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Apolipoprotein E polymorphism and changes in serum lipids during a family-based counselling intervention

Marika Salminen[1,2,*], Terho Lehtimäki[3], Yue-Mei Fan[3], Tero Vahlberg[4] and Sirkka-Liisa Kivelä[1,5,6]

[1]Institute of Clinical Medicine, Family Medicine, Lemminkäisenkatu 1, FI-20014 University of Turku, Turku, Finland: [2]Härkätie Health Centre, Lieto, Finland: [3]Laboratory of Atherosclerosis Genetics, Department of Clinical Chemistry, Centre for Laboratory Medicine, Tampere University Hospital and University of Tampere Medical School, Tampere, Finland: [4]Institute of Clinical Medicine, Biostatistics, University of Turku, Turku, Finland: [5]Satakunta Central Hospital, Pori, Finland: [6]Unit of Family Medicine, Turku University Hospital, Turku, Finland

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Abstract

Objective: To compare serum lipids and their changes during a family-based health education in children aged 6–17 years with or without the ϵ4 allele of the gene encoding apolipoprotein E (apoE).

Design: An intervention study.

Setting: A family-based prevention of risk factors of coronary heart disease in Eastern Finland. The programme consisted of two counselling meetings at children's schools and three at children's homes.

Subjects: Four hundred and thirty-nine children with a family history of cardiovascular diseases (CVD) participated in a family-based health education. The children were divided into two groups according to *apoE* genotype. The risk group consisted of 143 children having *apoE* ϵ4 allele (genotype ϵ3/4 or ϵ4/4) and the non-risk group of 296 children without *apoE* ϵ4 allele (ϵ2/3 or ϵ3/3). The final sample of the follow-up study included 354 (81%) children (114 and 240, respectively).

Results: Baseline differences were found in low-density lipoprotein cholesterol (LDL-C) ($P = 0.007$) and LDL-C/high-density lipoprotein cholesterol (HDL-C) ratio ($P = 0.030$) among boys and in total cholesterol (TC)/HDL-C ($P = 0.008$) and LDL-C/HDL-C ratios ($P = 0.006$) among girls. Differences between groups in changes during the follow-up were observed only for TC/HDL-C ratio (*P*-value adjusted for age = 0.049) among boys.

Conclusions: At baseline, children with *apoE* ϵ4 allele had on average a more unfavourable lipid profile than those without *apoE* ϵ4 allele. However, the effect of about 33 months' family-based health education on plasma lipids did not depend on *apoE* genotype in children with a family history of CVD.

Keywords
Apolipoprotein E
Cholesterol
Children
Diet
Intervention

Evidence is accumulating to indicate that responses to dietary interventions are at least partly under genetic control[1–4]. One of the most widely investigated candidates is polymorphism in the gene encoding apolipoprotein E (apoE), which has been shown to affect cholesterol absorption from the intestine. Subjects carrying *apoE* ϵ4 allele have higher cholesterol absorption efficiency than non-carriers[5]. The response of plasma lipids to diet depends on *apoE* genotype[1–4]. There is an association between specific alleles of *apoE* gene and the lipid-lowering effect of statins[6–8]. These findings suggest that *apoE* genotype may affect the response of plasma lipids during a family-based health education/counselling intervention among children belonging to families with a history of early-onset cardiovascular diseases (CVD).

Fewer studies about the associations between *apoE* polymorphism and the response of serum cholesterol have been made among children. In infancy, between the age of 7 and 13 months, a diet with reduced saturated fat and cholesterol contents effectively reduces age-associated increases in serum total cholesterol and non-high-density lipoprotein cholesterol concentration independently of *apoE* genotype[9]. Plant stanol esters reduce serum cholesterol concentration in healthy 6-year-old children irrespective of their gender or apoE4 phenotype[10].

