Use of determinations of 7-lathosterol (5α-cholest-7-en-3β-ol) and other cholesterol precursors in serum in the study and treatment of disturbances of sterol metabolism, particularly cerebrotendinous xanthomatosis

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Abstract The sterol composition of sera from patients with cerebrotendinous xanthomatosis (CTX) was investigated by gas chromatographic analysis of saponified extracts, using a polar (CP Wax 52CB) and an apolar (CP Sil 5CB) capillary column. Apart from already known sterols, the presence of increased amounts of 8-lathosterol (5α-cholest-8(9)-en-3β-ol) and significant amounts of 8-dehydrocholesterol (cholesta-5,8-dien-3β-ol) were noticed. The latter compound has not been detected previously in human serum and possibly represents a hitherto unknown cholesterol precursor. The apparently elevated levels of Δ^8-sterols in CTX serum suggests partial inhibition of migration of the 8,9 double bond to the 7,8 position in this condition. The concentration of 7-lathosterol, an indicator of cholesterol production rate, is also highly elevated in CTX serum and quickly returns to normal values after oral bile acid therapy. Determinations of serum lathosterol are not only useful in the follow-up of therapy of CTX patients, but also in the follow-up of hypercholesterolemic patients treated with either HMG-CoA reductase inhibitors or bile acid sequestrants. —**Wolthers, B. G., H. T. Walrecht, J. C. van der Molen, G. T. Nagel, J. J. Van Doormaal, and P. N. Wijnandts.** Use of determinations of 7-lathosterol (5α-cholest-7-en-3β-ol) and other cholesterol precursors in serum in the study and treatment of disturbances of sterol metabolism, particularly cerebrotendinous xanthomatosis. *J. Lipid Res.* 1991. **32:** 603–612.

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Biosynthesis of cholesterol is very complex, involving numerous intermediates and feed-back mechanisms, and the participation of lipoproteins and receptors in its regulation. Biosynthesis of cholesterol starts from acetyl-CoA and the first sterol structure formed is lanosterol with 30 carbon atoms (1, 2). The next steps, leading finally to cholesterol, are very complex and there is ample evidence that, apart from a distinct main route, alternative minor routes exist (1, 3–5). Endogenous cholesterol synthesis can be followed by determining unique precursors present in serum, e.g., mevalonic acid, an intermediate between acetyl-CoA and lanosterol, and lathosterol (5α-cholest-7-en-3β-ol), an intermediate between lanosterol and cholesterol.

Recent investigations concerning the determination of serum lathosterol have shown that this parameter is valuable in estimating the cholesterol production rate and can be used as a diagnostic tool. Vuoristo, Tilvis, and Miettinen (6) and Färkkilä and Miettinen (7) showed that in ileal dysfunction and coeliac disease, where cholesterol absorption by the intestinal tract is impaired, serum lathosterol is increased, reflecting enhanced endogenous cholesterol synthesis to meet demand for cholesterol. When patients adhere to the prescribed gluten-free diet (6), serum lathosterol decreases, proving the effectiveness of therapy. Björkhem et al. (5) measured several cholesterol precursors (including lathosterol) in serum in combination with activity of HMG-CoA reductase, the rate limiting step in cholesterol synthesis, in liver biopsies and demonstrated that these two parameters correlate significantly. Interestingly, it was shown that both parameters were highly increased when patients were treated with cholestyramine, a drug that markedly enhances endogenous cholesterol synthesis. Kempen et al.

Abbreviations: TLC, thin-layer chromatography; GC, gas chromatography; MS, mass spectrometry; CTX, cerebrotendinous xanthomatosis.

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(8) studied the effect of therapy with the HMG-CoA reductase inhibitor simvastatin (Mk-733), given to patients with familial hypercholesterolemia, on the serum lathosterol concentration. It was shown that this treatment resulted in marked lowering of serum lathosterol, indicating depressed cholesterol synthesis.

Our interest in cholesterol precursors was evoked during studies on the sterol composition of serum of cerebrotendinous xanthomatosis (CTX) patients. In this inherited disease, due to deficiency of cholesterol 26-hydroxylase, synthesis of bile acids from cholesterol is impaired, leading to accumulation of (useless) bile alcohols and cholestanol (9). In gas chromatograms of unsaponifiable lipids from sera of CTX patients, in which the cholestanol/cholesterol ratio was measured (9), small peaks were observed, some being unknown to us. Later investigations revealed that some of these were intermediates in the cholesterol–bile acid metabolic route (hydroxysterols such as 7α-hydroxycholesterol), which could be quantified as described by Koopman et al. (10). As expected, serum 7α-hydroxycholesterol levels reflecting enhanced 7α-hydroxylase activity (the rate-limiting step in bile acid synthesis) were increased, whereas 26-hydroxycholesterol levels were decreased due to the enzymatic defect in CTX. The present investigations, performed by GC and GC–MS analysis, were undertaken to clarify the nature of some more compounds, presumably sterols, present in CTX sera and to see whether these could be of additional interest in studying CTX. It may be expected that, owing to increased cholesterol production rate, cholesterol precursors in serum of CTX patients are also raised. Indeed, these investigations revealed that, in CTX, several cholesterol precursors, in particular lathosterol, are greatly increased in serum. Moreover, those precursors provided more insight into the biochemical pathways of cholesterol biosynthesis and could serve as diagnostic parameters in the treatment of CTX. Further, the use of serum lathosterol in the follow-up of other disturbances in sterol metabolism and their treatment was investigated. The results were compared with those obtained for CTX patients and with results previously reported by others.

