and young adults. The primary cross-sectional study was performed in 1980 and follow-up studies in 1983 and 1986 in five university cities and their surrounding rural communities in Finland. For the cross-sectional study in 1980, a population sample of boys and girls with an equal distribution of all age groups of 3, 6, 9, 12, 15, and 18 years and of all study municipalities was randomly chosen from the national population register according to their unique social security identification numbers. The participation rate was 83.1% of those invited, resulting in a study population of 3,596 subjects in 1980 (38). All participants of the 1980 survey were invited to follow-up studies in 1983 and 1986 (39).

TABLE 1. Clinical characteristics of the subjects according to diet group

	Diet Group						
	Low SAFA-Chol (n = 192)		Middle SAFA-Chol (n = 119)		High SAFA-Chol (n = 175)		
	Mean	SD	Mean	SD	Mean	SD	ANOVA
Age (years)	14.86	4.75	15.71	4.92	15.82	5.08	$P = 0.665$
Percent males	43		46		60		$P = 0.004$[a]
Pubertal stage[b]	6.7	3.2	7.0	3.2	7.2	3.2	$P = 0.385$
Body mass index (kg/m^2)	19.6	3.3	19.9	3.5	20.2	3.5	$P = 0.175$
Subscapular skinfold (mm)	10.3	5.8	10.4	5.6	10.7	6.9	$P = 0.813$
Triceps skinfold (mm)	10.8	5.2	10.1	4.5	10.5	5.3	$P = 0.507$
Biceps skinfold (mm)	6.6	3.5	5.9	3.3	6.2	3.5	$P = 0.157$
Systolic blood pressure (mm Hg)	112.8	13.2	113.4	13.54	115.1	13.6	$P = 0.245$
Diastolic blood pressure (mm Hg)	65.5	9.2	63.7	11.2	64.9	10.2	$P = 0.276$

SAFA, saturated fatty acids; Chol, dietary cholesterol; n, number of subjects.
[a]Chi square test.
[b]Sum of Tanner scores (means).

From a sample collected in 1986, consisting of 1012 subjects, apoE phenotype as well as dietary and lipid data were available. The subsample represented all six age groups (9–24 years old in 1986), both sexes (489 males and 523 females), and all municipalities. The subjects were divided into tertiles based on the intake of dietary SAFA (g/1000 kcal) and cholesterol (mg/1000 kcal). Group one (high SAFA-cholesterol group) consisted of subjects belonging to the highest tertiles of dietary cholesterol and SAFA intake (n = 175), group two (middle SAFA-cholesterol group) of subjects belonging to the middle respective tertiles (n = 119), and group three (low SAFA-cholesterol group) of subjects belonging to the lowest respective tertiles of dietary cholesterol and SAFA intake (n = 192). After this classification a total of 486 subjects remained for the study. Some basic variables of the subjects are presented in **Table 1.** Methods and procedures used for measurement of weight, height, and thicknesses of triceps, biceps, and subscapular skinfolds (40) and blood pressure measurements (41) have been described previously. The stage of puberty was assessed according to Tanner scores (42). The study protocol was approved by the ethical committees of all the participating universities.

ApoE phenotyping and sample storage

Phenotyping was performed with slight modifications from the original method of Menzel and Utermann (43) using delipidated plasma, isoelectric focusing, cysteamine treatment, and immunoblotting as described in detail (24). For apoE phenotyping, venous blood samples were drawn after an overnight fast from the antecubital vein into tubes containing EDTA. Plasma samples were stored at −20°C for up to 20 months until phenotyped.

