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Liver, biliary, and pancreas

Biliary lipid composition in monozygotic and dizygotic pairs of twins

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SUMMARY **The relative contribution of genetic factors to biliary and serum lipid composition was studied in 17 monozygotic and 18 dizygotic middle aged male pairs of twins. Cholesterol precursors, squalene and Methylated sterols which reflect the activity of cholesterol synthesis were also measured. Pairwise intraclass correlations were determined for monozygotic and dizygotic twin pairs and heritability estimates were calculated. Molar % of biliary cholesterol and percentage distribution of biliary cholic acid and particularly deoxycholic acid showed significant pairwise correlations within the monozygotic but not the dizygotic pairs. Similar correlations were found for total biliary methylsterols and of the methylsterol subfractions for the two methostenols but not for squalene, lanosterol and dimethylsterols. In serum, the precursor sterols, but not squalene, showed even higher pairwise correlations in the monozygotic twins than the corresponding precursors in bile. Molar per cent of bile acids and phospholipids and cholesterol saturation index were not correlated significantly in either twin pairs, but the pairwise correlations tended to be higher in the monozygotic than in the dizygotic pairs. Gall stones were found in seven monozygotic and three dizygotic subjects. Two monozygotic twin pairs were concordant for gall stones; all the dizygotic pairs were discordant. Overall, these data suggest that molar percentage of biliary cholesterol, bile acid composition, cholesterol synthesis, bile cholesterol saturation, and gall stone formation may be under a significant genetic control.**

Several environmental factors such as diet, treatment with steroid hormones and other medications, may contribute to the development of gall stones, particularly cholesterol cholelithiasis.[1] An increased familial frequency of gall stones has also been suggested, however.[2-7] Therefore, it is possible that gall stone formation is partly under genetic control. Cholesterol in bile is carried in vesicles as well as in micelles and a prerequisite for the development of cholesterol gall stones is lithogenic bile that contains more cholesterol than can be dissolved.[1 8 9] The relative contribution of genetic and environmental factors to the biliary cholesterol saturation is not known. Monozygotic twins have the same genes while dizygotic pairs are like any other full siblings. Therefore, anything under strong genetic control should have a high pairwise correlation in the monozygotic twins, but a 0·5-fold association should be seen within the dizygotic pairs of twins. Thus, the twin method offers a useful tool for the evaluation of genetic and environmental components in the pathogenesis of cholesterol cholelithiasis. The present study was carried out to determine serum and biliary lipids and heritability estimates for these metabolic components.

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Methods

SUBJECTS

Male twins (17 monozygotic and 18 dizygotic pairs) living apart in the Helsinki area were randomly selected from the Finnish Twin Cohort.[10] The age of

the subjects ranged from 43 to 58 years (mean 50). None of the subjects had any clinically manifest cardiovascular, endocrinological, renal or gastrointestinal disease. The body weight ranged from 56 kg to 103 kg with a mean of 78 kg (9·5, SD). The body mass index (weight/height^2) ranged from 19 to 32 with a mean of 25. The zygosity diagnosis was established by mailed questionnaire as previously described.[11-14] The determination of zygosity by this method has been validated by the analysis of 11 blood markers in another group of twins in the Helsinki area.[11,12] All the patients volunteered for the studies which was approved by the Ethical Committee of our hospital.

STUDY PROTOCOL

All the subjects were studied as outpatients with three visits to the outpatient clinic. The first visit was made for medical history, physical examination, and routine blood tests. One week later oral cholecystography was performed during the second visit. The third visit was two weeks after the initiation of the study when, during fasting, gall bladder bile, and serum samples were obtained simultaneously for lipid analysis. The duodenum was intubated under fluoroscopic control by a single lumen tube after a 12 hour fast and duodenal bile was aspirated using cholecystokinin to provoke gall bladder contraction.

