Cholesterol precursors and plant sterols in children with food allergy[1,2]

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ABSTRACT

Background: The data on lipid metabolism in allergic children is limited.

Objective: We investigated lipid and sterol metabolism in young children whose diets were restricted because of food allergy.

Design: Children in group A [$n = 21$; mean (± SD) age: 1.78 ± 0.73 y] were allergic to fish, eggs, and either cow milk or cereals; those in group B ($n = 31$, aged 1.45 ± 0.58 y) were allergic to fish, eggs, and both cow milk and cereals. Cholesterol precursor and plant sterol to cholesterol ratios ($10^2 \times$ μmol/mmol cholesterol) and apolipoprotein E phenotype distributions were analyzed in 36 subjects. The control group for cholesterol precursor and plant sterol measurements consisted of 18 healthy age-matched children.

Results: The mean serum cholesterol concentration was 3.6 ± 0.6 mmol/L, and HDL cholesterol was 1.03 ± 0.3 mmol/L in group A. Corresponding values in group B were 3.4 ± 0.7 and 1.09 ± 0.2 mmol/L. The daily cholesterol intake was low: 61.3 ± 36.0 mg in group A and 50.7 ± 48.5 mg in group B. Cholesterol precursor plant sterol concentrations were significantly higher in allergic subjects than in control subjects.

Conclusions: Allergic children with restricted diets have a low intake of cholesterol and relatively low serum cholesterol concentrations. Dietary intake of plant sterols was obviously increased because of supplementation with rapeseed oil, which is rich in plant sterols, leading to elevated plant sterol concentrations. Plant sterols may have inhibited cholesterol absorption, which in turn stimulated cholesterol synthesis in compensation, also explaining the increased precursor sterol ratios in serum in our subjects. *Am J Clin Nutr* 2003;77:51–5.

KEY WORDS Food allergy, cholesterol, cholesterol precursors, plant sterols, children

INTRODUCTION

Allergy is a common chronic disease in childhood, and cow-milk allergy is commonly the first manifestation of food allergy in early childhood. Oral ingestion of dietary proteins normally induces clinical tolerance. In allergy, antigenic challenge evokes adverse immune reactions and immunoglobulin secretion. These adverse reactions may impair the barrier function of the intestine. Enhanced absorption of antigens can further increase intestinal permeability and destruction of the intestinal mucosa (1). The dietetic treatment of food allergy consists of elimination of antigens, which preserves the intestinal integrity and reverses the disturbance of the humoral and cell-mediated immune responses (2). However, the restricted dietary regimens may impair the child's nutritional status and growth (3–5). On the other hand, inadequate elimination maintains intestinal inflammation and decreases nutrient absorption.

Cholesterol is an essential lipid for human cells. Dietary cholesterol esters are digested enzymatically in the small intestine, and the products of lipid digestion are absorbed. Within the intestinal mucosal cells, triacylglycerols, phospholipids, and cholesterol are packaged with specific proteins to form lipoprotein complexes called chylomicrons that carry dietary cholesterol to the liver (6). Enteral cholesterol absorption was shown to be regulated by apolipoprotein E (apo E), a structural protein of lipoproteins (6, 7). Human apo E occurs as 3 genetic isoforms, which can be separated into 3 allelic products: *E2*, *E3*, and *E4*. It was observed that *E4* carriers have higher serum cholesterol concentrations than do *E3* and *E2* carriers. This was also found in infants who were fed either breast milk or low-cholesterol formula (8). One reason is that the *E4* allele enhances cholesterol absorption from the gut (6, 7).

Inflammatory bowel diseases affecting the upper part of the gastrointestinal tract may interfere with cholesterol absorption and lead to decreased cholesterol concentrations. This is well documented for patients with celiac disease (9). Serum plant sterol (campesterol and sitosterol) concentrations reflect the effectiveness of cholesterol absorption (10). Endogenous cholesterol synthesis takes place mainly in the liver. Cholesterol synthesis is estimated to require ≈30 separate reactions involving different enzymatic steps (11). Measurement of the most abundant serum cholesterol precursors—Δ^8-cholestenol, lathosterol, and desmosterol—shows the effectiveness of endogenous cholesterol production. Effective cholesterol absorption has been shown to suppress cholesterol synthesis (10). If cholesterol absorption decreases, as in celiac disease, hepatic cholesterol synthesis increases (9).