Lipid analyses and sample storage

Serum cholesterol (44) and triglyceride (45) were determined by enzymatic methods (Boehringer, Mannheim, Germany) using the OLLI 3000 automatic analyzer. HDL cholesterol was measured enzymatically from the serum supernatant after precipitation of LDL and VLDL with dextran sulfate 500 (Pharmacia, Sweden) and $MgCl_2$ (46). The intra-assay coefficients of variation (CVs) for the determinations of total cholesterol, HDL cholesterol, and triglyceride were 1.6%, 1.7%, and 2.6%, respectively. The interassay CVs were 2.2%, 3.8%, and 4.4%, respectively. LDL cholesterol level was calculated according to Friedewald, Levy, and Fredrickson (47). For the assays of serum lipid levels, blood was allowed to clot at room temperature for 1 h. Serum samples were stored at −25°C for a maximum of 14 months until analyzed.

Dietary survey

The dietary survey was carried out by trained interviewers using the 48-h recall method (48, 49). The children or their parents were asked in detail about the child's food consumption during the 2 days preceding the interview. The food composition data base used in the computing at the Division of Nutrition, University of Helsinki is based on Finnish food composition tables and analytical data from the local food industry, and supplemented by data from foreign food composition tables when no other data were available. In the present study the intake of nutrients was calculated per 1000 kcal (fatty acids and dietary cholesterol) or as percentage of total energy intake (fat, protein, carbohydrate, sucrose) in order to eliminate the effect of age on total food consumption and energy intake. The composition (and units) of the diet in the three SAFA-cholesterol groups is given in **Table 2.**

TABLE 2. Energy intake and composition of diet for subjects belonging to the different diet groups

	Diet Group						
	Low SAFA-Chol (n = 192)		Middle SAFA-Chol (n = 119)		High SAFA-Chol (n = 175)		
Dietary Component	Mean	SD	Mean	SD	Mean	SD	ANOVA
Total energy, kcal	2042	764	2347	882	2566	1039	$P < 0.0001$
Cholesterol, mg/1000 kcal	89.2	25.6	154.8	17.6	278.0	135.3	$P < 0.0001$
Saturated fatty acids, g/1000 kcal	14.8	3.2	20.8	1.3	27.9	3.6	$P < 0.0001$
Polyunsaturated fatty acids, g/1000 kcal	6.5	3.2	5.8	2.0	5.1	2.1	$P < 0.0001$
Monounsaturated fatty acids, g/1000 kcal	12.6	3.8	15.1	3.7	17.3	3.5	$P < 0.0001$
Fat, E %[a]	33.6	6.5	38.4	4.7	44.1	4.9	$P < 0.0001$
P/S ratio[b]	0.45	0.30	0.29	0.11	0.20	0.10	$P < 0.0001$
Protein, E %	13.6	2.5	15.2	2.2	14.6	2.2	$P < 0.0001$
Carbohydrate, E %	52.8	7.1	46.5	5.1	41.3	5.6	$P < 0.0001$
Sucrose, E %	14.2	8.3	11.2	5.4	9.4	4.6	$P < 0.0001$

SAFA, saturated fatty acids; Chol, dietary cholesterol; n, number of subjects.
[a]E % = percentage of total energy intake.
[b]The ratio of polyunsaturated and saturated fatty acids.

Statistical analysis

ApoE allele frequencies were calculated by the gene counting method. The lipid values of different groups formed according to the apoE phenotype and dietary SAFA-cholesterol intakes were compared with a two- and one-way analysis of variance (ANOVA). Due to the small sample sizes, males and females were analyzed together. All statistical analyses were done using the General Linear Models procedure of SAS (50). Due to the small number of the phenotype E2/2 (n = 3) in the total series, this phenotype was not included in the statistical analyses. The average effects (α) of the $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ alleles on different lipid levels were calculated as follows (51):

$$\alpha_2 = \frac{f_{22}\mu_{22} + \tfrac{1}{2}f_{23}\mu_{23} + \tfrac{1}{2}f_{24}\mu_{24}}{f_{\epsilon 2}} - \mu$$

$$\alpha_3 = \frac{f_{33}\mu_{33} + \tfrac{1}{2}f_{23}\mu_{23} + \tfrac{1}{2}f_{34}\mu_{34}}{f_{\epsilon 3}} - \mu$$

$$\alpha_4 = \frac{f_{44}\mu_{44} + \tfrac{1}{2}f_{24}\mu_{24} + \tfrac{1}{2}f_{34}\mu_{34}}{f_{\epsilon 4}} - \mu$$

