Correlation between serum levels of some cholesterol precursors and activity of HMG-CoA reductase in human liver

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Abstract The possibility that the serum concentrations of various cholesterol precursors may reflect the activity of the hepatic HMG-CoA reductase was investigated in humans under different conditions. The serum levels of squalene, free and esterified lanosterol, (4α, 4β, 14α-trimethyl-5α-cholest-8, 24-dien-3β-ol), two dimethylsterols (4α, 4β-dimethyl-5β-cholest-8-en-3β-ol and 4α, 4β-dimethyl-5α-cholest-8, 24-dien-3β-ol), two methostenols (4α-methyl-5α-cholest-7-en-3β-ol and 4α-methyl-5α-cholest-8-en-3β-ol), two lathosterols (5α-cholest-7-en-3β-ol and 5α-cholest-8-en-3β-ol) and desmosterol (cholest-5, 24-dien-3β-ol) were measured in untreated patients (n = 7) and patients treated with cholestyramine (Questran^R, 8 g twice daily for 2–3 weeks, n = 5) or chenodeoxycholic acid (15 mg/kg body weight daily for 3–4 weeks, n = 8) prior to elective cholecystectomy. The activity of the hepatic microsomal HMG-CoA reductase was measured in liver biopsies taken in connection with the operation. High correlations were found between the relative concentration of the precursor (μg/100 mg of cholesterol) and the HMG-CoA reductase activity (pmol/min per mg of protein) in the case of free lanosterol (r = 0.93), free $\Delta^{8,24}$-dimethylsterol (r = 0.90), free Δ^8-dimethylsterol (r = 0.87), free Δ^8-methostenol (r = 0.86), free Δ^7-methostenol (r = 0.86), free, esterified, and total Δ^8-lathosterol (r = 0.76–0.83) and Δ^7-lathosterol (r = 0.79–0.81). With few exceptions, the correlations were higher for the free than for the esterified sterols. Considerably lower correlations than above were found for the relative concentration of free desmosterol (r = 0.62) and squalene (r = 0.20). It is suggested that the concentration of some of the cholesterol precursors in serum may be utilized for detection of cases with increased biosynthesis of cholesterol. Among the different precursors tested, total lathosterol is particularly attractive to use in view of its easy determination by simple gas–liquid chromatography without time-consuming thin-layer chromatography.—**Björkhem, I., T. Miettinen, E. Reihnér, S. Ewerth, B. Angelin and K. Einarsson.** Correlation between serum levels of some cholesterol precursors and activity of HMG-CoA reductase in human liver. *J. Lipid Res.* 1987. **28:** 1137—1143.

Supplementary key words lanosterol • dimethylsterol • methostenol • lathosterol • desmosterol • squalene • cholestyramine

It is well documented that the serum levels of different precursors to cholesterol are increased under conditions of increased synthesis of cholesterol (1–5). In addition, conditions leading to reduced synthesis of cholesterol seem to be associated with reduced serum levels of some of the precursors (6). Thus, it is possible that the serum levels of some precursors to cholesterol may reflect the activity of the rate-limiting enzyme in cholesterol biosynthesis, HMG-CoA reductase. This possibility was investigated in a more direct way in the present work by measurement of serum levels of squalene, free and esterified lanosterol, (4α, 4β, 14α-trimethyl-5α-cholest-8,24-dien-3β-ol), two dimethylsterols (4α, 4β-dimethyl-5α-cholest-8-en-3-ol and 4α, 4β-dimethyl-5α-cholest-8,24-dien-3-ol), two methostenols (4α methyl-5α-cholest-8-en-3β-ol and 4α-methyl-5α-cholest-7-en-3β-ol), two lathosterols (5α-cholest-7-en-3β-ol and 5α-cholest-8-en-3β-ol), and desmosterol (cholest-5, 24-dien-3β-ol) in untreated gallstone patients and in gallstone patients treated with cholestyramine or chenodeoxycholic acid prior to elective cholecystectomy. The activity of the hepatic HMG-CoA reductase was measured in liver biopsies taken in connection with the operation. A high correlation was obtained between the serum levels of some of the cholesterol precursors and the activity of the hepatic HMG-CoA reductase.

MATERIALS AND METHODS

Subjects

Only patients without diabetes mellitus, hyperlipoproteinemia, or diseases affecting liver, thyroid, and kidney function participated in the study. Informed consent was

Abbreviations: HMG, 3-hydroxy-3-methylglutaryl; DTT, dithiothreitol; GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

obtained from each patient before the operation. The study was approved by the Ethical Committee at Huddinge University Hospital.

