Inheritance of cholesterol metabolism of probands with high or low cholesterol absorption

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Abstract **Heredity of cholesterol absorption and synthesis was studied in siblings of hypercholesterolemic probands with low and high serum cholestanol to cholesterol ratio (assumed to indicate low and high absorption of cholesterol, respectively). Cholesterol synthesis was assayed with sterol balance technique and measuring serum cholesterol precursor to cholesterol ratios (synthesis markers of cholesterol), and cholesterol absorption with measuring dietary cholesterol absorption percentage and serum plant sterol and cholestanol to cholesterol ratios (absorption markers of cholesterol). In the siblings of the low absorption families, cholesterol absorption percentage and ratios of absorption markers were significantly lower, and cholesterol and bile acid synthesis, cholesterol turnover, fecal steroids and ratios of synthesis markers significantly higher than in the siblings of the high absorption families. The ratios of absorption and synthesis markers were inversely interrelated, and they were correlated with cholesterol absorption and synthesis in the siblings. In addition, low absorption was associated with high body mass index, low HDL cholesterol, and serum sex hormone binding globulin levels, suggesting that low absorption was associated with metabolic syndrome. Intrafamily correlations were significant for serum synthesis markers, cholestanol, triglycerides, and blood glucose level. In conclusion, cholesterol absorption efficiency and synthesis are partly inherited phenomena, and they can be predicted by the ratios of non-cholesterol sterols to cholesterol in serum.**—Gylling, H., and T. A. Miettinen. **Inheritance of cholesterol metabolism of probands with high or low cholesterol absorption.** ***J. Lipid Res.*** **2002.** 43: **1472–1476.**

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Owing to small absorption of dietary plant sterols, serum contains low concentrations of these sterols, mainly campesterol and sitosterol. In addition, serum also contains a low concentration of cholestanol, a 5α derivative of cholesterol, which is partly synthesized in the body, but is also present in diet in very small amounts. These three sterols are transported by serum lipoproteins, mainly by LDL like cholesterol. The ratio of the three sterols to cholesterol correlates positively to absorption efficiency and negatively to synthesis of cholesterol (1, 2). Accordingly, their increased serum ratio points to a high absorption percentage of cholesterol and low cholesterol synthesis. Furthermore, low cholesterol synthesis decreases the release of cholesterol precursor sterols, most likely mainly from the liver to circulation, such that the ratios of squalene, methyl sterols, cholestenol, desmosterol, and lathosterol to cholesterol are also reduced (1). In general, the ratios of the absorption markers, and synthesis markers of cholesterol are negatively related to each other (3). Accordingly, a selection of subjects from a population with high ratios of the absorption markers predicts high absorption and low synthesis of cholesterol in these cases (1). On the other hand, the higher the ratios of the synthesis markers, e.g., lathosterol, the higher is cholesterol synthesis. In addition, baseline synthesis rate of cholesterol predicts to some extent lowering of cholesterol to statins, inhibitors of cholesterol synthesis. For instance, in the highest cholestanol quartile of the Finnish subgroup to the Scandinavian Simvastatin Survival Study (4S), serum cholesterol lowering was slightly but significantly lower than in the lowest quartile with high cholesterol synthesis (3). From among the non-cholesterol sterols, methyl sterol ratios to cholesterol exhibited significant intrapair correlations in serum of monozygotic but not dizygotic twins (4). However, concentrations of demethylated precursor sterols in plasma of a large number of families and twins showed associations, e.g., with apoE polymorphism (5), but no familial studies have been performed. Our routine non-cholesterol sterol analyses have revealed several families with high serum plant sterol ratios associated with low ratios of synthesis markers, suggesting heredity of variables of cholesterol metabolism. To this end, this study was planned to show the possible association of cho-

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lesterol absorption and synthesis in siblings of probands with high versus low ratios of serum absorption marker sterols for cholesterol, suggesting high versus low cholesterol absorption. For this pupose, the first-degree relatives of those probands were studied for serum precursor sterols, cholesterol absorption percentage, and cholesterol synthesis with the sterol balance technique.