In this work, we investigated lipid and sterols reflecting cholesterol metabolism in young children kept on a restricted diet

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because of food allergy by measuring serum total and HDL cholesterol, cholesterol precursor and plant sterol concentrations, and apo E phenotypes.

SUBJECTS AND METHODS

Subjects

This study comprised 52 children aged 8–39 mo consecutively admitted for oral food challenge at the Skin and Allergy Hospital of Helsinki University Central Hospital and diagnosed to have challenge-proven food allergy to fish, eggs, and cow milk or cereals (rye, wheat, oats, and barley) or both. Food allergy was manifested by skin symptoms such as urticaria and excema (n = 34); gastrointestinal symptoms such as vomiting, loose stools, diarrhea, and abdominal pain (n = 4); and both skin and gastrointestinal symptoms (n = 14). Forty-eight children had atopic heredity (at least one of the first-degree relatives had an atopic disease such as atopic eczema or immunoglobulin E–mediated allergy). The subjects were not on steroids, antihistamines, or any other oral drugs at the time of the study.

Control values for serum cholesterol concentrations and an average cholesterol intake of children in Finland were picked up from the Finnish STRIP Baby Project (12, 13). Control values for cholesterol precursor and plant sterol concentrations were measured with gas chromatography in 18 healthy age-matched children.

The study was approved by the Ethical Committee of the Skin and Allergy Hospital of the Helsinki University Central Hospital. Written informed consent was obtained from each child's parents.

Diets

When food allergy was diagnosed, the children were put on an elimination diet excluding fish, eggs, and either cow milk or cereals (group A) or fish, eggs, and both cow milk and cereals (group B). The adequacy of the diet was followed and confirmed by a registered dietician. Group A comprised 21 children whose mean (±SD) age was 1.78 ± 0.73 y (range: 0.69–3.0 y); group B had 31 children aged 1.45 ± 0.58 y (range: 0.66–3.28 y).

If cow milk was eliminated from the diet, a tolerated formula [soy (n = 6), protein hydrolysate (n = 16), or amino acid formula (n = 10)] was substituted in infants < 2 y of age. For older subjects calcium supplementation was given (n = 12). The 3 youngest babies received breast milk. The mothers of the 3 breast-fed infants were on a cow-milk elimination diet because of the infant's allergy. During cereal elimination, gluten-containing cereals were excluded from the diet and were replaced with gluten-free rice, corn, millet, and buckwheat, or only millet. Forty subjects received supplementary rapeseed oil.

The food consumption was measured by means of a 3-consecutive-day food record kept by the parents. The portion sizes were estimated in household measures. The data were transferred from the food diaries to a computer, and nutrient intakes were analyzed by using the MICRO-NUTRICA computer program developed at the Research Centre of Social Insurance Institution, Turku, Finland.

Oral food challenge

The children all consumed a milk or cereal (or both) elimination diet for ≥ 2–4 wk before the oral food challenge. The challenge was started with a drop of the test antigen on the skin or lips. Thereafter increasing doses of cow milk or cereal were given at 2-h intervals on day 1: 1, 10, 50, and 100 mL cow milk or 1, 2, and 10 g cereal, and on day 2 normal milk or cereal intake appropriate for age was allowed. The challenge was discontinued and the subjects were examined by a pediatrician when any adverse reaction was noted. The time of the clinical reaction to the relevant antigen was defined as the time elapsing from the last given dose eliciting the specific reactions. The subjects were also observed over the 1-wk period of the challenge to detect symptoms developing several days after commencement of the challenge. A period of ≥ 2 wk without any symptoms was allowed to elapse before the next challenge.

Cholesterol and apo E measurements

After 3 (geometric $\bar{x}$: 7.2) months on strict elimination diets, blood samples were taken in the morning after the subjects had fasted for 8 h, and serum total cholesterol and HDL cholesterol concentrations were measured enzymatically (kit no. 236691; Boehringer Diagnostica GmbH, Mannheim, Germany). In a subgroup of 36 subjects, serum sterols and total cholesterol concentration were measured by gas chromatography (14) with a 50-m-long capillary column (Ultra 1; Hewlett Packard, Wilmington, DE). Because plant sterols are mainly transported in cholesterol-containing particles in serum, the absolute concentrations were adjusted for the serum cholesterol concentration ($10^2 \times$ μmol/mmol cholesterol) to eliminate the effect of changes in the serum cholesterol concentration. Apo E phenotypes were separated by isoelectric focusing (15).

