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Serum non-cholesterol sterols and cholesterol metabolism in childhood and adolescence

Helena Gylling[a,*], Matilda Korhonen[b], Annika Mutanen[c], Markku J. Nissinen[d], Mikko Pakarinen[c], Piia Simonen[b]

[a] Department of Internal Medicine, University of Helsinki and Helsinki University Hospital, PO BOX 700, 00029 HUS, Helsinki, Finland
[b] Heart and Lung Center, Department of Cardiology, University of Helsinki and Helsinki University Hospital, PO BOX 340, 00029 HUS, Helsinki, Finland
[c] Children's Hospital, Pediatric Surgery, University of Helsinki and Helsinki University Hospital, PO BOX 281, 00029 HUS, Helsinki, Finland
[d] Abdominal Center, Department of Gastroenterology, University of Helsinki and Helsinki University Hospital, PO BOX 700, 00029 HUS, Helsinki, Finland

HIGHLIGHTS

- We evaluated cholesterol metabolism in 0–15 year-old children without dyslipidemia.
- Serum non-cholesterol sterols depicted cholesterol synthesis and absorption.
- From the age of 1 year, cholesterol homeostasis was intact.
- From the age of 1–10 years cholesterol absorption prevailed cholesterol synthesis.
- This new finding emphasizes the importance of healthy diet from early childhood.

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ABSTRACT

Background and aims: The profile of cholesterol metabolism, i.e., high absorption *vs.* high synthesis, may have a role in the development of atherosclerosis, the early lesions of which can be present already in childhood. Since there is no information on cholesterol metabolism in children from birth to adolescence, we evaluated cholesterol metabolism in 0–15 year-old children and adolescents without dyslipidemia.

Methods: The study population consisted of 96 children (39 girls, 57 boys) divided into age groups < 1 (n = 14), 1–5 (n = 37), 6–10 (n = 24), and 11–15 (n = 21) years. Cholesterol metabolism was assessed by analysing serum non-cholesterol sterols, biomarkers of cholesterol synthesis and absorption, with gas-liquid chromatography.

Results: Serum non-cholesterol sterol ratios to cholesterol did not differ between gender. Cholesterol precursors squalene, cholestenol, and desmosterol were higher in the < 1 year than in the older age groups, whereas lathosterol was highest in the 11–15 year old. Plant sterols were low in the age group < 1 year, after which they did not differ between the groups. Cholestanol was not age-dependent. From the age of 1 year, cholesterol homeostasis was intact. Cholesterol absorption prevailed cholesterol synthesis from 1 to 10 years of age (e.g., lathosterol/cholestanol ratio 0.35 ± 0.03 and 0.45 ± 0.05 in 1–5 and 6–10 *vs.* 0.66 ± 0.08 in 11–15 year-old (mean ± SE, $p < 0.001$).

Conclusions: Serum non-cholesterol sterols had different individual profiles by age in childhood and adolescence. From 1 to 10 years of age, cholesterol absorption prevailed cholesterol synthesis. This novel finding emphasizes the importance of dietary aspects related to cardiovascular risk even from early childhood.

1. Introduction

Cholesterol is an essential lipid for the development, growth, and wellbeing of humans. In newborn children, cord blood cholesterol concentration is 1.5 mmol/l, on average [1–4]. During the first year of life, cholesterol concentration in serum increases to about 4–5 mmol/l [5], a level that remains throughout the childhood [6]. Serum cholesterol concentration, as well as cholesterol metabolism, is regulated by genetic, dietary, hormonal, and lifestyle factors, e.g., in newborns with heterozygous familial hypercholesterolemia, the concentration of cord

* Corresponding author. Biomedicum Helsinki C 4 22, P.O. BOX 700, 00029 HUS, Helsinki, Finland.
E-mail address: helena.gylling@hus.fi (H. Gylling).

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blood cholesterol is elevated up to about 2.4 mmol/l, and at the age of 1 year, serum cholesterol concentration is about 8 mmol/l [2]. Already in childhood and adolescence, elevated serum and LDL cholesterol concentration can lead to atherosclerotic changes in arterial wall [7]. In addition to LDL cholesterol, the whole-body cholesterol metabolism may have a role in the development of atherosclerosis. This was suggested in adult cohorts in which high cholesterol absorption efficiency increased the risk of coronary events regardless of LDL cholesterol concentration [8–11]. The growth and development in childhood and adolescence question the whole-body cholesterol metabolism, which may vary between different ages. To our knowledge, there is only a limited number of studies on limited age periods dealing with cholesterol metabolism in healthy children and adolescents [1–4,12–14], and there are no studies reaching all age groups from newborn to adolescence. Thus, there is no information on whether the metabolic profile of cholesterol differs between age groups or gender in children and adolescents. Therefore, the aim of this study was to evaluate cholesterol metabolism in healthy children from 0 to 15 years of age by analysing serum non-cholesterol sterols, which in general are used as biomarkers of cholesterol synthesis and cholesterol absorption [15–19].

2. Patients and methods

2.1. Study population

The study population consisted of consecutive 0-15 year-old day-surgery patients of our hospital during the years 2009–2015, who were otherwise healthy and did not have dyslipidemia, diabetes, thyroid, renal, hepatobiliary, gastrointestinal, or malignant disease. Written informed consent was received from all patients or their caregivers before any procedure. The study was performed according to the principles of the Declaration of Helsinki. The Ethics Committee of the Helsinki University Central Hospital had approved the study protocol.