MATERIALS AND METHODS

Proband population

From a large number (n = 330) of coronary subjects with baseline measurement of serum non-cholesterol sterols, six and seven subjects with respective lowest and highest ratios of serum cholestanol to cholesterol were selected as probands. Definition for low and high absorption families was performed by the serum cholestanol to cholesterol ratios, such that the respective ratios were 98 ± 5 (mean ± SE) and 162 ± 12 10^2 mmol/mol of cholesterol with no overlapping between the groups.

Sibling population

Probands' sisters and brothers were questioned by mail about their willingness to participate in the study. Of the 75 siblings 37, 14 from the low and 23 from the high absorption families, responded positively and were sent an invitation letter to participate in the study. None of them took lipid-lowering drugs or other products known to affect specifically cholesterol metabolism. A blood sample was drawn after an overnight fast, and a careful clinical examination was performed, and personal and family histories were recorded. The participants were advised to keep their normal habitual diet and possible drugs unchanged. They were asked to keep food diary for the next week, and consume a capsule (contained chromic oxide, C^{14}-labeled cholesterol and tritiated sitostanol) three times a day with each major meal. During the last three days of the week, the participants were asked to provide stool samples to test tubes and bring them to laboratory, and give another blood sample. Owing to varying age and willingness, cholesterol absorption could be performed only in ten participants.The proband group had been studied earlier, and most of them had been given statin treatment by doctors at the Outpatient Department after the baseline blood samples, such that absorption and fecal studies could not be performed. All subjects volunteered to the study, the protocol of which was accepted by the Ethics Committee of the hospital.

Laboratory methods

Table 1 shows the demographic data of the probands with low and high cholesterol absorption and of their respective siblings. Dietary diaries were calculated for different variables using a recent national method for their computerized analysis (6). Serum lipid analysis for total, HDL cholesterol (HDL-C) and LDL-C, total triglycerides, and phospholipids were made with the routine commercial kits (Boehringer Mannheim, Germany; Roche, Switzerland; Wako Chemicals, Germany). Ultracentrifugation was performed in the sibling's samples according to ealier methods (7) separating VLDL, IDL, LDL, and HDL followed by measurement of total, free, and esterified cholesterol, phospholipids, and triglycerides with commercial kits. In addition, blood glucose (hexokinase method; Roche, Switzerland), fasting serum insulin (radioimmunoassay; Pharmacia & Upjohn, Sweden), and serum sex hormone binding globulin (SHBG), an indicator of insulin resistance (8) (fluoroimmunoassay, Wallac, Finland), were quantified with routine commercial kits. Plasma total homocysteine level was assayed with high-pressure liquid chromatography. Serum cholesterol, squalene, and non-cholesterol sterols mere measured by gas-liquid chromatography (GLC) on a 50 m long capillary column (9) from non-saponifiable material in serum. Each GLC run separates cholesterol, squalene, cholestenol, desmosterol, lathosterol (three sterols reflecting cholesterol synthesis), cholestanol, campesterol, sitosterol, and avenasterols (four sterols reflecting cholesterol absorption). The squalene and non-cholesterol sterol levels were standardized by cholesterol of the same GLC run, because this procedure eliminates differences caused by variable lipoprotein contents transporting cholesterol and non-cholesterol sterols. Thus, the values are expressed in terms of 10^2 × mmol/mol of cholesterol and expressed in the text as ratio.

Cholesterol absorption percentage and fecal sterols of both cholesterol and plant sterol origin, and bile acids were measured from a pooled sample of the three daily stool samples by earlier methods (10–13). Mean daily intestinal cholesterol pool was calculated dividing fecal neutral sterols by (1-fractional absorption of cholesterol). Biliary cholesterol secretion was the difference between the intestinal pool and daily dietary cholesterol. Fractional absorption multiplied by the intestinal pool, biliary secretion, or dietary cholesterol revealed the absorption of total, biliary, and dietary cholesterol, respectively. Cholesterol synthesis was calculated by the sterol balance technique subtracting dietary cholesterol intake from the fecal sum of neutral sterols of cholesterol origin plus bile acids. Cholesterol turnover was cholesterol synthesis plus absorbed dietary cholesterol. All values are expressed in terms of mg/day.