MATERIALS AND METHODS

Materials

5α-Cholestan-3β-ol, 5β-cholestan-3β-ol, 5β-cholestan-3α-ol, cholesterol, and 7-lathosterol were from Steraloids Inc., Wilton, NH. 5α-Cholest-8(14)-en-3β-ol and desmosterol were from Makor Chemicals Ltd., Jerusalem, Israel. 7-Dehydrocholesterol, campesterol, sitosterol, chenodeoxycholic acid, and deoxycholic acid were from Sigma Chemical Company, St. Louis, MO. Stigmasterol was from Janssen, Beerse, Belgium. 8-Lathosterol was provided by Professor D. N. Kirk (Queen Mary and Westfield College, London, UK) from The MRC Steroid Reference Collection. Trisil/TBT (a mixture of N-trimethylsilyl-imidazole, N,O-bistrimethylsilyl-acetamide and trimethylchlorosilane, 3:3:2 by volume) was from Pierce Europe, Oud Beijerland, the Netherlands. All other chemicals were of analytical grade and supplied by Merck Darmstadt, Germany. The 25-m polar capillary column (CP-Wax 52-CB, i.d. 0.32 mm) was from Chrompack Middelburg, the Netherlands, and the 25-m apolar (OV 1) column was an Ultra 1 (cross-linked methylsilicone gum phase) column, i.d. 0.2 mm, film thickness 0.33 μm, from Hewlett-Packard, Amstelveen, the Netherlands.

Methods

Sterols in serum, lathosterol in particular, were determined as follows. One hundred μl of a methanolic solution containing 5β-cholestan-3α-ol (internal standard) at a concentration of 84.8 μmol/l, was pipetted into a test tube and the solvent was evaporated to dryness under a stream of N_2 at 60°C. Next, 50 μl of serum was added, followed by 1 ml of a freshly prepared mixture consisting of 6 ml of 33% aqueous KOH solution (w/v) and 94 ml of 96% ethanol. After mixing and incubating at 56°C for 15 min, the solution was cooled and 1 ml of distilled water and 2 ml of hexane were added. The mixture was vortexed for 30 sec and the hexane layer was transferred to another tube. After evaporation to dryness under a stream of N_2 at 60°C, derivatization was carried out by addition of 100 μl of Trisil/TBT and the mixture was allowed to stand for 30 min at 80°C. After adding 4 ml of hexane and 4 ml of 0.1 N HCl, the mixture was vortexed for 30 sec. The aqueous layer was removed and the hexane layer was washed with 4 ml of water. The hexane layer was transferred to another tube and evaporated to dryness under a stream of N_2 at 40°C. The residue was redissolved in 200 μl of hexane.

GC procedure

One μl of sample was injected into a 5890 Hewlett Packard gas chromatograph equipped with a split/splitless injection device for capillary columns and flame ionization detection. The gas chromatograph was connected to an interface, coupled to an IBM PC, loaded with Nelson integration software (Perkin-Elmer). Helium was used as carrier gas. Injector and detector temperatures were set at 305° and 310°C, respectively. Further conditions: the helium flow through the Ultra 1 column was 0.5 ml/min, split ratio 1:20; temperature programming of the oven: starting temperature 240°C, final temperature 310°C, programming rate 2°C per min. For the CP-Wax 52 CB column, flow rate through the column was 2.5 ml/min, split ratio 1:6, oven temperature 230°C (isothermal).

Quantification was carried out by comparing the peak area of the sterol of interest with that of the internal standard, assuming that the response factor of the internal standard was the same as for the sterol. Retention indexes (methylene units, M.U. values) were established by injecting separately a mixture of alkanes (C_{30}, C_{31}, C_{32}, C_{33}, C_{34}, C_{36}, C_{38}, C_{40}) under exactly the same conditions as the samples and by linear interpolation of retention indexes of sterols between those of adjacent alkane peaks.

GC-MS

GC-MS analyses were carried out on a VG 70-250S magnet sector instrument, connected to a Hewlett-Packard 5880 gas chromatograph. GC conditions were similar to those described above. However, the injection mode was splitless. Electron impact (EI) spectra were recorded at 70 eV at a resolution ($m/_{\Delta}m$) of 1000. Source and interface temperature were 250°C.

Patients

CTX. Stored serum samples, kept at −20°C, of CTX patients were used. Diagnosis had been based on urinary excretion of typical bile alcohols. Thirteen samples were from untreated patients, while 13 samples were from patients, who were treated daily with either 750 mg chenodeoxycholic or cholic acid administered orally.

Four patients were studied in detail. These patients belonged to one family: a brother, 38 years old (JJ) and three sisters, 40 years old (WO), 42 years old (WC), and 45 years old (TJ). They had participated in former investigations, described elsewhere, after informed consent (11). Patients were divided into two groups of two patients each. Before starting the study, they did not receive any medication. Two patients started with a daily oral dose of 2 × 250 mg of deoxycholic acid for 2 weeks, then no medication for 1 week, and finally a daily dose of 2 × 250 mg of chenodeoxycholic acid for 2 weeks. The schedule for the other two patients consisted of daily oral doses of 2 × 250 mg chenodeoxycholic acid for 2 weeks, 1 week with no medication, followed by 2 weeks of oral medication with 2 × 250 mg of deoxycholic acid daily. Serum samples of each patient were collected at the start of the trial (day 0), and days 14, 21, 35. All samples were stored at −20°C until analysis.

