ASBMB JOURNAL OF LIPID RESEARCH

Occurrence of isomeric dehydrocholesterols in human plasma

Magnus Axelson[1]

Department of Clinical Chemistry, Karolinska Hospital, S-104 01 Stockholm, Sweden

Abstract Three isomeric dehydrocholesterols were found in plasma from healthy subjects and patients with abnormal production or metabolism of cholesterol. These chemically labile steroids were isolated by a mild liquid-solid extraction procedure using octadecylsilane-bonded silica as sorbent. Sterol-protein interactions were minimized by diluting plasma with aqueous isopropanol. The dehydrocholesterols were identified by high-performance liquid chromatography–ultraviolet spectroscopy and gas chromatography–mass spectrometry as cholesta-5,7-dien-3β-ol (7-dehydrocholesterol), 5α-cholesta-6,8(9)-dien-3β-ol (isodehydrocholesterol), and tentatively as cholesta-5,8(9)-dien-3β-ol. There was a strong positive correlation between plasma levels of the two former compounds, isodehydrocholesterol levels usually being about 1.4 times higher than those of 7-dehydrocholesterol. The median concentration of 7-dehydrocholesterol in plasma from healthy subjects was 52 ng/ml. Similar concentrations were found in colectomized patients (median concentration 47 ng/ml) and patients with extrahepatic cholestasis and alcoholic liver cirrhosis (median concentrations 79 and 67 ng/ml, respectively). Patients with ileal resection or under treatment with cholestyramine had elevated levels (median concentrations 142 and 160 ng/ml, respectively) whereas patients with primary biliary cirrhosis had subnormal levels (median concentration 26 ng/ml). The results are consistent with a positive correlation between levels of the dehydrocholesterols in plasma and the rate of cholesterol synthesis. The sterols were also analyzed in human skin and bile and the results indicate that the liver may be an important source of isodehydrocholesterol.—**Axelson, M.** Occurrence of isomeric dehydrocholesterols in human plasma. *J. Lipid Res.* 1991. **32:** 1441–1448.

Supplementary key words 7-dehydrocholesterol • vitamin D_3 • cholesterol biosynthesis • human skin • human bile

7-Dehydrocholesterol is the precursor of vitamin D_3 and cholesterol in mammals. The presence of this compound in skin is well documented (1), however, studies on its occurrence in plasma have given conflicting results. Whereas relatively high levels of the compound in plasma were reported several decades ago (2, 3), recent studies using more advanced techniques have failed to confirm this (4).

In the course of studies on the biosynthesis and metabolism of vitamin D_3 in humans (5), a simple method for the analysis of 7-dehydrocholesterol in plasma was developed. The results show that 7-dehydrocholesterol is a normal constituent in plasma. In addition, two isomers of this compound were discovered in similar concentrations. One of these could be definitively identified as 5α-cholesta-6,8(9)-dien-3β-ol (isodehydrocholesterol) whereas the other was tentatively characterized as being cholesta-5,8(9)-dien-3β-ol. This report describes the isolation of these compounds from plasma, their identification by chromatographic and spectrometric techniques, and gives information about their levels in plasma under normal and some pathological conditions.

MATERIALS AND METHODS

Subjects and samples

Healthy subjects. Blood from 35 apparently healthy men and women, 21–62 years old (median age 33), was collected in tubes with or without heparin. After centrifugation, plasma/serum was separated and stored at −20°C until analyzed. All subjects were not fasting, but most samples were collected in the morning.

Patients. Blood was obtained from patients with intestinal diseases, liver diseases, or under treatment with cholestyramine. The following groups of patients were studied: Group A: three men and three women, 28–50 years old (median age 37), subjected to a complete colectomy due to polyposis coli. One patient had ileostomy and the others had an ileo-rectal anastomosis. Group B: six men and six women, 30–59 years old (median age 40), with Crohn's disease subjected to resection of terminal ileum (>60 cm) with or without colectomy. The operations on patients in groups A and B were performed more than 2 years before this investigation. Group C: one man and nine women, 39–70 years old (median age 63), with low-fat diet and treated with 8 g of cholestyramine (Ques-

Abbreviations: ODS, octadecylsilane; HPLC, high-performance liquid chromatography; GLC-MS, gas-liquid chromatography-mass spectrometry; TMS, trimethylsilyl.

[1]Correspondence address: Department of Clinical Chemistry, Karolinska Hospital, Box 60 500, S-104 01 Stockholm, Sweden.

Downloaded from www.jlr.org by guest, on June 18, 2012

tran[R], Bristol) twice daily for 6-8 weeks prior to sampling. Group D: three men and eight women, 49-87 years old (median age 68), with large bile duct obstruction secondary to carcinoma or gallstones and with grossly elevated bile acid levels in blood. Group E: one man and ten women, 40-70 years old (median age 65), with primary biliary cirrhosis. Group F: six men and three women, 51-66 years old (median age 57), with moderate or severe forms of alcoholic liver cirrhosis. The diagnosis of patients in groups E and F was made on the basis of full clinical and chemical investigation including liver biopsy in most cases. Most of these patients had elevated levels of bile acids in blood.

Bile samples. Bile fistula bile and gall bladder bile samples were obtained from four patients undergoing gallstone surgery at the Karolinska Hospital. The samples were collected in connection with other research projects, which had been approved by the local Ethics Committee.

Skin samples. Normal human skin samples were obtained from two Caucasian women at breast surgery. They were stored at $-70°C$ until analyzed.