The purpose of the present study was to analyse whether serum lipids and their responses during a family-based health education/counselling intervention (about 33 months) are associated with genetic variation at the *apoE* locus among children and adolescents (6 to 17 years of age) with a family history of CVD.

*Corresponding author: Email majosa@utu.fi

Methods

Subjects

The intervention group of children and adolescents for the family-based health education/counselling was selected from the population aged 6 to 17 years living in Kainuu (10 municipalities in Eastern Finland) and having a family history of early-onset (first attack before the age of 55 years among men and 65 years among women) coronary heart disease (CHD), myocardial infarction (MI) or brain infarction (BI) in their parents or grandparents, or having a family history of hypercholesterolaemia (FH) (high-risk families). The parents, sisters and brothers of children in the intervention group also participated in the health counselling.

The names of adult residents in Kainuu having had early-onset MI, CHD or BI or a family history of FH during 1987–1995 were collected from hospital discharge registers (*International Classification of Diseases*, 9th revision; codes 272, 410–414)[11] (Fig. 1). They were informed by letter about the project and asked to report the names and addresses of their children and grandchildren aged 6–17 years living in Kainuu. The names and addresses of 600 children were received.

Participation was voluntary and written informed consent was given by the children themselves (15 years or over) or by their parents (under 15 years). The Ethical Committee of Oulu University approved the plan.

Basic examinations were carried out from September 1997 to May 1998. Two trained public health nurses took measurements of blood pressure, triceps skinfold thickness, height and weight. With the help of the parents, all participating children filled out structured questionnaires pertaining to diet and nutrition, exercise and other health issues. Answers were complemented with interviews carried out by the public health nurses of Kainuu Heart Association, and the interview included questions on smoking and the use of alcohol and drugs.

DNA extraction and apoE genotyping

DNA was extracted from peripheral blood leucocytes using a commercially available kit (Qiagen Inc., Valencia, CA, USA). *ApoE* genotypes were determined by polymerase chain reaction and restriction enzyme digestion[12].

ApoE genotype of 439 children was determined. The children were divided into two groups according to *apoE* genotype: *apoE* ε4 allele carriers (ε3/4 or ε4/4, $n = 143$) (71 boys and 72 girls) and *apoE* ε4 allele non-carriers (ε2/3 or ε3/3, $n = 296$) (150 boys and 146 girls). *ApoE* genotypes ε2/2 and ε2/4 were not found. In this study, children with *apoE* ε4 allele are called the risk group (RG) and those without *apoE* ε4 allele the non-risk group (NRG) (Fig. 2). The genotype analyses were carried out at the Department of Clinical Chemistry at the University of Tampere and Tampere University Hospital (Laboratory of Atherosclerosis Genetics).