Normals. Serum was collected from 22 healthy volunteers with ages ranging from 22 to 50 years.

Patients with familial hypercholesterolemia. Sera of four patients, treated with the bile acid sequestrant cholestyramine, 3 × 10 g daily, were investigated. Sera of two other patients, who were treated with simvastatin (10 mg daily), were collected before the start of therapy and at different periods of time during medication. Sera were stored at −20°C until analysis.

RESULTS

Qualitative analysis of sterols in serum of CTX patients

Sera of CTX patients, processed as described in Materials and Methods, were analyzed both on a polar capillary column (CP Wax 52 CB) and on an apolar column (OV 1). Gas chromatograms obtained on the two stationary phases for a representative patient are shown in **Fig. 1A and 1B**. Apart from a predominant cholesterol peak, several minor peaks were observed; their identity was further investigated by means of GC-MS analysis. The identity of the peaks could be established by comparison of retention times and mass spectra of reference compounds with these parameters derived from serum analysis.

The identification of all peaks on the OV 1 chromatogram was straightforward (Fig. 1). Peak 4 showed a mass spectrum and retention time consistent with those of reference 5α-cholest-8(9)-en-3β-ol (8-lathosterol), kindly provided by Professor D. N. Kirk. The mass spectrum of this compound is depicted in **Fig. 2A**. Although 5α-cholest-8(14)-en-3β-ol exhibits the same mass spectrum (12), its retention time is markedly different, as shown by comparison of retention times of both reference compounds; this is in accord with a previously published study (13). Retention time and mass spectrum (see Fig. 2B) of peak 6 established the presence of 5α-cholest-7-en-3β-ol (7-lathosterol). Although the mass spectra of 7- and 8-lathosterol are rather similar, there was a marked difference in intensity of the fragment *m/z* 255. Noteworthy is the proven presence of substantial amounts of cholesta-5,7-dien-3β-ol (7-dehydrocholesterol, peak 5), as the retention time of this peak coincided with and the mass spectrum was closely similar to that of the reference compound (Fig. 2D).

All peaks on the CP Wax 52 CB chromatogram (Fig. 1B) could be identified in a similar way, with the exception of peak 5, whose mass spectrum is depicted in Fig. 2C. It shows close resemblance with the mass spectrum of reference 7-dehydrocholesterol, shown in Fig. 2D. Peak 7 was 7-lathosterol, contaminated with an unknown substance, contributing a high fragment *m/z* 364 to the mass spectrum.

The fact that 7-lathosterol, 8-lathosterol, and 7-dehydrocholesterol are present in CTX serum gave rise to the assumption that peak 5 in the CP Wax chromatogram might be 8-dehydrocholesterol (cholesta-5-8(9)-dien-3β-ol). Substantial evidence that this assumption is correct can be enumerated. First, the correspondence between its mass spectrum (Fig. 2C) and that of 7-dehydrocholesterol (Fig. 2D) is striking; both compounds possess the same characteristic mass fragments, e.g., *m/z* 456, 366, 351, and