Where f_{22}, f_{23}, etc., are the expected phenotype frequencies assuming Hardy-Weinberg equilibrium; $f_{\epsilon 2}$, $f_{\epsilon 3}$, and $f_{\epsilon 4}$ are the allele frequencies; $f\mu_{22}$, μ_{23}, etc., are the means for the phenotypes; and μ is the grand mean of the sample.

RESULTS

We studied the relations of the apoE phenotype and diet with serum lipids in a sample of 486 free-living subjects (243 males and 243 females) aged 9–24 years, divided into three groups according to their dietary intake of cholesterol and SAFA. Some clinical characteristics of the subjects are presented in Table 1. There were no statistically significant differences among the diet groups in body mass index (kg/m^2), weight, height, age, systolic or diastolic blood pressure, or thicknesses of the triceps, biceps, and subscapular skinfolds (Table 1). Composition of the diet in different diet groups is given in Table 2. The mean content of dietary cholesterol, SAFA, and energy intake from the dietary fats increased when moving from the low SAFA-cholesterol to high SAFA-cholesterol group, and conversely, the content of dietary polyunsaturated and monounsaturated fatty acids as well as that of carbohydrates decreased, respectively (Table 2).

The statistical significance of the association of serum total and LDL-cholesterol concentrations with apoE phenotype increased according to dietary SAFA-cholesterol group as follows: low SAFA-cholesterol ($P = 0.024$ for total cholesterol and $P = 0.015$ for LDL-cholesterol, respectively), middle SAFA-cholesterol ($P = 0.020$ for total cholesterol and $P = 0.012$ for LDL cholesterol, respectively), and high SAFA-cholesterol group ($P = 0.0022$ and $P = 0.00073$, respectively) (**Table 3** and **Table 4**). The mean serum concentrations of total and LDL-cholesterol varied significantly in all SAFA-cholesterol groups, but the stepwise increase in phenotype order of E3/2 < E4/2 < E3/3 < E4/3 < E4/4 was seen only in the SAFA-cholesterol groups of middle and high intakes of dietary SAFA and cholesterol (Tables 3 and 4). The average effects of apoE alleles on serum lipids in different SAFA-cholesterol groups are shown in **Table 5.** The serum cholesterol lowering effect of $\epsilon 2$ allele in the low, middle, and high SAFA-cholesterol groups was: −0.22 mmol/l, −0.33 mmol/l, and −0.52 mmol/l, respectively, while the $\epsilon 4$ allele increased these levels by +0.27, +0.36, and +0.52 mmol/l, respectively (Table 5). The results for serum LDL-cholesterol are parallel to those of serum total cholesterol (Table 5). The statistical significances of the

TABLE 3. Serum total cholesterol concentrations according to apoE phenotype and diet group

Diet Group	ApoE Phenotype E3/2	E4/2	E3/3	E4/3	E4/4	All Subjects	ANOVA
			mean ± SD				
Low SAFA-Chol	4.66 ± 0.80	3.89 ± 0.48	4.51 ± 0.79	4.99 ± 1.44	5.10 ± 0.87	4.68 ± 1.06	$P = 0.024$
n	11	4	110	60	7	192	
Middle SAFA-Chol	4.41 ± 0.80		4.62 ± 0.90	4.94 ± 0.94	6.09 ± 1.95	4.74 ± 0.96	$P = 0.020$
n	9		71	36	3	119	
High SAFA-Chol	4.39 ± 0.55	4.47 ± 0.81	4.82 ± 0.81	5.02 ± 0.75	5.96 ± 1.67	4.90 ± 0.86	$P = 0.0022$
n	9	3	103	53	7	175	
All subjects	4.50 ± 0.72	4.14 ± 0.66	4.65 ± 0.84	5.02 ± 0.75	5.96 ± 1.67	4.77 ± 0.97	$P < 0.001$
ANOVA, P value[a]	0.654	0.289	0.022	0.954	0.461	0.083	
n	29	7	284	149	17	486	

SAFA, saturated fatty acids; Chol, dietary cholesterol; n, number of subjects.
[a]Difference among dietary groups.