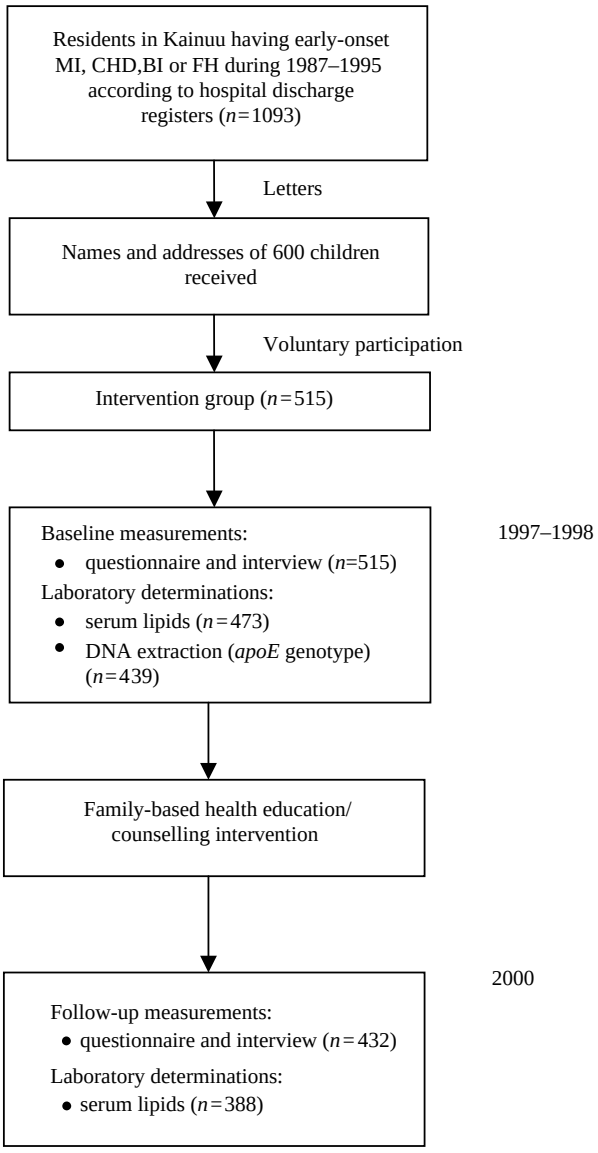

Fig. 1 Flow chart of the intervention. MI – myocardial infarction; CHD – coronary heart disease; BI – brain infarction; FH – familial hypercholesterolaemia; *apoE* – apolipoprotein E gene

Laboratory determinations

Blood samples were drawn in the laboratories of the 10 health centres in Kainuu after a period of fasting of at least 10 h. The sera were separated and the samples were sent to the laboratory of Kainuu Central Hospital, where they were kept frozen at − 20°C until analysed. Serum total cholesterol (TC) concentration was analysed using the cholesterol esterase method (Konelab, Espoo, Finland)[13–16]. Very-low-density lipoproteins (VLDL) and low-density lipoproteins (LDL) were precipitated with a phosphowolframate–magnesium chloride reagent and separated by centrifugation. After this, high-density lipoprotein cholesterol (HDL-C) was determined

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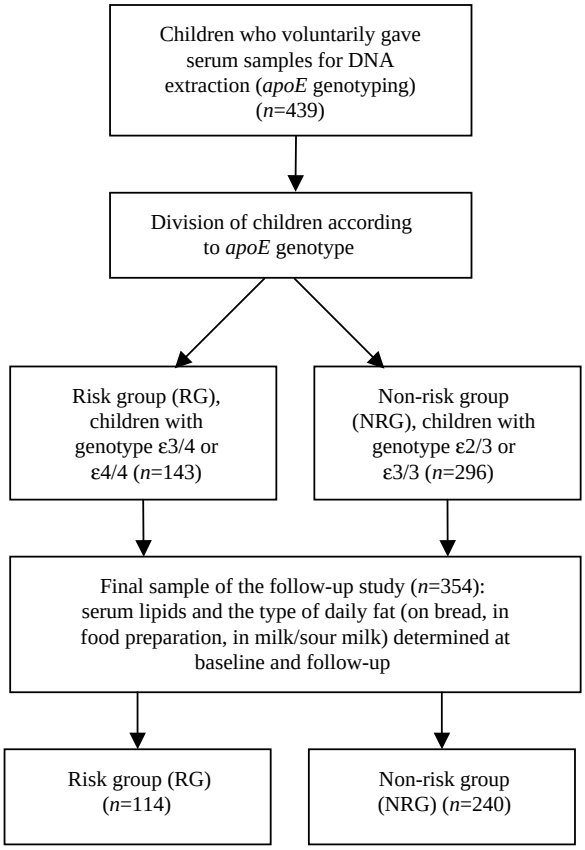

Fig. 2 Flow chart of the material in this study. *apoE* – apolipoprotein E gene

enzymatically. Triglycerides were analysed using the glycerol phosphorylase method (Konelab). LDL cholesterol (LDL-C) was calculated using Friedewald's formula[17]. Concentrations are expressed in SI units ($mmol\,l^{-1}$).