DIETS

The patients were studied on their regular home diet. They were advised to keep a food record for nine days and the dietary consumption was determined by a modified food record method.[15] The consumption of dietary constituents was calculated from the record as previously described.[16] This method has been shown to measure dietary constituents such as dietary cholesterol intake reasonably accurately.[17] In fact, we found that the values by direct quantitation and the dietary recall method were not significantly different from each other in a small group of various patients.[18] The mean daily caloric consumption was 31·1 kcal/kg (6·4 SD). On average, 14·2 (1·5)% of the calories were obtained from protein, 39·5 (5·3)% from fat, 43·2 (5·7)% from carbohydrates, and 3·0 (4·6)% from alcohol. Dietary cholesterol intake ranged from 200 to 867 mg/day with a mean of 439 mg/day (132 SD), and the ratio of polyunsaturated to saturated fat (P/S ratio) averaged 0·24 (0·11).

SERUM AND BILIARY LIPIDS

Serum total cholesterol and triglycerides were determined according to the routine methods of our hospital laboratory.[19,20] Total biliary bile acids and bile acid distribution was determined by gas liquid chromatography.[21] Biliary phospholipids were measured by Bartlett's method[22] using the Fiske-Subbarow reagent.[23] Serum and biliary squalene, serum free methylsterols and free cholesterol (Fig. 1) were isolated and measured by thin layer chromatography gas chromatography as previously described.[24] The respective biliary sterol analyses were performed from non-saponifiable material. Five major methylated precursor sterols were identified in increasing order of retentions: (1) Δ^8-methostenol (4α-methyl-5α-cholest-8-en-3-β-ol), (2) Δ^8-demethylsterol (4α, 4β-dimethyl-5β-cholest-8-en-3β-ol), (3) methostenol (4α-methyl-5α-cholest-7-en-3β-ol), (4) lanosterol (4α, 4β, 14α-trimethyl-5α-cholest-8, 24 dien-3β-ol), and (5) $\Delta^{8,24}$-dimethylsterol (4α, 4β-dimethyl-5α-cholest-8, 24-dien-3β-ol). To minimise the effect of different lipid concentrations, the cholesterol precursors are mainly expressed in terms of μg/mg of the serum free or total biliary cholesterol.

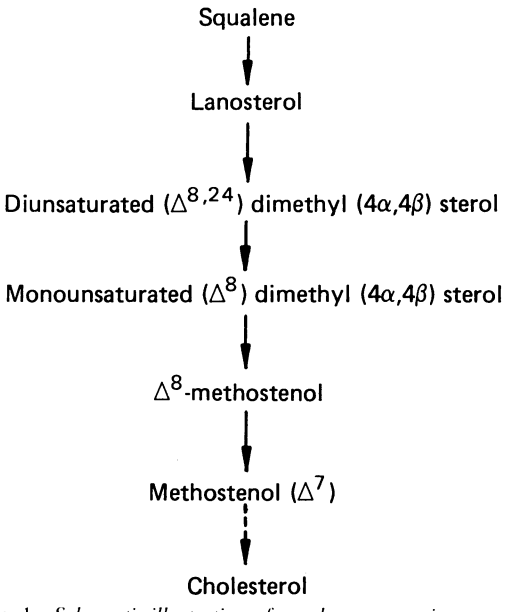

Fig. 1 *Schematic illustration of squalene conversion to cholesterol as determined in the present study.*

BILIARY CHOLESTEROL SATURATION

Lipid composition of bile was calculated as the molar percentages of cholesterol, bile acids, and phospholipids. In addition, the cholesterol saturation index of bile samples was determined using the solubility limits for cholesterol proposed by Admirand and Small.[8]

STATISTICAL ANALYSIS

The monozygotic and dizygotic twin pairs were compared calculating pairwise intraclass correla-