Glassware and chemicals

All glassware was silanized and cleaning was carried out in an ultrasonic bath. Solvents were of analytical reagent grade. 7-Dehydrocholesterol and vitamin D_3 were purchased from Sigma Chemical Company (St. Louis, MO). 5α-Cholesta-6,8(9)-dien-3β-ol (isodehydrocholesterol) and cholesta-4,6-dien-3-one were kind gifts from Professors D. N. Kirk, (MRC Steroid Reference Collection) and I. Björkhem, Huddinge Hospital, respectively. (1a,2a(n)-^{3}H)Vitamin D_3 (10 Ci/mmol) was from the Radiochemical Centre (Amersham, England) and radioactivity was determined in an LKB/Wallac 1215 Rackbeta scintillation counter with Picofluor as the scintillation liquid. Octadecylsilane (ODS)-bonded silica (preparative C_{18}, particle size 55-105 μm) was from Waters Associates (Milford, MA). Column beds (1.5 × 0.8 cm, dry weight about 0.4 g) of this material were prepared in glass columns equipped with gauze-covered valves of Teflon. The sorbent was washed with 5 ml each of methanol, methanol-chloroform 1:1 (v/v), methanol and water prior to use. Appropriate flow rates were obtained by application of nitrogen pressure.

Analytical procedures

Extraction of sterols. After addition of an internal standard (^{3}H-labeled vitamin D_3, about 10,000 cpm), 0.5 ml of plasma or serum was diluted with 1.0 ml of water. To the solution was then added 0.75 ml of isopropanol (2-propanol) and the mixture was held for 5 min in an ultrasonic bath. It was then allowed to equilibrate for another 10 min without ultrasound. Extraction was performed by passing the mixture through a column of ODS-bonded silica at room temperature (flow rate about 1 ml • min^{-1}). The column was washed with 10 ml of water and 10 ml of 85% aq. methanol. A gentle stream of nitrogen was then passed through the sorbent for 1 min prior to elution of sterols with 4 ml of methanol. To the eluate was added 1 ml of water. After a wash of the column with 5 ml each of methanol-chloroform 1:1 (v/v), methanol, and 1 ml of 85% aq. methanol, the diluted eluate was reextracted on the same column. The column was washed with 2 ml of 85% aq. methanol followed by a gentle nitrogen stream through the sorbent for 2 min. Hydrophobic sterols were then eluted with 8 ml of hexane-chloroform 95:5 (v/v). The eluate was taken to dryness under a stream of nitrogen and the residue was redissolved in 100 μl of methanol. All solvents were degassed prior to use.

The described method was also used in the experiments with 0.5-ml aliquots of human bile. Human skin samples (about 0.25 cm^2, 0.2 g) were cut into small pieces and were extracted with 6 ml of ethanol in an ultrasonic bath for 15 min. After addition of the internal standard and centrifugation, the ethanol extract was transferred to another tube and diluted with 3 ml of water. Skin sterols were then extracted/purified on a column of ODS-bonded silica as described for plasma.

High-performance liquid chromatography (HPLC). Reversed-phase HPLC was carried out on a column of LiChrospher (250 × 4 mm, LiChroCart, Hibar, 100RP-18, 5 μm, Merck, Darmstadt, GFR) using a pump (Constametric III) and a variable wave-length detector (spectra Monitor D from LDC/Milton Roy, Riviera Beach, FL) set at 282 nm and a Rheodyne Model 7125 injector with a 100 μl loop. The mobile phase was a mixture of methanol-ethanol-water 160:40:5 (by vol) and the flow rate was 1 ml • min^{-1}.

The amount of 7-dehydrocholesterol in plasma was determined by comparing its peak with that of known amounts of the reference compound. Since a radioactive version of this compound was not available, ^{3}H-labeled vitamin D_3, added prior to extraction and determined in the effluent from the HPLC column, served to correct for losses and differences in injection volumes. The amount of isodehydrocholesterol was determined by comparing its peak area with that of 7-dehydrocholesterol and using a molar response at 282 nm of 0.33 relative to that of the latter compound.

For identification purposes, appropriate fractions of the effluent from the reversed-phase HPLC column were collected and reanalyzed by straight-phase HPLC. The latter was carried out on a column (250 × 4.5 mm) of LiChrospher (Hibar, Si 100,5 μm, Merck) connected to a pump (Constametric III) and a fixed-wavelength (254 nm) detector (LDC/Milton Roy) or a Waters 990 photodiode array detector (Waters Associates) monitoring wavelengths from 220 to 300 nm. The mobile phase was hexane-isopropanol 99:1 (v/v) and the flow rate was 1 ml • min^{-1}.

Downloaded from www.jlr.org by guest, on June 18, 2012

Gas-liquid chromatography-mass spectrometry (GLC-MS). Fractions collected from the HPLC column were taken to dryness under a stream of nitrogen and treated with a mixture (0.1 ml) of trimethylchlorosilane-hexamethyldisilazane-pyridine 1:2:3 (by vol) for 30 min at 60°C. The reagents were then evaporated under nitrogen and the trimethylsilyl (TMS) ethers were dissolved in hexane. GLC-MS analyses were carried out using a Finnigan 1020 instrument housing a fused-silica column (30 × 0.32 mm) coated with a 0.25-μm layer of SE-30, DB-1 (J & W Scientific, Inc., Rancho, Cordova, CA) ending in the ion source. An on-column injection device was used. The oven temperature was about 50°C during the injection and, after 6 min, was rapidly increased to 190°C and was then programmed from 190° to 285°C at a rate of 5°C • min^{-1}.

