Plasma Levels of Lathosterol and Phytosterols in Relation to Age, Sex, Anthropometric Parameters, Plasma Lipids, and Apolipoprotein E Phenotype, in 160 Dutch Families

H.J.M. Kempen, P. de Knijff, D.I. Boomsma, H.A. van der Voort, J.A. Gevers Leuven, and L. Havekes

In this study, the relation of plasma levels of lathosterol (an indicator of whole body cholesterol synthesis) and plant sterols (indicator of cholesterol absorption) with age, sex, weight, height, plasma lipids, and lipoproteins, and with apolipoprotein (apo) E phenotype, was investigated in a group of 160 nuclear families consisting of twins living with their parents. Lathosterol was higher in fathers than in mothers, but not different between boys and girls. In each of these four groups, there was a strong correlation with plasma and low-density lipoprotein (LDL)-cholesterol and -triglyceride, as well as with body weight, but not with height or high-density lipoprotein (HDL)-cholesterol. In adults, lathosterol was inversely correlated with plant sterols. Lathosterol was higher in children with E4/3 phenotype than in those with E3/3 or E3/2; in adults, lathosterol did not differ among the various E phenotypes. The plasma levels of the two plant sterols, campesterol and β-sitosterol, were highly correlated with each other, and also with plasma or LDL-cholesterol, in each of the four groups. Plant sterols were higher in adults or children with E4/3 phenotype as compared with those with other phenotypes. In multivariate analysis (performed separately for two groups of adults and children) plasma cholesterol, plasma plant sterols, plasma triglycerides, and weight were found to make significant contributions to the variation of lathosterol in all groups, and E phenotype and sex only in one group, while age did not contribute in any group. For plant sterols, plasma cholesterol and lathosterol were significant independent predictors in all groups, sex and E phenotype only in one or two of the four groups, and age, weight, height, and HDL-cholesterol in none of the groups. Thus, although lathosterol and plant sterols were weakly related to E phenotype in some of the groups, these findings do not support a major role for the E phenotype in determining rates of cholesterol synthesis or absorption, as claimed by others.

THE RATES OF cholesterol synthesis and cholesterol absorption in man are of considerable interest in both fundamental and clinical respects. The latter has been underscored in recent years by the advent of potent and selective inhibitors of the enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, rate-limiting for the overall process of cholesterol synthesis.[1,2] These inhibitors have proven to be capable of lowering serum levels of low-density lipoprotein (LDL) with minimal side effects,[3] and have found wide acceptance already for the treatment of severe hypercholesterolemia.

In 1988, we reported on the use of the serum concentration of lathosterol, a late precursor of cholesterol, as an indicator of the rate of whole body cholesterol synthesis in healthy males on normal western diets.[4] In that report, we confirmed the pioneering work of Miettinen et al,[5-7] showing that serum levels of lathosterol and other sterol precursors changed in the same direction as the (expected) rate of cholesterol synthesis upon various manipulations, including small intestinal resection, cholestyramine feeding, or bile acid administration. Additional support for the validity of serum lathosterol as indicator for whole body cholesterol synthesis was gained from the demonstration that serum lathosterol was strongly decreased (more than serum LDL) in familial hypercholesterolemia (FH) patients during treatment with simvastatin, an HMG-CoA reductase inhibitor.[4]

Miettinen et al also reported that levels of serum plant sterols campesterol and β-sitosterol correlate with the fractional absorption of dietary cholesterol.[8,9] So far, this has not been reproduced by other laboratories.

In this study, we quantitated the plasma levels of lathosterol and phytosterols in a large group of healthy people to assess their relationships with sex, height, weight, and apolipoprotein (apo) E phenotype. The latter was considered of particular interest in view of the reports from Miettinen's laboratory reporting that both cholesterol synthesis and fractional cholesterol absorption were different in humans with different apo E phenotype.[10,11]

Our data are obtained as part of a parent-twin study, in which 160 nuclear families (parents and children) participated. The present report is restricted to a presentation of the descriptive statistics and the intercorrelations with anthropometric and lipoprotein parameters; the quantitative genetic inferences will be reported elsewhere.

SUBJECTS AND METHODS

Subjects

This study is part of a project in which cardiovascular risk factors were studied in 160 adolescent twin pairs and their parents. Addresses of twins (between 14 and 20 years of age) living in Amsterdam and neighboring cities were obtained from the City Council population registries. Twins still living with both their biological parents were contacted by letter and asked to participate in the study. A family was included in the study only if the twins and both parents were willing to cooperate. Between 30% and 40% of the families contacted complied. In addition, a small number of families who heard of the study from other twins also volunteered to participate. All subjects were paid $20 to reimburse expenses related to their participation. At the time of the data collection, 83 families lived in and 77 outside Amsterdam. There were 35 families

From the Gaubius Institute, Leiden, The Netherlands; and the Department of Psychonomy, Free University, Amsterdam, The Netherlands.