Other measurements

Questions on diet and nutrition included three issues: (1) fat (type of fat used on bread, in food preparation and baking, type of milk/sour milk used, frequency of eating fatty snacks, sweet breads and ice cream, observable fat in food, and type of ice cream); (2) fibre (frequency of eating vegetables, root vegetables, fruits and berries, and type and slices of bread eaten daily); and (3) salt (type of salt, frequency of adding salt to food).

Exercise was measured with questions about the frequency and exertion of exercise. The measure of cigarette smoking involved the number of cigarettes, cigars or pipes smoked daily.

Family-based health education/counselling intervention

All children participated in a family-based health education/counselling intervention throughout the study. Health education/counselling began in September 1997 and the follow-up measurements were completed in the spring of 2000 (Table 1). The intervention consisted of two individual counselling sessions with children at school (1 h) and three counselling sessions with children and their families in their homes (2–3 h). Two trained public health nurses worked as counsellors with each having their designated children and families. Children and other family members were individually counselled about diet and nutrition (fatty acids, fibre content, salt), overweight, exercise, cigarette smoking, and drugs and alcohol during the family sessions. Diet and nutrition were stressed in the intervention. All family members made an effort to identify their own risk factors for CHD. When the risk factors of the family members were identified, information was given and the nurse attempted to motivate the family members to modify or change their unhealthy behaviours. Concrete plans to control the risks were made together; for example, trying to switch to milk with zero fat or to change butter to oils and plant margarines. During the following sessions, evaluation of the goals identified by the family members was an important theme. Family members were encouraged to maintain the identified favourable health behaviours and change the unfavourable ones. Additional information was given in order to help the family members control their risk factors. Reading materials provided by voluntary organisations were distributed during the whole intervention period. The contents of the materials included the effects of nutrition, cholesterol concentration, alcohol, exercise and smoking on blood pressure and CHD, and the effects of nutrition

Table 1 Family-based health education/counselling intervention

Phase	Time span for the session
First school counselling session and baseline measurements • individual counselling concerning health habits	Sept 1997–May 1998
First family counselling session • identification of unfavourable health habits • information about general risk factors of coronary heart disease • possibilities and concrete plans (goals) to change unfavourable health habits	Aug. 1998–Jan. 1999
Second family counselling session • additional information about identified risk factors • evaluation of identified goals • encouragement to maintain favourable health habits and to change unfavourable ones	Jan. 1999–Aug. 1999
Third family counselling session • additional information about identified risk factors • evaluation of identified goals • encouragement to maintain favourable health habits and to change unfavourable ones	Aug. 1999–Dec. 1999
Second school counselling session and follow-up measurements • individual plans for maintenance of positive health habits	Jan. 2000–Jun. 2000

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and exercise on weight control. The use of fats and fibre was stressed in the handouts on nutrition.

Among children with baseline TC concentration between 5.0 and 6.5 mmol l^{-1}, TC concentrations were measured at health centres at a 6-month interval for follow-up purposes. Children whose serum TC concentration was 6.5 mmol l^{-1} or above at baseline ($n = 20$) were treated in Kainuu Central Hospital. In addition to participating in the family-based health education/counselling ($n = 16$), the children were counselled and treated by regular health services. Nobody used lipid-lowering drugs.

Follow-up measurements were carried out from January 2000 to June 2000. Measures similar to those in the basic examination were used, except for determining *apoE* genotype. Of the 439 original participants, 85 dropped out during the follow-up period (29 from RG and 56 from NRG) because of relocation, parents' divorce, family member's serious illness or other reasons. Only children with *apoE* genotype determined at baseline and with complete baseline and follow-up data about lipids, as well as diet and nutrition, were included in the analyses ($n = 354$) (81%). Of the 354 children and adolescents included in the analyses, 114 (57 boys and 57 girls) belonged to RG and 240 (121 boys and 119 girls) to NRG.