Retention indices (Kovats) were calculated by comparison of retention times with those of the normal C_{30}-C_{38} hydrocarbons analyzed under the same conditions. The temperature of the ion source was 290°C and the electron energy was 40 eV. Repetitive scanning (20 scans • min^{-1}) over the *m/z* range 50-800 was started after a suitable delay. The instrument was tuned so that intensities of fragment ions with *m/z* values above 200-300 were enhanced relative to those of lighter fragments.

Analyses of cholesterol and 25-hydroxyvitamin D_3. Plasma concentrations of cholesterol were determined by a dry slide technique using a Kodak Ektachem 700 Analyzer (Eastman Kodak Co., Rochester, NY). Since plasma levels of vitamin D_3 are usually very low (6), its major circulating metabolite, 25-hydroxyvitamin D_3, was measured using an HPLC-UV detection method (7).

Statistical analysis

Statistical evaluation of data included calculation of Spearman's rank correlation coefficient and Kolmogorov-Smirnov two-sample test (8). The median and interquartil range were used as measures of central tendency and variation, respectively.

RESULTS AND DISCUSSION

Isolation of 7-dehydrocholesterol from plasma

The occurrence of 7-dehydrocholesterol in plasma was reported many years ago (2, 3). However, later studies failed to confirm these results (4). The discrepancy in results was thought to be due to the nonspecific methods used in the original studies, although degradation of the labile sterol could not be excluded with the latter.

In order to elucidate whether 7-dehydrocholesterol exists in plasma, a mild and sensitive analytical method was developed. Conjugated forms were not studied, since hydrolysis may cause degradation of the steroid. Hydrophobic sterols are mainly transported in blood bound to lipoproteins. All previous extraction procedures have therefore involved precipitation of proteins and extraction with organic solvents (4, 9). Relatively large volumes of organic solvents are often used, but steroids can be trapped by proteins, resulting in incomplete recoveries.

After the observation by Tamazawa et al. (N. Tamazawa, H. Ichimiya, M. Axelson and J. Sjövall, unpublished results) that isopropanol has specific effects on lipoproteins, conditions for solid extraction of plasma sterols were tested. Essentially quantitative sorption ($>90\%$) of hydrophobic sterols (tested with 7-dehydrocholesterol, ^{3}H-labeled cholesterol, and vitamin D_3) on a small column of ODS-bonded silica was obtained when plasma had been diluted with 3.5 volumes of 43% aqueous isopropanol. At lower concentrations of isopropanol, adsorption was not quantitative and at higher concentrations the viscosity of the sample increased and precipitation of proteins was observed. Analogous experiments with methanol or ethanol resulted in precipitation of proteins.

After extraction, hydroxysterols and more polar compounds were washed from the column with water and aqueous methanol. Hydrophobic sterols were then eluted with methanol. Occasionally droplets were formed when the eluate was concentrated, but this was avoided after introducing a reextraction step.

Since the final extract was expected to contain sterols with polarities similar to that of free 7-dehydrocholesterol, HPLC conditions were optimized for analysis of the latter. Thus, UV-detection was carried out at 282 nm (10) and a mobile phase consisting of methanol, ethanol, and water was selected. The retention time of 7-dehydrocholesterol with this system was similar to that obtained with methanol alone, but the presence of water significantly improved the separation of 7-dehydrocholesterol from other UV-absorbing compounds.

Recoveries of 7-dehydrocholesterol (29-1150 ng) added to plasma were essentially quantitative and similar to those of ^{3}H-labeled vitamin D_3 as determined by reversed-phase HPLC and UV-detection. Losses due to degradation of these labile steroids were expected to be low, since the sorbent is highly inert and the temperature and pH were not altered during the extraction. The precision of the method was evaluated by analysis of 7-dehydrocholesterol in nine samples from a plasma pool. The coefficient of variation was about 10% at a concentration of 45 ng/ml plasma. The detection limit was usually about 5 ng/ml.

Identification of dehydrocholesterols in plasma

When plasma was analyzed by this method, a UV-absorbing compound with the same retention time as 7-dehydrocholesterol was observed in the HPLC chromatograms (**Fig. 1**). Based on the mobilities on different HPLC