Statistics

One-factor analysis of variance was used to determine differences between the groups in diet records and serum total and HDL-cholesterol concentrations, expressed as means ± SDs. The concentrations of cholesterol precursors and plant sterols were log transformed because the variables were not normally distributed, and the results are expressed as geometric mean values with 95% CIs. Statistical significance is defined as $P \leq 0.05$. Statistical analyses were carried out with STATVIEW 4.0 software (Abacus Concepts Inc, Berkeley, CA).

RESULTS

Diet records

Diet records are given in **Table 1**. The average diet of the children in this study was well within the Nordic Nutrition Recommendations (16). The mean daily total energy intakes in groups A and B were 3826 and 4334 kJ, respectively. The share of total fat and saturated and monounsaturated fatty acids exceeded the recommended amounts. When compared with the average intakes of healthy Finnish children (17, 18), our subjects received lower amounts of proteins and carbohydrates but higher amounts of fats. The quality of the fat, expressed as energy intakes of polyunsaturated fatty acids, monounsaturated fatty acids, and saturated fatty acids was high. The ratio of polyunsaturated to monounsaturated to saturated fatty acids was 0.6:1.2:1.0 in group A and 0.9:1.5:1.0 in group B. No significant difference was found in fat intake or saturated fat intake between groups A and B. Cholesterol intakes were low but comparable with those observed in the Finnish STRIP Baby Project (83.6 ± 36.6 mg/d in the intervention group and 112.8 ± 44.2 mg/d in the control group; 18).

TABLE 1
Fat, fatty acid, carbohydrate, protein, and cholesterol intakes in the 2 study groups

Intake	Group A[1,2] (n = 21)	Group B[1,3] (n = 31)	Nordic recommendations for children aged 1–3 y
Fat (% of energy)	36.8 ± 5.4	37.9 ± 10.4	30–35
Saturated	12.1 ± 3.0	10.3 ± 3.7	10
Monounsaturated	15.0 ± 3.0	15.5 ± 6.4	10–15
Polyunsaturated	7.7 ± 2.1	9.2 ± 3.9	5 - 10
Carbohydrate (% of energy)	50.0 ± 5.7	52.1 ± 9.9	50–55
Protein (% of energy)	13.2 ± 2.3	10.9 ± 3.2	10–15
Cholesterol (mg/d)	61.3 ± 36.0	50.7 ± 48.5	—
Energy (kJ)	3826 ± 1050	4334 ± 1784	3172–7210

[1] $\bar{x}$ ± SD.
[2] Subjects were consuming an elimination diet that restricted fish, eggs, and either cow milk or cereals.
[3] Subjects were consuming an elimination diet that restricted fish, eggs, and both cow milk and cereals.

Serum lipids and sterols

The mean serum cholesterol concentration was 3.6 ± 0.6 mmol/L in group A and 3.4 ± 0.7 mmol/L in group B. The mean HDL-cholesterol concentrations were 1.03 ± 0.3 mmol/L in group A and 1.09 ± 0.2 mmol/L in group B. These values were not significantly different in the 2 groups. Compared with age-matched healthy Finnish children (12), 80% of the subjects in diet group A and 90% of the subjects in group B had low serum total cholesterol concentrations, and 45% and 26%, respectively, had low serum HDL-cholesterol concentrations. The ratios of serum total cholesterol to HDL cholesterol were 3.5 and 3.1 in groups A and B, respectively. The mean serum cholesterol concentration was 3.7 ± 0.61 mmol/L in a small group of 18 healthy children whose total cholesterol concentrations were measured together with the measurements of cholesterol precursor and plant sterol concentrations.

The ratios of cholesterol precursor to cholesterol adjusted for cholesterol concentrations (geometric $\bar{x}$ values with 95% CIs) in groups A and B and in control subjects are given in **Table 2**. The values varied between individuals in groups A and B but were not significantly different. The groups A and B were combined, and the statistical difference was tested between the allergic subjects and the control group. Δ^8-Cholestenol (P = 0.005), desmosterol (P = 0.059), lathosterol (P = 0.040), sitosterol (P = 0.005), and campesterol (P = 0.013) concentrations were significantly higher in the allergic subjects than in the control subjects. When individual values of plant sterols were analyzed, we noticed that those subjects who received supplementary rapeseed oil had higher plant sterol concentrations than those who did not (data not shown).

