Cholesterol Absorption and Synthesis in Children With Type 1 Diabetes

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OBJECTIVE — The levels of the surrogate markers of cholesterol absorption (cholestanol and plant sterols) and synthesis (cholesterol precursors) in serum have suggested that in adult type 1 diabetes, cholesterol absorption is high and synthesis is low compared with type 2 diabetic or control subjects. Accordingly, these findings were further studied in children with type 1 diabetes.

RESEARCH DESIGN AND METHODS — Forty-eight children with diabetes were compared with 79 age- and sex-matched control subjects. The serum ratios of cholesterol absorption and synthesis markers were measured with gas-liquid chromatography. The study population was divided into triads (combining the two lowest triads) by serum cholestanol ratios of the control subjects indicating low to high cholesterol absorption efficiency.

RESULTS — The ratios of the absorption and synthesis markers were similar in case and control subjects, and they were negatively related to each other in control subjects, being less consistent in diabetic patients. Thus, high cholesterol absorption was associated with low synthesis. Plant sterol ratios increased significantly with increasing cholestanol triads in both groups, but the values in the lowest triads were higher in case versus control subjects.

CONCLUSIONS — Homeostasis between cholesterol absorption and synthesis is maintained in control children and somewhat less consistently in those with diabetes. The higher plant sterol ratios in diabetic versus control subjects in the lowest cholestanol triads suggest that cholesterol absorption is higher in children with diabetes versus control subjects but only within the range of low cholesterol absorption.

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Of the noncholesterol sterols in human sera, cholestenol, desmosterol, and lathosterol, especially when divided by serum total cholesterol and expressed as ratios to cholesterol, reflect cholesterol synthesis (1). The ratios of cholestanol (a metabolite of cholesterol) and plant sterols, campesterol and sitosterol, are surrogate markers of cholesterol absorption (1). Thus, in homogenous populations, synthesis of cholesterol is positively related to the ratios of the synthesis markers and negatively to those of the absorption markers (1). Furthermore, during homeostasis of cholesterol metabolism, high absorption of cholesterol suppresses its synthesis; therefore, the ratios of the surrogate synthesis and absorption markers should also relate negatively to each other (1).

Recent studies (2,3) have suggested that in adults with type 1 diabetes, cholesterol synthesis is low and cholesterol absorption high compared with healthy individuals or subjects with type 2 diabetes. These clinical findings have been corroborated in the experimental model of streptozotocin-induced diabetes, in which expression of *ABCG 5/8* genes in enterocytes and hepatocytes were reduced (4). This finding resembles the situation in hereditary sitosterolemia, in which the mutations of *ABCG 5/8* genes result in enhanced absorption and reduced biliary secretion of sterols (5,6). Affected sitosterolemic individuals exhibit markedly increased serum plant sterol levels and less markedly increased cholesterol concentrations, the findings expected to increase the risk for developing premature atherosclerosis (7). In nonsitosterolemic subjects, high serum plant sterol levels have been related to the occurrence of atherosclerosis in several (8–13), but not in all (14), clinical studies. Furthermore, in children with type 1 diabetes, ultrasound studies of arterial wall have suggested some signs of early atherosclerosis (15).

These observations could suggest that sterol absorption is also high in children with type 1 diabetes. Accordingly, the purpose of the present study was to examine whether the noncholesterol sterol ratios, especially those of the absorption markers, are different in children with type 1 diabetes compared with age-matched control subjects.

RESEARCH DESIGN AND METHODS

— The study population was comprised of 48 children with type 1 diabetes (15 girls and 33 boys) and 79 healthy control subjects (40 girls and 39 boys) (Table 1). The type 1 diabetic children were consecutively recruited from the outpatient clinic of the Department of Pediatrics, Turku University Central Hospital, and the inclusion criteria were age 7–14 years, duration of diabetes >3 months, normotension, and no chronic disease other than diabetes. The mean (±SD) duration of diabetes was 4.3 ± 3.0 years. None of the children were taking regular medications other than daily insulin, the dose of which was 0.96 ± 0.25 IU/kg (range 0.62–1.66) and none had microalbuminuria or retinopathy. The mean HbA_{1c} (A1C) was 8.8 ± 1.3% (range 6.2–12.7; reference range 4.2–6.0) and urinary albumin-to-creatinine ratio was 0.84 ± 0.55 mg/mmol. Participants did not differ in any clinical charac-