ASBMB JOURNAL OF LIPID RESEARCH JLR

Downloaded from www.jlr.org by guest, on June 18, 2012

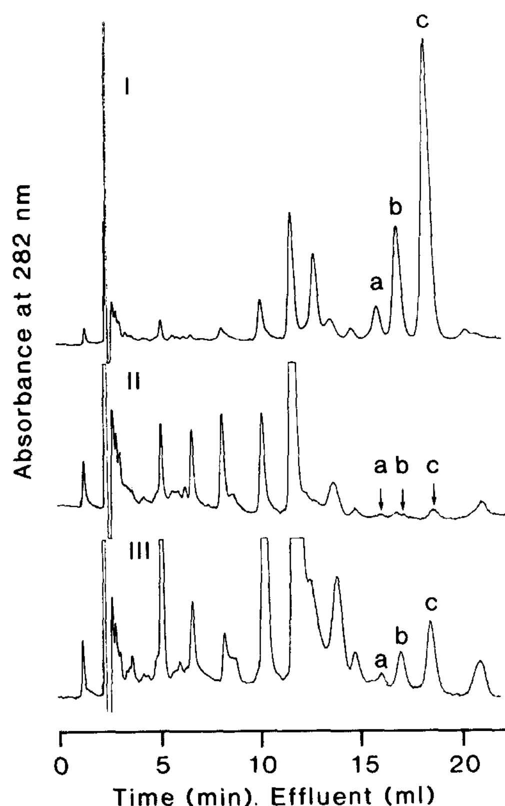


Fig. 1. Reversed-phase HPLC analyses of nonpolar sterols in plasma from (I) a 50-year-old woman with resection of about 2 m terminal ileum and the entire colon; (II) a 60-year-old woman with alcoholic liver cirrhosis; and (III) a 22-year-old healthy man. The arrows indicate the retention times of (a) cholesta-4,6-dien-3-one, (b) isodehydrocholesterol, and (c) 7-dehydrocholesterol. The equivalents of about 25 μl (I), 50 μl (II), and 100 μl (III) of plasma were injected and the concentrations of 7-dehydrocholesterol were 1900 ng/ml (I), 18 ng/ml (II), and 87 ng/ml (III).

columns (**Table 1**), the UV spectrum (**Fig. 2**), the GLC retention time (of the TMS ether derivative), the mass spectrum (**Fig. 3**), and comparisons with the reference steroid, this compound could be definitively identified as 7-dehydrocholesterol.

In addition, the plasma extracts also contained two sterols with similar mass spectra (Fig. 3), but different chromatographic properties, suggesting they were isomers of 7-dehydrocholesterol. One of these was also observed in the HPLC chromatograms (Fig. 1). Its UV spectrum showed a maximum at 274 nm (Fig. 2), indicating a 6,8(9)-diene structure (10). This isomer was definitively identified as 5α-cholesta-6,8(9)-dien-3β-ol (isodehydrocholesterol) by having the same properties as the synthetic compound (Table 1). This may be the first time that isodehydrocholesterol has been found in biological material. It should be noted that it was almost impossible to distinguish between isodehydrocholesterol and 7-dehydrocholesterol by GLC-MS.

The other isomer of 7-dehydrocholesterol was discovered by GLC-MS. The mass spectrum showed a molecular ion at *m/z* 456 and intense fragment ions at *m/z* 351 (M-15-90) and 325 (M-131) characteristic of the TMS ether of a 3-hydroxysterol with two double bonds in the B-ring (Fig. 3). The ion at *m/z* 253 was due to the steroid skeleton with loss of the substituent. This isomer was not absorbing light at 282 nm and the maximum of the UV spectrum was shown to be below 220 nm, indicating that the two double bonds were not conjugated (10). Whereas these results were only compatible with cholesta-5,8(9)-dien-3-ol, migration of double bonds could have occurred during GLC-MS analysis. Assuming that the 3-hydroxy group was in the β-position (for biological reasons) a steroid with a 5,8(9)-dien structure may have isomerized to 5β-cholesta-6,8(9)-

TABLE 1. Chromatographic and spectrometric properties of three dehydrocholesterols found in plasma; properties of vitamin D_3 are shown for comparison

Chromatographic-Spectrometric Properties[a]	Dehydrocholesterols in Plasma			Vitamin D_3
	I[b]	II[c]	III	
HPLC, retention time (min)				
Reversed phase (methanol-ethanol-water)	18.5	17.0	16.4-17.0 (16.7)[d]	10.7
Straight phase (hexane-isopropanol)	15.5	16.3	12.6-16.0 (14.9)[d]	12.9
UV maximum (nm)	282	274	<220	265
GLC-MS (as TMS ethers)				
Retention index (Kovats)	3170	3160	3130	2970/3110[e]
Molecular ion (*m/z*)	456	456	456	456

[a] Abbreviations and experimental conditions, see Materials and Methods.
[b] Properties identical to those of 7-dehydrocholesterol.
[c] Properties identical to those of isodehydrocholesterol.
[d] Detected by GLC-MS in this HPLC fraction (a UV-absorbing peak at 220 nm).
[e] Pyro/isopyro forms (Ref. 11).

Downloaded from www.jlr.org by guest, on June 18, 2012

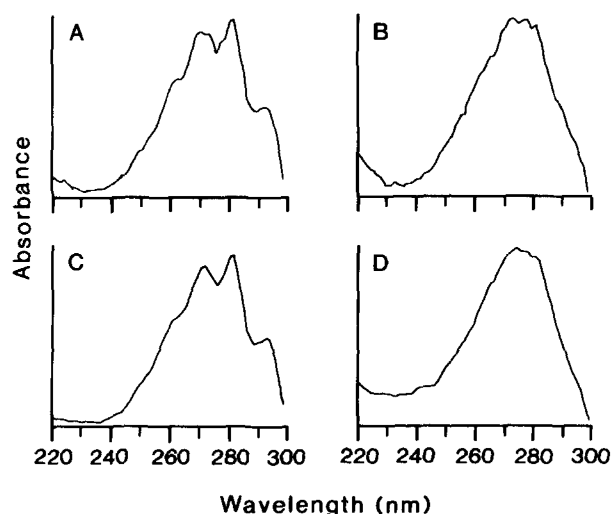


Fig. 2. UV spectra of two sterols in plasma identified as 7-dehydrocholesterol (A) and isodehydrocholesterol (B). UV spectra of the corresponding reference compounds (C and D) are shown for comparison.

dien-3β-ol in the mass spectrometer. The TMS ether of the latter is expected to have a shorter GLC retention time than the corresponding 5α-steroid, isodehydrocholesterol (1) as seen in Table 1. Isomerization to a steroid with a 5,7-diene structure could be excluded on the basis of GLC retention times (11).