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Address reprint request to H.J.M. Kempen, PhD, F. Hoffmann-La Roche AG, Department PRPM, Bldg 60/106, CH-4002 Basel, Switzerland.

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with monozygotic boys, 35 with monozygotic girls, 31 with dizygotic boys, 30 with dizygotic girls, and 29 with dizygotic twins of opposite sex. During the first visit, the mother was asked to tell which of the twins was born first. This person was then further denoted as "oldest" of the twins, the other twin as "youngest."

The health status of the subjects was recorded for all of the participants. At the time of their active participation they were apparently healthy. Some of them had chronic conditions like hypertension, diabetes, or asthma, for which they received drug treatment. Drug consumption was recorded. None of the subjects used hypolipidemic drugs. We have chosen not to omit these subjects, since none of the drugs used are known to influence rates of cholesterol synthesis or sterol absorption.

Blood samples could not be obtained from two of the fathers.

Participants recorded their food intake (items and quantities) during 1 weekday and 1 weekend day. These records were converted into macro-nutrients and micronutrients using the Dutch Food Composition Table.[12] For the purpose of this report, intake of dietary fiber (average of the 2 days) was used as an indirect indicator of plant food intake.

Methods

Fasting blood was taken between 8:30 and 10:30 AM by venapuncture, using Becton-Dickinson Vacutainers containing sodium-EDTA. After centrifugation for 10 minutes at 3,000 rpm, the plasma was aspirated and kept at 4°C for lipid determinations within the next 5 days. Cholesterol and triglyceride concentrations were determined using enzymatic methods (Boehringer CHOD-PAP kit, no. 236691 and Triglyceride kit no. 701904). High-density lipoprotein (HDL)-cholesterol was measured after precipitation of very-low-density lipoprotein (VLDL) and LDL using $MnCl_2$ and phosphotungstate.[13] LDL-cholesterol was calculated by the formula of Friedewald et al.[14] The remaining portions of plasma were stored at −80°C, in 2.5-mL tubes with tightly fitting screw caps.

For quantitation of the concentrations of lathosterol and plant sterols campesterol and β-sitosterol, one tube was thawed. Gas chromatography was performed essentially as reported before,[4] now using a CPSil 5CB column (30 m × 0.25 mm) in a Chrompack model 438 S gas chromatograph. In brief, to 150 μL serum, 10 μg 5-cholestane (in 50 μL ethanol) and 1 mL 0.4 mmol/L KOH in 95% ethanol were added. The mixture was left for 60 minutes at 37°C to saponify the lipid esters, and then extracted with 2 mL hexane after addition of 1 mL distilled water. The hexane was removed by evaporation and the residue derivatized using 25 μL of the "Deriva-Sil" preparation (Chrompack, no. 8260). After mixing and leaving the tubes for at least 15 minutes at room temperature, 1 μL of this mixture was injected using a split ratio 1:20. The injector port and detector were at 290°C, the oven temperature was first kept for 1 minute at 265°C, then raised by 0.5°C/min for 17 minutes, then increased by 25°C/min until 318°C, where it was kept for 10 minutes. In these conditions, the retention times of lathosterol, campesterol, and β-sitosterol were 9.57, 10.54, and 12.51 minutes, respectively. The procedure was found to have an intraassay coefficient of variation of 5%, 4%, and 4% for lathosterol, campesterol, and sitosterol, respectively (n = 10).

Apo E phenotyping was performed by isoelectric focusing of delipidated plasma followed by immunoblotting, as recently described.[15]

Statistics

Student's *t* test for comparing two samples, single and multiple regressions, and ANOVA procedures were performed using the programs contained in the package NCSS, version 5.01 (Dr J.L. Hintze, Kaysville, UT).

RESULTS

The mean ± SD values of age, anthropometric measures, and plasma levels of lipids and sterols are given in Table 1, separately for the fathers, mothers, sons, and daughters in the 160 families. Figure 1 shows the distribution of plasma levels of lathosterol, campesterol, and β-sitosterol; these distributions display a slight positive skewness in both sexes within each generation.

We addressed the question whether a sex difference existed in the parameters of interest. A valid statistical evaluation of this question requires that observations within groups are independent. For this purpose, fathers of the odd-numbered families were compared with mothers of the even-numbered families, and oldest boys with oldest girls. Separately, the statistical evaluation was performed for the opposite selection (fathers of even, mothers of odd families, youngest children). In this manner, two independent replicate samples were obtained from the total number of participants. The results of the two comparisons are also given in Table 1, showing similar outcomes.