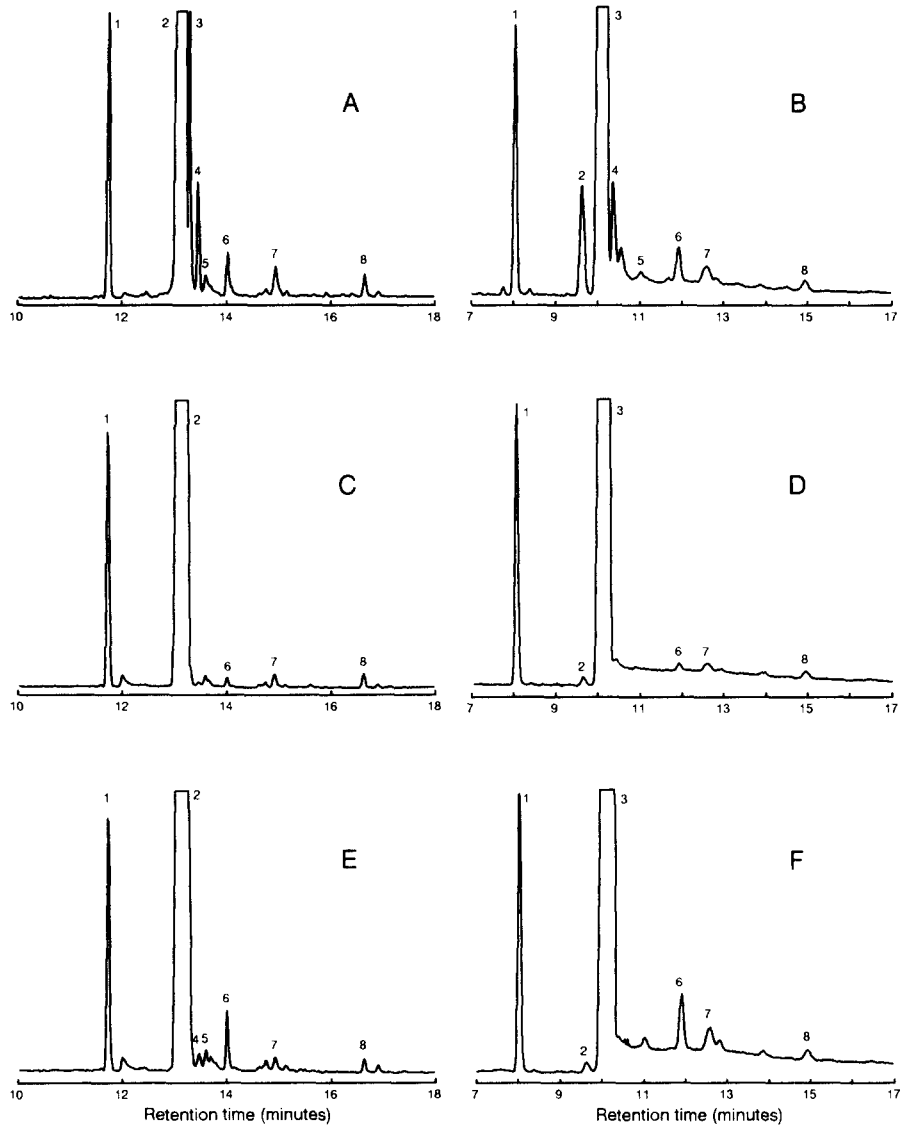

Fig. 1. Chromatograms of human sera, showing peaks of several sterols. A,B: serum of CTX patients (on OV-1 and CP-Wax 52 CB columns, respectively); C,D: serum of a normal subject (on OV-1 and CP-Wax 52 CB columns, respectively); E,F: serum of a patient with hypercholesterolemia, treated with cholestyramine (on OV-1 and CP-Wax 52 CB columns, respectively). Identity of peaks, as checked with GC–MS: OV-1 chromatograms: 1, 5β-cholestan-3α-ol (internal standard); 2, cholesterol; 3, 5α-cholestan-3β-ol; 4, 8-lathosterol; 5, 7-dehydrocholesterol; 6, 7-lathosterol; 7, campesterol; 8, sitosterol. CP-Wax chromatograms: 1, internal standard; 2, 5α-cholestan-3β-ol; 3, cholesterol; 4, 8-lathosterol; 5, 8-dehydrocholesterol; 6, 7-lathosterol; 7, 7-dehydrocholesterol + campesterol; 8, sitosterol.

325. Moreover, the retention time of peak 5 (on CP Wax) differs from that of 7-dehydrocholesterol in the same way as that of 8-lathosterol differs from the retention time of 7-lathosterol. Additional evidence comes from closer inspection of the mass spectra in Fig. 2C and 2D (see Discussion). At first glance, no peak that could possibly be 8-dehydrocholesterol on the basis of its mass spectrum could be discovered in the OV 1 chromatogram of CTX serum. However, the retention time of 8-dehydrocholesterol on OV 1 can be predicted, if those of 8-lathosterol, 7-lathosterol, and 7-dehydrocholesterol are known, which is the case. It then appears that the retention time should be expected just behind that of cholesterol and therefore the peak will not show up in gas chromatograms of sera, which are dominated by cholesterol. However, subsequent mass spectrometric analysis of sterols from CTX serum also identified 8-dehydrocholesterol in the OV 1 chromatogram at the expected position, as shown in **Fig. 3**. Fig. 3A shows the total ion current of the mass spectrometric analysis, per-

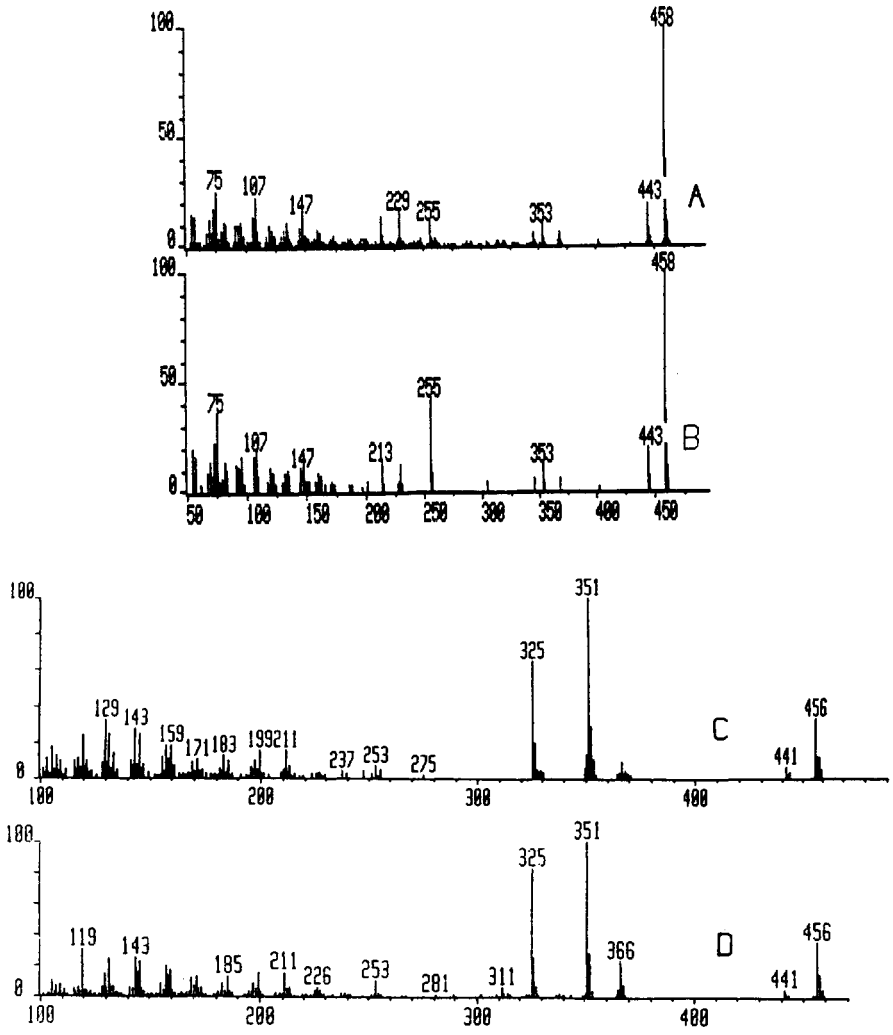

Fig. 2. Mass spectra of TMS-derivatives of some sterols. A, 8-lathosterol (reference compound); B, 7-lathosterol (reference compound); C, 8-dehydrocholesterol (spectrum found in CTX serum); D, 7-dehydrocholesterol (reference compound).