Although other types of steroids could be rearranged and get two double bonds in the B-ring when analyzed by GLC-MS, none of them were expected to have the properties of the observed compound or were likely to exist in humans. Furthermore, neither 7-dehydrocholesterol nor isodehydrocholesterol formed this isomer when analyzed by GLC-MS. For these reasons the third isomer was tentatively identified as cholesta-5,8(9)-dien-3β-ol, a steroid that has not previously been described in humans.

Another UV-absorbing steroid was also detected by HPLC analysis (Fig. 1). This compound was identified as cholesta-4,6-dien-3-one, having a UV maximum at 285 nm (10, 12). Its mass spectrum has been described previously (12). The compound can be formed enzymatically

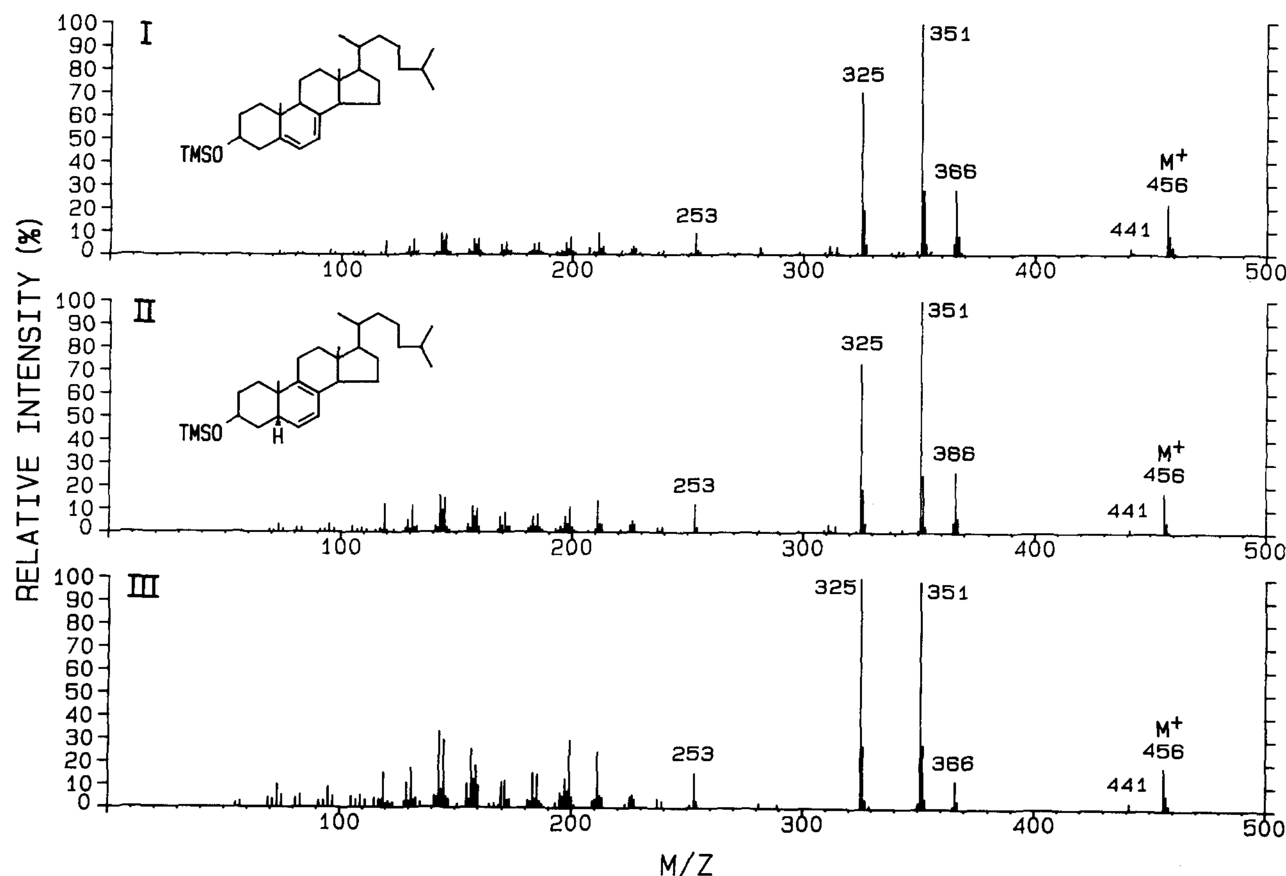


Fig. 3. Mass spectra of trimethylsilyl ethers of three isomeric dehydrocholesterols isolated from plasma. Compounds I and II were identified as 7-dehydrocholesterol and isodehydrocholesterol, respectively. The structure of compound III was not completely elucidated.