TABLE 1. Demographic data, variables of glucose metabolism, and plasma total homocysteine level of study population

	Probands			Siblings		
Variables	Low Absorbers	High Absorbers	All	Low Absorbers	High Absorbers	All
Number of patients	6	7	13	14	23	37
Age, years	58 ± 3	58 ± 1	58 ± 3	58 ± 2	52 ± 3[a]	54 ± 3[a]
Weight, kg	82 ± 5	78 ± 3	80 ± 3	75 ± 4	68 ± 2[a]	70 ± 2[a]
BMI, kg/m^2	29 ± 1	25 ± 1[b]	27 ± 1	25 ± 1[a]	24 ± 1	24 ± 1[a]
Blood glucose, mmol/l	5.4 ± 0.6	4.4 ± 0.2	4.9 ± 0.3	5.2 ± 0.2	4.8 ± 0.1	5.0 ± 0.1
Serum insulin, pmol/l	—	—	8.3 ± 1.8	6.6 ± 0.7	6.4 ± 0.5	6.5 ± 0.4
Serum SHBG, nmol/l	—	—	37 ± 5	52 ± 8	89 ± 15[b]	75 ± 10[a]
Plasma total homocysteine, μmol/l	—	—	—	9.2 ± 0.6	11.5 ± 1.1	10.6 ± 0.7

Mean ± SE. BMI, body mass index; SHBG, sex hormone binding globulin.
[a] Significantly different from probands.
[b] Significantly different from low absorbers.

TABLE 2. Serum and lipoprotein lipids of study population

Variables	Probands			Siblings		
	Low Absorbers (n = 6)	High Absorbers (n = 7)	All (n = 13)	Low Absorbers (n = 14)	High Absorbers (n = 23)	All (n = 37)
Serum cholesterol (C), mmol/l	6.9 ± 0.4	6.1 ± 0.3	6.5 ± 0.2	5.3 ± 0.2[b]	5.6 ± 0.2	5.5 ± 0.2[b]
HDL-C, mmol/l[a]	1.15 ± 0.09	1.34 ± 0.11	1.25 ± 0.07	1.61 ± 0.10[b]	1.92 ± 0.10[b,c]	1.80 ± 0.07[b]
Serum triglycerides, mmol/l	1.8 ± 0.3	1.2 ± 0.2	1.5 ± 0.2	1.4 ± 0.2	1.1 ± 0.1	1.2 ± 0.1
LDL-C, mmol/l[a]	4.9 ± 0.4	4.2 ± 0.3	4.5 ± 0.2	3.0 ± 0.2[b]	3.2 ± 0.2[b]	3.1 ± 0.2[b]
VLDL-C, mmol/l	—	—	—	0.38 ± 0.08	0.28 ± 0.03	0.31 ± 0.04
IDL-C, mmol/l	—	—	—	0.15 ± 0.01	0.11 ± 0.01[c]	0.12 ± 0.01
LDL-C, mmol/l	—	—	—	3.1 ± 0.2	3.2 ± 0.2	3.2 ± 0.2
HDL-C, mmol/l	—	—	—	1.34 ± 0.07	1.59 ± 0.07[c]	1.50 ± 0.05
Serum phospholipids, mg/dl	—	—	—	199 ± 7	222 ± 6[c]	213 ± 5
VLDL-PL, mg/dl	—	—	—	21 ± 4	18 ± 2	18 ± 2
IDL-PL, mg/dl	—	—	—	5 ± 1	4 ± 1	4 ± 1
LDL-PL, mg/dl	—	—	—	72 ± 5	78 ± 5	76 ± 3
HDL-PL, mg/dl	—	—	—	89 ± 3	110 ± 4[c]	102 ± 3

Mean ± SE.
[a] Enzymatic analyses from serum. The other lipoprotein lipid values are from ultracentrifuge fractionation without recovery corrections.
[b] Significantly different from probands.
[c] Significantly different from low absorbers.

