Apolipoprotein E phenotypes in Finnish youths: a cross-sectional and 6-year follow-up study

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Abstract Apolipoprotein E (apoE) polymorphism is a genetic determinant of plasma lipid levels and of coronary heart disease (CHD) risk. We determined the apoE phenotypes and plasma lipid levels in 1577 youths aged 3 to 18 years in 1980. The subjects were randomly selected from five areas of Finland. ApoE phenotyping was performed directly from plasma by isoelectric focusing and immunoblotting. The apoE allele frequencies in the population sample were $\epsilon 2 = 0.039$, $\epsilon 3 = 0.767$, and $\epsilon 4 = 0.194$. There were no differences in the apoE phenotype distribution between East and West Finland or between sexes. The concentrations of serum total cholesterol, low density lipoprotein cholesterol, and apolipoprotein B increased with apoE phenotype in the order of E2/2, E3/2, E4/2, E3/3, E4/3, and E4/4. This increase was already seen in 3-year-old children; it was observed in both sexes, but was clearer in males than in females. The mean levels of high density lipoprotein (HDL) cholesterol, apolipoprotein A-I, triglyceride, Lp[a] lipoprotein, and the activity of lecithin:cholesterol acyltransferase did not differ between the apoE phenotypes. The observed differences in serum cholesterol remained fairly stable during the 6-year follow-up from 1980 to 1986, while the mean serum cholesterol concentration in the whole study population decreased by 6.3%. This study confirms the reported higher frequency of the $\epsilon 4$ allele in Finns as compared to most other populations; this may contribute to the high rates of CHD in Finland as compared to most other populations. The results do not, however, explain the higher rate of CHD in East Finland in comparison to the western part of the country. —**Lehtimäki, T., T. Moilanen, J. Viikari, H. K. Åkerblom, C. Ehnholm, T. Rönnemaa, J. Marniemi, G. Dahlen, and T. Nikkari.** Apolipoprotein E phenotypes in Finnish youths: a cross-sectional and 6-year follow-up study. *J. Lipid Res.* 1990. **31:** 487-495.

Supplementary key words apolipoprotein E alleles • plasma lipids • cholesterol • apolipoprotein B • isoelectric focusing • immunoblotting

Apolipoprotein E is a structural and functional constituent of plasma chylomicrons and very low density lipoproteins (VLDL) and their lipolytic degradation products, i.e., chylomicron remnants and intermediate density lipoproteins (IDL). ApoE is also found in some subfractions of HDL (1, 2). ApoE serves as a ligand for LDL- and apoE-receptors, thus allowing a specific uptake of its carrier lipoprotein particles by the liver (2–4).

In plasma, three major apoE isoforms, E2, E3, and E4, have been detected (5–11). These isoforms are coded by three alleles, $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$, respectively, at a single genetic locus (12). As a result, six apoE phenotypes are possible (6–11).

In several populations, including the Finns, the $\epsilon 4$ allele is associated with a high concentration of plasma total cholesterol, low density lipoprotein (LDL) cholesterol, and apolipoprotein B (apoB) (6–9, 13). High frequencies of apoE4 have also been reported in subjects with angiographically documented coronary artery disease or myocardial infarction (11, 14, 15), although some studies have failed to establish such an association (16).

Finland has one of the highest incidences of CHD in the world (17). The rate of CHD in eastern Finland is almost twice that in the western part of the country (18), and only a part of the difference can be explained on the basis of the classical risk factors (18). Multinational epidemiologic studies have demonstrated that total cholesterol levels in the blood of the children are positively correlated with the incidence of adult CHD (19, 20). The role of genetic factors in the high prevalance of CHD in Finland and its regional differences is poorly known.

To survey CHD risk factors in Finnish children, a multicenter study was launched in 1980, with follow-up studies in 1983 and 1986 (21). The study carried out in 1986 included an analysis of plasma apoE phenotypes and

Abbreviations: IDL, intermediate density lipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; CHD, coronary heart disease; LCAT, lecithin:cholesterol acyltransferase; IEF, isoelectric focusing; TBS, Tris-buffered saline; CV, coefficient of variation.

determinations of the concentrations of plasma total cholesterol, HDL-cholesterol, triglycerides, apolipoprotein A-I (apoA-I), apoB, Lp[a] lipoprotein, as well as the plasma lecithin:cholesterol acyltransferase (LCAT) activity. This study reports the results of the apoE phenotype analyses, carried out in 1577 children and young adults (aged 9–24 year in 1986), and the relationship of the apoE phenotype to some other parameters of lipid metabolism.