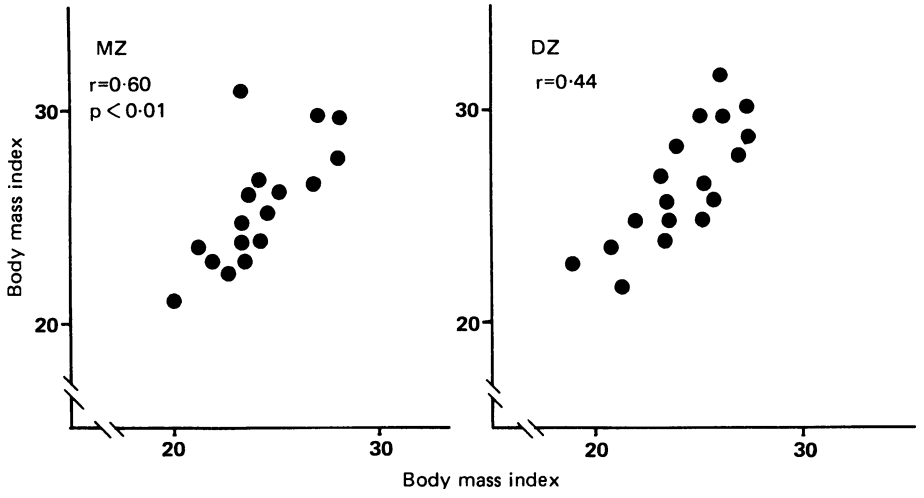

Fig. 2 *Pairwise intraclass correlation of body mass index in monozygotic (MZ) and dizygotic (DZ) twins.*

tions (r). Heritability estimates (h^2) were calculated according to Jensen.[25] The upper limit of heritability was estimated from the following formulas: (1) $h^2 = r_{MZ}$ – that is, heritability in monozygotic cotwins reared apart, (2) $h^2 = 2(r_{MZ} - r_{DZ})$ – that is, heritability from monozygotic and dizygotic cotwins reared together. Heritability estimates are given by formula 2 except in cases when heritability estimates exceeds r_{MZ}. Also, the role of some environmental factors (mainly dietary) in the regulation of bile and serum lipid metabolism was estimated by calculating the correlations between the intrapair differences of the dietary components and the intrapair differences of the lipid metabolism in monozygotic twins. The intrapair difference indicates a difference of a variable between the members of a twin pair. This was mainly done for testing the possibility that some environmental factors may be more similar among the monozygotic than dizygotic cotwins and may therefore confound the pairwise analyses for the estimation of heritability. The correlation coefficients were calculated taking into account the observed log-normal distribution of the study parameters.

Results

The pairwise correlation for the body mass index was somewhat higher for monozygotic ($r=0{\cdot}60$, $p<0{\cdot}01$) than dizygotic ($r=0{\cdot}44$) twins, indicating that the monozygotic pairs were more alike with regard to their body habitus than the dizygotic twins (Fig. 2). Table 1 shows the biliary and serum lipids and biliary cholesterol saturation among all the subjects. The biliary contents of cholesterol precursor sterols, especially that of lanosterol, were several times higher than the corresponding free sterols in serum.

BILIARY LIPIDS

The pairwise correlation coefficients for biliary lipids and bile cholesterol saturation percentages in the monozygotic and dizygotic twin pairs are shown in Table 2. Molar percentage of cholesterol, but not bile acids and phospholipids, was significantly correlated in the monozygotic pairs. These correlations were insignificant in the dizygotic twins. The percentages of cholic acid and especially deoxycholic acid (Fig. 3), but not of chenodeoxycholic acid, showed a signifi-

Table 1 *Biliary and serum lipids and biliary cholesterol saturation among all the subjects*

	*Bile**	*Serum†*
Triglycerides	–	1·5 (0·1)††
Squalene§	75 (7)‡	40 (3)
Sum of the methylsterols§	1234 (70)	84 (5)
Lanosterol§	681 (40)	29 (1)
Δ⁺⁺-dimethylsterol§	189 (12)	19 (1)
Δ⁺-dimethylsterol§	64 (3)	8 (1)
Δ⁺-methostenol§	194 (13)	16 (1)
Methostenol§	106 (7)	13 (1)
Cholesterol‖	9·0 (0·3)‖	6·0 (0·1)††
Phospholipids‖	18·5 (0·5)‖	–
Bile acids‖	72·5 (0·6)‖	–
Cholic acid %¶	47·1 (0·9)	–
Deoxycholic acid %¶	14·2 (1·1)	–
Chenodeoxycholic acid %¶	38·7 (0·7)	–
Cholesterol saturation %**	96 (4)	–

*Determined in 60 subjects; †Determined in all 70 subjects; ‡The results are given as mean (SE); §Expressed as μg/mg of cholesterol; in serum for free, in bile for total sterols; ‖Molar percentage; ¶Percent of total bile acids; **Calculated according to Admirand and Small[5]; ††mmol/l.