Statistics

Means and SEs were calculated and the differences between the four groups were analyzed by ANOVA and Student's two-sided *t*-test. Correlation coefficients were calculated by Pearson's product-moment correlation. $P < 0.05$ was considered statistically significant.

RESULTS

Table 1 shows the demographic values for probands and siblings in the low and high absorption groups. The siblings were 8 years younger than the probands, but the difference was significant only in the high absorbing families. Body mass index was higher in the low absorbing probands, and higher overall in probands than in siblings. Both diastolic and systolic blood pressure values were higher in the probands than siblings, but the low and high absorbers did not differ from each other. Blood glucose and serum insulin values were similar in the subgroups, even though SHBG values were high in the siblings, especially in the high absorbing ones. Plasma concentration of total homocysteine did not differ between the groups.

Lipids and lipoproteins

Table 2 shows the major lipids of probands and siblings and ultracentrifugation analysis of the siblings. It can be seen that serum total and LDL-C levels did not differ between the low and high absorbers, even though they were higher in the probands, but HDL-C level was significantly lower in the low than high absorbers. However, no consistent difference in triglycerides was seen between low and high absorbers, even after ultracentrifugation (data not shown), such that they only tended to be higher in the low than high absorbers, especially in VLDL and IDL. In contrast to cholesterol, serum phopholipids were lower in the low than high absorbers due to difference in HDL. Esterification percentage of cholesterol was significantly lower only in HDL fraction of the low than high absorbers.

Squalene and non-cholesterol sterol ratios

Squalene. The ratio of serum squalene only tended to increase in siblings as compared with probands, but the differences were not significant. The levels were not affected by the absorption state (**Table 3**).

Cholesterol precursors. The ratios of serum cholestenol, desmosterol, and lathosterol were significantly higher in the low than high absorbers of both the probands and siblings, respectively. The low absorbing siblings tended to have lower ratios than their probands (significant for des-

TABLE 3. Serum squalene and non-cholesterol sterol to cholesterol ratios (10^2 mmol/mol of cholesterol) in study population

Compounds	Group	Low Absorbers	High Absorbers	All
Squalene	Proband	30 ± 4	30 ± 4	30 ± 2
	Sibling	36 ± 4	34 ± 3	35 ± 2
Cholestenol	Proband	27 ± 6	10 ± 3[b]	18 ± 4
	Sibling	18 ± 2	13 ± 1[b]	15 ± 1
Desmosterol	Proband	88 ± 5	66 ± 7[b]	76 ± 5
	Sibling	75 ± 4[a]	64 ± 2[b]	68 ± 2
Lathosterol	Proband	215 ± 35	86 ± 7[b]	146 ± 24
	Sibling	170 ± 15	131 ± 7[a,b]	146 ± 8
Cholestanol	Proband	98 ± 8	168 ± 12[b]	136 ± 12
	Sibling	120 ± 8[a]	148 ± 7[b]	137 ± 6
Campesterol	Proband	146 ± 20	263 ± 40[b]	209 ± 28
	Sibling	271 ± 29[a]	385 ± 27[a,b]	341 ± 22[a]
Sitosterol	Proband	105 ± 17	166 ± 20[b]	137 ± 16
	Sibling	129 ± 14	160 ± 11	148 ± 9
Avenasterol	Proband	35 ± 3	46 ± 7	41 ± 4
	Sibling	49 ± 3	53 ± 4	51 ± 3

Mean ± SE.
[a] Significantly different from probands.
[b] Significantly different from low absorbers.

mosterol), while in the high absorbing siblings the lathosterol ratio was higher than in the respective probands.