formed on sterols from CTX serum by OV 1 chromatography. Peaks with scan numbers 582 and 591 represent 8-lathosterol and 7-lathosterol, respectively. Fig. 3B shows the traces of characteristic mass fragments *m/z* 325, 351, and 456, selected from the mass spectra; these three masses peaked at scan numbers 579 and 587, the latter peak corresponding with 7-dehydrocholesterol. The peaks at scan number 579 (closely behind the top of the cholesterol peak) therefore have to be ascribed to 8-dehydrocholesterol, almost coinciding with cholesterol as predicted.

Table 1 summarizes MU values of the TMS-derivatives of reference sterols on the polar and apolar columns, together with the calculated value, derived from GC and GC–MS analysis of CTX serum for (tentative) 8-dehydrocholesterol. All together, convincing evidence that 8-dehydrocholesterol is actually present in CTX serum can be inferred from the data. Peak area measurements of 8-lathosterol and 8-dehydrocholesterol in CTX serum in the CP Wax chromatogram (Fig. 1B) allowed a rough estimation of their concentrations (60 and 25 μmol/l, respectively), and the same was possible in the OV 1 chromatogram for 7-lathosterol and 7-dehydrocholesterol (20 and 12 μmol/l, respectively).

Quantitative measurements of serum 7-lathosterol

The qualitative study described above revealed that 7-lathosterol in serum, separated on an OV 1 capillary column, was not contaminated by another substance, as its peak was sharp and its mass spectrum was identical to reference 7-lathosterol, in contrast to CP Wax chromatography, where its peak was broad and the mass spectrum was contaminated. We concluded that by relatively simple pretreatment of sterols from serum, followed by gas chro-

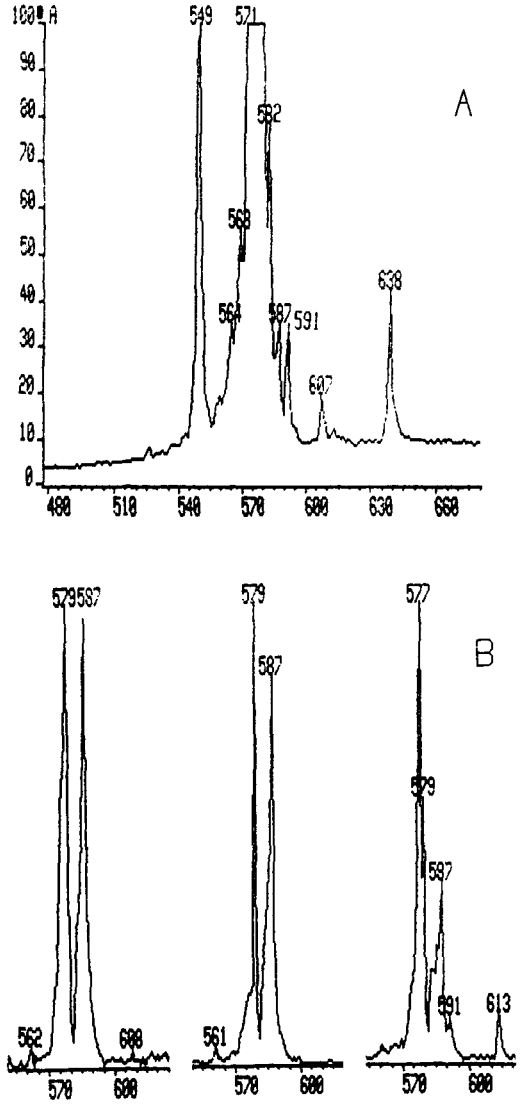

Fig. 3. GC–MS analysis of a serum extract of a CTX patient. An OV-1 capillary column was used. The aim of the analysis was to prove the presence of 8-dehydrocholesterol in serum. A. Total ion current. The x-axis represents the number of the MS-scan. The highest peak (scan number 571) is cholesterol; the peak with scan number 582 is due to 8-lathosterol; 587 is 7-dehydrocholesterol; and 591 is 7-lathosterol. B. Mass chromatograms. After GC–MS analysis, the intensities of mass fragments, representative for dehydrocholesterols, viz *m/z* 325 (left trace), 351 (middle trace), and 456 (right trace), were depicted for each recorded mass spectrum by means of the GC–MS system. At all three masses, two peaks were observed with peaks at scan number 579 (just behind the top of cholesterol) and at scan number 587. The latter peak is known to be 7-dehydrocholesterol; the first peak is presumably 8-dehydrocholesterol.

matography on an OV 1 capillary column, serum lathosterol could be determined quantitatively. Most papers published so far on this subject included extensive prepurification of serum unsaponifiable lipids by thin-layer chromatography, which we considered unnecessary.