SUBJECTS AND METHODS

Subjects and sample collection

The basic cross-sectional study of the Multicentre Study on Atherosclerosis Precursors in Finnish Children and Adolescents was carried out in October–November 1980 in five university cities and their surrounding rural communities. The study included 3596 boys and girls in the age groups of 3, 6, 9, 12, 15, and 18 years, randomly chosen from the national population register with equal numbers of boys and girls in each of the six age groups. The population sample represented fairly well the whole country. A detailed description of the population sample is given in reference 21. The study protocol was approved by the ethical committees of the participating universities.

All children participating in the 1980 study were invited to the two follow-up studies in 1983 and 1986. Serum total cholesterol, HDL-cholesterol, and triglyceride were measured in all 3 studies. In 1986, apoE phenotype analyses were carried out in 1577 boys and girls aged 9, 12, 15, 18, 21, and 24 years. They were a random subsample of the children and adolescents participating in the 1986 follow-up (n = 2803), with roughly equal numbers of boys and girls in each of the six age groups representing all the study areas (5 urban; 12 rural).

Venous blood samples were drawn after an overnight (10–12 h) fast into tubes containing EDTA and plasma was separated after cooling by low-speed centrifugation. When serum samples were used, blood was allowed to clot at room temperature for 60 min before separation of serum. The samples were stored at −20°C for up to 20 months until analyzed.

ApoE phenotyping

ApoE phenotyping was performed at the Department of Biomedical Sciences, University of Tampere.

Sample delipidation. Ten μl plasma was delipidated with 2.5 ml ethanol diethyl ether 3:1 (vol/vol) for 24 h at −20°C. The precipitate was centrifuged and washed with 2.5 ml ether. The final precipitate was dissolved in 200 μl 0.1 mmol/l Tris-HCl, pH 10, containing 6 mol/l urea, 1% sodium decyl sulfate (Merck), and 1 μl β-mercaptoethanol (Fluka), and left for 0.5 h at +4°C before isoelectric focusing (IEF).

Cysteamine treatment. Ninety μl 0.1 mol/l Tris-HCl, pH 8.2, and 20 μl 10% cysteamine solution (Sigma cat. no. M-6500) were added to 10 μl plasma, incubated for 20 h at room temperature, and delipidated as above. The precipitate was solubilized without β-mercaptoethanol.

Isoelectric focusing. Analytical IEF was performed for 3.5 h at 750 volts at 4°C on 5% polyacrylamide gels (1.0 mm × 16 cm × 16 cm) containing 8 mol/l urea and 2% Ampholine, pH 4–6 (LKB, Sweden), using a Bio-Rad Protean II cell vertical electrophoresis unit, with 20 wells per gel. Ten-μl samples were pipetted into each gel slot and filled with 5% sucrose containing 25 μl/ml LKB Ampholine, pH 4–6. Sulfuric acid (0.2%) was used as the anodal solution and 0.4% ethylenediamine as the cathodal buffer. When cysteamine was used, the treated sample was pipetted next to the β-mercaptoethanol-treated sample.

Immunoblotting. After IEF the proteins were transferred to nitrocellulose membranes using an LKB 2117-2500 electroblotting unit. Transfer was achieved by 18 V/cm in 1 h at room temperature. A 20% (vol/vol) solution of methanol in 39 mmol/l glycine and 4.8 mmol/l Tris was used as a blotting buffer. After transfer, the nitrocellulose membrane was soaked overnight in 10 mmol/l Tris-HCl buffer, pH 7.4, containing 0.9% sodium chloride (TBS) and 3% gelatin-TBS. The nitrocellulose membrane was then incubated and gently agitated for 1.5 h at room temperature with (1:1000) polyclonal rabbit anti-human apoE antibody in 1% gelatin-TBS (6). After incubation, the blot was washed for 40 min in 100 ml TBS containing 25 μl Tween-20. The washed nitrocellulose paper was then incubated with IgG-horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibody (Bio-Rad cat. no. 170-6515) in a dilution of 1:2000 (vol/vol) in 1% gelatin-TBS for 2 h and washed as previously. ApoE isoforms were then visualized in a substrate solution containing 60 mg 4-chloro-1-naphthol reagent (Merck) in 20 ml cold (−20°C) methanol, 100 ml TBS, and 60 μl 30% hydrogen peroxide (6).

A blot containing the six different apoE phenotypes with cysteamine-treated duplicates is shown in **Fig. 1**. The recognition of apoE phenotypes in the blots was based on comparison with IEF patterns from known apoE phenotypes and with earlier published IEF patterns. The results were interpreted by two persons, with 97.5% concordance.