The differences in cholesterol precursor and plant sterol concentrations were also tested in subjects receiving different substitute formulas. No statistically significant differences were observed in these variables between the children fed on the different substitute formulas, although children receiving formulas based on hydrolyzed protein and amino acids tended to have a lower cholesterol intake than did those receiving soy formula or calcium supplementation.

Apo E phenotype distributions were analyzed in a subgroup of 36 children with food allergy. The allele frequencies were as follows: 0.286 for *E4*, 0.657 for *E3*, and 0.057 for *E2*. The phenotype distribution and allelic frequencies were similar to those observed in a large group of adult Finns (15). In **Table 3**, concentrations of serum cholesterol, its precursors, and plant sterols are given according to the apo E phenotype. Subjects carrying the phenotypes 4/3 and 3/3 had the highest sitosterol concentrations. The lowest campesterol concentrations were seen in subjects carrying phenotype 3/2. There was a tendency, although not significant, for higher serum cholesterol concentrations in subjects carrying the *E4* allele than in subjects not carrying *E4* allele.

DISCUSSION

Cholesterol is an essential lipid molecule for human cells because it is a precursor of steroid hormones, vitamin D metabolites, and bile acids and is also important for neural myelinization and brain growth (11, 19). Cholesterol is therefore especially important for a growing child. Endogenous cholesterol is synthesised in the liver from acetyl-CoA through a complex enzymatic pathway (11). In young infants, the liver enzyme activities needed for cholesterol synthesis are possibly immature. In this study we tried to find out whether the relatively low serum cholesterol concentrations observed in our subjects could be a result of insufficient hepatic cholesterol synthesis. Other possible reasons for low cholesterol concentrations could be diminished absorption of cholesterol or a low amount of cholesterol or saturated fat in the diet.

TABLE 2
Serum cholesterol precursor and plant sterol concentrations in the 2 study groups and the control subjects[1]

	Group A (n = 21)	Group B (n = 31)	Control subjects (n = 18)	P
Δ^8-Cholestenol (10^2 × μmol/mmol cholesterol)	10.5 (6.8, 16.2)	12.9 (10.2, 16.3)	6.6 (4.3, 8.9)	0.005
Desmosterol (10^2 × μmol/mmol cholesterol)	86.2 (76.6, 97.2)	87.6 (78.6, 97.8)	77.3 (75.0, 79.6)	0.059
Lathosterol (10^2 × μmol/mmol cholesterol)	95.9 (72.5, 126.8)	95.4 (91.7, 123.5)	82.6 (80.3, 84.9)	0.040
Sitosterol (10^2 × μmol/mmol cholesterol)	202.4 (164.7, 248.6)	200.9 (161.6, 250.1)	140.2 (137.9, 142.5)	0.005
Campesterol (10^2 × μmol/mmol cholesterol)	363.6 (275.1, 481.1)	396.2 (322.5, 486.9)	244.3 (262.0, 266.6)	0.013

[1] Geometric $\bar{x}$; 95% CI in parentheses. There were no significant differences between group A and group B. P for comparison of data from allergic subjects combined compared with control subjects.

TABLE 3
Serum cholesterol, HDL-cholesterol, cholesterol precursor, and plant sterol concentrations by different apolipoprotein E phenotypes in a subgroup of 36 allergic subjects[1]

	E4/3 (n = 18)	E3/3 (n = 13)	E4/2 (n = 2)	E3/2 (n = 2)
Cholesterol (mmol/L)	3.8 ± 0.7[2]	3.2 ± 0.6	3.5, 3.3	2.95, 3.3
HDL cholesterol (mmol/L)	1.12 ± 0.26	1.03 ± 0.18	0.7, 0.8	0.9, 1.1
Δ^8-Cholestenol (10^2 × μmol/mmol cholesterol)	12.1 (9.84, 14.4)[3]	13.7 (11.4, 16.0)	12, 13	13, 11
Desmosterol (10^2 × μmol/mmol cholesterol)	93.0 (90.8, 95.4)	90.3 (88.0, 92.6)	139, 80	125, 85
Lathosterol (10^2 × μmol/mmol cholesterol)	101.9 (99.6, 104.2)	112.7 (110.4, 114.9)	121, 88	91, 123
Sitosterol (10^2 × μmol/mmol cholesterol)	192.8 (190.5, 195.5)	184.2 (181.9, 186.5)	152, 144	104, 107
Campesterol (10^2 × μmol/mmol cholesterol)	360.7 (358.4, 363.0)	379.7 (377.4, 381.9)	378, 310	117, 200