We undertook a study on serum lathosterol concentration in a number of clinical situations related to disturbances in sterol metabolism in comparison with healthy controls. **Fig. 4** shows results for the following cases: *1*) 22 normal controls; *2*) 13 untreated CTX patients; *3*) 13 CTX patients on oral bile acid therapy; and *4*) 4 patients with familial hypercholesterolemia and treated with cholestyramine. Representative chromatograms on both stationary phases for a normal control (Fig. 1C and 1D) and a cholestyramine-treated patient (Fig. 1E and 1F) are shown. The lathosterol values of untreated CTX patients were, with a few exceptions, highly elevated as were those of cholestyramine-treated patients, whereas all treated CTX patients showed lathosterol values within the normal range. Normal reference values were 5.70 ± 2.46 μmol/l in accord with published data (8).

In order to explore further the possibility that serum lathosterol might be a suitable parameter to monitor bile acid therapy in CTX patients, four patients were studied who started such therapy according to a strict protocol. The therapy protocol is described in the Materials and Methods section. In a former study, serum cholestanol and urinary bile alcohol excretion of these patients had been determined. The study showed that deoxycholic acid actually depressed endogenous bile acid synthesis in these patients (11). In the present investigation serum lathosterol was also determined in the collected sera; both cholestanol and lathosterol levels are depicted in **Fig. 5**. After 2 weeks of therapy, serum lathosterol values approached normal values, in contrast to those of cholestanol, which decreased more slowly.

Finally, serum lathosterol and cholesterol were determined in two patients with familial hypercholesterolemia, who started therapy with the HMG-CoA reductase inhibitor, simvastatin. Results are given in **Table 2** and presented as percentage of initial values (just before therapy)

TABLE 1. Methylene units of TMS-derivatives of sterols on OV-1 and CP-Wax 52 CB capillary columns

Sterol	OV-1	CP-Wax 52 CB
5β-Cholestan-3β-ol	30.51	31.96
5β-Cholestan-3α-ol	30.60	32.31
8(14)-Lathosterol	31.32	33.55
Cholesterol	31.36	33.66
Δ^8-Dehydrocholesterol[a]	31.42	34.07
5α-Cholestan-3β-ol	31.49	33.40
8(9)-Lathosterol	31.56	33.81
Desmosterol	31.68	34.55
Δ^7-Dehydrocholesterol	31.73	34.62
7-Lathosterol	31.88	34.36
Campesterol	32.40	34.61
Stigmasterol	32.69	34.80
Sitosterol	33.27	35.35

[a]Methylene units of presumed 8-dehydrocholesterol derived from combined GC and GC–MS data (see text).

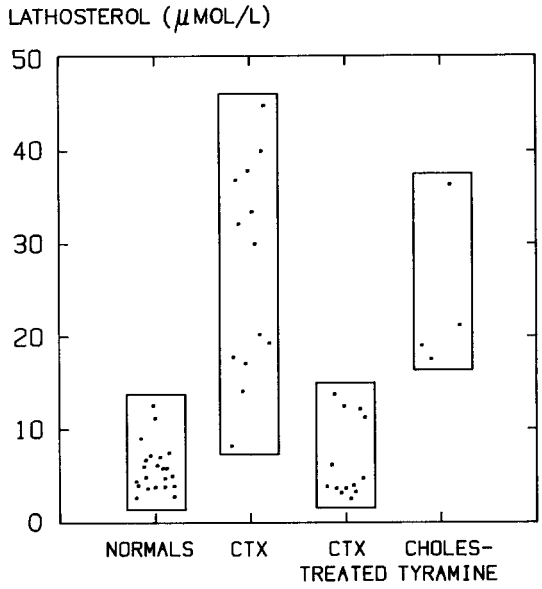

Fig. 4. Concentration of lathosterol in serum of normal subjects (n = 22), untreated CTX patients (n = 13), CTX patients receiving oral bile acid therapy (n = 13), and cholestyramine-treated patients with hypercholesterolemia (n = 4). Statistical data (mean ± SD): normals, 5.70 ± 2.47 μmol/l; untreated CTX, 26.05 ± 11.03 μmol/l; treated CTX, 6.34 ± 4.06 μmol/l; cholestyramine-treated, 22.63 ± 8.31 μmol/l. Statistical comparison (Mann-Whitney U-test: two-sided rank sum test for unpaired data): normals/treated CTX, $P = 0.62$ (not significant); normals/untreated CTX, $P < 0.001$; normals/cholestyramine-treated, $P < 0.002$.

in **Fig. 6**. Both parameters decreased, the percentage decrease of lathosterol being substantially higher than that of cholesterol. Similar results have been described by Kempen et al. (8) who studied hypercholesterolemic patients treated with simvastatin (Mk-733).

DISCUSSION

Briefly summarized, the present study can be regarded as *1*) a qualitative study of the sterol composition in sera of CTX patients, and *2*) a quantitative study of serum lathosterol in a number of clinical conditions.

As to the qualitative study, this work can be conceived as an extension of former studies of Tint and Salen (14) on the presence of cholesterol precursors in samples obtained from CTX patients. Whereas these workers investigated bile and feces, we focused attention on analysis of serum. Tint and Salen (14) showed, by administering radioactive mevalonate to patients, that a number of cholesterol precursors, notably lanosterol, 24,25-dihydrolanosterol, and lathosterol, were synthesized in markedly increased amounts in CTX patients. These experiments led to the conclusion that the main pathway of cholesterol synthesis involved early reduction of the 24,25-double bond of lanosterol and that an alternative route, late reduction of this bond (resulting in the production of Δ^{24}-cholesterol (desmosterol)), was of no significance.