The following three groups of patients participated in the study. *1*) Six females and one male with gallstones, ages 33–74 years (mean age 54 years) (untreated group). *2*) Five females with gallstones, ages 41–65 years (mean age 55 years) were treated with cholestyramine (QuestranR, Bristol) in a dose of 8 g twice daily for 2–3 weeks preoperatively (cholestyramine-treated group). *3*) Eight females with gallstones, ages 38–69 years (mean age 59 years) were treated with chenodeoxycholic acid 15 mg/kg body weight per day for 3–4 weeks before surgery (chenodeoxycholic acid-treated group).

Experimental procedure

The patients were hospitalized in the surgical ward 1–2 days before the operation. They were given the regular hospital diet. The blood samples were collected in the morning on the day of operation after a 12-hr fast. To avoid the influence of diurnal variation on cholesterol precursors (4), the blood sampling was performed at the same time in the morning in each study group. The cholecystectomy was performed between 8 and 9 A.M. After opening of the abdomen, a 2- to 3-g liver biopsy was cut out from the left liver lobe. A small specimen was sent for histological examination. The rest of the biopsy was immediately placed in ice-cold homogenizing medium and transported to the laboratory within 10 min.

Preparation of microsomes and assay of HMG-CoA reductase activity

The liver homogenate (10% w/v) was prepared in 50 mM Tris-Cl, pH 7.4, containing 50 mM NaCl, 0.3 M sucrose, 10 mM EDTA, and 10 mM DTT, and the microsomal fraction was isolated as described previously (7). HMG-CoA reductase was assayed essentially as described by Brown, Goldstein, and Dietschy (8) with some modifications (7). Two independent assays were performed on each liver biopsy and the mean was calculated. The standard error was about 8%.

Quantitation of precursor sterols

The precursor sterols were quantitated by gas–liquid chromatography (GLC) as described in detail in previous studies (1, 3, 4). This procedure involves separation of free and esterified sterols and squalene present in a chloroform–methanol extract of serum lipids by preparative thin-layer chromatography (TLC). For isolation of squalene, hexane–toluene 90:10 was used as solvent (1). The isolated squalene was quantitated with GLC (cf. below). The plate was then redeveloped with heptane–ethyl ether 44:55 for isolation of the free cholesterol and the fractions containing free methyl sterols and esterified sterols. After saponification of the ester fraction, the material was chromatographed by TLC for isolation of the esterified cholesterol and methyl sterol fractions. After silylation, the resulting four sterol fractions were quantitated with GLC on a 35-m-long SE-30 capillary column under the following conditions. Starting temperature was 170°C, with a temperature program of +20°C/min up to 265°C. Injector temperature was 300°C and detector temperature was 320°C. The carrier gas (helium) flow was 2.5 ml/min at 14 psi.

Typical gas–liquid chromatographic patterns of the free and esterified methyl sterol fractions and the free cholesterol fraction of human serum are shown in **Fig. 1.** The five major free methyl sterols are: lanosterol, $\Delta^{8,24}$-dimethylsterol, Δ^{8}-dimethylsterol, Δ^{8}-methostenol and Δ^{7}-methostenol. Frequently a small amount of dihydrolanosterol was found in front of the Δ^{7}-methostenol peak. The patterns of the free and esterified methyl sterol fractions were markedly different. Several other minor