TABLE 4. Serum LDL-cholesterol concentrations according to apoE phenotype and dietary group

Diet Group	ApoE Phenotype E3/2	E4/2	E3/3	E4/3	E4/4	All Subjects	ANOVA
			mean ± SD				
Low SAFA-Chol	2.75 ± 0.77	1.98 ± 0.19	2.71 ± 0.71	3.15 ± 1.40	3.31 ± 0.78	2.86 ± 1.01	P = 0.015
n	11	4	110	60	7	192	
Middle SAFA-Chol	2.44 ± 0.63		2.81 ± 0.81	3.05 ± 0.87	4.18 ± 1.96	2.89 ± 0.88	P = 0.012
n	9		71	36	3	119	
High SAFA-Chol	2.43 ± 0.49	2.50 ± 0.53	2.97 ± 0.73	3.24 ± 0.68	3.73 ± 0.84	3.04 ± 0.74	P = 0.00073
n	9	3	103	53	7	175	
All subjects	2.56 ± 0.65	2.21 ± 0.43	2.83 ± 0.75	3.16 ± 1.6	3.64 ± 1.04	2.93 ± 0.89	P = NS
ANOVA, P value[a]	0.453	0.122	0.040	0.720	0.485	0.108	
n	29	7	284	149	17	486	

SAFA, saturated fatty acids; Chol, dietary cholesterol; n, number of subjects.
[a]Difference among dietary groups.

association of serum HDL-cholesterol and triglyceride concentrations with apoE phenotype also varied (but in opposite directions) according to SAFA-cholesterol group as follows; low SAFA-cholesterol (P = 0.878 for HDL cholesterol and P = 0.00081 for triglycerides), middle SAFA-cholesterol (P = 0.198 for HDL cholesterol and P = 0.230 for triglyceride), and high SAFA-cholesterol group (P = 0.013 for HDL cholesterol and P = 0.846 for triglycerides), in one-way ANOVA (Table 5). The association between apoE phenotypes and serum HDL-cholesterol and triglyceride concentrations is significantly influenced by the different SAFA-cholesterol categories (two-way ANOVA interaction between SAFA-cholesterol group and apoE phenotype: P = 0.05 for HDL cholesterol and P = 0.02 for triglycerides). The average effect of the ϵ2 allele was to raise serum triglyceride levels in the subject belonging to low SAFA-cholesterol group, while such an effect was not seen in middle and high SAFA-cholesterol groups. The alleles ϵ3 and ϵ4 had almost neutral effects on serum triglycerides in all fat groups (Table 5). The average effect of the ϵ2 and ϵ4 alleles was to raise serum HDL-cholesterol in the high SAFA-cholesterol group only.

DISCUSSION

This study demonstrates that the influence of apoE alleles on serum lipid concentrations varies according the consumption of SAFA and dietary cholesterol in a population-based sample of subjects consuming their ordinary diet. The results from some previous dietary intervention studies also suggest that there are differences in response of serum cholesterol to dietary fat and cholesterol between different apoE phenotypes (26–28, 34, 35, 52, 53). On the other hand, on the basis of some intervention studies, it remains unclear whether this kind of interaction between the intake of dietary fats and apoE phenotype on serum lipids exists (29–33, 54). These controversial results may be due to the heterogeneity of the various study populations and to small sample sizes, differences in the selection of subjects, age distributions, gender distributions, and diets. Due to the absence or combination of the rare phenotypes E2/2, E3/2, E4/2, and E4/4 in several intervention studies, it has not been possible to draw conclusions of the effects of these phenotypes on lipid metabolism. In the present study there were no significant age or pubertal differences among the dietary groups (Table 1), although there was a small difference in sex distribution among the dietary tertiles. However, the main results were similar in both sexes and therefore they were combined.