Downloaded from www.jlr.org by guest, on June 18, 2012
ASBMB JOURNAL OF LIPID RESEARCH

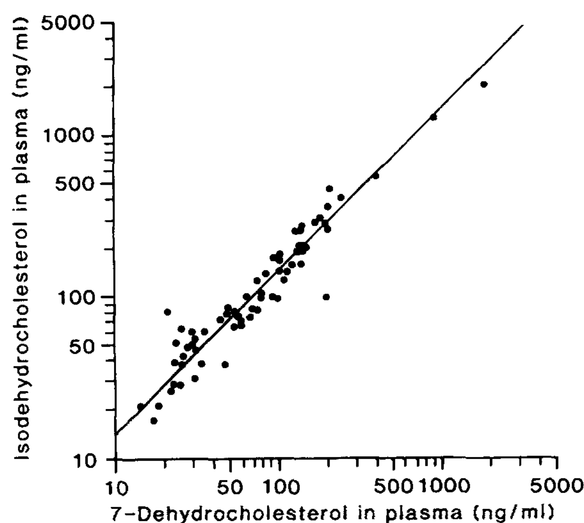


Fig. 4. Correlation between the concentrations of 7-dehydrocholesterol and isodehydrocholesterol in plasma from healthy subjects and patients with an abnormal cholesterol/vitamin D_3 synthesis or metabolism. Correlation coefficient was 0.91 and y = 1.4 x.

in vivo (13) or as an artefact from the bile acid precursor 7α-hydroxy-4-cholesten-3-one during purification or storage of plasma samples (12, 13). High levels of cholesta-4,6-dien-3-one have been reported in plasma from patients with cerebrotendinous xanthomatosis (14). The levels of patients in this study were usually low and only about 10% of those of the corresponding 7α-hydroxylated compound. Details on this compound will be reported elsewhere.

Concentrations of dehydrocholesterols in plasma

7-Dehydrocholesterol is an established precursor of vitamin D_3 and cholesterol in humans (1). Because of this, the concentrations of 7-dehydrocholesterol in plasma from healthy subjects and patients with an abnormal production or metabolism of these sterols were determined. The levels of isodehydrocholesterol were also determined. As shown in **Fig. 4,** there was a strong positive correlation between the levels of this sterol and those of 7-dehydrocholesterol (r = 0.91, $P < 0.0001$). The third sterol, tentatively identified as cholesta-5,8(9)-dien-3β-ol, was not quantified, since it was not detected by conventional HPLC analysis. However, the concentrations of this isomer were similar to those of the other dehydrocholesterols in the samples analyzed by GLC-MS. The results are summarized in **Table 2.**

When the levels of 7-dehydrocholesterol were compared with those of cholesterol or 25-hydroxyvitamin D_3, no relationships could be seen (Table 2). However, the levels of 7-dehydrocholesterol seemed to be determined by its rate of formation, as judged from the known production of cholesterol in these patients. This would then be in agreement with several other cholesterol precursors found in higher concentrations in plasma (9).

Thus, elevated levels were observed in patients with ileal resection or under treatment with cholestyramine. These patients are expected to have an increased rate of cholesterol synthesis (15). Colectomized patients had normal plasma levels consistent with an unaffected cholesterol production. Significantly reduced levels of 7-dehydrocholesterol were seen in primary biliary cirrhosis, but not in alcoholic liver cirrhosis or extrahepatic cholestasis. Low activities of the rate-limiting enzyme in cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase have been reported in cholestasis (16), indicating that factors other than the production rate may affect the plasma levels of 7-dehydrocholesterol. It has been noted that the correlation between plasma levels of cholesterol precursors and hepatic activities of HMG-

TABLE 2. Concentrations of dehydrocholesterols in plasma of healthy subjects and patients with an abnormal production/metabolism of cholesterol and vitamin D_3; the levels of cholesterol and 25-hydroxyvitamin D_3 are shown for comparison

Subjects[a]	Sterol concentration in plasma[b]		Cholesterol	25-Hydroxyvitamin D_3[c]
	7-Dehydrocholesterol	Isodehydrocholesterol		
	ng/ml	*ng/ml*	*mg/ml*	*ng/ml*
Healthy	52:39-75	68:54-86[d]	2.0:1.9-2.3	22:17-27
Colectomized	47:31-123	57:38-160	2.2:2.0-2.2	28:26-36
Ileal resection	142:137-201****[e]	269:199-325***	1.2:1.1-1.5****	11:7-16*[f]
Cholestyramine-treated	160:103-246****	232:142-415**	2.7:2.2-3.1**	24:17-27
Extrahepatic cholestasis	79:30-105	99:38-174	2.2:2.0-2.6	8:6-17*
Primary biliary cirrhosis	26:23-36***	51:38-72	2.4:2.1-2.7*	12:7-17
Alcoholic liver cirrhosis	67:28-74	84:48-100	1.4:1.3-1.7**	4:2-9**[f]

[a]Details on patients are given in Materials and Methods.
[b]Concentration expressed as median: lower quartil-upper quartil.
[c]Vitamin D_3 not determined due to low levels.
[d]Determined only in seven subjects.
[e]Levels significantly different from normal: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.
[f]Some patients treated with vitamin D_3.

Downloaded from www.jlr.org by guest, on June 18, 2012

CoA reductase can be improved by expressing the levels relative to those of cholesterol rather than in absolute concentrations (9). Possibly such a calculation will correct levels for the rate of elimination. In **Fig. 5** individual plasma levels of 7-dehydrocholesterol are expressed in this way. The results suggest that 7-dehydrocholesterol, as some other cholesterol precursors (9), may be used as an indicator of increased cholesterol synthesis. Whether or not the levels also reflect a decreased production of cholesterol remains to be elucidated.