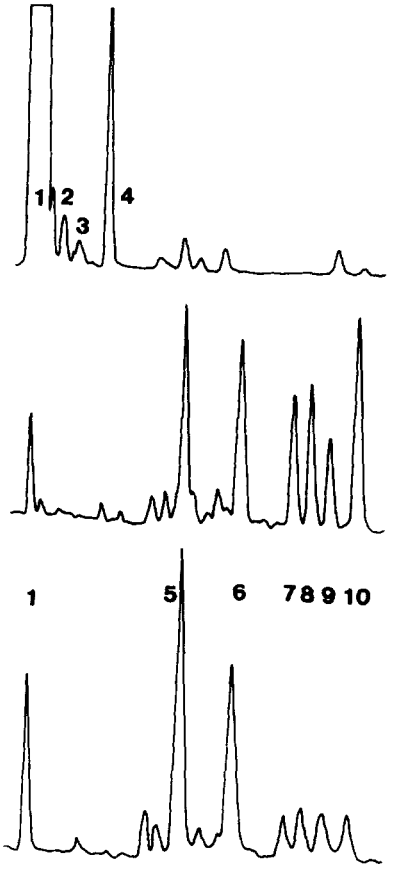

Fig. 1. Gas–liquid chromatographic patterns of free and esterified sterols in human serum. The upper tracing is from the free cholesterol fraction: 1, cholesterol; 2, Δ^{8}-lathosterol; 3, desmosterol; 4, Δ^{7}-lathosterol. The middle tracing is from the free methylsterol fraction: 1, cholesterol; 5, Δ^{8}-methostenol; 6, Δ^{7}-methostenol (including dihydrolanosterol when present); 7, Δ^{8}-dimethylsterol; 8, lanosterol; 9, unknown; 10, $\Delta^{8,24}$-dimethylsterol. The lower tracing is from the esterified methyl sterol fraction showing predominance of methostenols.

unidentified methylsterol peaks occurred in the GLC analyses. Δ^7-Lathosterol, Δ^8-lathosterol, and desmosterol could be identified in the cholesterol fraction. The patterns of the free and esterified cholesterol fractions were quite similar, the esterification percentage of desmosterol being usually higher and that of lathosterol lower than that of cholesterol.

RESULTS

As expected, the patients treated with chenodeoxycholic acid had decreased levels of HMG-CoA reductase as compared to the untreated patients (52 ± 12 vs. 98 ± 15 pmol/min per mg protein, means $\pm$ SEM). This difference was statistically significant ($P < 0.05$, Student's t-test). The patients treated with cholestyramine had markedly increased levels of HMG-CoA reductase as compared to the controls (404 ± 35 pmol/min per mg protein). This difference was highly significant ($P < 0.00005$).

In general, the relative concentrations of the precursors were higher for the cholestyramine-treated patients than for the untreated patients, whereas there was little or no difference between the chenodeoxycholic acid-treated patients and the untreated patients. As an example, the relative concentration of free lanosterol in serum was 53 ± 7 μg/100 mg of cholesterol in the cholestyramine-treated patients and $11 \pm 2\mu$g/100 mg of cholesterol in the untreated patients. This difference was highly significant ($P < 0.0005$). The concentration of free lanosterol in the chenodeoxycholic acid-treated group (16 ± 2 μg/100 mg of cholesterol) was, however, not statistically different from the corresponding concentration in the untreated group ($P > 0.05$).

The above concentrations were expressed relative to cholesterol since these were almost invariably found to correlate somewhat better to the HMG-CoA reductase activity than did the absolute concentrations expressed in μg/100 ml of serum.

The correlations between the relative concentrations of

TABLE 1. Correlations between hepatic HMG-CoA reductase activity and relative concentrations of different precursors to cholesterol in serum (n = 20)

Cholesterol Precursor	Concentration Range	Correlation to HMG-CoA Reductase	Correlation to Total Serum Cholesterol (mg/dl)
	μg/100 mg of cholesterol	*r*	*r*
Δ^7-Lathosterol			
Free	61–1464	0.80	0.19
Esterified	9–384	0.81	0.18
Total	70–1848	0.80	0.19
Δ^8-Lathosterol			
Free	5–131	0.83	0.04
Esterified	0–78	0.76	0.21
Total	5–209	0.81	0.10
Desmosterol			
Free	20–67	0.61	0.28
Esterified	22–75	0.54	0.15
Total	42–139	0.63	0.05
Δ^7-Methostenol			
Free	6–112	0.86	0.06
Esterified	1.3–24	0.69	0.17
Total	7.7–128	0.85	0.02
Δ^8-Methostenol			
Free	11–90	0.85	0.13
Esterified	2.8–17	0.54	0.14
Total	15.1–106	0.83	0.11
Δ^8-Dimethylsterol			
Free	6–70	0.86	0.04
Esterified	0.2–5	0.25	0.11
Total	6.6–72	0.86	0.04
$\Delta^{8,24}$-Dimethylsterol			
Free	7.4–104	0.89	0.10
Esterified	0.8–8	0.13	0.14
Total	8.4–108	0.88	0.11
Lanosterol			
Free	6–79	0.93	0.04
Esterified	0.1–9	0.13	0.12
Total	6.2–83	0.89	0.06
Squalene (μg/dl)	3.9–72	0.20	0.08