Statistical analyses

At baseline, the mean ages between groups were compared with the two-sample *t*-test. Cumulative logistic regression was used to analyse differences at baseline in the type of fat used on bread and in food preparation and the type of milk/sour milk (ordinal dependent variables consisting of three categories). At baseline, differences in serum lipids between groups were evaluated with analysis of variance using age as the covariate.

Changes in nutrition behaviour (ordinal dependent variables) of children in the intervention group and differences in changes between groups (RG and NRG) in the type of fats used daily were tested using cumulative logistic models[18] with generalised estimation equations. The differences in changes in serum lipids between groups were tested using analysis of variance for repeated measurements, where age or age and quality of fat used (fat used on bread and in food preparation and type of milk/sour milk) were used as covariates. Due to a skewed distribution, triglyceride concentrations were log-transformed for statistical analyses. All analyses, except for nutrition behaviour variables, were done separately for boys and girls. *P*-values less than 0.05 were considered statistically significant. Statistical analyses were done using SAS System for Windows, release 8.02 (SAS Institute, Cary, NC, USA).

Results

Descriptive data

At baseline, mean age of the subjects was 11.4 (standard deviation (SD) 3.0) years for boys and 10.4 (SD 3.0) years for girls in RG; 10.5 (SD 2.9) years for boys and 11.1 (SD 2.9) years for girls in NRG. The mean age of boys in RG was higher than that in NRG ($P = 0.039$), while there was no statistically significant difference among girls. There were no statistically significant differences between RG and NRG in the daily use of fat (type of fat used on bread and in food preparation, type of milk/sour milk used) for either boys or girls.

Changes in nutrition behaviour during the intervention

The effects of the intervention on the nutrition behaviour of all children are shown in Table 2. There were no significant differences between RG and NRG in changes in the type of fats used daily (fat used on bread and in food preparation, type of milk/sour milk used) for either boys or girls.

Baseline differences in serum lipids between risk and non-risk groups

The lipid values in RG and NRG are shown in Table 3. At baseline, there were statistically significant differences between RG and NRG in serum LDL-C levels (analysis of covariance adjusted by age, $P = 0.007$) and LDL-C/HDL-C ratio ($P = 0.030$) among boys. In girls, similar differences between groups were found in TC/HDL-C ($P = 0.008$) and LDL-C/HDL-C ($P = 0.006$) ratios. In both genders the values tended to be lower in NRG (see Table 3).

Lipid changes during the intervention

In boys, analysis of variance for repeated measures (adjusted by age) showed a significant difference between RG and NRG only in change of TC/HDL-C ratio: the increase was higher in RG than in NRG during the intervention. No differences were found in the changes of

Table 2 Changes in nutrition behaviour during baseline (1997–1998) and follow-up measurements (2000) in the intervention group ($n = 354$)

	Change		
	COR*	95% CI	*P*-value
Type of fat used on bread	1.79	1.16–2.74	0.008
Type of fat used in food preparation	2.95	2.33–3.73	<0.001
Type of fat used in baking	2.67	1.99–3.57	<0.001
Type of milk (or sour milk) used	3.94	2.98–5.20	<0.001
Frequency of eating observable fat	0.97	0.76–1.24	0.805
Frequency of eating greasy snacks	1.36	0.98–1.90	0.066
Frequency of eating sweet bread	1.41	1.12–1.77	0.003
Frequency of eating ice cream	1.81	1.39–2.35	<0.001
Type of ice cream eaten	2.29†	1.78–2.95	<0.001
Slices of rye bread eaten daily	1.11	0.87–1.42	0.391
Frequency of eating fruits	0.79	0.62–1.01	0.059
Frequency of eating berries	0.73	0.58–0.93	0.011

COR – cumulative odds ratio, year 2000 vs. 1997–1998 (favourable categories were compared with unfavourable categories); CI – confidence interval.
* Adjusted for gender and apolipoprotein E genotype.
† Odds ratio, dichotomous dependent variable.