The rare phenotypes (E2/2, E3/2, and E4/4) were verified by another focusing using parallel β-mercaptoethanol- and cysteamine-treated samples as described above. The reliability of the method was confirmed by phenotyping 20 samples, also at the National Public Health Institute, Helsinki, with the same results.

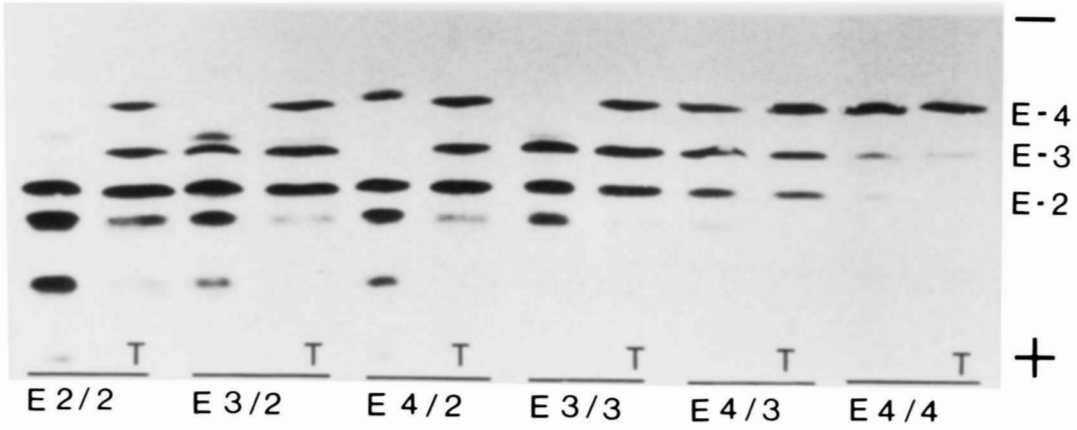

Fig. 1. ApoE phenotypes after isoelectric focusing of delipidated plasma, followed by immunoblotting. The untreated samples are to the left and the cysteamine-treated (T) to the right. The locations of the main apoE isoform bands are indicated. The cathode (–) is at the top and the anode (+) at the bottom.

Lipid and apolipoprotein analyses

The serum concentrations of lipids, apoA-I, apoB, and LCAT activity were measured in the laboratory of Rehabilitation Research Centre of the Social Insurance Institution, Turku. Serum cholesterol (22) and triglyceride (23) were determined by enzymatic methods (Boehringer, Mannheim, West Germany) using OLLI 3000 automatic analyzer. HDL-cholesterol was measured enzymatically from serum supernatant after precipitation of LDL and VLDL with dextran sulfate 500 (Pharmacia, Sweden) and $MgCl_2$ (24). The interassay coefficients of variation (CVs) of the determinations of total cholesterol, HDL-cholesterol, and triglycerides were 1.6%, 1.7%, and 2.6%, respectively. The day-to-day CVs were 2.2% for total cholesterol, 3.8% for HDL-cholesterol, and 4.4% for triglyceride. LDL-cholesterol was calculated according to Friedewald et al. (25). None of the subjects had serum triglycerides above 4 mmol/l making the calculation of LDL-cholesterol possible in all subjects.

Serum apolipoproteins A-I and B were determined by immunoturbidimetry (26). The interassay CVs of the determinations of apoA-I and apoB were 3.7% and 2.3%, respectively. The day-to-day CVs were 4.7% for apoA-I and 5.0% for apoB.

Serum LCAT activity was determined using exogenous substrate according to the method of Alcindor et al. (27) as previously described (28). The intra-assay CV of the method for LCAT determination was 8.8%. Lp[a] lipoprotein was determined by radioimmunoassay, according to the same principles as described for an immunoenzymatic assay (29), using research kits from Pharmacia Diagnostics, Uppsalä, Sweden. The detection limit of the Lp[a] lipoprotein method was 30 mg/l. Values under this limit were taken as 15 mg/l when mean Lp[a] lipoprotein concentration was calculated.

Statistical analysis

Statistical analysis was made using the BMDP software package (BMDP Statistical Software Inc., Los Angeles, CA). The lipid and apolipoprotein values of different apoE phenotypes were compared using one-way or two-way analysis of variance (ANOVA). The significance of differences in serum lipids and apolipoproteins between the different apoE phenotypes was examined with a modified *t*-test. The apoE allele frequencies were compared by a chi-square test. The variances of the apoE gene frequencies were calculated as described (6).