Possible origin of isodehydrocholesterol

The origin of isodehydrocholesterol in plasma is not known. However, the significant correlation between levels of this compound and those of 7-dehydrocholesterol, shown in Fig. 4, indicates a close metabolic relationship. Isodehydrocholesterol is not a known intermediate in cholesterol biosynthesis and 7-dehydrocholesterol is generally considered to be formed from Δ^8-lathosterol via Δ^7-lathosterol (1) as shown in **Fig. 6.** In theory, isodehydrocholesterol could be formed in a number of ways. Whereas isomerization of 7-dehydrocholesterol seems to be a biologically unlikely reaction, Δ^8-lathosterol is a possible precursor of both dehydrocholesterols. Enzymes directly introducing a double bond in the 6(7)-position have not been described, but the introduction of a double bond in the 5(6)-position is an established reaction in cholesterol synthesis (1,17). Δ^7-Lathosterol is the supposed

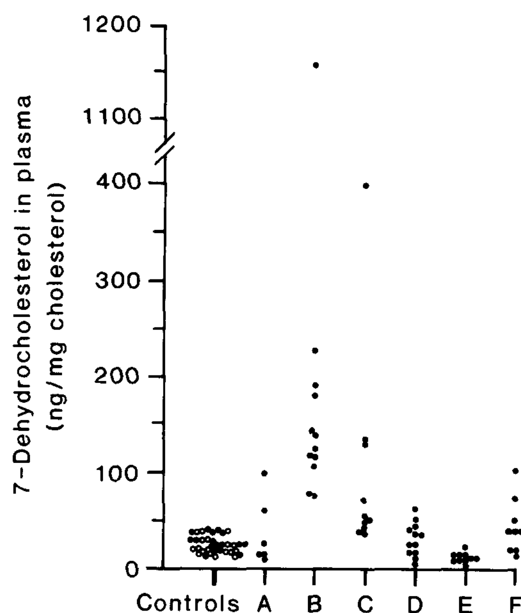


Fig. 5. Concentrations of 7-dehydrocholesterol in plasma from healthy subjects (filled circles, men; open circles, women) and colectomized patients (A), patients with ileal resection (B), under cholestyramine treatment (C), with extrahepatic cholestasis (D), primary biliary cirrhosis (E), or alcoholic liver cirrhosis (F).

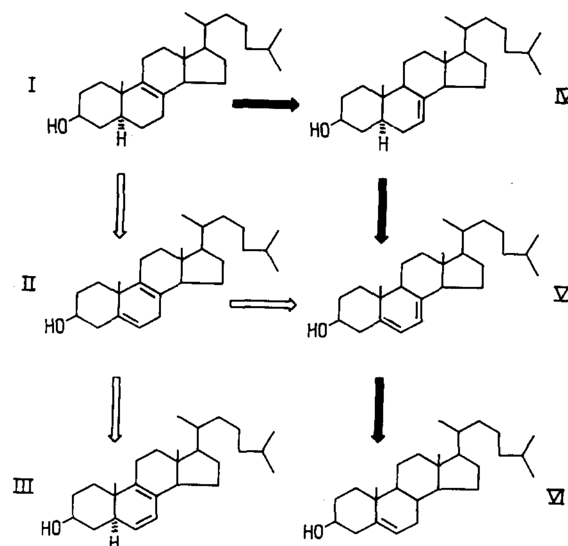


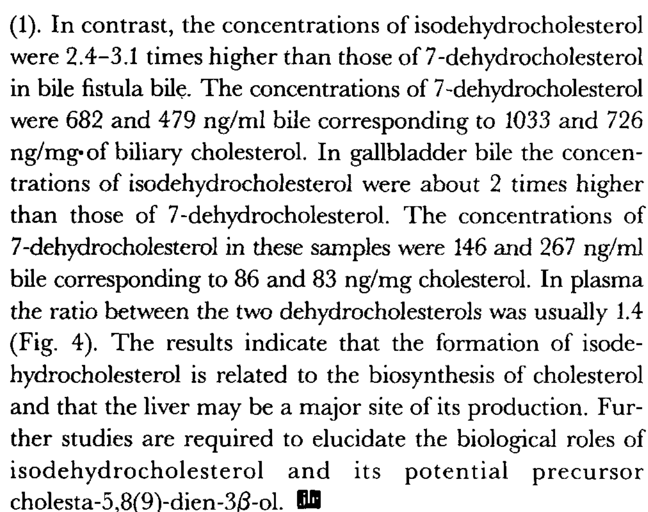
Fig. 6. A simplified scheme for the proposed conversion of Δ^8-lathosterol (I) into isodehydrocholesterol (III) via cholesta-5,8(9)-dien-3β-ol (II). The classical biosynthetic pathway from Δ^8-lathosterol to cholesterol (VI) via Δ^7-lathosterol (IV) and 7-dehydrocholesterol (V) is shown by filled arrows.

substrate of this enzyme, but possibly Δ^8-lathosterol could also serve as such. This reaction would then yield cholesta-5,8(9)-dien-3β-ol, which also seems to be present in plasma. The latter finding indicates that such a reaction occurs in vivo. Isomerization would then yield isodehydrocholesterol, but 7-dehydrocholesterol is also a potential isomerization product. A simplified scheme for these reactions is shown in Fig. 6. Although 6(7)-dehydrogenation has been described for 7α-hydroxylated cholesterol metabolites (13), the finding of normal levels of isodehydrocholesterol in patients with extremely low activities of cholesterol 7α-hydroxylase (16) suggests that a 7-oxygenated sterol is not a precursor of isodehydrocholesterol. Thus, the present data seem to support the conversion of Δ^8-lathosterol to isodehydrocholesterol via cholesta-5,8(9)-dien-3β-ol.