the precursors and the HMG-CoA reductase activity are listed in **Table 1.** In general, the correlations were higher for the free than for esterified methyl sterols. The correlations for the total methyl sterols (sum of free and ester) were between those for the free and esterified sterols. Very high correlations were found in the case of free lanosterol ($r = 0.93$), free $\Delta^{8,24}$-dimethylsterol ($r = 0.89$), free Δ^{8}-dimethylsterol ($r = 0.86$), free Δ^{8}-methostenol ($r = 0.85$), and free Δ^{7}-methostenol ($r = 0.86$). Also the correlations for Δ^{7}- and Δ^{8}-lathosterol, free, esterified, and total, were high ($r = 0.76$–0.83). Considerably lower correlations were found for free desmosterol ($r = 0.61$) and squalene ($r = 0.20$). As expected, there was little or no correlation between the levels of the different precursors and the serum concentration of total cholesterol.

Table 2 summarizes the correlations between the absolute concentration of the precursors and the HMG-CoA reductase activity. In general, the correlations were somewhat lower than those obtained with relative concentrations.

The correlation between the activity of the hepatic HMG-CoA reductase and the relative level of serum free lanosterol is shown in **Fig. 2.** It is evident that the high degree of correlation is due mainly to the cholestyramine-treated patients with high activities of HMG-CoA reductase. Thus, there was a high correlation between the two parameters within this group of patients ($r = 0.90$), whereas there was a weaker correlation between the two parameters within the group of chenodeoxycholic acid-treated patients ($r = 0.74$) and the group of untreated patients ($r = 0.27$).

One of the patients in the untreated group was very obese with a relative body weight of 136%, higher than any other patient. Another patient in this group was very lean with a relative body weight of only 66%, lower than that of any other patient. When these two patients were excluded, the correlation between the HMG-CoA reductase and the concentration of free lanosterol increased to $r = 0.57$ in the untreated group and $r = 0.95$ in the treated and untreated groups.

TABLE 2. Correlations between hepatic HMG-CoA reductase activity and absolute concentrations of different precursors to cholesterol in serum (n = 20)

Cholesterol Precursor	Concentration Range	Correlation to HMG-CoA Reductase	Correlation to Total Serum Cholesterol (mg/dl)
	μg/100 ml of serum	*r*	*r*
Δ^{7}-Lathosterol			
Free	15–461	0.80	0.12
Esterified	7.7–345	0.84	0.11
Total	33.7–806	0.82	0.11
Δ^{8}-Lathosterol			
Free	1–62	0.80	0.21
Esterified	0–55	0.81	0.04
Total	2–117	0.82	0.13
Desmosterol			
Free	5.3–36	0.36	0.72
Esterified	15–75	0.44	0.50
Total	20.3–111	0.50	0.61
Δ^{7}-Methostenol			
Free	2–54	0.77	0.33
Esterified	1.2–33	0.60	0.42
Total	4–87	0.74	0.37
Δ^{8}-Methostenol			
Free	2.9–41.3	0.75	0.38
Esterified	2.6–24.1	0.48	0.41
Total	5.8–65.4	0.68	0.43
Δ^{8}-Dimethylsterol			
Free	1.8–36.1	0.75	0.37
Esterified	0.2–3.7	0.36	0.26
Total	2–39.8	0.75	0.38
$\Delta^{8,24}$-Dimethylsterol			
Free	3.2–56	0.79	0.37
Esterified	0.7–6.9	0.16	0.24
Total	4.1–62	0.79	0.37
Lanosterol			
Free	1.6–42	0.77	0.41
Esterified	0.1–9.8	0.14	0.34
Total	1.7–47.2	0.70	0.47
Squalene (μg/dl)	3.9–72	0.20	0.05

