

## ARTICLES

# Apolipoprotein E Phenotype Regulates Cholesterol Absorption in Healthy 13-Month-Old Children—The STRIP Study

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### ABSTRACT

High serum cholesterol concentration is one of the key risk factors in development of atherosclerosis, which may begin early in life and later progress to symptomatic coronary heart disease. In adults, apoE polymorphism strongly influences cholesterol metabolism, as subjects with apoE 3/4 or 4/4 (collectively called apoE4) phenotype absorb cholesterol effectively and thus also have higher cholesterol absorption-reflecting plant sterol concentrations in serum than subjects with other apoE phenotypes. Because of the inverse correlation of absorption and synthesis of cholesterol, concentrations of cholesterol synthesis-reflecting serum cholesterol precursor sterols are lower in subjects with apoE4 than in subjects with other phenotypes. To analyze whether apoE phenotype affects cholesterol absorption and synthesis in early childhood, we measured serum plant sterol (campesterol and sitosterol) and cholesterol precursor sterol (desmosterol and lathosterol) concentrations in healthy 13-month old children using gas-liquid chromatography. The 36 study children

were participants in a randomized prospective trial (the Special Turku Coronary Risk Factor Intervention Project) aimed at decreasing exposure of the children to environmental atherosclerosis risk factors. The 16 apoE4 children had 30% to 50% higher cholesterol-adjusted campesterol and sitosterol concentrations in serum than the 20 apoE 3/3 children ( $p = 0.002$  and  $p = 0.02$ , respectively). The concentrations of cholesterol precursor sterols in serum did not differ between the two groups of children. We conclude that the young apoE4 children may absorb cholesterol and plant sterols more effectively than the children with apoE 3/3 phenotype without compensatory reduction in endogenous synthesis of cholesterol. (*Pediatr Res* 50: 688–691, 2001)

#### Abbreviations

**E%**, percent of daily total energy intake  
**non-HDL cholesterol**, total cholesterol – HDL cholesterol

Plant sterols, components of vegetal cellular membranes, are structurally closely related to cholesterol. Only small amounts of poorly absorbed plant sterols are found in human plasma or serum (1, 2), and they are entirely derived from the diet, since they are not synthesized endogenously (1). In middle-aged

subjects, serum cholesterol-adjusted plant sterol concentrations reflect cholesterol absorption (2, 3). Subjects with apoE 3/4 or 4/4 phenotype absorb cholesterol more effectively (4), and have thus higher serum plant sterol concentrations than subjects with other apoE phenotypes (5).

In addition to cholesterol and plant sterols, plasma also contains cholesterol precursor sterols, *e.g.* lathosterol and desmosterol, which reflect the activity of endogenous cholesterol synthesis (2, 6). Because the rate of cholesterol synthesis is lower in subjects with apoE 3/4 or 4/4 phenotypes than in those with other phenotypes (7), serum lathosterol concentrations are lower in subjects with apoE 3/4 or 4/4 phenotype than in subjects with other apoE phenotypes

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(4). As cholesterol absorption and synthesis are inversely correlated with each other (8), serum concentrations of plant sterols and cholesterol precursor sterols are also inversely correlated in healthy men (9) and in patients with familial hypercholesterolemia (10).

Because a high serum cholesterol concentration is one of the most important risk factors in development of atherosclerosis (11), factors regulating serum cholesterol concentration since birth are important in planning of proper strategies for the prevention of atherosclerosis development. We studied extent to which apoE phenotype regulates cholesterol absorption and synthesis in early childhood by determining serum plant sterol and cholesterol precursor sterol concentrations in 36 children (16 with apoE 3/4 or 4/4 phenotype; 20 with apoE 3/3 phenotype) at the age of 13 months.