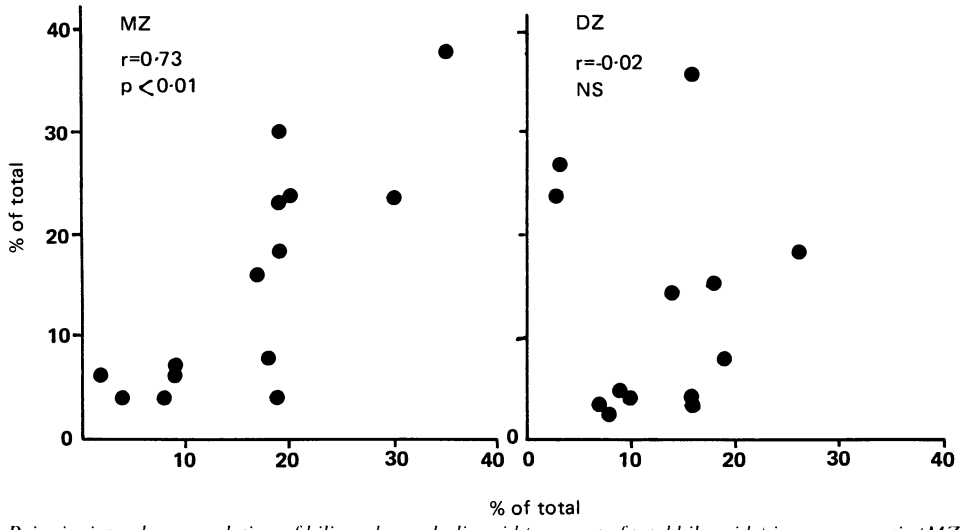

Fig. 3 *Pairwise intraclass correlation of biliary deoxycholic acid (per cent of total bile acids) in monozygotic (MZ) and dizygotic (DZ) twins.*

cant pairwise correlation in the monozygotic twins, whereas no correlation was found in the dizygotic pairs. Heritability estimates were high for the molar percentage of biliary cholesterol (0·47) and the percentages of biliary bile acids (from 0·28 to 0·73). Of the biliary cholesterol precursor sterols the two methostenols and the sum of methylsterols were significantly correlated in the monozygotic twin pairs. The calculated heritability estimates for biliary methylsterols ranged from 0·22 to 0·54. The bile cholesterol saturation index showed insignificant pairwise correlations in the monozygotic and dizygotic twins, the heritability estimate being 0·46.

Table 2 *Pairwise intraclass correlation coefficients for biliary lipids and cholesterol saturation in monozygotic and dizygotic pairs of twins*

Biliary lipid	*Correlation coefficient*		*Heritability estimate*
	Monozygotic twins (r)	*Dizygotic twins* (r)	
Cholesterol M %*	0·47§	−0·09	0·47
Bile acids M %*	0·08	−0·07	0·08
Phospholipids M %*	−0·13	−0·01	0·00
Cholic acid %†	0·58§	−0·25	0·58
Deoxycholic acid %†	0·73§	−0·02	0·73
Chenodeoxycholic acid %†	0·28	−0·34	0·28
Squalene	0·36	0·33	0·06
Lanosterol	0·43	0·46	0·00
Δ⁷-dimethylsterol	0·41	0·30	0·22
Δ⁸-dimethylsterol	0·38	0·03	0·38
Δ⁸-methostenol	0·54§	0·05	0·54
Methostenol	0·54§	−0·15	0·54
Sum of the methylsterols	0·48§	0·32	0·32
Cholesterol saturation‡	0·46	−0·01	0·46

*M % = molar percentage; †Percentage of individual bile acid from total bile acids; ‡Calculated according to Admirand and Small[9]; §p<0·05 or less.

GALL STONES

Two of the subjects had had cholecystectomy because of gall stones in their past history. In addition, previously unknown gall stones were observed in eight patients during the present studies. All the other subjects had normally visualised cholecystograms. Gall stones, including cholecystectomies for gall stones, were found in seven monozygotic and three dizygotic subjects. Two monozygotic twin pairs were concordant for gall stones – that is, both members of the pair had gall stones whereas all the dizygotic pairs were discordant. Therefore, the pairwise concordance for gall stones was 40% for monozygotic and 0% for dizygotic twins.