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Abbreviations: GLC, gas-liquid chromatography; STRIP, Special Turku Coronary Risk Factor Intervention Project for children.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Table 1—*Clinical variables of the study population*

Variables	Control subjects			Diabetic subjects		
	Girls	Boys	Total	Girls	Boys	Total
n	40	39	79	15	33	48
Age (years)	10.4 ± 0.1	10.6 ± 0.2	10.5 ± 0.1	11.5 ± 0.4	11.2 ± 0.3	11.3 ± 0.3
BMI (kg/m^2)	17.8 ± 0.5	18.2 ± 0.6	18.0 ± 0.4	19.6 ± 0.6*	19.0 ± 0.4	19.2 ± 0.3*
Blood glucose (mmol/l)	ND	ND	ND	11.9 ± 1.7	12.7 ± 1.0	12.4 ± 0.8
A1C (%)	5.37 ± 0.05	5.33 ± 0.04	5.35 ± 0.03	8.79 ± 0.27*	8.79 ± 0.25*	8.79 ± 0.19*
Systolic blood pressure (mmHg)	107.7 ± 1.4	110.8 ± 1.3	109.2 ± 1.0	111.3 ± 2.1	110.4 ± 1.7	110.6 ± 1.3
Diastolic blood pressure (mmHg)	62.4 ± 0.9	64.6 ± 1.1	63.4 ± 0.7	64.5 ± 1.3	65.1 ± 1.3	64.9 ± 1.0
Insulin dose (IU)	—	—	—	47.9 ± 5.1	41.6 ± 3.2	43.6 ± 2.7
Years of diabetes	—	—	—	5.26 ± 0.83	3.93 ± 0.50	4.34 ± 0.43

Data are means ± SE. *$P < 0.05$ vs. control subjects. ND, not determined.

teristics from the whole eligible diabetes clinic population of the same age.

Based on blood glucose and A1C, the metabolic control of diabetes was rather poor but comparable to a previous population-based study of children with diabetes of the same age (16). The families were advised by their physicians to comply with diet according to the Nordic nutrition recommendations (17).

Of the control subjects, 59 were participating in an ongoing trial aiming at decreasing children's exposure to known atherosclerotic risk factors according to the Special Turku Risk Factor Intervention for children (STRIP) (18), and 20 were friends of the diabetic children of this study or were children of the staff members. The STRIP children followed a diet similar to the type 1 diabetic subjects. We calculated the carbohydrate and fat intake from dietary recalls presented in detail earlier (18). In the intervention group, the polyunsaturated-to-saturated fatty acid ratio was higher than in control subjects (0.56 vs. 0.46, $P < 0.05$), whereas total fat and carbohydrate intakes were similar.

All subjects were nonsmokers. Written informed consent was acquired from the legal guardians of the children, and they were also encouraged to get an approval from the child. The study was conducted according to the Declaration of Helsinki, and the study protocol was approved by the Joint Commission on Ethics of the University of Turku and the Turku University Central Hospital.

Fasting blood samples were obtained in the morning. Blood glucose, A1C, HDL cholesterol, and serum triglycerides were measured with routine laboratory methods of the hospital. Serum sterols and squalene were analyzed with gas-liquid chromatography (GLC) (19). Total cholesterol, squalene, and noncholesterol sterols were measured from nonsaponifiable material of serum with GLC using a 50-m-long capillary column (Ultra-2; Agilent Technologies, Wilmington, DE). The procedure used 5α-cholestane as an internal standard, and it measured the concentrations of cholesterol, squalene, cholestenol, desmosterol, lathosterol (sterols reflecting cholesterol synthesis), cholestanol, campesterol, sitosterol, and avenasterol (sterols reflecting cholesterol absorption). Since the synthesis and absorption sterol ratios to cholesterol better reflect the absolute whole-body synthesis and absorption percentage of cholesterol than the concentrations (1), all values have been related to cholesterol obtained from the same GLC run, and the term ratio is used for the cholesterol-standardized values. To avoid extra decimals because of low concentrations of the noncholesterol sterols, the values are multi-