RESULTS

We determined the apoE phenotypes of 1577 children and adolescents (aged 9–24 years in 1986) from various areas in Finland. There were no east–west or other regional differences in apoE phenotype or allele distributions (**Table 1**). The apoE gene frequencies observed in the whole population sample were $\epsilon 2 = 0.039$, $\epsilon 3 = 0.767$, and $\epsilon 4 = 0.194$.

Serum lipid and apolipoprotein concentrations and LCAT activity are given in **Table 2**. The mean concentrations of total cholesterol, LDL-cholesterol, and apoB were highest in the E4/4 homozygotes. The lowest mean values of total and LDL-cholesterol were seen in the small group of E2/2 homozygotes. The difference in serum total and LDL-cholesterol concentrations between the two extremes, E2 and E4 homozygotes, was 1.06 and 1.07 mmol/l, respectively. **Table 3** gives the results for the children who were 3 years old in 1980 and whose apoE phenotypes became available in 1986. Already in these 3-year-old children, LDL- and total cholesterol values increased with the apoE phenotype in the order E3/2, E3/3,

TABLE 1. ApoE phenotype and allele frequencies (± SD) in Finnish children and adolescents

	West[a]		East[b]		Whole Country	
Phenotype	N	%	N	%	N	%
E2/2	2	0.4	0	0.0	5	0.3
E3/2	28	5.8	41	5.6	85	5.4
E4/2	12	2.5	11	1.5	28	1.8
E3/3	277	57.4	438	60.1	926	58.7
E4/3	144	30.0	221	30.3	483	30.6
E4/4	19	3.9	18	2.5	50	3.2
Total	482		729		1577	
Allele frequencies						
$\epsilon 2$	0.045 ± 0.0173		0.035 ± 0.0140		0.039 ± 0.0096	
$\epsilon 3$	0.753 ± 0.0193		0.781 ± 0.0156		0.767 ± 0.0106	
$\epsilon 4$	0.202 ± 0.0134		0.184 ± 0.0105		0.194 ± 0.0074	

Differences between East and West Finland were not statistically significant by chi-square test.
[a]West, areas of Tampere and Turku.
[b]East, areas of Kuopio and Oulu. Helsinki was excluded from the comparison because of its genetically mixed population.

E4/3, and E4/4. Mean HDL-cholesterol values were similar in all apoE phenotypes, resulting in a clearly lower HDL/total cholesterol ratio in E4-containing phenotypes. No differences among the phenotypes were present in triglyceride, Lp[a] lipoprotein, and apoA-I concentrations or LCAT activity (Table 2).

There were no differences in apoE allele or phenotype distributions between female and male subjects (data not shown). The plasma lipids and apoA-I and apoB concentrations according to sex are presented in **Table 4**. The differences between different apoE phenotypes in serum total cholesterol, LDL-cholesterol, and apoB concentra-

TABLE 2. Serum lipids, apoA-I, apoB, Lp[a], and LCAT activity in children and adolescents according to apoE phenotypes