2.2. Study design

Fasting blood samples were drawn after a 12-h fast in the morning before the surgery. The subjects of the study population were weighed and height was measured, and medical history and possible drug treatment were recorded from the medical records. All subjects had been on their habitual home diet, but more detailed dietary information or the breastfeeding status was not recorded.

2.3. Methods

Serum cholesterol and non-cholesterol sterols and squalene were quantified by capillary gas-liquid chromatography (GLC) with flame ionization detection and using a 50-m capillary column (Ultra 2, Agilent Technologies, Wilmington, DE) with 5α-cholestane as the internal standard [20]. The following non-cholesterol sterols were analysed: squalene, cholestenol (5α-cholest-8-en-3β-ol), desmosterol, and lathosterol (cholesterol precursors), cholestanol (a metabolite of cholesterol), and campesterol, sitosterol, stigmasterol, and avenasterol (plant sterols). To eliminate the effects of variation of non-cholesterol sterol transporters (mainly LDL) on the serum sterol concentrations, the non-cholesterol sterol concentrations were standardized and divided by the cholesterol concentration of the same GLC run and expressed as ratios to cholesterol (10^2 x μmol/mmol of cholesterol). In general, the ratios to cholesterol of the serum cholesterol precursors reflect cholesterol synthesis, while those of plant sterols (campesterol, sitosterol, and avenasterol) and cholestanol reflect cholesterol absorption efficiency. We also calculated the synthesis marker/absorption marker ratios cholestenol/cholestanol and lathosterol/cholestanol, which reflect cholesterol metabolism, the lathosterol/desmosterol ratio reflecting the activity of the two pathways of cholesterol synthesis, and the campesterol/cholestanol ratio, a biomarker of dietary phytosterol intake [21].

2.4. Statistics

Statistical analyses were performed with SPSS for Windows 22.0 (SPSS, Chicago, IL). The normality and homogeneity of variance assumptions were checked before further analyses. Comparison of continuous variables between groups were performed by analysis of variance followed by independent samples *t*-test or Mann-Whitney test if the normality and homogeneity of variance were not reached after logarithmic transformation. Non-continuous variables were tested with Chi-square or Fisher's exact test. Spearman correlation coefficients were calculated. To control the overall level, Bonferroni adjustment was used. A p-value of < 0.05 was considered statistically significant. The results are expressed as mean ± SE.

3. Results

The study population consisted of 96 children and adolescents. Thirty-nine subjects were girls and 57 were boys (Table 1). The mean age of the study population was 6.6 ± 0.5 (SE) years ranging from 0.10 to 15.94 years, and there was no difference between girls and boys.

Table 1
Gender, age, weight, serum cholesterol concentration and serum non-cholesterol sterol ratios to cholesterol in the study population divided in different age groups.

Variables	Age < 1 year n = 14	Age 1–5 years n = 37	Age 6–10 years n = 24	Age 11–15 years n = 21	*p*
Girls/boys, n	3/11	17/20	11/13	8/13	0.670
Age, years	0.36 ± 0.06	3.82 ± 0.22	8.27 ± 0.29	13.62 ± 0.34	< 0.001
Weight, kg	7.6 ± 1.2	16.5 ± 0.6	29.7 ± 1.6	51.1 ± 2.9	< 0.001
Cholesterol, mmol/l	3.38 ± 0.20	3.75 ± 0.1	4.14 ± 0.14[b]	3.94 ± 0.14	0.008
Squalene[a]	21.6 ± 1.9	13.0 ± 0.9[b]	12.7 ± 0.7[b]	16.3 ± 1.7	< 0.001
Cholestenol[a]	20.7 ± 1.6	11.3 ± 0.7[b]	12.7 ± 1.0[b]	15.9 ± 1.3[b]	< 0.001
Desmosterol[a]	158.5 ± 13.6	77.7 ± 2.1[b]	79.7 ± 2.4[b]	91.5 ± 3.1[b]	< 0.001
Lathosterol[a]	66.3 ± 5.7	60.1 ± 3.5	77.2 ± 6.4	100.9 ± 8.6[c]	< 0.001
Campesterol[a]	154.4 ± 35.3	344.9 ± 18.9[b]	361.6 ± 19.0[b]	313.3 ± 23.1[b]	< 0.001
Sitosterol[a]	140.2 ± 40.4	197.8 ± 8.9	204.1 ± 11.8	174.2 ± 14.1	0.068
Stigmasterol[a]	19.0 ± 2.3	28.7 ± 1.4[b]	30.8 ± 3.0[b]	26.1 ± 1.4	0.006
Avenasterol[a]	24.3 ± 4.1	53.0 ± 2.7[b]	52.8 ± 3.0[b]	46.1 ± 3.0[b]	< 0.001
Cholestanol[a]	170.1 ± 8.3	176.3 ± 5.0	180.5 ± 8.6	168.3 ± 7.2	0.628

Mean ± SE.
[a] 10^2 μmol/mmol cholesterol.
[b] Significantly different from age < 1 year.
[c] Significantly different from all other age groups.