TABLE 5. Average effects of apoE alleles (mmol/l) in different dietary groups

	Apolipoprotein E Allele ϵ2	ϵ3	ϵ4	ANOVA
Total cholesterol				
Low SAFA-Chol	−0.22	−0.06	+0.27	P = 0.024
Middle SAFA-Chol	−0.33	−0.07	+0.36	P = 0.020
High SAFA-Chol	−0.52	−0.06	+0.52	P = 0.0022
LDL-cholesterol				
Low SAFA-Chol	−0.31	−0.06	+0.26	P = 0.015
Middle SAFA-Chol	−0.45	−0.05	+0.32	P = 0.012
High SAFA-Chol	−0.61	−0.04	+0.41	P = 0.00073
HDL-cholesterol				
Low SAFA-Chol	−0.07	+0.01	±0.00	P = 0.878
Middle SAFA-Chol	+0.17	−0.01	+0.04	P = 0.198
High SAFA-Chol	+0.14	−0.01	+0.11	P = 0.013
Triglycerides				
Low SAFA-Chol	+0.34	−0.02	−0.01	P = 0.0008
Middle SAFA-Chol	−0.09	+0.09	+0.11	P = 0.231
High SAFA-Chol	−0.09	+0.01	+0.02	P = 0.846

The average effects of apoE alleles on serum lipids were calculated according to the formula of Boerwinkle et al. (51) (see Statistical methods). SAFA, saturated fatty acids; Chol, dietary cholesterol.

Several mechanisms by which different isoforms of apoE influence plasma cholesterol concentrations have been described. ApoE phenotype has been reported to affect cholesterol absorption (55–58), endogenous cholesterol synthesis (55, 59–61), cholesterol elimination as bile acids (56, 57), removal of chylomicron remnants (62), conversion of IDL to VLDL (63, 64), LDL kinetics (64), hepatic clearance of dietary fat (65) and binding to lipoprotein receptors, which have all been reported to enhance lipoprotein metabolism (6).

These findings have lead to the assumption that apoE polymorphism may also influence the response of plasma lipids to dietary fat (8). It has been shown that reduction of dietary fat and cholesterol in humans causes a marked decrease in cholesterol absorption, increased cholesterol synthesis and LDL receptor activity, and reduced production rate of apoB and LDL cholesterol (57). In hamsters, the addition of dietary saturated fatty acids to the diet reduces cholesterol synthesis, suppresses receptor-dependent LDL transport, and increases LDL cholesterol production rate (66). In addition, in hamsters dietary saturated fatty acids tend to potentiate the suppressive effect of dietary cholesterol on cholesterol synthesis and decrease receptor-dependent LDL uptake into the liver (67, 68). A change from the low-fat, low-cholesterol diet to the high-fat and high-cholesterol diet might cause opposite changes in humans also. Interestingly, Miettinen et al. (57) found a significant correlation between dietary fat intake and cholesterol absorption efficiency ($r = 0.445$); this correlation was especially high in the apoE4 group ($r = 0.645$). This dietary fat-related reduction of cholesterol absorption may contribute to the findings that the differences in the serum cholesterol level caused by apoE phenotypes can disappear on a low-fat low-cholesterol diet (26, 27) and that the difference may not be detectable in populations habitually consuming diets low in fat and cholesterol (7). These studies together with our results (63–69) imply that the combined apoE-phenotype–dietary fat regulation of LDL receptor expression may be the mechanism explaining the various associations of apoE polymorphisms with serum lipids in different SAFA-cholesterol groups seen in the present study.