For their age, the adults and children had the expected weight and height, males being heavier and taller than females. Plasma cholesterol and LDL-cholesterol did not differ between adult men and women, but were lower in boys than in girls. Plasma triglyceride was higher in fathers than in mothers, but not different between boys and girls. HDL-cholesterol was higher in women than in men, irrespective of generation. Plasma lathosterol was higher in fathers than in mothers, but not different between boys and girls. Plant sterols did not display a sex difference in either generation.

We also tested whether these parameters differed between generations, regardless of sex. For that purpose, the parents of odd-numbered families were compared with the oldest children of even-numbered families. Parents had significantly higher body weight, as well as higher plasma cholesterol, triglyceride, LDL-cholesterol, and lathosterol levels than children. Height, HDL-cholesterol, or plant sterols did not differ between generations.

Finally, to test for interaction between sex and generation in a two-way ANOVA, we randomly selected one subject out of each family, resulting in a group of 160 unrelated adults and children. This was then repeated three times, each time yielding a different set. In these four sets, the ANOVA for lathosterol showed a significant effect of sex only once, an effect of generation four times, and an interaction between sex and generation two times. No main effect or interaction of sex and generation appeared for either of the plant sterols in any of these four sets.

The simple correlation coefficients of lathosterol or plant sterols with each other or with age, weight, height, or plasma lipids, are given in Table 2, separately for both sexes and generations. In this case, the data of all fathers and mothers could be used, while for the children the data of the oldest or youngest twin again were taken separately.

In boys and girls, lathosterol was positively correlated with age, but this was significant only in one of the two sets. In parents, age had no effect. Lathosterol was also positively related with weight (although just not significant in

Table 1. Age, Anthropometric Parameters, and Plasma Lipid and Sterol Levels, in Parents and Children of Twin Families

	Men (n = 158)	Women (n = 160)	P^1	Boys (n = 161)	Girls (n = 159)	P^1
Age	48.1 ± 6.7	45.6 ± 5.9	*/†	16.7 ± 1.8	16.7 ± 2.2	NS/NS
(yr)	35.3-65.1	34.5-59.5		13.9-21.4	13.1-22.1	
Weight	80.7 ± 9.4	67.9 ± 10.1	‡/‡	61.5 ± 10.3	56.6 ± 8.2	*/†
(kg)	56.0-104.5	46.5-114.5		31.6-83.8	40.5-83.7	
Height	177.6 ± 6.7	165.2 ± 5.3	‡/‡	176.2 ± 8.9	166.5 ± 6.9	‡/‡
(cm)	160.5-196.6	152.0-178.0		146.5-195.0	150.0-187.0	
Chol	5.84 ± 1.03	5.63 ± 1.12	NS/NS	4.05 ± 0.65	4.47 ± 0.86	‡/†
(mmol/L)	3.26-10.45	3.35-9.40		3.00-6.70	2.17-7.10	
Trig	1.39 ± 0.71	0.95 ± 0.45	‡/‡	0.67 ± 0.30	0.71 ± 0.28	NS/NS
(mmol/L)	0.35-4.07	0.17-3.19		0.20-2.32	0.24-2.02	
HDL-chol	1.14 ± 0.28	1.41 ± 0.31	‡/‡	1.23 ± 0.22	1.38 ± 0.29	‡/†
(mmol/L)	0.60-2.40	0.70-2.51		0.61-1.78	0.86-2.56	
LDL-chol	4.07 ± 0.97	3.79 ± 1.06	NS/NS	2.53 ± 0.63	2.76 ± 0.76	*/*
(mmol/L)	1.87-8.44	1.73-7.66		1.23-5.28	0.99-4.63	
Latho	7.43 ± 2.84	6.08 ± 2.53	†/†	3.85 ± 1.52	4.02 ± 1.58	NS/NS
(μmol/L)	2.11-15.17	2.09-15.76		1.40-10.82	1.06-10.84	
Campe	12.68 ± 5.33	12.31 ± 5.49	NS/NS	12.13 ± 5.52	12.31 ± 5.77	NS/NS
(μmol/L)	3.56-37.0	2.88-34.95		3.36-36.1	4.16-44.7	
Sito	6.78 ± 2.88	6.96 ± 3.09	NS/NS	6.50 ± 2.70	6.98 ± 3.33	NS/NS
(μmol/L)	2.73-25.2	2.04-18.80		2.02-19.03	1.78-21.76	

NOTE. Numerical data are means ± SD, and range. Statistical tests for a sex difference were done for the fathers of the odd-numbered families versus the mothers of the even-numbered families, and for the oldest boys versus the oldest girls, as well as for the opposite sets (fathers of even-numbered *v* mothers of odd-numbered families, youngest boys *v* youngest girls). The results of both tests are given, separated by a slash.