Our study has demonstrated that, in CTX, $\Delta^{8(9)}$-cholesterol precursors are also present. With regard to 8-lathosterol, its concentration is considerably higher than that of 7-lathosterol (see Fig. 1A). This is remarkable, as in previous studies not involving CTX, the reverse was found. Gylling and Miettinen (15) found, in familial hypercholesterolemia, a ratio 8-lathosterol/7-lathosterol of about 1:10. Björkhem et al. (5) reported in patients with gallstones, untreated or treated with either chenodeoxycholic acid or cholestyramine, a similar ratio, as did Färkkilä and Miettinen (7) in ileal dysfunction; finally Vuoristo et al. (6) found considerably more 7-lathosterol than 8-lathosterol in coeliac disease. Our chromatograms on OV 1 of sterols from serum of a normal subject (Fig. 1C) and of a cholestyramine-treated patient (Fig. 1E) suggest the same.

A further curious finding was the demonstration of the presence of 8-dehydrocholesterol in CTX serum. Although cholesterol precursors in serum have been studied

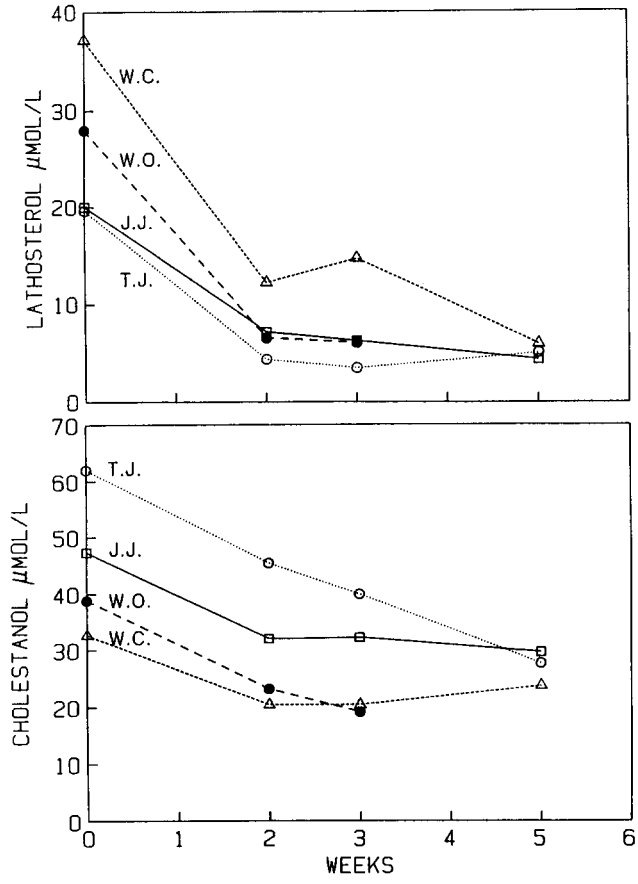

Fig. 5. Concentrations of 5α-cholestan-3β-ol and lathosterol in serum of four CTX patients just before and during oral bile acid therapy. The treatment protocol is described in Materials and Methods.

TABLE 2. Serum concentrations of lathosterol and cholesterol for two patients with familial hypercholesterolemia, before and during simvastatin therapy

	Days of Simvastatin Therapy							
	Patient A					Patient B		
	0	42	84	126	168	0	38	80
Lathosterol (μmol/l)	9.9	3.2	4.2	4.2	4.3	10.7	5.8	2.2
Cholesterol (mmol/l)	7.5	4.9	5.6	5.4	4.7	7.4	5.5	5.2

for many years, the presence of this compound in human sera has never been reported. We are convinced that we have proved its presence beyond doubt. Retention indexes as well as mass spectrometric data support this view. Additional evidence stems from close inspection of the mass spectrum, as obtained from GC–MS analysis of sterols from CTX serum on a CP Wax column, in comparison with that of 7-dehydrocholesterol. In **Table 3**, the relative intensities of a number of mass fragments of the TMS derivatives of both compounds, analyzed on the same GC–MS equipment, are given. The (presumed) 8-dehydrocholesterol shows significantly lower relative intensities for *m/z* 366 and 325. Comparison of published mass spectra of underivatized 8-dehydrocholesterol (16) and 7-dehydrocholesterol (17) shows a realtively lower intensity particularly of *m/z* 325 for the Δ^8 isomer (see Table 3). Apparently mass fragment *m/z* 351 is more stable and more easily formed in 8-dehydrocholesterol. This fragment arises from loss of H_2O and the 19-methyl group of the parent compounds (or $((CH_3)_3$ Si-OH + 19-methyl) of the TMS derivatives), while ring B opens and a 8(9) double bond is present (17). For this, in 7-dehydrocholesterol the 7(8) double bond has to migrate, whereas in 8-dehydrocholesterol the double bond is already in the right place. Therefore it is to be expected that, in the latter, the *m/z* fragment 351 is more dominant than in 7-dehydrocholesterol. The fact that 8-dehydrocholesterol is present in serum of CTX patients is no doubt related to the elevated concentration of the other Δ^8 precursor, i.e., 8-lathosterol, suggesting a more or less serious block in the migration of the 8,9 double bond to the 7,8 position during cholesterol synthesis in this condition. According to recent views with regard to the final steps in cholesterol biosynthesis, major migration of the 8,9 double bond occurs while a 4α-methyl group is still present on the precursor molecule (conversion of 8-methostenol into 7-methostenol (3, 5)). In this way primarily 7-lathosterol is ultimately formed, which by the action of 5-desaturase is converted into 7-dehydrocholesterol and finally, by reduction of the 7,8 double bond, into cholesterol. Our experiments have shown that 5-desaturase can also act on 8-lathosterol, which means that prior movement of the 8,9 double bond to the 7,8 position (brought about by 8→7-isomerase) is not a prerequisite for action of the first enzyme.