Previous data on the effects of dietary fat on the association between apoE phenotypes and serum lipids are mainly based on intervention studies with extraordinary, metabolically controlled diets. The present work is, to our knowledge, the first study demonstrating significant differences in the effects of apoE alleles on serum lipids in a representative population sample of free-living people consuming their habitual diets. Our study supports the hypothesis of Utermann (7) that the effect of $\epsilon 4$ allele on serum total cholesterol differs among populations and that these differences may be due to the different dietary habits of these populations.

Dietary recall methods covering 24 or 48 h have been shown to be suitable for assessing food consumption of groups of children and adolescents (70–73). The validity of the recall method in measuring the average intakes levels of groups is well established (72, 74). Even though there is a large intra-individual variation in dietary intake, the energy-adjusted intake of nutrients can be estimated fairly well using the recall method (75). The number of days required for the estimation of energy-adjusted mean intakes of an individual within $\pm$ 20% of the subject's usual intake (95% confidence limit) is only 2 to 4 days for protein, carbohydrate, total fat, and saturated fatty acids in the population of the present study (75). For other fatty acids, dietary cholesterol, and sugar, more days are needed. So, if there had been more representative information about dietary cholesterol from present children and young adults, the apoE–diet–lipid associations had probably been even stronger.

The difference in the intake of saturated fatty acid energy between low and high SAFA-cholesterol groups was 13.1%. Using the equation of Mensink and Katan (76) it would be predicted that this difference would lead to a difference of 0.73 mmol/l in serum total cholesterol. The difference in total cholesterol between the low and high SAFA-cholesterol group was 0.22 mmol/l in all subjects. In the apoE phenotype E3/2, E4/2, E3/3, E4/3, and E4/4 subgroups, the corresponding differences were 0.27, 0.58, 0.31, 0.03, and 0.86 mmol/l, respectively. In male subjects, Clifton and Nestle (77) have shown that age influences the response of plasma total cholesterol to dietary fat plus cholesterol. In their trial, the change in LDL cholesterol with the fat/cholesterol supplement was 0.16 mmol/l in those <50 and 0.54 mmol/l in those >50 years old. Because the baseline cholesterol levels are low in children and young adults, it is possible that their absolute changes in serum cholesterol (in mmol/l) are smaller during dietary intervention than in adults with higher baseline levels. The relative changes in serum cholesterol are, however, similar than in adults. Therefore, it is possible that the formula of Mensink and Katan (76) is not directly applicable in these young age cohorts. It is also important to note that the present study is not an intervention study and that the subjects in different SAFA-cholesterol groups are not the same.

Interest in the apoE polymorphism is in part due to the observations that apoE phenotypes are associated with different kinds of hyperlipidemias (12, 78–81). The E2 isoform is significantly more frequent in patients with hypertriglyceridemia and almost every patient with type III hyperlipidemia carries at least on $\epsilon 2$ allele (see ref. 8, 80, 81). However, in most population studies there are no consistent relationships between apoE phenotypes and serum triglycerides or HDL-cholesterol (see ref. 8). It is quite surprising, since apoE is mainly associated with plasma

triglyceride-rich lipoprotein fractions in the circulation and regulates the metabolism of these lipoproteins (6–8). In this study, the average effect of the ε2 allele was to raise serum triglyceride levels by +0.34 mmol/l in the subjects belonging to the low SAFA-cholesterol group, while such an effect was not seen in middle and high SAFA-cholesterol groups or in the whole population sample. This result raises the possibility that diet can be one confounding factor behind the negative association between apoE alleles and serum triglycerides, seen frequently in normal populations (8).

In summary, in the Finnish population, where the frequency of apoE4 isoform is high (14, 24) and the diet is rich in SAFA and cholesterol (48, 49), this physiological diet–gene interaction may be a significant inducer of hypercholesterolemia and subsequently affect the high incidence of CHD seen in this population (36). Therefore, in public health and dietetic implications it seems to be even more important in Finland than in countries with lower frequency of E4 to emphasize in dietary guidelines for the general public the need to decrease the consumption of high-fat milk products and butter as these products are the main sources of SAFA and dietary cholesterol in Finland.

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