Abbreviations: chol, total plasma cholesterol; trigl, total plasma triglycerides; latho, total plasma lathosterol, campe, total plasma campesterol; sito, total β-sitosterol.

[1]Student's *t* test for significance of sex difference.

*$P < .05$.

†$P < .005$.

‡$P < .0005$.

one set for girls). It was not correlated with height in any group. It was highly correlated with total and LDL-cholesterol and also with triglycerides, but not with HDL-cholesterol, in both generations and both sexes. Finally, lathosterol was inversely correlated with plant sterols, which was significant only in the adults.

Plant sterols tended to be correlated negatively with age (only significant in boys), with weight (not significant in fathers), and with height (only significant in children). They correlated positively with plasma and LDL-cholesterol (although for sitosterol not significant in fathers), but not with triglycerides. In the fathers, plant sterols were correlated also with HDL-cholesterol. Finally, the two plant sterols were highly correlated with each other.

The possibility was considered that plasma plant sterol levels were related to the dietary intake of these sterols. Although we were unable to quantify the plant sterol content of the diet, and also could not calculate this from the food records, an indirect estimation of the relative contribution of plants to the total food intake was available in the form of dietary fiber. In none of the groups, plasma phytosterols were significantly correlated with fiber intake (data not shown).

We next examined whether plasma lipids, as well as lathosterol or plant sterols, would differ according to the apo E phenotype. This was done separately for two sets of adults and two sets of children, using a two-way ANOVA to check for a possible interaction between E phenotype and sex. Males did not significantly differ from females in their frequency distribution among E phenotypes, in each of the sets (χ-square test). As shown in Table 3, plasma cholesterol and LDL-cholesterol levels were found to be lower in subjects with the E3/2 phenotype than with the E3/3 or E4/3 phenotype (significant in one set of adults and in both sets of children). The latter two phenotypes did not differ from each other. HDL-cholesterol tended to be higher in E3/2 subjects, while triglycerides levels were not different among apo E phenotypes.

For lathosterol, we found no effect of the apo E phenotype in the two sets of adults, while in one set of children E4/3 subjects had significantly higher lathosterol values than in the E3/3 or E3/2 subjects.

The plant sterols were higher in subjects with the E4/3 phenotype as compared with those with the E3/3 or E3/2 phenotypes, although this was significant in only one of the two sets of adults and children.