	ApoE Phenotype						
Variable	E2/2	E3/2	E4/2	E3/3	E4/3	E4/4	Total
				mmol/l ± SD			
Total cholesterol[a]	4.00 ± 1.27	4.37 ± 0.71[d]	4.46 ± 0.83	4.75 ± 0.91	4.95 ± 0.98[d]	5.06 ± 1.18[b]	4.80 ± 0.95
N =	(5)	(85)	(27)	(924)	(482)	(49)	(1572)
LDL-cholesterol[a]	2.12 ± 1.14[b]	2.48 ± 0.65[d]	2.53 ± 0.69[b]	2.92 ± 0.83	3.12 ± 0.90[d]	3.19 ± 0.98[b]	2.96 ± 0.86
N =	(5)	(85)	(27)	(918)	(479)	(49)	(1563)
HDL-cholesterol	1.54 ± 0.15	1.47 ± 0.27	1.56 ± 0.37	1.45 ± 0.26	1.43 ± 0.26	1.44 ± 0.43	1.44 ± 0.28
N =	(5)	(85)	(28)	(926)	(483)	(50)	(1577)
HDL/chol-ratio[a]	0.41 ± 0.12[d]	0.34 ± 0.07[d]	0.35 ± 0.06[c]	0.31 ± 0.06	0.30 ± 0.06[d]	0.30 ± 0.09	0.31 ± 0.07
N =	(5)	(85)	(27)	(921)	(481)	(49)	(1568)
Triglyceride	0.74 ± 0.20	0.91 ± 0.44	0.84 ± 0.38	0.85 ± 0.37	0.90 ± 0.47	0.91 ± 0.34	0.87 ± 0.41
N =	(5)	(85)	(28)	(926)	(482)	(50)	(1576)
ApoA-I, mg/dl	172.0 ± 0.00	149.2 ± 0.20	145.4 ± 0.17	149.0 ± 0.23	148.3 ± 0.24	144.8 ± 0.19	148.3 ± 0.23
N =	(1)	(37)	(14)	(419)	(193)	(24)	(688)
ApoB, mg/dl[a]	96.0 ± 0.00	77.8 ± 20.9[b]	83.9 ± 23.8	87.1 ± 23.4	94.2 ± 26.3[d]	97.8 ± 24.5[b]	88.9 ± 0.25
N =	(1)	(37)	(14)	(419)	(191)	(24)	(686)
Lp[a], mg/dl	50.4 ± 42.3	106.2 ± 155.5	112.0 ± 170.0	102.1 ± 133.9	108.0 ± 144.5	102.4 ± 162.5	104.2 ± 139.8
N =	(3)	(48)	(18)	(611)	(312)	(30)	(1022)
LCAT[e]		22.22 ± 3.7	21.1 ± 3.6	21.6 ± 4.3	21.4 ± 3.7	21.5 ± 4.5	21.6 ± 4.1
N =		(35)	(13)	(385)	(168)	(24)	(625)

[a]There was a significant ($P < 0.001$) variation between the different phenotypic groups in one-dimensional analysis of variance.
[b]$P < 0.05$; [c]$P < 0.01$; [d]$P < 0.001$: the significance of difference between the phenotypic group in comparison to group E3/3 by modified *t*-test.
[e]Percent cholesterol esterified/h.

TABLE 3. Serum LDL- and total cholesterol in 1980 in 3-year-old children with different apoE phenotypes

Variable	ApoE Phenotype E3/2	E3/3	E4/3	E4/4	Total
			mmol/l ± SD		
LDL-cholesterol[a]	2.56 ± 0.71	3.23 ± 0.64	3.56 ± 0.69	3.71 ± 0.65	3.30 ± 0.72
N =	(20)	(148)	(81)	(7)	(259)
Total cholesterol[a]	4.26 ± 0.69	4.99 ± 0.72	5.30 ± 0.70	5.48 ± 0.71	5.05 ± 0.77
N =	(20)	(148)	(82)	(7)	(260)

[a]There was a significant ($P < 0.001$) variation between the different phenotype groups in one-way analysis of variance.

tions were clearer in male than in female subjects. No differences between the phenotypes or sexes were present in triglyceride concentrations (data not shown). The distributions of lipoproteins between various age groups will be reported in detail later.

When subjects in each of the phenotype groups were divided into four strata formed using cut-off points between LDL-cholesterol quartiles of the whole study population, the percentage distribution of subjects belonging to the LDL-cholesterol quartiles within the different apoE phe-

TABLE 4. Serum lipids, apoA-I, and apoB in female and male subjects according to apoE phenotype