In order to gain more information about the origin of isodehydrocholesterol, the occurrence of this compound in human skin and bile was also investigated. Skin and liver are important organs for cholesterol production (1), and major biosynthetic pathways to cholesterol have mainly been elucidated by studies on skin (1). Furthermore, since isodehydrocholesterol is a potential precursor of vitamin D_3, it was of interest to study the content of the former sterol in skin. However, the concentrations of isodehydrocholesterol in skin were only about 3–6 μg/g tissue (corresponding to about 1.5–3 $\mu g/cm^2$) being much lower (about 2–4%) than those of 7-dehydrocholesterol. This may indicate that the production of isodehydrocholesterol in skin is low, and could explain why the compound has been overlooked in studies on skin sterols

Downloaded from www.jlr.org by guest, on June 18, 2012

ASBMB JOURNAL OF LIPID RESEARCH


(1). In contrast, the concentrations of isodehydrocholesterol were 2.4–3.1 times higher than those of 7-dehydrocholesterol in bile fistula bile. The concentrations of 7-dehydrocholesterol were 682 and 479 ng/ml bile corresponding to 1033 and 726 ng/mg of biliary cholesterol. In gallbladder bile the concentrations of isodehydrocholesterol were about 2 times higher than those of 7-dehydrocholesterol. The concentrations of 7-dehydrocholesterol in these samples were 146 and 267 ng/ml bile corresponding to 86 and 83 ng/mg cholesterol. In plasma the ratio between the two dehydrocholesterols was usually 1.4 (Fig. 4). The results indicate that the formation of isodehydrocholesterol is related to the biosynthesis of cholesterol and that the liver may be a major site of its production. Further studies are required to elucidate the biological roles of isodehydrocholesterol and its potential precursor cholesta-5,8(9)-dien-3β-ol.

The excellent technical assistance of Ms. G. Anderson and Ms. K. Nauclér is gratefully acknowledged. This work was supported by the Swedish Medical Research Council (grant 03x-7890) and Karolinska Institutet.

Manuscript received 31 December 1990 and in revised form 17 April 1991.

REFERENCES

1. Myant, N. B. 1981. The Biology of Cholesterol and Related Steroids. William Heinemann Medical Books Ltd., London.
2. Koehler, E., and E. Hill. 1953. 7-Dehydrocholesterol in human serum. *Fed. Proc.* **12:** 232–233.
3. Henry, R. J. 1964. Lipids. *In* Clinical Chemistry—Principles and Technics. R. J. Henry, editor. Harper & Row, New York. 832–883.
4. Devgun, M. S., and C. R. Paterson. 1986. Measurement of blood levels of 7-dehydrocholesterol in man by high-performance liquid chromatography. *J. Chromatogr.* **353:** 169–173.
5. Axelson, M. 1987. The cholecalciferol sulphate system in mammals. *J. Steroid Biochem.* **26:** 369–373.
6. Seamark, D. A., D. J. H. Trafford, and H. L. J. Makin. 1981. The estimation of vitamin D and its metabolites in human plasma. *J. Steroid Biochem.* **14:** 111–123.
7. Axelson, M. 1985. Liquid–solid extraction of vitamin D_3 metabolites from plasma for analysis by HPLC, GC/MS and protein binding techniques. *Anal. Lett.* **18:** 1607–1622.
8. Siegel, S. 1956. Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill, New York.
9. Björkhem, I., T. Miettinen, E. Reihnér, S. Ewerth, B. Angelin, and K. Einarsson. 1987. Correlation between serum levels of some cholesterol precursors and activity of HMG-CoA reductase in human liver. *J. Lipid Res.* **28:** 1137–1143.
10. Dorfman, L. 1953. Ultraviolet absorption of steroids. *Chem. Rev.* **53:** 47–144.
11. Bell, P. A. 1978. The chemistry of the vitamins D. *In* Vitamin D. D. E. M. Lawson, editor. Academic Press, London. 1–50.
12. Axelson, M., B. Mörk, and J. Sjövall. 1988. Occurrence of 3β-hydroxy-5-cholestenoic acid, 3β,7α-dihydroxy-5-cholestenoic acid, and 7α-hydroxy-3-oxo-4-cholestenoic acid as normal constituents in human blood. *J. Lipid. Res.* **29:** 629–641.
13. Skrede, S., M. S. Buchman, and I. Björkhem. 1988. Hepatic 7α-dehydroxylation of bile acid intermediates, and its significance for the pathogenesis of cerebrotendinous xanthomatosis. *J. Lipid Res.* **29**: 157–164.
14. Björkhem, I., S. Skrede, M. S. Buchmann, C. East, and S. Grundy. 1987. Accumulation of 7α-hydroxy-4-cholesten-3-one and cholesta-4,6-dien-3-one in patients with cerebrotendinous xanthomatosis: effect of treatment with chenodeoxycholic acid. *Hepatology.* **7:** 266–271.
15. Grundy, S. M., E. H. Ahrens, Jr., and G. Salen. 1971. Interruption of the enterohepatic circulation of bile acids in man: comparative effects of cholestyramine and ileal exclusion on cholesterol metabolism. *J. Lab. Clin. Med.* **78:** 94–121.
16. Salen, G., G. Nicolau, S. Shefer, and E. H. Mosbach. 1975. Hepatic cholesterol metabolism in patients with gallstones. *Gastroenterology.* **69:** 676–684.
17. Goad, L. J. 1984. Cholesterol biosynthesis and metabolism. *In* Biochemistry of Steroid Hormones. H. L. J. Makin, editor. Blackwell Scientific Publications, Oxford.

ASBMB
JOURNAL OF LIPID RESEARCH
Downloaded from www.jlr.org by guest, on June 18, 2012