SERUM LIPIDS

Table 3 shows the pairwise correlation coefficients for serum total cholesterol and triglyceride and for the serum cholesterol precursors, methylsterols, and squalene. Serum cholesterol and triglyceride levels were significantly correlated within the monozygotic pairs; for the dizygotic twins the pairwise correlation was significant only for plasma cholesterol. The calculated heritability estimates were 0·26 for serum cholesterol and 0·60 for serum triglyceride. All the serum methylsterols except methostenol were significantly correlated within the monozygotic but

not in the dizygotic twins. The calculated heritability estimates for serum methylsterols ranged from 0·43 to 0·60. Serum squalene was not significantly correlated in either pair of twins.

DIETARY INTAKE

The pairwise correlation coefficients for various dietary components were insignificant in both the monozygotic and dizygotic twins: dietary cholesterol $r_{MZ}=-0·13$, $r_{DZ}=0·42$; P/S ratio $R_{MZ}=0·31$, $r_{DZ}=0·06$; percentage of calories derived from fat $r_{MZ}=0·34$; $r_{DZ}=-0·35$; from carbohydrates $r_{MZ}=0·25$; $r_{DZ}=0·28$; from protein $r_{MZ}=-0·04$; $r_{DZ}=-0·28$; and from alcohol $r_{MZ}=0·16$; $r_{DZ}=0·42$.

CORRELATIONS BETWEEN THE INTRAPAIR DIFFERENCES IN MONOZYGOTIC TWINS

Because the monozygotic twins share the same genes and still show some intrapair differences in biliary and serum lipids these differences must be the result of environmental factors. To elucidate the relative importance of these factors, the intrapair differences of the monozygotic twins in biliary and serum lipid components were correlated with those in environmental parameters studied. The intrapair difference between the monozygotic twins in the body mass index, calories derived from fat, carbohydrates, protein, and alcohol, dietary cholesterol, and P/S ratio showed only the following significant correlations with the intrapair differences between serum and bile lipids: dietary cholesterol intake *v* molar percentage of biliary phospholipids ($r=0·45$) ($p<0·05$) and percentage of biliary chenodeoxycholic acid ($r=0·47$) ($p<0·05$); calories derived from fat *v* serum total cholesterol ($r=0·51$; $p<0·05$).

Table 3 *Pairwise intraclass correlation coefficients for serum lipids in monozygotic and dizygotic pairs of twins*

Serum lipid	*Correlation coefficient*		
	Monozygotic twins (r)	*Dizygotic twins* (r)	*Heritability estimate*
Total cholesterol	0·67*	0·54*	0·26
Total triglyceride	0·60*	0·11	0·60
Squalene	0·15	0·41	0·00
Lanosterol	0·63*	0·14	0·63
$\Delta^{7,24}$-dimethylsterol	0·57*	0·19	0·57
Δ^{7}-dimethylsterol	0·50*	0·10	0·50
Δ^{7}-methostenol	0·47*	0·14	0·47
Methostenol	0·43	0·09	0·43
Sum of the methylsterols	0·60*	0·16	0·60

*$p<0·05$ or less.

Discussion

The critical basis for twin studies is the correct diagnosis of zygosity. The zygosity of the twins in the Finnish Twin Cohort had previously been diagnosed by mailed questionnaire[13,14] and had also been validated by 11 blood group markers.[11,12] Therefore, the subjects studied in the present work were most probably correctly classified into monozygotic and dizygotic pairs of twins.

In accordance with previous reports,[26] we found serum total cholesterol and triglyceride concentrations to correlate closely within the monozygotic twins. The relative genetic contribution to the regulation of serum cholesterol concentration, however, remained fairly weak (25%). In fact, the effect of environmental factors on serum cholesterol concentration is well known and dietary fat intake accordingly showed a positive correlation with serum total cholesterol when the intrapair differences in these parameters were plotted among the monozygotic twins. A strong pairwise correlation of serum triglyceride concentration within the monozygotic ($r=0·60$) but not in the dizygotic pairs ($r=0·11$) suggests a somewhat higher genetic control for serum triglyceride regulation in our series than has been noticed previously.[26] Body mass index, however, showed a somewhat higher pairwise correlation in the monozygotic than dizygotic twins, and the degree of obesity is a well known factor regulating serum triglyceride concentrations.