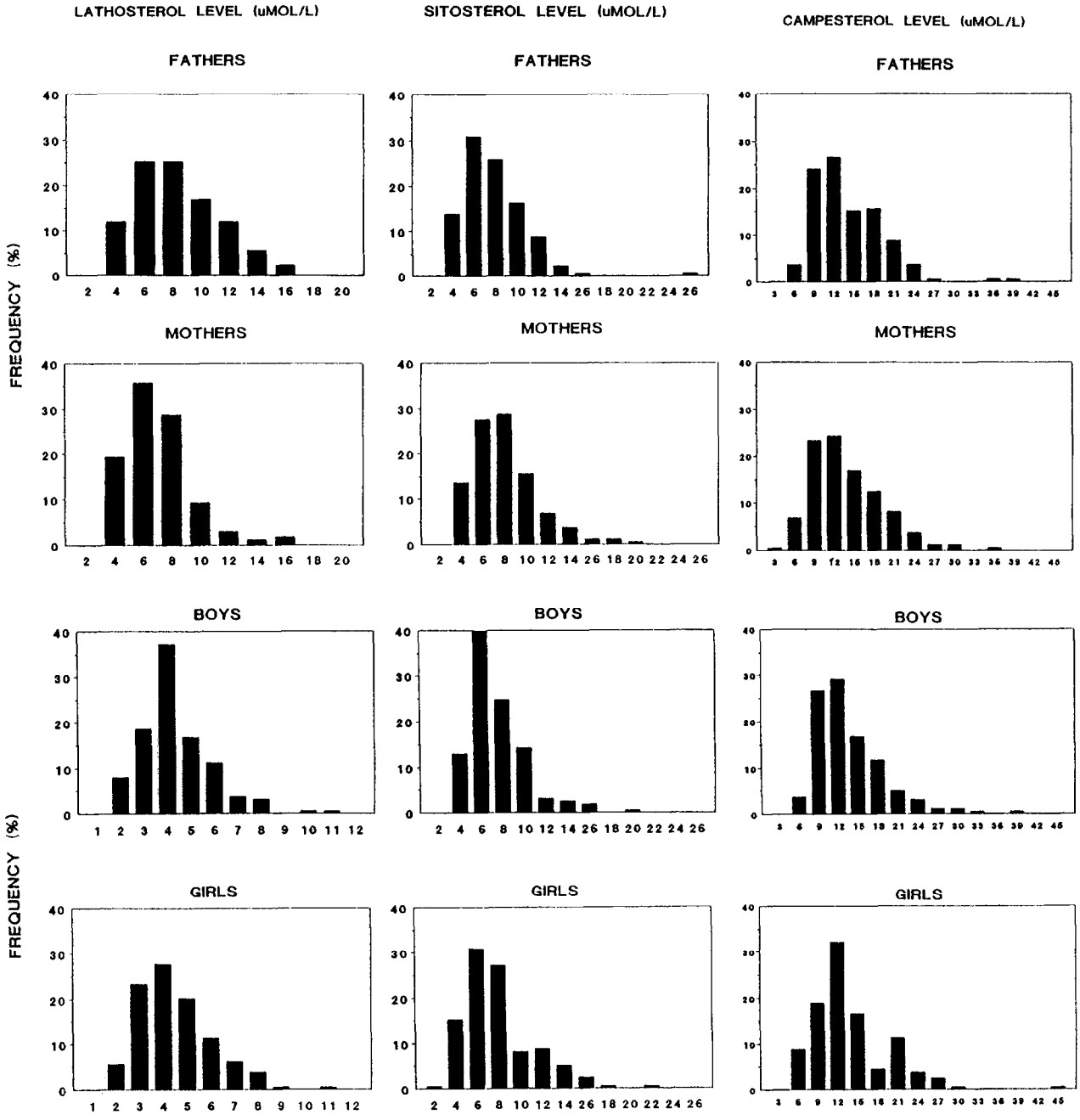

Fig 1. Frequency distribution of plasma levels of lathosterol, campesterol, and β-sitosterol in the 638 subjects (158 fathers, 160 mothers, 161 sons, 159 daughters) participating in the study.

No significant interactions were found between E phenotype and sex for any of the parameters, in either generation.

To assess the possibility that the higher level of plant sterols in the E4/3 subjects might be due to a higher dietary intake of plant sterols by these subjects, we checked if the intake of dietary fiber differed among the various E phenotypes. This was found not to be the case (results not shown).

It was finally questioned whether these univariate relations of lathosterol or the plant sterols with age, sex, weight, height, plasma lipids, and E phenotype would be independent of each other. This was tested by multiple regression analysis, again separately for two sets in each generation. The sum of the suffixes was taken as a numeric variable for the E phenotype.

As shown in Table 4, lathosterol remained correlated with weight, plasma cholesterol, and triglycerides, while the correlation with age disappeared in all groups. The sex difference seen between fathers and mothers (Table 1) also disappeared. The negative correlation with plant sterols (shown in Table 1 for campesterol, but virtually the same

Table 2. Univariate Correlations of Lathosterol and Phytosterol Levels With Each Other, and With Age, Anthropometric Parameters, Plasma Cholesterol, Triglyceride, HDL-Cholesterol, and LDL-Cholesterol Levels

			Boys		Girls	
	Men (n = 158)	Women (n = 159)	Set 1 (n = 82)	Set 2 (n = 78)	Set 1 (n = 78)	Set 2 (n = 82)
With age						
Latho	−0.042	0.155	0.147	0.328*	0.297*	0.135
Campe	−0.149	−0.048	−0.232*	−0.259*	0.000	0.073
Sito	−0.084	−0.044	−0.204*	−0.268*	0.013	0.162
With weight						
Latho	0.308***	0.195*	0.268*	0.236*	0.204	0.283*
Campe	−0.066	−0.179*	−0.243*	−0.303*	−0.056	−0.301*
Sito	−0.131	−0.217*	−0.198	−0.322*	−0.065	−0.253*
With height						
Latho	0.075	−0.052	0.063	0.104	0.081	0.051
Campe	−0.001	−0.104	−0.237*	−0.334†	−0.044	−0.377†
Sito	−0.007	−0.097	−0.154	−0.336†	−0.044	−0.283*
With plasma cholesterol						
Latho	0.495‡	0.456‡	0.450‡	0.428‡	0.416‡	0.396‡
Campe	0.222†	0.316‡	0.537‡	0.483‡	0.278*	0.470‡
Sito	0.129	0.286‡	0.531‡	0.488‡	0.358†	0.544‡
With plasma triglycerides						
Latho	0.444‡	0.357‡	0.326†	0.342†	0.364†	0.391‡
Campe	−0.021	−0.061	−0.117	−0.281*	−0.026	0.113
Sito	−0.112	−0.093	−0.079	−0.286*	−0.017	0.131
With HDL-cholesterol						
Latho	−0.113	−0.043	−0.033	−0.121	0.141	−0.081
Campe	0.188*	0.084	0.105	0.265*	0.105	0.234*
Sito	0.208*	0.040	0.037	0.239*	0.170	0.289*
With LDL-cholesterol						
Latho	0.415‡	0.428‡	0.410‡	0.411‡	0.403‡	0.419‡
Campe	0.191*	0.324‡	0.550‡	0.465‡	0.272*	0.440‡
Sito	0.115	0.309‡	0.560‡	0.478‡	0.332*	0.501‡
With campesterol						
Latho	−0.007	−0.190*	−0.010	−0.149	−0.148	−0.116
Sito	0.861‡	0.860‡	0.925‡	0.939‡	0.924‡	0.934‡
With sitosterol						
Latho	−0.203*	−0.200*	−.046	−0.176	−0.083	−0.086