	ApoE Phenotype E2/2	E3/2	E4/2	E3/3	E4/3	E4/4
			mean ± SD			
Total chol. (mmol/l)[a,b]						
Females	4.26 ± 1.18	4.55 ± 0.67	4.81 ± 0.86	4.85 ± 0.91	5.16 ± 1.08	5.13 ± 1.10
N =	(2)	(48)	(14)	(475)	(251)	(30)
Males	3.83 ± 1.55	4.13 ± 0.70	4.08 ± 0.64	4.65 ± 0.91	4.73 ± 0.80	4.94 ± 1.32
N =	(3)	(37)	(13)	(449)	(231)	(19)
LDL-chol. (mmol/l)[a]						
Females	2.25 ± 1.07	2.59 ± 0.63	2.73 ± 0.81	2.98 ± 0.82	3.27 ± 1.00	3.16 ± 0.83
N =	(2)	(48)	(14)	(472)	(251)	(30)
Males	2.04 ± 1.41	2.34 ± 0.66	2.32 ± 0.48	2.87 ± 0.84	2.97 ± 0.74	3.24 ± 1.20
N =	(3)	(37)	(13)	(446)	(228)	(19)
HDL-chol. (mmol/l)[c]						
Females	1.70 ± 0.08	1.54 ± 0.23	1.65 ± 0.34	1.49 ± 0.25	1.49 ± 0.25	1.54 ± 0.51
N =	(2)	(48)	(14)	(476)	(252)	(30)
Males	1.44 ± 0.05	1.38 ± 0.31	1.47 ± 0.40	1.40 ± 0.27	1.36 ± 0.25	1.30 ± 0.18
N =	(3)	(37)	(14)	(450)	(231)	(20)
HDL/Total chol-ratio[a]						
Females	0.41 ± 0.10	0.35 ± 0.06	0.35 ± 0.07	0.31 ± 0.06	0.30 ± 0.06	0.30 ± 0.07
N =	(2)	(48)	(14)	(474)	(251)	(30)
Males	0.42 ± 0.16	0.34 ± 0.08	0.35 ± 0.06	0.31 ± 0.07	0.29 ± 0.06	0.29 ± 0.12
N =	(3)	(37)	(13)	(447)	(230)	(19)
ApoB (mg/dl)[a,d]						
Females		80.0 ± 22.0	91.0 ± 26.0	90.0 ± 24.0	99.0 ± 29.0	91.0 ± 21.0
N =	(0)	(22)	(9)	(208)	(94)	(11)
Males	96.0 ± 0.00	75.0 ± 20.0	71.0 ± 14.0	84.0 ± 23.0	89.0 ± 23.0	104.0 ± 26.0
N =	(1)	(15)	(5)	(211)	(97)	(13)
ApoA-I (mg/dl)[c,d]						
Females		156.0 ± 17.0	148.0 ± 19.0	153.0 ± 23.0	156.0 ± 25.0	148.0 ± 19.0
N =	(0)	(22)	(9)	(209)	(94)	(11)
Males	172.0 ± 0.00	139.0 ± 21.0	140.0 ± 12.0	144.0 ± 22.0	141.0 ± 20.0	141.0 ± 19.0
N =	(1)	(15)	(5)	(210)	(99)	(13)

Two-way analysis of variance performed using the six phenotypic groups as a first and sex groups as a second dimension.
[a]There was a significant ($P < 0.001$) variation between the different phenotypic groups.
[b]$P < 0.01$, [c]$P < 0.001$, the significance of difference between the sex groups.
[d]Analysis performed without apoE2/2 phenotype group.

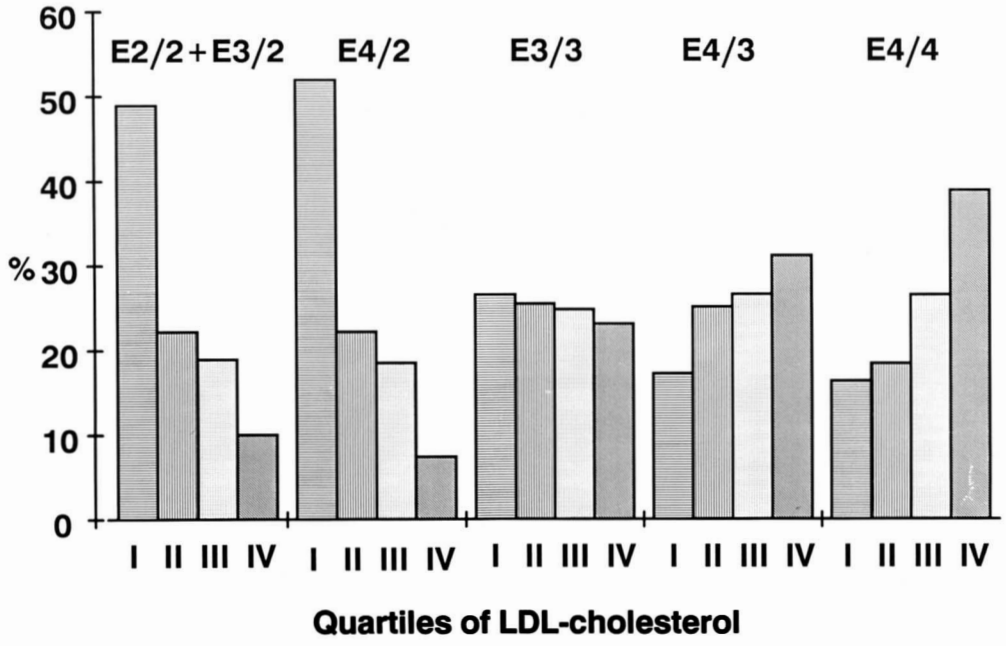

Fig. 2. Percentage distribution of subjects belonging to serum LDL-cholesterol quartiles within apoE phenotype groups E4/4, E4/3, E3/3, E4/2 and E3/2 or E2/2. LDL-cholesterol values in the quartiles formed according to the whole study population were: I < 2.40 mmol/l, II 2.40–2.89 mmol/l, III 2.89–3.40 mmol/l, IV > 3.40 mmol/l.