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Table 3 Change in serum lipids (mmol l^{-1}) during the follow-up period and *P*-values for interaction between groups (RG and NRG) and period by gender

	Baseline measurement, mean (SD)		Follow-up, mean (SD)			
	RG	NRG	RG	NRG	*P*-value*	*P*-value†
Boys						
TC	4.8 (0.8)	4.6 (0.8)	4.6 (0.9)	4.4 (0.8)	0.327	0.247
HDL-C	1.4 (0.3)	1.5 (0.4)	1.3 (0.3)	1.4 (0.3)	0.723	0.822
LDL-C	2.9 (0.7)	2.7 (0.7)	2.8 (0.8)	2.5 (0.7)	0.553	0.428
TC/HDL-C	3.4 (0.6)	3.1 (0.8)	3.6 (0.9)	3.2 (0.9)	0.049	0.059
LDL-C/HDL-C	2.1 (0.6)	1.9 (0.6)	2.2 (0.7)	1.9 (0.7)	0.168	0.163
TG	0.9 (0.4)	0.8 (0.5)	1.1 (0.5)	0.9 (0.5)	0.064‡	0.073‡
Girls						
TC	5.0 (0.9)	4.7 (0.9)	4.8 (0.9)	4.4 (0.8)	0.288	0.384
HDL-C	1.5 (0.3)	1.6 (0.3)	1.5 (0.3)	1.5 (0.3)	0.401	0.549
LDL-C	3.1 (0.9)	2.8 (0.8)	2.9 (0.9)	2.5 (0.7)	0.287	0.365
TC/HDL-C	3.5 (1.2)	3.1 (0.7)	3.4 (1.1)	3.0 (0.7)	0.773	0.763
LDL-C/HDL-C	2.2 (1.1)	1.8 (0.6)	2.1 (0.9)	1.7 (0.6)	0.885	0.813
TG	0.9 (0.4)	0.9 (0.4)	1.0 (0.6)	1.0 (0.5)	0.458‡	0.517‡

RG – risk group (children with apolipoprotein E (*apoE*) genotype ϵ3/4 or ϵ4/4); NRG – non-risk group (children with *apoE* genotype ϵ2/3 or ϵ3/3); SD – standard deviation; TC – mean total cholesterol; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; TG – triglycerides.
* *P*-values for interaction between groups and period adjusted for age.
† *P*-values for interaction between groups and period adjusted for age and the quality of fats used.
‡ Values are log-transformed for statistical analyses.

lipid values during the intervention between RG and NRG, either in boys or girls, when adjusting for both age and the quality of daily fat.

Discussion

The goal of this family-based education intervention was to modify or change unfavourable health habits of all family members and to reduce high TC and LDL-C levels and overall risks of CHD, MI, BI or FH among the members of those families. The intervention used a health education approach within each family and specifically concentrated on counselling children and adolescents about nutrition, exercise and cigarette smoking. The intervention has been shown to be effective in modifying and changing the nutritional habits of children, especially the use of fats[19]. Our previous analyses performed separately for boys and girls divided into three age groups (6–9, 10–12, 13–17 years) showed positive effects of the intervention on TC and LDL-C levels among the youngest (6–9 years) children[20]. In the present study, the sample comprised children with *apoE* genotype determined at baseline and with complete baseline and follow-up data about lipids and diet and nutrition ($n = 354$). Analyses showed favourable changes in nutrition behaviour and lipid profile of these children during the intervention. The children were divided into two groups according to *apoE* genotype, *apoE* ϵ4 allele carriers and non-carriers, to analyse whether serum lipids and their responses during the intervention are associated with genetic variation at the *apoE* locus. Because the changes in lipid profiles of boys and girls were partly different, results are presented separately for boys and girls.