Absorption sterols. In addition to the ratio of serum cholestanol, those of campesterol and sitosterol, but not of avenasterol, were higher in the high than low absorbers in both the probands (by definition for cholestanol) and siblings, respectively. In the low absorbers, the ratios of serum cholestanol and campesterol were lower in the probands than siblings, this difference being significant only for serum campesterol in the high absorbers.

In general, as in the probands, the ratios of the absorption markers were negatively related to those of the synthesis markers, but in the siblings their respective ratios to serum cholesterol, for instance, were positive (e.g., $r = 0.798$ for campesterol; $P < 0.01$) and negative (e.g., $r = -0.519$ for cholestenol; $P < 0.05$). Intrafamily correlations were significant for the ratios of cholestenol ($P = 0.047$), desmosterol ($P = 0.014$), lathosterol ($P = 0.009$), triglycerides ($P = 0.043$), and glucose ($P = 0.037$), but not for squalene, total or HDL-C, or body mass index, and only tended to be significant for plant sterol ratios ($P = 0.072$ for campesterol), but was that for cholestanol ($P = 0.015$).

Fecal and diet data

Cholesterol absorption efficiency (48.7 ± 2.4% vs. 36.9 ± 2.6%) was significantly higher in the high than low absorbers, a finding supporting the ratios of the cholesterol absorption sterols in serum (**Table 4**). Correspondingly, fecal output of cholesterol as neutral sterols of cholesterol origin and as bile acids was significantly increased in the low absorbers, resulting in increased cholesterol synthesis (increases also bile acid synthesis from newly formed cholesterol), as compared with the high absorbers. Despite the low cholesterol absorption efficiency in the low absorbers, their dietary intake of cholesterol was lower than in the high absorbers, but the respective dietary intake of plant sterols was also higher. Owing to a markedly high biliary secretion of cholesterol in the low absorbers, their absolute cholesterol absorption was within the limits of the high absorbers despite low absolute absorption of dietary cholesterol.

TABLE 4. Cholesterol absorption and metabolism in siblings of low and high absorbers

Variables	High Absorbers (n = 23)	Low Absorbers (n = 14)
Cholesterol absorption, %	48.7 ± 2.4	36.9 ± 2.6[a]
Dietary cholesterol, mg/d	285 ± 28	189 ± 19[a]
Fecal steroids, mg/d		
Neutral sterols	596 ± 169	1086 ± 223[a]
Bile acids	293 ± 27	649 ± 164[a]
Total	889 ± 64	1735 ± 384[a]
Campesterol	58 ± 7	101 ± 14[a]
Sitosterol	178 ± 14	271 ± 42[a]
Cholesterol synthesis, mg/d	608 ± 67	1546 ± 384[a]
Cholesterol turnover, mg/d	746 ± 66	1615 ± 380[a]
Intestinal cholesterol, mg/d		
Total pool	1170 ± 77	1670 ± 288
Biliary pool	883 ± 77	1481 ± 286[a]
Cholesterol absorption, mg/d		
Total mass	565 ± 38	584 ± 70
Dietary	138 ± 16	69 ± 6[a]
Biliary	427 ± 37	514 ± 72

Mean ± SE.
[a] Significantly different from high absorbers.

TABLE 5. Correlation coefficients of cholesterol synthesis (mg/d) and absorption (%) with ratios of serum markers of cholesterol absorption and synthesis (10^2 mmol/mol of cholesterol) in siblings of high and low absorption probands

Variables	Cholesterol Synthesis	Cholesterol Absorption
Cholesterol absorption	−0.721[b]	1.000
Squalene	+0.758[b]	−0.425[b]
Cholestenol	+0.688	−0.559[b]
Desmosterol	+0.737	−0.685
Lathosterol	+0.828[a,b]	−0.699
Campesterol	−0.653[a]	+0.751[a]
Sitosterol	−0.615	+0.812[a,b]
Cholestanol	−0.709[a,b]	+0.657

All correlation coefficients (n = 37), except between squalene and absorption, $P < 0.05$.
[a] Significantly different for high absorbers.
[b] Significantly different for low absorbers.