## METHODS

**Subjects.** The children in this study were all 13-month-old participants of the STRIP project (Special Turku Coronary Risk Factor Intervention Project for children), which is a randomized, prospective trial aimed at decreasing exposure of the intervention children to known environmental atherosclerosis risk factors. In the project, launched in 1990, 1062 infants were randomized to the intervention ( $n = 540$ ) or control ( $n = 522$ ) group at the age of 7 months as described (12). The children in the intervention group received dietary counseling aiming at low intake of saturated fat and cholesterol at regular intervals. We recommended that the intervention families add 2–3 teaspoonfuls of vegetable oil (preferably low-erucic acid rapeseed oil) or vegetable margarine to the child's daily diet to replace the saturated fat. Not only cholesterol absorption rate, but also the amount of plant sterols in the diet may affect serum plant sterol concentrations (13). The main dietary sources of plant sterols in western diets are vegetable oils and margarines (14). To confirm equal dietary intake of plant sterols, we selected for the present study only intervention children from STRIP. We randomly selected thirty-seven 13-month-old in-

tervention children, who, according to 3-day dietary records, fulfilled the criteria of recommended vegetable oil or margarine intake (Table 1). Seventeen of the children had the apoE 3/4 or 4/4 phenotype (later called children with apoE4 phenotype), while the remaining 20 children had the apoE 3/3 phenotype. One boy with the apoE 3/4 phenotype was excluded from analyses because he had an extremely low serum cholesterol concentration (1.70 mmol/L) caused by asymptomatic hypobetalipoproteinemia. The mean (SD) daily vegetable fat supplement of the apoE4 children was 13.4 (5.6) g, and that of the apoE 3/3 children 12.5 (5.6) g ( $p = 0.62$ ).

**Counseling.** The intervention families visited the study pediatrician and dietitian at the child's age of 7, 8, 10, and 13-months old. The families were advised to feed the children low-saturated fat, low-cholesterol diets, which after discontinuation of breast milk or formula at the age of 12 months contained, for example, skim milk; therefore, the children's daily diets were supplemented with 2–3 teaspoonfuls of vegetable oil or margarine. The suggested total fat intake of the children was 30% to 35% of their daily E %. The counseling dealt mainly with quality of fat in child's diet, aimed at replacing part of saturated fatty acids with poly- and monounsaturated fatty acids to approach a polyunsaturated/monounsaturated/saturated fatty acid ratio of 1/1/1 and a daily cholesterol intake <200 mg. Control families visited the project pediatrician and dietitian at child's ages of 7 and 13 months old. They were counseled like families at the Finnish well-baby clinics to change the child from breast milk or formula to cow's milk with 1.9% or 2.9% fat at the age of one year, and they received no individualized dietary advice. The intervention and control families recorded child's food consumption for 3 days (one weekend day) about a week before each visit using a food diary. The intake data were analyzed using Micro-Nutrica® program (Research and Development Unit, Social Insurance Institution, Turku, Finland) (15). Daily intake of plant sterols was estimated using a database developed in the National Public Health Institute and added to the Micro-Nutrica® pro-

**Table 1.** Characteristics of the study subjects and their daily intake of energy and fat according to 3-day dietary record in children with apoE 3/3 ( $n = 20$ ) and apoE 3/4 or apoE 4/4 phenotype ( $n = 16$ )

Variable	Children with apoE 3/3	Children with apoE 3/4 or apoE 4/4	<i>p</i> -value
Female, <i>n</i>	6	8	
Male, <i>n</i>	14	8	
Height, cm	78.3 (2.7)	77.5 (2.1)	0.34
Weight, kg	10.3 (1.2)	10.0 (1.0)	0.50
Relative weight, %*	+0.5 (10.4)	+1.0 (7.6)	0.87
Dietary intakes			
Total energy, MJ†	4.43 (0.85)	4.12 (0.77)	0.29
Total fat, E%	25.7 (5.8)	30.0 (4.8)	0.02
Saturated fat, E%	7.4 (1.9)	8.4 (2.6)	0.17
Monounsaturated fat, E%	9.9 (2.5)	12.1 (1.9)	0.006
Polyunsaturated fat, E%	6.4 (2.4)	7.2 (1.6)	0.31
P/S ratio‡	0.88 (0.27)	0.92 (0.34)	0.67
Cholesterol, mg	82 (33)	85 (47)	0.85
Plant sterols, mg	168 (53)	182 (51)	0.42

Values are means (SD).