[1] For E4/2 and E3/2 phenotypes with 2 persons, actual values were expressed. There were no children with E4/4 or E2/2 phenotype. There was no significant difference between phenotypes.
[2] $\bar{x}$ ± SD.
[3] Geometric $\bar{x}$; 95% CI in parentheses.

Within the intestinal lumen, dietary cholesterol is solubilized into mixed micelles. Micelles enter the intestinal mucosal cells and degrade, and the free cholesterol is packed into lipoproteins called chylomicrons. Apo E phenotypes influence cholesterol absorption. The apo E allele frequencies observed in our subjects were comparable with those detected in the adult Finnish population. Thus, abnormal distribution of apo E alleles seemed not to explain low serum cholesterol concentrations in allergic children.

The effects of bowel inflammation on serum lipids and plant sterols were studied previously in celiac patients. In an acute phase of celiac disease (villous atrophy of the jejunal mucosa in biopsy caused by allergic bowel inflammation), cholesterol absorption is low, leading to enhanced cholesterol synthesis in the liver. A gluten-free diet leads to improvement in clinical symptoms and in jejunal villous atrophy. An appropriate diet also improves cholesterol absorption and decreases cholesterol synthesis (9). Cholesterol absorption efficiency also regulates cholesterol synthesis in healthy children receiving plant stanol ester margarine; this margarine lowers the absorption of cholesterol, leading to compensatory activation of cholesterol synthesis (20). In the present study, plant sterol concentrations reflecting cholesterol absorption were higher in our allergic subjects than in the control subjects. The children who received rapeseed oil supplementation had the highest serum plant sterol concentrations. This allows us to assume that, although no intestinal biopsies were taken, our subjects did not have allergic bowel inflammation at the time of the investigation of cholesterol metabolism.

The restricted dietary regimens, including low cholesterol and saturated fat intake, used in a treatment of food allergy contributed to relatively low serum cholesterol concentrations. Cholesterol precursor concentrations were significantly higher in the allergic subjects than in the control subjects. This means that the liver enzyme activities required for cholesterol synthesis are well matured, and there is a compensatory activation of cholesterol synthesis taking place in the liver of the subjects. Dietary intake of plant sterols was obviously increased because of supplementation of rapeseed oil, which is rich in plant sterols, leading to elevated plant sterol concentrations in most of our subjects. Plant sterols may have inhibited cholesterol absorption, which in turn up-regulated cholesterol synthesis in compensation, also explaining the increased precursor sterol ratios in serum. Our earlier plant sterol feeding studies and those with plant sterol ester consumption by Hallikainen et al (21) caused identical changes.

A low-saturated-fat, low-cholesterol diet may reduce not only serum total cholesterol but also HDL-cholesterol concentrations (22). The same observation was made in a large Finnish study of infants with a diet low in saturated fat and cholesterol (18). It is therefore not surprising that our allergic subjects, whose cholesterol intake was very low, had low HDL-cholesterol concentrations. HDL cholesterol is an important lipoprotein in reverse cholesterol transport and is protective against atherosclerosis. One way to assess a risk of atherosclerosis is to calculate the ratio of total cholesterol to HDL cholesterol: the lower the ratio, the lower the risk of coronary artery disease. In our subjects, total cholesterol–HDL-cholesterol ratios were very low compared with the ratios in adult populations, in which the risk of coronary artery disease is evident (23). Thus, our study population does not seem to have an increased risk of this harmful effect of low HDL cholesterol.

In conclusion, allergic children with restricted diets have a low intake of cholesterol and relatively low serum cholesterol concentrations. Dietary intake of plant sterols was obviously increased because of supplementation with rapeseed oil, which is rich in plant sterols, leading to elevated plant sterol concentrations. Plant sterols may have inhibited cholesterol absorption, which in turn stimulated cholesterol synthesis in compensation, also explaining the increased precursor sterol ratios in serum in our subjects.

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