Cholesterol absorption efficiency was positively related to the ratios of the serum absorption markers ($P < 0.05$ to $P < 0.001$), indicating that the higher the serum plant sterol and cholestanol ratios, the higher is cholesterol absorption efficiency (**Table 5**). Cholesterol synthesis (mg/d) was correspondingly positively related to the ratios of the synthesis markers ($P < 0.01$ to $P < 0.001$), and negatively to the absorption markers ($P < 0.01$) and cholesterol absorption efficiency ($P < 0.001$). Thus, the higher the ratios of the synthesis markers the higher is absolute synthesis of cholesterol, and the lower is cholesterol absorption in both sibling groups. It is owing to small numbers that the correlations were separately less frequently significant in the high or low absorbing siblings.

DISCUSSION

The present results show convincingly that cholesterol absorption efficiency and cholesterol synthesis are inherited phenomena. Second, our earlier interpretation that the high serum cholestanol, campesterol, and sitosterol ratios to serum cholesterol (or low serum precursor sterol ratios) indicate high cholesterol absorption efficiency and low cholesterol synthesis was proven to be true. The findings on variables of cholesterol metabolism in these coronary families are probably regulated not only by heredity, but environmental factors may also play some role. It remains to be shown whether, in a random normal population, selection of subjects into absorption categories using high versus low ratios of serum absorption markers (or low vs. high synthesis markers) of cholesterol also indicate identification of families with respective high versus low absorption (or low vs. high synthesis) of cholesterol, as in the present hypercholesterolemic coronary

population. However, despite differences in synthesis and absorption of cholesterol, serum total and LDL-C levels were quite similar e.g., in the two sibling groups, indicating that removal and/or synthesis of LDL apoB apparently regulated LDL-C level. The lack of intrafamily correlation of total and LDL-C might be explained by the small number of subjects, since usually lipid association is found in family studies (14). On the other hand, lower HDL-C level and a trend of higher serum triglyceride value and body weight in low compared with high absorbers confirm our earlier findings in a large number of coronary patients (15), and resemble the findings observed in metabolic syndrome (16). In addition, low serum SHBG levels support the presence of metabolic syndrome in the low absorbers.

The intake of fat, cholesterol, and plant sterols, the three dietary variables interfering with cholesterol metabolism, were different in the two sibling groups. Dietary fat intake was, however, due to different body weight, and the fat intake was comparable in terms of g/kg of body weight. Lower dietary chlesterol intake in the low than high absorbers lowered its absorption and could have enhanced cholesterol synthesis. It is difficult to imagine, however, that the increase of synthesis by almost 900 mg/day in Table 4 could be a cosequence of about 70 mg/day reduction of dietary cholesterol absorption. Additionally, low absorbers were eating larger amounts of plant sterols, which could have decreased cholesterol absorption and, accordingly, increased cholesterol synthesis. Adjusted for body weight, plant sterol intake was, however, similar in the two groups, suggesting that its contribution to different cholesterol absorption was probably minimal.

It has been shown earlier that weight reduction in obese subjects with diabetes increases the at baseline low cholesterol absorption efficiency (17), suggesting in concordance with the present results that low cholesterol absorption efficiency seems to be associated with insulin resistance. What will then be the final explanation: primarily altered absorption or altered synthesis of cholesterol, for the inheritance of cholesterol metabolism, remains unknown. Current observations suggest that cholesterol absorption is regulated by ABG G/5 G/8 genes (18) through the expression of sterolins (19), which then regulate at least plant sterol, and apparently also cholesterol absorption.

NOTE ADDED IN PROOF

It has recently been shown (Berge et al. 2002. *J. Lipid Res.* **43:** 486–494) that variation in the plasma concentrations of noncholesterol sterols is highly heritable, and polymorphism in ABCG8 contributes to genetic variation in the plasma plant sterol concentrations.

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