Table 2—*Serum total cholesterol concentrations and ratios of squalene and noncholesterol sterols to cholesterol in the study population*

Variables	Control subjects			Diabetic subjects		
	Girls	Boys	Total	Girls	Boys	Total
n	40	39	79	15	33	48
Cholesterol (mg/dl)	176.8 ± 5.0*	159.1 ± 4.8	168.1 ± 3.6	164.3 ± 7.2	162.3 ± 5.5	163.0 ± 4.4
Synthesis markers of cholesterol ($10^2 \times$ μmol/mmol)						
Squalene	20.9 ± 0.8	22.6 ± 1.0	21.8 ± 0.6	26.4 ± 1.5†	28.8 ± 1.5*	28.0 ± 1.2‡
Cholestenol	6.1 ± 0.5	7.5 ± 0.7	6.8 ± 0.4	5.0 ± 0.4§	6.6 ± 0.4	6.1 ± 0.3
Desmosterol	74.0 ± 1.8	70.9 ± 2.4	72.5 ± 1.5	70.0 ± 1.9	76.3 ± 2.5	74.3 ± 1.9
Lathosterol	124.4 ± 6.4	134.3 ± 7.7	129.3 ± 5.0	100.6 ± 6.5†§	127.0 ± 6.0	118.7 ± 4.9
Absorption markers of cholesterol ($10^2 \times$ μmol/mmol)						
Campesterol	373.3 ± 18.8	400.7 ± 33.2	386.8 ± 18.9	433.7 ± 30.0	450.3 ± 36.6	445.1 ± 26.7
Sitosterol	184.9 ± 7.5	198.2 ± 13.7	191.5 ± 7.7	208.6 ± 12.5	206.6 ± 13.9	207.2 ± 10.3
Avenasterol	50.8 ± 1.6	52.7 ± 3.2	51.7 ± 1.8	56.5 ± 3.3	56.8 ± 3.8	56.7 ± 2.8
Cholestanol	171.8 ± 4.6	181.7 ± 6.1	176.7 ± 3.8	196.6 ± 8.6†§	172.6 ± 5.9	180.1 ± 5.1

Data are means ± SE. *$P < 0.05$ vs. control boys; †$P < 0.05$ vs. control girls; ‡$P < 0.05$ vs. control total; §$P < 0.05$ vs. diabetic boys.

Table 3—*Correlation coefficients of BMI and serum noncholesterol sterols in the study population*

Noncholesterol sterols	BMI	Cholestanol	Campesterol	Sitosterol
Control subjects (n = 79)				
Cholestanol	−0.503*			
Campesterol	−0.460*	0.792*		
Sitosterol	−0.462*	0.817*	0.946*	
Avenasterol	−0.289†	0.602*	0.695*	0.798*
Lathosterol	0.579*	−0.555*	−0.470*	−0.452*
Diabetic subjects (n = 48)				
Cholestanol	−0.050			
Campesterol	−0.128	0.514*		
Sitosterol	−0.152	0.623*	0.914*	
Avenasterol	−0.105	0.657*	0.746*	0.868*
Lathosterol	0.354‡	−0.319†	−0.236	−0.229

*$P < 0.001$; †$P < 0.05$; ‡$P < 0.01$.

plied with 100 (10^2 × μmol/mmol cholesterol).

Statistical analysis

The differences between groups were calculated with two-sided Student's t test or with ANOVA. Logarithmic conversion was performed with skewed distributions. Correlation coefficients were calculated with Pearson and Spearman rank correlation in appropriate cases. A P value <0.05 was considered significant.