NOTE. Date are correlation coefficients (Pearson). For the children, the correlations for the oldest twins (set 1) and youngest twins (set 2) are given separately.

*$P < .05$.

†$P < .005$.

‡$P < .0005$.

for β-sitosterol) now was found also in the children. Lathosterol was not correlated with the apo E phenotype, except in the set of children, of which the E4/3 subjects were found to have a higher lathosterol already in the ANOVA.

For plant sterols, the positive correlation with cholesterol and the inverse correlation with lathosterol both were maintained. There was a positive correlation with the apo E phenotype, although this was only significant in one of the two sets of adults or children. The correlations with age, weight, height, or HDL-cholesterol were decreased to insignificant values. However, a significant sex effect now appeared in one of the two sets of adults and children.

The findings were quite similar for both generations. The total variance in lathosterol was explained for 33% to 44%, mainly by body weight, plasma cholesterol, triglycerides, and plant sterols. The total variance in plant sterols was explained for 19% to 44%, mainly by plasma cholesterol and lathosterol, and to a smaller extent by apo E phenotype and sex.

DISCUSSION

Lathosterol

This study shows that plasma lathosterol is highly correlated with plasma cholesterol (positive), and to a lesser extent with plant sterols (inverse), body weight (positive), and plasma triglycerides (positive). The correlation with age in the children appeared to be due to that with weight, since it disappeared in the multiple regression model. In the latter, the correlation with body weight was maintained in both generations. This would agree with the status of lathosterol as indicator of cholesterol synthesis put forward by Miettinen et al[5-7] and ourselves,[4] since Nestel et al[16] and Goodman et al[17] have documented that whole body cholesterol synthesis is highly correlated with body weight.

Table 3. Plasma Concentrations of Cholesterol, Triglyceride, HDL- and LDL-Cholesterol, as well as of Lathosterol and Phytosterols in Adults and Children, According to Apo E Phenotype

	Set	E2/3	E3/3	E3/4	$F_{E\text{-}fenc}$	F_{Inter}
Adults						
n	1	16	100	35		
	2	32	85	32		
Chol	1	5.52 ± 0.28	5.73 ± 0.11	6.13 ± 0.19	2.35	NS
	2	5.35 ± 0.18	5.69 ± 0.11	5.92 ± 0.18	4.32*	NS
Trigl	1	1.02 ± 0.15	1.13 ± 0.06	1.14 ± 0.10	0.39	NS
	2	1.35 ± 0.10	1.13 ± 0.06	1.31 ± 0.10	0.29	NS
HDL-chol	1	1.43 ± 0.08	1.27 ± 0.03	1.31 ± 0.05	2.32	NS
	2	1.28 ± 0.05	1.25 ± 0.03	1.18 ± 0.05	0.22	NS
LDL-chol	1	3.63 ± 0.27	3.94 ± 0.11	4.31 ± 0.18	3.05	NS
	2	3.46 ± 0.16	3.92 ± 0.10	4.16 ± 0.16	5.87†	NS
Latho	1	6.52 ± 0.67	6.42 ± 0.27	7.11 ± 0.45	0.78	NS
	2	6.42 ± 0.48	7.08 ± 0.30	6.91 ± 0.49	0.17	NS
Campe	1	11.22 ± 1.37	12.15 ± 0.55	15.84 ± 0.92	6.65†	NS
	2	11.52 ± 0.91	11.90 ± 0.56	13.57 ± 0.91	1.64	NS
Sito	1	6.31 ± 0.81	6.86 ± 0.32	8.40 ± 0.55	3.07*	NS
	2	6.03 ± 0.46	6.51 ± 0.28	7.27 ± 0.46	1.51	NS
Children						
n	1	33	83	37		
	2	30	87	39		
Chol	1	3.93 ± 0.14	4.34 ± 0.09	4.53 ± 0.13	8.71*	NS
	2	3.86 ± 0.13	4.24 ± 0.08	4.44 ± 0.12	7.82†	NS
Trigl	1	0.69 ± 0.05	0.69 ± 0.03	0.75 ± 0.05	0.49	NS
	2	0.64 ± 0.05	0.64 ± 0.03	0.78 ± 0.04	3.56*	NS
HDL-chol	1	1.41 ± 0.05	1.29 ± 0.03	1.27 ± 0.04	3.47*	NS
	2	1.40 ± 0.04	1.28 ± 0.03	1.30 ± 0.04	2.44	NS
LDL-chol	1	2.20 ± 0.12	2.75 ± 0.07	2.94 ± 0.11	15.8†	NS
	2	2.17 ± 0.12	2.67 ± 0.07	2.79 ± 0.10	8.84†	NS
Latho	1	3.86 ± 0.87	3.85 ± 0.17	4.37 ± 0.25	1.52	NS
	2	3.37 ± 0.26	3.75 ± 0.15	4.26 ± 0.23	3.62*	NS
Campe	1	10.93 ± 0.96	11.94 ± 0.61	14.99 ± 0.91	6.45†	NS
	2	9.99 ± 0.99	12.18 ± 0.58	13.11 ± 0.88	2.48	NS
Sito	1	6.03 ± 0.51	6.47 ± 0.32	8.44 ± 0.48	8.82†	NS
	2	5.79 ± 0.54	6.66 ± 0.32	7.09 ± 0.48	1.78	NS