notypes was significantly different ($P < 0.001$ in chi-square test) (**Fig. 2**). Forty eight% of the subjects with E3/2 phenotype (n = 85) and 52% of those with E4/2 phenotype (n = 27) belonged to the lowest (<2.40 mmol/l) LDL-cholesterol quartile and 8.2% and 7.4%, respectively, to the highest (>3.40 mmol/l) quartile. Of all apoE4/4 phenotype subjects (n = 49), 37% belonged to the highest (>3.40 mmol/l) LDL-cholesterol quartile.

The between-phenotype differences in total and LDL-cholesterol concentrations remained remarkably stable during the 6-year follow-up from 1980 to 1986, although there was a 6.3% decrease in the mean total cholesterol level during this time period (**Table 5**). The relative changes (%) in serum total and LDL-cholesterol from

TABLE 5. Six-year follow-up of serum LDL- and total cholesterol in children and adolescents with different apolipoprotein E phenotypes

	ApoE Phenotype						
	E2/2	E3/2	E4/2	E3/3	E4/3	E4/4	Total
				mmol/l ± SD			
LDL-cholesterol							
1980[a]	2.09 ± 0.65	2.71 ± 0.58	3.02 ± 0.62	3.23 ± 0.78	3.47 ± 0.76	3.60 ± 0.81	3.28 ± 0.79
N =	(5)	(85)	(27)	(909)	(470)	(49)	(1545)
1986[a]	2.12 ± 1.13	2.48 ± 0.65	2.53 ± 0.69	2.92 ± 0.83	3.12 ± 0.90	3.19 ± 0.98	2.96 ± 0.86
N =	(5)	(85)	(27)	(909)	(470)	(49)	(1545)
% Change[b]	+1.44	−8.49	−16.2	−9.60	−10.10	−11.39	−9.76
Total cholesterol							
1980[a]	4.12 ± 0.81	4.57 ± 0.68	4.98 ± 0.64	5.05 ± 0.86	5.28 ± 0.82	5.44 ± 0.88	5.10 ± 0.85
N =	(5)	(81)	(26)	(861)	(443)	(43)	(1459)
1983[a]	4.10 ± 1.32	4.52 ± 0.75	4.91 ± 0.78	5.00 ± 0.89	5.16 ± 0.91	5.34 ± 0.91	5.02 ± 0.90
N =	(5)	(81)	(26)	(861)	(443)	(43)	(1459)
1986[a]	4.00 ± 1.27	4.38 ± 0.71	4.44 ± 0.85	4.74 ± 0.90	4.95 ± 0.99	5.06 ± 1.21	4.78 ± 0.94
N =	(5)	(81)	(26)	(861)	(443)	(43)	(1459)
% Change[b]	−2.91	−4.16	−10.84	−6.14	−6.25	−6.99	−6.27

[a]There was a significant ($P < 0.001$) variation between the different phenotype groups in one-way analysis of variance.
[b]Change in serum LDL- or total cholesterol from 1980 to 1986; +, increase; −, decrease.

year 1980 to 1986 were greatest in the apoE4/2 phenotype subjects (−10.8% and −16.2%), respectively (Table 5). Serum LDL-cholesterol increased by 1.44% in the group of E2/2, whereas in the other apoE phenotypes LDL-cholesterol decreased by 8.49–16.2% during these 6 years (Table 5). ApoE4/2 (n = 27) and E2/2 (n = 5) were not distributed equally in different age groups. A total of 179 children went through puberty during the 6-year follow-up.

DISCUSSION

Our results confirm earlier finding by Ehnholm et al. (6) of a higher frequency of ε4 and a lower one of ε2 among Finns as compared to frequencies published from other populations (**Table 6**). The present analysis was carried out in a randomly selected and regionally representative sample of 1577 Finnish youths, and can thus be considered reliable. The results also confirm the connection of E4 with high and of E2 with low total and LDL-cholesterol levels, shown earlier in several studies (7, 8). This is the first study to demonstrate that this association can be seen already among unselected normolipidemic children and adolescents.