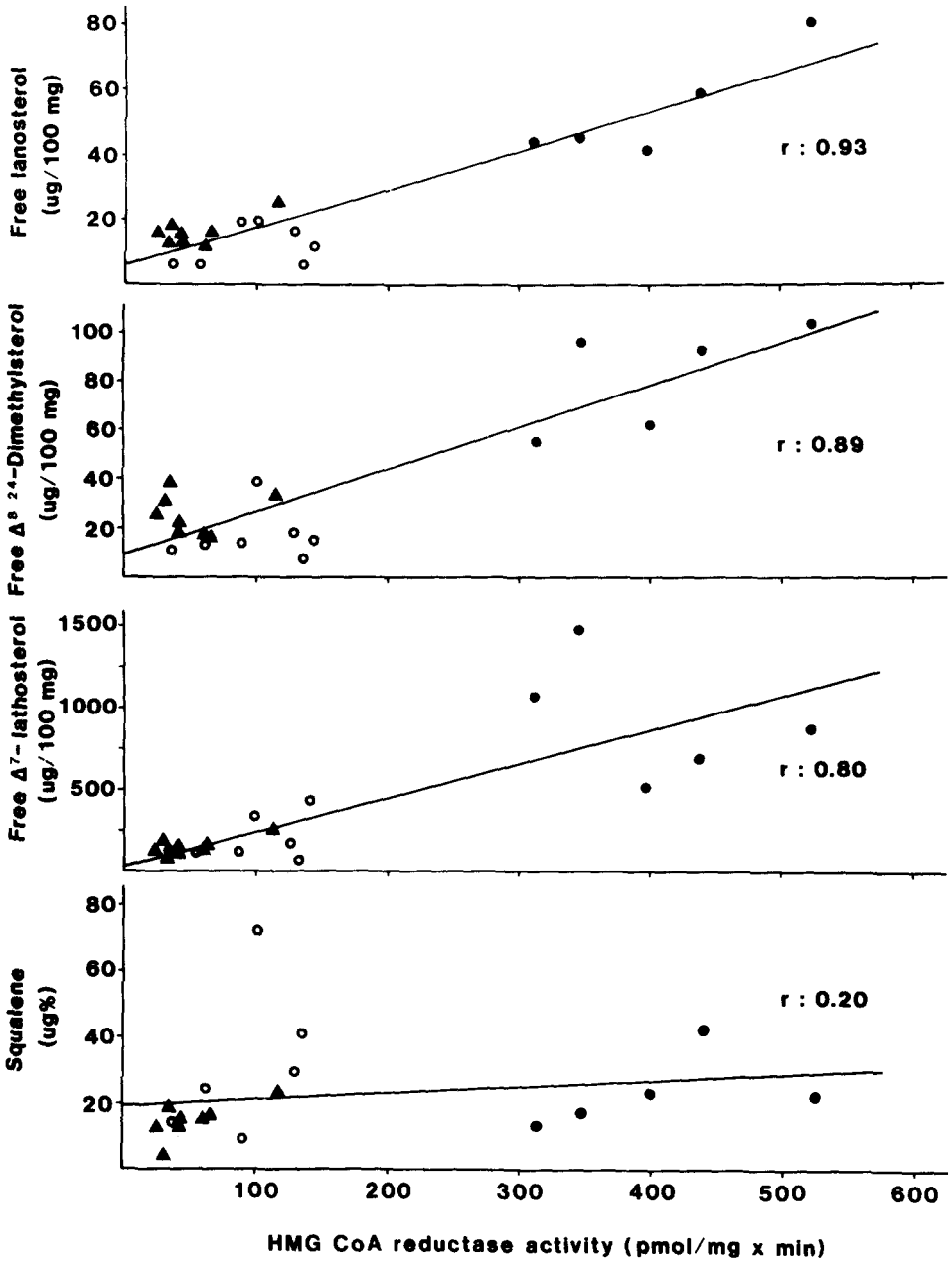

Fig. 2. Correlation between the hepatic HMG-CoA reductase activity and the relative level of serum free lanosterol, free $\Delta^{8,24}$-dimethylsterol, free Δ^{7}-lathosterol, or squalene (μg/mg of cholesterol) in untreated patients (○), chenodeoxycholic acid-treated patients (▲), and cholestyramine-treated patients (●).

In Fig. 2, the correlation between the hepatic HMG-CoA reductase activity and the relative serum levels of free $\Delta^{8,24}$-dimethylsterol and Δ^{7}-lathosterol is also shown. In contrast to the high correlations obtained in these comparisons, there was no correlation between the hepatic HMG-CoA reductase and the serum level of squalene.

DISCUSSION

The general changes in the pattern of cholesterol precursors obtained after treatment with cholestyramine are in good agreement with previous reports (1–3). Thus, the concentrations of the free methyl sterols were increased

markedly with a lesser increase in the esterified sterols. The highest increase was seen in Δ^8-lathosterol (about sixfold), $\Delta^{8,24}$-dimethylsterol (about fivefold), and methostenol (about fivefold). Desmosterol and squalene were affected only to a very small degree.