At present the reason for an apparent block in Δ^8 conversion in CTX is not yet clear. One explanation could be that, due to the accelerated cholesterol production in CTX, this enzymatic step becomes rate-limiting, in contrast to normal situations. However, a similar trend should then be expected in cholestyramine-treated patients. Neither Björkhem et al. (5) nor we (Fig. 1E) observed such a trend. On the other hand, Strandberg et al. (3) demonstrated that, in serum of cholestyramine-treated rats, the 8-lathosterol/7-lathosterol ratio became about 1:1, whereas in control rats this ratio was about 1:5.

Another suggestion is that, in CTX, accumulation of products such as hydroxycholesterols, cholestanol, or bile alcohols, caused by the enzymatic defect in CTX as a result of 26-hydroxylase deficiency, is responsible for inhibiting 8→7-isomerase to a certain extent.

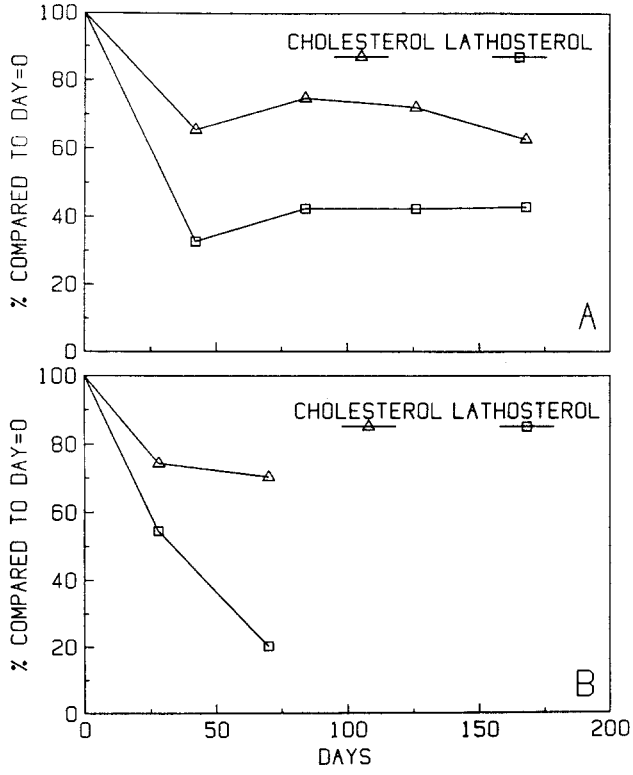

Fig. 6. Concentrations of serum lathosterol in two patients (A and B) with familial hypercholesterolemia, before and during treatment with simvastatin.

TABLE 3. Abundances (in % of base peak, m/z = 351) of TMS-derivatized and underivatized 7-dehydrocholesterol and 8-dehydrocholesterol

Mass Fragment	TMS Derivatives		Underivatized (Free)[a]	
	7-Dehydro	8-Dehydro	7-Dehydro	8-Dehydro
m/z				
456	20	25		
384			75	60
366	20	5	20	?
351	100	100	100	100
325	80	60	60	20
253	15	5	40	20

[a]From the literature (16, 17).

As to the quantitative study, the relatively simple analysis of serum lathosterol by mere extraction of saponified serum, omitting time-consuming TLC prepurification, followed by determination of the TMS-derivative on OV 1 capillary columns, has proven of considerable value. In all cases investigated, the GC lathosterol peak by shape and width suggested a pure substance. Mass spectrometric analysis, performed in a variety of situations, always confirmed the purity of the peak. This corroborates statements made by several others (5, 6, 8) that, at least for the determination of serum lathosterol, TLC purification is not necessary. As the present study has demonstrated, determination of serum lathosterol is very useful for *1*) follow-up of bile acid therapy in CTX patients, and *2*) follow-up of hypercholesterolemic patients treated with either cholestyramine or HMG-CoA reductase inhibitors such as simvastatin. Normal values of serum lathosterol agree closely with those found by Kempen et al. (8), who did not use TLC, and others, who incorporated this step (6).

Finally, we want to comment on the results obtained by Kempen et al. (8) for hypercholesterolemic patients treated with Mk-733 (simvastatin). In order to conclude that this drug lowers cholesterol production rate, they suggested that the ratio total lathosterol/total cholesterol in serum is a better parameter than lathosterol alone. They showed that in untreated hypercholesterolemic patients both lathosterol and cholesterol levels are higher than those in normal individuals, whereas the ratio was the same. This is in accord with the observation that in hypercholesterolemic patients the cholesterol production rate is normal. The increased serum lathosterol in hypercholesterolemia, then, has to be ascribed to the decreased clearance of low density lipoproteins from serum; however, the effect of therapy is correctly represented by the decrease of the lathosterol/cholesterol ratio in serum during treatment. We fully agree with this, as we too found raised values of both lathosterol and cholesterol in serum in untreated patients, followed by a larger decrease of lathosterol than of cholesterol during treatment with simvastatin.

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