The amino acid differences in the main apoE isoproteins have been characterized (2, 30), and these are responsible for the markedly lower affinity of apoE2 to apoE binding receptors as compared to that of E3 and E4 (31). In several populations the ε2 allele is associated with low plasma total cholesterol, LDL-cholesterol, and apoB and the ε4 allele with high plasma total cholesterol, LDL-cholesterol, and apoB levels (6–8). Our results explain, in part, the high mean cholesterol levels prevailing in Finns (32).

Wynder et al. (19) studied total and HDL-cholesterol levels in 5331 13-year-old children from 13 countries. The highest total cholesterol levels were observed in children from Norway and Finland. Observed cholesterol patterns tended to reflect the CHD mortality rates in these countries, with the highest cholesterol levels in children from countries with the highest CHD mortality.

The hypothesis that the ε4 allele favors and the ε2 allele protects against the development of atherosclerosis has been raised based on clinical studies of survivors of myocardial infarction (10, 16), of patients with angiographically documented CHD (10, 14, 15), and octogenarians (33). The results of an association of apoE phenotype with the occurrence of CHD are conflicting at present (10, 11, 14–16), even though some studies suggest that E3/2 phenotype is less frequently seen in CHD patients than in healthy controls (10). The high frequency of E4 and the low E2 frequency in the Finnish population on one hand, and the exceptionally high rate of CHD in Finns on the other hand, support the concept that CHD is associated with apoE phenotype and imply that genetic factors contribute to the high rate of CHD in Finland.

Despite the declining trend in CHD mortality in Finland during the last 15 years, the difference in CHD mortality between East and West Finland has persisted (18). According to the immigration theory, eastern and western Finns are assumed to have different origins (34). However, there were no regional differences in apoE phenotype or allele distribution in Finland. We therefore concluded that other factors, genetic or environmental, must exist which can explain the east-west difference in CHD and also in serum total cholesterol, demonstrated in several studies (35).

TABLE 6. Apolipoprotein E gene frequencies (± SD) in different populations

Country	Allele ε2	Allele ε3	Allele ε4	N	Reference
Finland	0.039 ± 0.0096	0.767 ± 0.0106	0.194 ± 0.0074	1577	this study
Finland	0.041 ± 0.0151	0.733 ± 0.0170	0.227 ± 0.0125	615	(6)
France[a]	0.123 ± 0.0202	0.751 ± 0.0217	0.126 ± 0.0155	434	(33)
Germany[a]	0.077 ± 0.0126	0.773 ± 0.0137	0.150 ± 0.0081	1031	(41)
Germany[a]	0.078 ± 0.0128	0.783 ± 0.0139	0.139 ± 0.0080	1000	(10)
Scotland[a]	0.080 ± 0.0202	0.770 ± 0.0220	0.150 ± 0.0130	400	(11)
USA[a]	0.110 ± 0.0521	0.720 ± 0.0567	0.170 ± 0.0352	61	(12)
USA[a]	0.075 ± 0.0116	0.786 ± 0.0126	0.135 ± 0.0071	1209	(13)
Canada[a]	0.078 ± 0.0397	0.770 ± 0.0434	0.152 ± 0.0259	102	(9)
New Zealand[a]	0.120 ± 0.0200	0.720 ± 0.0216	0.160 ± 0.0130	426	(42)
Singapore[a]	0.122 ± 0.0314	0.782 ± 0.0333	0.096 ± 0.0155	188	(8)
Japan[a]	0.038 ± 0.0409	0.853 ± 0.0442	0.109 ± 0.0234	92	(7)
Japan[a]	0.081 ± 0.0243	0.849 ± 0.0255	0.067 ± 0.0100	319	(8)

[a]The frequency distribution of the present study differed from those cited at the level $P < 0.001$ by chi-square test.

There was a decline in cholesterol levels in the study population during the follow-up from 1980 to 1986. The change is considered to be caused mainly by hormonal changes during puberty. Similar changes have also been described in several other studies (36–38). Part of the decline in serum cholesterol in this study group may also have been due to decreasing dietary intake of saturated fat while the total fat intake remained at 38% of energy (39). A corresponding decreasing trend in serum total cholesterol has been reported in the adult Finnish population as well (40). The slightly greater magnitude of cholesterol decline in E4-containing phenotypes (Table 5) suggests a different response of these phenotypes to dietary or to hormonal changes during puberty.

In conclusion, we have confirmed an internationally high frequency of apoE4 and a low frequency of apoE2 genes in Finns. The results do not, however, explain the higher rate of CHD in East Finland in comparison to the western part of the country. Also, an association of apoE polymorphism with serum cholesterol levels was demonstrated in unselected children and adolescents. This association persisted over a 6-year follow-up.

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