It has been reported that treatment with chenodeoxycholic acid decreases the concentration of most sterol precursors in serum, in particular that of free lanosterol (6). This could not be confirmed in the present study. On the other hand, the effect on the HMG-CoA reductase by the treatment with chenodeoxycholic acid was relatively small in our study. If our hypothesis is correct that the levels of some cholesterol precursors reflect the activity of the hepatic HMG-CoA reductase, only small effects would thus be expected in the present experiments with chenodeoxycholic acid.

From the results obtained, it is evident that the best correlations between the relative concentrations of the precursors and the activity of the hepatic HMG-CoA reductase were obtained at high activities of the enzyme. It is obvious that the relative concentration in serum of free lanosterol seems to be a most valuable marker for HMG-CoA reductase under conditions of increased enzymatic activity.

A leakage of enzymatically formed precursors to cholesterol into the circulation under conditions of increased biosynthesis of cholesterol would not be expected if the activity of the rate-limiting enzyme is much lower than the activities of all the other enzymes involved in the conversion.

Thus, it seems reasonable to believe that a certain degree of accumulation of the precursors must occur within the cell before leakage into the circulation. Such an accumulation may occur if there is some limitation in the capacity to metabolize the intermediate into cholesterol within the cell. From this point of view it is interesting to note that HMG-CoA reductase does not seem to be the only enzyme in cholesterol biosynthesis subjected to a regulation and it has been reported that activity of squalene oxidocyclase and lanosterol demethylase may be suppressed by low density lipoproteins (for a review, see ref. 9). **Fig. 3** shows the proposed conversion of squalene to cholesterol in humans. The present results are in agreement with the hypothesis that there is a high capacity to convert squalene into lanosterol and desmosterol into cholesterol and a relatively lower capacity for other interconversions. However, the mechanism of transfer of the sterols from the cells may also be of critical importance for the serum levels. If a certain degree of accumulation of the cholesterol precursor within the cell is required before there is a significant leakage into the circulation, this may explain our failure to demonstrate a high correlation between the hepatic HMG-CoA reductase activity and levels of cholesterol precursors in serum at low activities of the HMG-CoA reductase.

A reason for the low correlation of the esterified methyl sterols with the HMG-CoA reductase activity is that these sterols are not esterified in serum (10). Methostenols, major esterified methylsterols in serum, are effectively esterified by acyl CoA:cholesterol acyltransferase in the liver, while dimethylsterols and lanosterol are poor substrates of that enzyme (11). Demethylated sterols, on the other hand, are esterified by lecithin:cholesterol acyltransferase in serum (12), indicating that an increase in the free sterol content should be followed by a proportional change in the esterified sterol level. This is obvious-

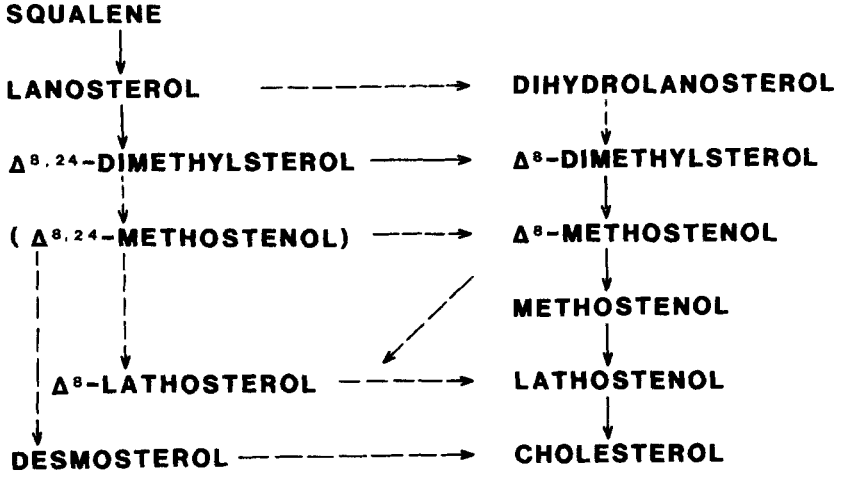

Fig. 3. Proposed conversion of squalene to cholesterol in humans (13). Major pathways are illustrated with continuous arrows; the minor and hypothetical pathways are shown by broken-line arrows.

ly a major reason for the similar correlation of the free, esterified, and total serum lathosterols with the HMG-CoA reductase activity. Since total lathosterol can be easily quantitated by GLC directly after saponification, this steroid fraction is particularly attractive to use as a marker for the hepatic HMG-CoA reductase activity.

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