NOTE. Two-way ANOVA was used with E phenotype and sex (1 = male, 2 = female) as factors. For the adults, the analysis was performed separately for the set of fathers of odd plus mothers of even families (set 1) and for the set of fathers of even plus mothers of odd families (set 2). For the children, set 1 is the collection of oldest twins, set 2 that of the youngest twins. Subjects with the E2/2, E4/4, and E4/2 phenotype are omitted from the analysis, since they are too small in number (3, 5, and 10 adults and 1, 6, and 4 children, respectively). Data are means ± SEM for each phenotype, in mmol/L or (for lathosterol and the plant sterols) μmol/L.

*$P < .05$, †$P < .005$. Subsequent testing for contrasts (using Fisher's least squares difference procedure) allowed the conclusions as stated in the text.

The latter investigators also found that cholesterol synthesis is not correlated with plasma levels of cholesterol or triglyceride when body weight is accounted for. The fact that in our multiple model lathosterol remained correlated with the plasma levels of these macrolipids suggests that the lathosterol level is also partly determined by a partitioning phenomenon ("extraction" of lathosterol from organs to plasma in proportion to the total amount of plasma lipid acting as a physicochemical acceptor), as discussed before.[4]

The difference in lathosterol level between the sexes in the parents disappeared when body weight and plasma lipids are taken into account. For whole body cholesterol synthesis, a sex difference has been reported in tabular form,[18] but synthesis was not expressed per kilogram body weight in that report.

We were particularly interested in the effect of the apo E phenotype on plasma lathosterol levels. Cholesterol synthesis in the liver might be higher in subjects with the E2/2 or E2/3 isoform pattern, as in these subjects the liver would take up chylomicron remnants (carrying the absorbed cholesterol from the intestine) less efficiently.[19] Conversely, hepatic synthesis might be lower in subjects with E4/4 or E4/3. Indeed, Kesaniemi et al,[10] reported a lower lathosterol to cholesterol ratio in middle-aged people with E4/4 or E4/3 than in people with E3/3 or E3/2. However, this was not borne out in our study, which comprised a much larger number of each of the phenotypes than the Finnish study. In fact, in one set of children lathosterol was even higher in those with E4/3 than those with E3/3 or E3/2 (Table 3).

The absence of an association of apo E phenotype with lathosterol in adults is in agreement with the finding of Palmer et al[20] that whole body cholesterol metabolism was not correlated with the apo E phenotype in adults.

The studies of Kesaniemi et al,[10] Palmer et al,[20] and our own data all show that subjects with E2/E3 have the same rate of cholesterol synthesis and/or the same level of plasma lathosterol as those with E3/3. Furthermore, we have reported recently that subjects homozygous for the E2 isoform also have no different lathosterol level, unless in addition they show the traits of type III hypercholesterolemia.[21] Therefore, if E2 carriers really have a lower cholesterol influx into the liver via chylomicron remnants, this is probably balanced by an increased uptake of LDL via enhanced LDL-receptor activity, and not by increase in cholesterol synthesis. An increased LDL uptake indeed is evident from the observation that LDL-cholesterol was clearly lower in the E2/3 carriers than in the E3/3 or E3/4 subjects (Table 3), in agreement with a number of earlier studies.[22-25]

Plant Sterols

The most important correlations were with plasma or LDL-cholesterol (positive) and with lathosterol (inverse), the latter of which was apparent most clearly and consistently in the multiple regression analysis.