RESULTS — The mean age of the control and diabetes groups was similar, ~10–11 years (Table 1). BMI was higher in diabetic than in control subjects due to higher values in diabetic than control girls. Serum cholesterol (4.4 ± 0.1 and 4.3 ± 0.1 mmol/l) and triglycerides (0.70 ± 0.04 and 0.64 ± 0.03 mmol/l) were similar between the groups, but HDL cholesterol was lower in control (0.88 ± 0.02 mmol/l) than in diabetic (1.02 ± 0.03 mmol/l, $P < 0.01$) subjects. Systolic and diastolic blood pressure were similar between the groups.

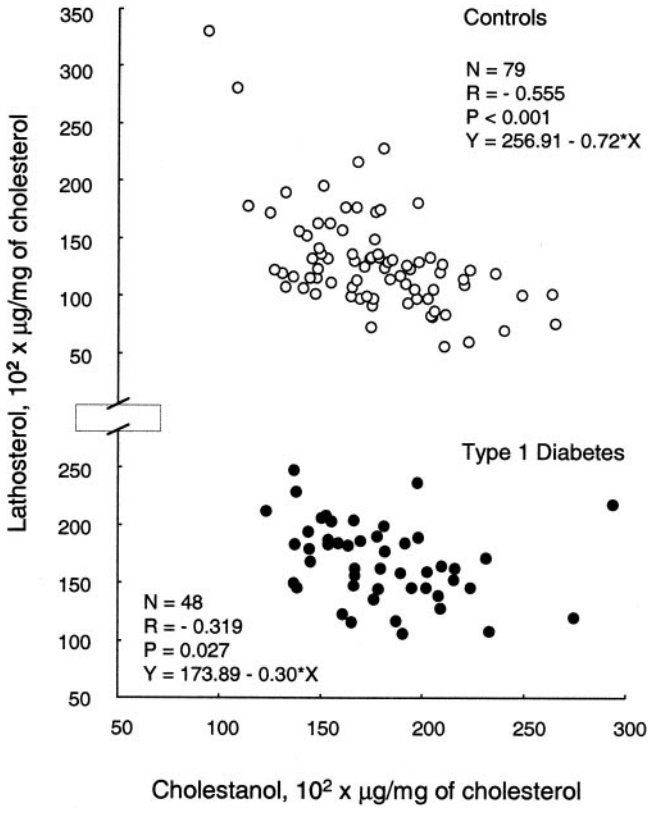

**Figure 1—*The correlations between serum cholestanol and lathosterol ratios to cholesterol, surrogate markers of cholesterol absorption, and synthesis in children without* (n = *79) and with* (n = *48) type 1 diabetes.*

Serum cholesterol, squalene, and noncholesterol sterols

Control subjects. Serum cholesterol concentration was higher in the control girls than boys, but squalene and noncholesterol sterol ratios were similar (Table 2).

Diabetic subjects. Of the synthesis markers, cholestenol and lathosterol ratios were lower in girls than boys (Table 2). Of the absorption markers, cholestanol was higher in girls than boys.

Diabetic versus control subjects. The noncholesterol sterol ratios were similar in diabetic and control subjects (Table 2). The squalene ratio was higher in diabetic girls and boys versus the respective control subjects. However, in diabetic girls, the cholestanol ratio was significantly higher than in control girls and that of lathosterol ratio was lower, respectively.

Relations of BMI with absorption and synthesis markers

BMI was negatively related to the absorption sterols and positively related to all synthesis markers (shown for lathosterol only) in control subjects but only for lathosterol in diabetic subjects (Table 3). After standardization of BMI, the correlations were still detectable. The ratios of the absorption sterols were positively related to each other in both control and diabetic subjects.

Absorption of cholesterol was negatively related to that of synthesis, as shown for the ratios of cholestanol versus lathosterol in Fig. 1. Cholestenol and desmosterol (data not shown) and lathosterol (Table 3) were unrelated to plant sterol ratios in diabetic subjects.