\* Relative weight expresses weight as deviation from mean weight of Finnish children of same height and sex.

† To convert MJ to kcal multiply by 239.

‡ P/S, ratio of polyunsaturated fatty acids to saturated fatty acids.

**Table 2.** Concentrations of serum lipids (mmol/l) in children with apoE 3/3 phenotype (n = 20) and with apoE 3/4 or apoE 4/4 phenotype (n = 16)

Variable	Children with apoE 3/3	Children with apoE 3/4 or 4/4	p-value
Total cholesterol	3.78 (0.61)	4.00 (0.57)	0.28
HDL cholesterol	0.84 (0.19)	0.82 (0.19)	0.81
HDL cholesterol/total cholesterol	0.23 (0.05)	0.20 (0.04)	0.20
Non-HDL cholesterol	2.94 (0.60)	3.18 (0.46)	0.21

Values are means (SD).

gram. Sterol values of foods analyzed chemically ranged from 70% to 98% of the calculated sterol values (16).

**Biochemical determinations.** Blood was drawn from an antecubital vein under cutaneous anesthesia (Emla, Astra, Södertälje, Sweden) from nonfasting children at the age of 13 months. Serum was separated by centrifugation ( $3400 \times g$ , for 12 minutes) after clotting at room temperature and was stored at  $-25^{\circ}\text{C}$  for less than one month. Serum cholesterol concentration and HDL cholesterol concentration after precipitation of LDL and VLDL with dextran sulfate 500,000 were measured using a fully enzymatic method. Samples for the measurement of serum concentrations of cholesterol, plant sterols, and cholesterol precursor sterols were stored at  $-70^{\circ}\text{C}$  until determined with gas liquid chromatography (17, 18) at the Research Laboratory of the Department of Medicine, Helsinki University Central Hospital, using 50 m capillary SE-30 column (Hewlett Packard® Ultra I). ApoE phenotypes were determined using isoelectric focusing and immunoblotting of delipidated serum (19).

**Statistical analysis.** The results are expressed as means (SD). For statistical analysis the SAS 6.12 program package was used (SAS Institute, Cary, NC). Two-sided *p*-values  $<0.05$  were regarded as significant. Differences between the apoE4 children and the apoE 3/3 children were tested with a two-sample *t* test. Pearson correlation coefficient was calculated for correlation between variables.

**Ethics.** The Joint Commission on Ethics of the Turku University and the Turku University Central Hospital have approved STRIP. An informed consent was obtained from the parents of all children.

## RESULTS

The measured serum lipid concentrations (cholesterol, HDL cholesterol, HDL cholesterol/cholesterol, and non-HDL cho-

lesterol) did not differ significantly between the 16 children with apoE4 and the 20 children with apoE 3/3 (Table 2).

Serum concentrations of campesterol and sitosterol were 54% and 35% higher, respectively, in the apoE4 children than in the apoE 3/3 children (Table 3). When the concentrations of campesterol and sitosterol were adjusted for serum cholesterol, the values in the apoE4 children exceeded those of the apoE 3/3 children by 45% and 27%, respectively. However, serum concentrations of cholesterol precursor sterols or their cholesterol-adjusted concentrations did not differ between the two groups of children (Table 3).

Plant sterol concentrations in serum showed no correlation with cholesterol precursor sterols in the two groups of children studied either separately or combined (data not shown).

## DISCUSSION

This study shows that the children with apoE4 phenotype have higher serum concentrations of plant sterols in early childhood than the children with apoE 3/3 phenotype. This finding may reflect higher absorption of dietary cholesterol in the apoE4 children than in the apoE 3/3 children. However, contrary to findings in adults, the children with the two phenotypes had similar cholesterol precursor concentrations. Consequently, the concentrations of serum plant sterols and cholesterol precursor sterols did not correlate with each other in these children.