Baseline LDL-C levels and LDL-C/HDL-C ratio among boys, and TC/HDL-C and LDL-C/HDL-C ratios among girls, differed significantly between RG (children with *apoE* ϵ4 allele) and NRG (children without *apoE* ϵ4 allele) in favour of NRG. These findings are in accordance with many earlier reports[9,21–25].

The only significant difference in the changes of serum lipid concentrations between the groups during the follow-up period was found in TC/HDL-C ratio among boys. This difference was in favour of NRG, and it was significant only when age was used as a covariate. When age and the quality of daily fat (fat used on bread and in food preparation, type of milk/sour milk) at baseline were used as covariates, the difference in changes between groups was not significant. Different and partially contradictory results have been obtained before in studies about the associations of apoE variation with plasma lipid responses to dietary variations in adults[1,3,26–28]. An interesting finding has been the presence of an apparent gender effect – the apoE effect has generally been found in men but not in women[29]. Fewer corresponding studies have been made among school-aged or younger children[10,30], which suggest that the ϵ4 allele is not associated with a greater sensitivity of serum cholesterol concentrations to dietary changes compared with other *apoE* alleles.

The education in our study was not very intensive. During 33 months there were two counselling sessions at children's schools and three at their homes, amounting to about 8–11 h of individual counselling per child and/or family. The intervention had favourable effects on nutrition behaviour and serum lipid levels of children, however. The North Karelia Youth programme, which was a 2-year school- and community-based intervention aiming to influence health behaviour and CVD risk factors in 13- to 15-year-old children, also had favourable effects

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on nutrition behaviour and cholesterol levels of children[31]. Another intervention also carried out in North Karelia, Finland, showed that the high serum cholesterol levels in Finnish children are to a great extent caused by the dietary pattern and can be decreased by dietary modification. During a 12-week dietary fat modification made in families, children's TC levels decreased by 15% and then increased almost to initial levels during the 5-week switch-back period[32].

Because there are only two previous studies about the associations of apoE variation with plasma lipid responses to dietary variations in children, it is hard to assess whether a more intensive intervention would give different results regarding the effect of *apoE* genotype. One of these earlier studies comprised either a 3-month face-to-face parent/child counselling programme or a 3-month parent/child auto-tutorial programme for children aged 4–10 years with elevated LDL-C concentrations[30]. The other study was a placebo-controlled, double-blind, cross-over study which comprised two 3-month study periods and a 6-week wash-out period to analyse the cholesterol-lowering effect of plant sterol esters in normocholesterolaemic children aged 6 years[10]. The results of these earlier studies[10,30] are in accordance with our findings. However, plasma TC and LDL-C levels in children aged 4–11 years with less family history of CVD have been found to be significantly more responsive to a change in dietary cholesterol than the levels in children with stronger family history of CVD[30].

The results of our study may have been influenced by the large variation in the children's age (6–17 years). There are age- and gender-related changes in serum lipid levels of children. Serum cholesterol concentration decreases during puberty. This is due to the decline in LDL-C level among boys and girls and the decline in HDL-C level among boys[33]. The mean age of boys in RG was on average 1 year greater than that in boys in NRG. This may have had some influence on the results.

The variables describing nutrition behaviour are ordinal because the data concerning nutrition were collected with questionnaires instead of diaries. Therefore, we do not know the actual saturated fat and cholesterol contents of the diets at baseline and follow-up, and the results do not allow any conclusions to be made on the relative contributions of the reductions in intake of these components. However, we did analyse the differences in changes between RG and NRG in the type of fats used daily, and no significant differences were found either among boys or girls.

The only significant difference in changes between *apoE* ε4 allele carriers and non-carriers was found in TC/HDL-C ratio among boys during this 33-month family-based health education/counselling intervention. The response of plasma lipids levels to diet does not seem to depend on *apoE* genotype in children with a family history of CVD. More studies in populations of children with a family history of CVD are needed to test this hypothesis and to confirm our results.

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