Relations of blood pressure with absorption and synthesis markers

An interesting additional observation suggested that the higher the blood pressure, the lower the cholesterol absorption. Thus, the ratios of absorption sterol markers were negatively related to blood pressure in the whole study population (e.g., for campesterol versus systolic blood pressure $r = -0.309$, $P = 0.007$). On the other hand, the systolic and diastolic blood pressures were positively as-

sociated with cholesterol synthesis (e.g., for lathosterol $r = 0.395$ and 0.362, $P = 0.0003$) and negatively associated for absorption sterol markers in control subjects (e.g., for campesterol $r = -0.338$ and -0.274, $P < 0.001$). In diabetic subjects, the association of blood pressure with synthesis and absorption was lacking.

Triads by cholestanol ratios

Since the correlation between cholesterol absorption and synthesis markers was highest between cholestanol and lathosterol (Table 3), serum cholestanol ratios of the control subjects were ranked to triads and applied for the diabetes group also. The mean ratios of the combined two lowest triads were compared with those of the highest one in Table 4. Serum cholesterol concentrations were similar in the control and diabetic triads. Cholesterol absorption, indicated by campesterol, sitosterol, and avenasterol (ratios of the two lowest triads), was significantly higher in diabetic than in control subjects. In the third triad, the values of both groups were similar but significantly higher than in the combined lower triads. From among the synthesis markers, only the lathosterol ratio was lower in diabetic than in control subjects of the combined triads, while in the third triad the values were similar. The squalene ratio was higher in diabetic than in control subjects in both triad divisions. The ratios of lathosterol/absorption markers were also calculated, and they were significantly lower in diabetic than in control subjects in the combined triads (data not shown).

CONCLUSIONS — The present study showed that noncholesterol sterols reflecting synthesis of cholesterol were inversely related to those of cholesterol absorption in healthy children but less consistently in children with type 1 diabetes. Even though the mean sterol ratios were not markedly affected by diabetes, some differences occurred when compared with control subjects. When the study population was ranked to triads defined by the control cholestanol-to-cholesterol ratio, cholesterol absorption was higher in diabetic than in control subjects within the low cholesterol absorption triad. If anything, cholesterol synthesis showed an opposite behavior, as indicated by the low lathosterol ratio in the first two triads. Absorption and synthesis appeared to be similar in the highest triad of the whole study population for each respective marker.

Children with type 1 diabetes and those control subjects participating in STRIP shared the same dietary recommendations. Regarding the nature of type 1 diabetes, we anticipate that children with type 1 diabetes followed the recommendations. It turned out that the intervention children of STRIP had higher polyunsaturated-to-saturated fatty acid ratio versus control children, suggesting somewhat higher consumption of vegetable fat and possibly also plant sterols. Theoretically, this should have decreased serum cholesterol levels and increased the plant sterols. Neither of these changes occurred, suggesting that different intake of carbohydrate, fat, and cholesterol was not detectable between diabetic and control subjects.

The negative association between cholesterol synthesis and absorption, revealed by the surrogate synthesis and absorption markers, suggests that in healthy children the homeostasis of cholesterol metabolism is like adult humans, i.e., high cholesterol absorption inhibits cholesterol synthesis (1). This homeostasis was partly attenuated in children with type 1 diabetes, suggesting that diet supporting the preferable plant oil consumption may have been used more consistently by the diabetic children of the lowest triads. Another factor, which might affect plant sterol contents between control and diabetic subjects was the slightly higher BMI in the diabetes group, but standardization of the triads by BMI had virtually no effect on the respective campesterol ratios.

In two previous studies (2,3), plant sterol and cholestanol ratios were markedly increased in sera of adult type 1 diabetic subjects compared with control or type 2 diabetic subjects. In addition, cholesterol synthesis markers appeared to be low, most likely indicating reduced overall synthesis of cholesterol (2,3). The findings were interpreted to indicate reduced expression of *ABCG 5/8* genes in enterocytes and hepatocytes resulting in enhanced sterol absorption and reduced biliary secretion and downregulation of cholesterol synthesis. Similar conclusions were made in streptozotocin-induced experimental diabetes (4). The role of *ABCG 5/8* genes in regulation of noncholesterol sterol ratios in children may also depend on the polymorphism of these genes because they have different effects on cholesterol synthesis and absorption (20). In addition to the genetic regulation, another possibility for high cholesterol ab-