The serum contents of methylsterols indicate the activity of hepatic cholesterol synthesis in many clinical conditions.[27-29] In fact, in man the correlation of serum unesterified methylsterol concentrations with the hepatic hydroxymethyl glutaryl CoA reductase is highly significantly positive.[30] Thus, the significant pairwise correlations of almost all individual methylsterols in the monozygotic, but not in the dizygotic pairs suggest that the activity of cholesterol synthesis, most probably in the liver, is under strong genetic regulation. Serum squalene was not correlated in either twin pairs probably because it appears to occur only transiently in parallel with acute changes in cholesterol synthesis.[27-31] The cholesterol precursor contents of the monozygotic twins showed less frequent pairwise correlations in the bile than in serum and the significant association was found for the most polar methylsterols, methostenols only. This raises a question of genetic contribution to their biliary secretion. In fact, it is of interest to note that methostenols are markedly enriched in cholesterol gall stones while the less polar precursor sterols, especially lanosterol, remain mainly soluble in the bile.[32]

The significant pairwise correlations of the molar percentage of cholesterol in the fasting duodenal bile

in the monozygotic, but not the dizygotic twins suggests that biliary cholesterol metabolism is to some extent genetically controlled, probably through cholesterol synthesis as indicated by the precursor sterols. The high pairwise correlation of the relative cholic and deoxycholic acid contents in the monozygotic, but not in the dizygotic twins indicates genetic control in the metabolism of these two bile acids. In fact, because deoxycholic acid is a bacterial conversion product, formed mainly in the colon, the finding suggests that colonic bacterial flora or colonic absorption of deoxycholate is genetically controlled. The intestinal bacterial flora, however, is also strongly influenced by the diet. Even though the dietary review did not show any higher pairwise correlation among the monozygotic than the dizygotic pairs, the influence of some dietary factor(s), such as the type of fibre, is not totally excluded. As the dietary intakes were estimated by the dietary recording method instead of direct weighing of the food products, it is possible that the diet analysis missed some important findings.

Cholesterol gall stone formation is frequently associated with supersaturation of biliary cholesterol[1 8 33–36] and cholesterol stones are the most frequent gall stones in Western societies[37] including two populations of this country.[38] The present study shows that the intrapair correlation coefficient for the molar percentage and the saturation index of cholesterol are higher in the monozygotic than in the dizygotic pairs. This suggests that the development of supersaturated bile is also genetically controlled, possibly through genetically controlled cholesterol metabolism or biliary secretion. This was also suggested by a previous study[39] where a higher cholesterol saturation of bile was found in younger sisters of patients with cholesterol gall stones than in those of non gall stone controls. Genetic regulation of bile cholesterol saturation, however, does not necessarily indicate genetic control of gall stone formation. As several investigators have pointed out[40–43] there are a number of subjects with supersaturated bile who do not develop cholelithiasis, and others who develop cholelithiasis without supersaturation, as shown by the National Gallstone Study.[44] Genetic control of the critical physiochemical abnormality in the initiation of gall stone, cholesterol crystal formation,[40–42] is not known but this possibility is not ruled out. At any rate, genetic contribution to the gall stone formation was suggested by the findings of the present study that the monozygotic pairs showed a 40% concordance for gall stones compared with a complete discordance in the dizygotic twins.

The similar pairwise correlations of dietary components in the monozygotic and dizygotic twins indicate that the monozygotic pairs of the present study, intentionally selected from pairs living apart, did not have more similar dietary habits than the dizygotic twin pairs. This finding further supports the importance of genetic contribution in the regulation of cholesterol metabolism. Furthermore, of the many environmental factors regulating cholesterol and bile acid metabolism those determined in this study, diet, and the degree of obesity, are probably the most important. The intrapair differences between these environmental factors among the monozygotic pairs, however, showed surprisingly weak correlations with the intrapair differences between the measured parameters of cholesterol and bile acid metabolism. This may still be additional evidence pointing towards significant genetic control in the regulation of these metabolic pathways.

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