The correlation with total cholesterol does not seem to be due to a "partioning effect," since the plant sterols were not correlated with triglycerides (as lathosterol was). The correlations with plasma and LDL-cholesterol are consistent with the notion, supported strongly by the recent study of Miettinen et al in free-living human volunteers,[9] that plant sterols are proportional to fractional cholesterol absorption, which in turn is inversely related to the hepatic LDL-receptor activity and so positively to the plasma cholesterol level.

In addition, the independent inverse correlation with lathosterol suggests that cholesterol absorption also is a (suppressive) determinant of hepatic cholesterol synthesis.

Table 4. Multiple Regressions of Lathosterol and the Phytosterols on the Various Predictive Parameters Identified in Tables 1 to 3

		Adults		Children	
Dependent	Independent	Set 1	Set 2	Set 1	Set 2
Latho	Age	−0.034	−0.057	−0.027	−0.016
	Weight	0.242†	0.026	0.192*	0.172*
	Sex	0.022	−0.167*	−0.058	−0.043
	Choles	0.393‡	0.472‡	0.455‡	0.429‡
	Trigl	0.217*	0.212*	0.175*	0.192*
	Campe	−0.260†	−0.285‡	−0.214*	−0.311‡
	E-pheno	0.092	0.032	0.005	0.212*
	R^2	0.389	0.428	0.318	0.389
Campe	Age	−0.141	−0.142	−0.093	−0.003
	Weight	−0.056	−0.070	−0.024	−0.054
	Height	0.024	−0.072	−0.022	−0.124
	Sex	−0.027	−0.249†	−0.043	−0.275†
	Choles	0.409‡	0.338‡	0.380‡	0.481‡
	E-pheno	0.182*	0.118	0.167*	0.123
	HDL-chol	0.124	0.130	−0.006	0.000
	Latho	−0.261†	−0.313†	−0.236†	−0.358‡
	R^2	0.238	0.215	0.254	0.401
Sito	Age	−0.157	−0.063	−0.101	0.070
	Weight	−0.163*	−0.046	−0.038	−0.109
	Height	0.072	−0.079	0.028	−0.072
	Sex	−0.037	−0.170*	0.042	−0.226*
	Choles	0.392‡	0.282†	0.414†	0.544†
	E-pheno	0.140	0.151	0.203*	0.043
	HDL-chol	0.120	0.086	0.011	−0.031
	Latho	−0.274†	−0.354‡	−0.249†	−0.382‡
	R^2	0.249	0.189	0.307	0.442

NOTE. For E-phenotype the number of subscripts was taken as numerical variable. Data represent partial correlation coefficients, and total explained variance (R^2). Adults and children were evaluated each as two sets, as described in Table 3.

*$P < .05$.

†$P < .005$.

‡$P < .0005$.

We confirmed the effect of apo E phenotype on plant sterol levels as reported by Kesaniemi et al[10] and Gylling et al.[11] Analysis of the food intake data made it highly unlikely that this effect was due to a difference in dietary intake of plant sterols. In the latter studies, the differences between E phenotypes were not statistically significant, whereas significance was found (also after adjustment for the other covariates) in two of the four sets that we have analyzed. In those two sets, the effect was solely due to the E4 allele; subjects with the E2/3 phenotype did not differ from the E3/3 subjects. This is in complete agreement with the finding for fractional cholesterol absorption,[10,11] which also was higher in subjects carrying the E4 isoform, and not different between the E2 (E2/2 or E2/3) and E3/3 subjects. So far, no biochemical explanation is available for this effect of the E4 allele on plant sterols or fractional cholesterol absorption.

In the univariate analysis, plant sterols were negatively correlated with weight, in agreement with the recent report that cholesterol absorption is reduced in obesity.[26] However, this correlation disappeared in the multivariate model, when lathosterol was also incorporated. This indicates that the relation with weight was probably indirect, mediated by the inverse association between plant sterols and lathosterol and the positive one between lathosterol and weight.

We did not observe a sex difference in the raw data for plant sterols, but in the multivariate analysis, sex appeared as a significant factor for both campesterol and β-sitosterol in two of the four sets. The effect was opposite to that reported by Tilvis and Miettinen,[8] who found women to have higher plant sterols than men. However, the disagreement might be apparent only, since their data were not corrected for other confounding factors like weight and height.

We believe that the data in the present study may serve as a reference basis, and can be used in making statements about lathosterol and plant sterols in selected populations, eg, in various groups of hyperlipidemic patients.[21]

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