Table 4—*Serum cholesterol concentrations and ratios to cholesterol of squalene and noncholesterol sterols in the study population ranked to triads by control cholestanol-to-cholesterol ratio*

Group	Cholesterol (mg/dl)	Squalene ($10^2 \times$ μmol/mmol)	Cholestanol ($10^2 \times$ μmol/mmol)	Cholesterol ($10^2 \times$ μmol/mmol)	Desmosterol ($10^2 \times$ μmol/mmol)	Lathosterol ($10^2 \times$ μmol/mmol)	Campesterol ($10^2 \times$ μmol/mmol)	Sitosterol ($10^2 \times$ μmol/mmol)	Avenasterol ($10^2 \times$ μmol/mmol)
Triads 1 and 2									
Control ($n = 53$)	170.5 ± 4.6	21.4 ± 0.8	158.2 ± 3.0	6.9 ± 0.5	74.5 ± 1.5	141.9 ± 6.3	320.7 ± 13.3	162.3 ± 5.8	46.1 ± 1.5
Diabetes ($n = 33$)	160.5 ± 5.1	28.0 ± 1.5*	161.5 ± 3.2	6.1 ± 0.4	76.1 ± 2.5	123.3 ± 5.8*	400.1 ± 23.7*	188.5 ± 9.0*	51.8 ± 2.2*
Triad 3									
Control ($n = 26$)	163.2 ± 5.6	22.6 ± 1.0	214.5 ± 4.1†	6.6 ± 0.7	68.3 ± 3.2	103.5 ± 5.3†	521.8 ± 39.5†	250.8 ± 14.7†	63.2 ± 3.5†
Diabetes ($n = 15$)	168.4 ± 8.3	28.1 ± 1.8*	220.8 ± 7.4†	6.1 ± 0.5	70.4 ± 2.4	108.7 ± 8.9	544.2 ± 61.5†	248.4 ± 23.4†	67.6 ± 6.7†

Data are means ± SE. *$P < 0.05$ vs. respective control subjects; †$P < 0.05$ vs. respective values in triads 1 and 2.

sorption was suggested to result from increased mass of intestinal mucosa during poor glycemic control, as assessed with increased plasma postheparin diamine oxidase activity (21). However, in our preliminary data of adult type 1 diabetic subjects, glycemic control and serum absorption markers were not associated. The present findings showed virtually similar results revealed earlier in adults (2,3), even though, surprisingly, the noncholesterol sterol ratios were similar in diabetic and in control subjects in the highest absorption triad. In addition, the ratios of cholestenol and desmosterol were quite similar in all triads. The markedly increased squalene ratio in children with diabetes is in contrast to the low cholesterol synthesis suggested by the other cholesterol precursors.

Reasons are not known why the differences in cholesterol absorption and synthesis between diabetic and control subjects are seen only in the two lowest triads. Thus, the surrogate markers of cholesterol metabolism indicated that cholesterol absorption was lowest in the first two triads of both groups, even though it was higher in diabetic versus control subjects. The respective values were higher in the third than in the first and second triads, but no difference was seen between the case and control subjects. The findings emphasize the importance of measuring the percentage of dietary cholesterol absorption and cholesterol synthesis by the sterol balance technique.

The negative correlation of absorption sterol ratios to both systolic and diastolic blood pressure of control children is interesting, since this novel finding suggests that high cholesterol absorption reduces the expression of high blood pressure. It is suggested by the positive correlation with the synthesis markers that the association might be reflected by cholesterol metabolism. In view of the relatively poor correlation between cholesterol synthesis and absorption markers in children with diabetes, the lacking correlation of their blood pressure with synthesis and absorption markers is understandable.

Taken together, the data suggest that the homeostasis between cholesterol absorption and synthesis is less consistently maintained in children with diabetes than in control subjects. Although the mean ratios of the surrogate markers of cholesterol absorption and synthesis were similar in the two groups, plant sterol ratios were higher in diabetic versus control subjects in the two lowest triads defined by the cholestanol-to-cholesterol ratio, suggesting that within the range of low cholesterol absorption, it is still higher in children with diabetes than in control subjects.

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