Adult subjects with apoE4 absorb cholesterol more effectively than others, thus showing high cholesterol-adjusted serum plant sterol concentrations (4, 5). Similarly, in this study, the young children with apoE4 phenotype had higher cholesterol-adjusted serum campesterol and sitosterol concentrations than the children with the apoE 3/3 phenotype, even though dietary plant sterol intakes of children in the two groups did not

**Table 3.** Serum concentrations of non-cholesterol sterols ( $10^2 \mu\text{mol/l}$ ) and sterol to cholesterol ratio ( $10^2 \mu\text{mol/l}$  of cholesterol) in children with apolipoprotein (apo) E3/3 (n = 20) and apo E3/4 or apo E4/4 phenotype (n = 16)

Sterol	Children with apoE 3/3	Children with apoE 3/4 or apoE 4/4	p-value
Sterols reflecting cholesterol absorption			
Campesterol	737 (290)	1137 (375)	0.0009
Campesterol/cholesterol	205 (81)	297 (80)	0.002
Sitosterol	504 (145)	681 (231)	0.009
Sitosterol/cholesterol	140 (38)	178 (52)	0.02
Sterols reflecting cholesterol synthesis			
Desmosterol	284 (59)	298 (70)	0.53
Desmosterol/cholesterol	79 (14)	78 (13)	0.88
Lathosterol	483 (182)	472 (154)	0.86
Lathosterol/cholesterol	132 (42)	123 (32)	0.51

Values are means (SD).

differ. The dietary intakes of total and monounsaturated fat were higher in the apoE4 children than in the apoE 3/3 children. On the other hand, no correlations were detected between these dietary intakes and the cholesterol-adjusted serum concentrations of campesterol or sitosterol in either group of children ( $r$  always  $<0.35$ ,  $p$  always  $>0.13$ ). Therefore, it is unlikely that the increased cholesterol absorption efficiency (reflected by plant sterol levels) in the apoE4 children would be caused by their higher dietary intake of total and monounsaturated fat (7). Our findings also support the hypothesis that increased cholesterol absorption is a mechanism by which the apoE4 phenotype influences serum cholesterol concentrations in childhood as has been previously demonstrated (19–22). Also, in the present study, there was a tendency toward higher serum total cholesterol concentrations in the apoE4 children, although the difference was not significant, probably owing to the small number of study subjects (36 children). In fact, a similar difference in serum cholesterol concentration (0.22 mmol/l) between apoE4+ and apoE4-children was significant ( $p = 0.0001$ ) in the original STRIP with 723 children (23).

In adults serum cholesterol-adjusted concentrations of plant sterols and cholesterol precursor sterols are inversely correlated with each other, reflecting an inverse correlation with cholesterol absorption and synthesis (9, 10). However, in this study, the 13-month-old children did not show any correlation between serum cholesterol-adjusted plant sterol and cholesterol precursor sterol concentrations. Further, the children with the apoE4 phenotype and those with the apoE 3/3 phenotype showed no difference in serum absolute or cholesterol-adjusted precursor sterol concentrations, even though cholesterol absorption, as reflected by serum plant sterols, was higher in the children with the apoE4 phenotype compared with the children with the apoE 3/3 phenotype. Because the daily intake of cholesterol was less than 90 mg in both groups of children and thus far below the common intake values of adults (24), the low intake and small amount of cholesterol absorbed might not suppress cholesterol synthesis to such an extent that changes in the precursor concentrations would become visible (24–26). Such a phenomenon may occur especially in children below the age of 2 years, because the growth velocity of the children is still extremely fast, and high rates of endogenous cholesterol synthesis are needed for construction of the new cell membranes and other membranous structures in the cells.

In summary, this study shows that the healthy children with apoE4 phenotype had higher absolute and cholesterol-adjusted concentrations of plant sterols in serum than the children with the apoE 3/3 phenotype at the age of 13 months, but both absolute and cholesterol-adjusted concentrations in serum of sterols reflecting cholesterol synthesis did not differ between the two phenotypes. These findings suggests that the apoE4 children absorb cholesterol more effectively than the children with apoE 3/3 phenotype, but the increased absorption of

cholesterol was not accompanied by a compensatory reduction in cholesterol synthesis.

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