Diurnal variation of cholesterol precursors squalene and methyl sterols in human plasma lipoproteins[1]

T. A. Miettinen

Second Department of Medicine, University of Helsinki, 00290 Helsinki 29, Finland

Abstract Animal cholesterol synthesis shows a marked diurnal variation, a phenomenon, at the moment, not known to occur in man. Since cholesterol precursors in serum reflect overall cholesterol synthesis in many conditions, a 24-hr profile of squalene and methyl sterols was studied in plasma lipoproteins in order to demonstrate whether these cholesterol precursors could exhibit a diurnal cycling in healthy human subjects. During the 24-hr period, lipoproteins of density < 1.006 g/ml transported 30–50% of the plasma squalene. Free methyl sterols were found mainly in low density lipoproteins (LDL) and esterified methyl sterols in LDL and high density lipoproteins (HDL). Postprandial hyperlipidemia at noon was associated with an inconsistent increase of the squalene and free methyl sterol concentrations in the lipoproteins of density < 1.006 g/ml. In terms of μg per mg of cholesterol, the precursor contents were, however, low in each lipoprotein during the daytime. During the night and early morning, the values were several times higher. Thus the peak plasma squalene and methyl sterol contents occurred at midnight and 4 AM. The highest variation was found for squalene in the density class < 1.006 g/ml and for lanosterol and diunsaturated dimethyl sterol in LDL and HDL. For different methyl sterols, the mean diurnal variation was 3.5- to 6.9-fold in LDL, 2.0- to 4.5-fold in HDL, and 2.6- to 3.6-fold in the density class < 1.006 g/ml. The respective values for squalene were 2.2, 1.4, and 2.9. Esterified methyl sterols varied slightly in the density class < 1.006 g/ml only, and the percentage esterification exhibited a diurnal fluctuation that was the reciprocal of that of free methyl sterol levels. The rapid and marked diurnal fluctuation of squalene and free methyl sterols in plasma lipoproteins suggests that these precursors are metabolized on and off lipoproteins. The variation is most likely caused by changes in cholesterol synthesis, inferring that circadian rhythm also regulates human cholesterol production.—**Miettinen, T. A.** Diurnal variation of cholesterol precursors squalene and methyl sterols in human plasma lipoproteins. *J. Lipid Res.* 1982. **23:** 466–473.

Supplementary key words circadian rhythm • cholesterol synthesis • lanosterol

In vitro and in vivo studies have shown convincingly that cholesterol synthesis exhibits a marked diurnal variation in experimental animals (1–3). This variation occurs in the liver and with a somewhat lesser amplitude in the intestinal mucosa (3, 4). The rate of cholesterol biosynthesis is highest after midnight and lowest during the morning and early afternoon. The rhythm variation is associated with food intake, the peak being reached about 6 hr after the presentation of food (3, 5).

Closer investigation has revealed that the circadian rhythm is caused by diurnal changes in the activity of the cholesterol biosynthesis rate-limiting enzyme hydroxymethylglutaryl coenzyme A reductase (HMG-CoAR) (6, 7). The circadian rhythm of hepatic and intestinal HMG-CoAR seems to persist even during enhanced cholesterol synthesis caused by interrupted enterohepatic circulation of bile acids with cholestyramine in the rat (4, 5).

At the moment it is not known exactly whether cholesterol synthesis exhibits diurnal variation in man. This is mainly due to methodological difficulties in obtaining serial tissue samples for in vitro studies. Assuming a similar rapid variation in the hepatic and intestinal HMG-CoAR activity in man as in experimental animals, the production rate of all cholesterol precursors from mevalonate onwards, including squalene and methyl sterols, should also exhibit a marked diurnal variation. This may result in a cyclic accumulation of the precursors in the hepatic and intestinal epithelial cells, and subsequently in increased release into blood lipoproteins. Measurements of cholesterol precursors in serum would thus offer a practically noninvasive method of studying diurnal changes in human cholesterol synthesis. Earlier studies have indicated that serum methyl sterol levels in fasting subjects correspond to the overall cholesterol synthesis measured with the sterol balance technique under many clinical and experimental conditions (8, 9). In the present study, squalene and methyl sterols in different serum lipoprotein fractions from normal human subjects under normal conditions were quantitated over a 24-hr period. A several-fold diurnal variation was found in the

Abbreviations: HMG-CoAR, hydroxymethylglutaryl coenzyme A reductase; VLDL, very low density lipoprotein (includes chylomicrons whenever present); LDL, low density lipoprotein; HDL, high density lipoprotein; GLC, gas-liquid chromatography.

[1] Some of the results have been presented in the VII International Symposium on Drugs Affecting Lipid Metabolism, Milan, May 28–31, 1980. Abstract Book, p. 10 (Fondazioni Giovanni Lorenzini).

Downloaded from www.jlr.org by guest, on June 19, 2012

precursor levels. The findings suggest that cholesterol synthesis also follows a diurnal rhythm in man.

SUBJECTS AND METHODS

Seven normolipidemic (cholesterol 168 ± 8 mg/dl, triglycerides 61 ± 9 mg/dl), non-obese, healthy subjects aged 16 to 49 years volunteered to give a blood sample every 4 hr starting at 8 AM. The subjects used no drugs. Breakfast was consumed after the 8 AM sample, lunch after the noon sample, dinner after the 4 PM sample, and evening tea and snacks after the 8 PM sample. A food record indicated that the fat intake provided about 35% of calories.

Lipoprotein isolation

Very low density lipoprotein (VLDL, d < 1.006 g/ml) was isolated by ultracentrifugation (10) from 6 ml of plasma. After careful collection of the supernatant, which contained any chylomicrons present, the infranatant was sampled for lipid analysis. In three cases a portion of the infranatant was used for the separation of high density lipoprotein (HDL) with heparin–manganese precipitation (11). Low density lipoprotein (LDL) cholesterol was calculated as the difference between the infranatant and HDL cholesterol.

Lipid analysis

Lipids were extracted with chloroform–methanol from VLDL, infranatant, or HDL after addition of 5α-cholestane and coprostanol as internal standards. An aliquot of the extract was used for the quantitation of triglyceride levels. The remainder of the extract was used for the determination of squalene, free methyl sterols, and cholesterol. This was performed using the thin-layer chromatography–gas–liquid chromatography (GLC) procedure described earlier (9, 12). In short, the extract, to which a small amount of α-tocopherol was added, and a reference mixture (squalene, α-tocopherol, lanosterol, coprostanol, and cholesterol) were applied to a 0.5-mm thick Silica Gel-G plate. The plate was developed for almost its full length in hexane–benzene 90:10 (v/v). The squalene area containing 5α-cholestane was separated and the plate was redeveloped in heptane–diethyl ether 53:46 (v/v). The lipid fractions were localized with Rhodamine under UV light. Free cholesterol and the methyl sterol mixture (including coprostanol) from the area between cholesterol and α-tocopherol (excluded) were eluted for quantitation. To isolate esterified sterols, the ester fraction was eluted and saponified with 2M NaOH in 90% ethanol for 1 hr at 80°C. Nonsaponifiable lipids were rechromatographed on the Silica Gel-G plate in heptane–diethyl ether as described above for the free sterols. The sterol fractions were eluted as in the case of unesterified sterols.

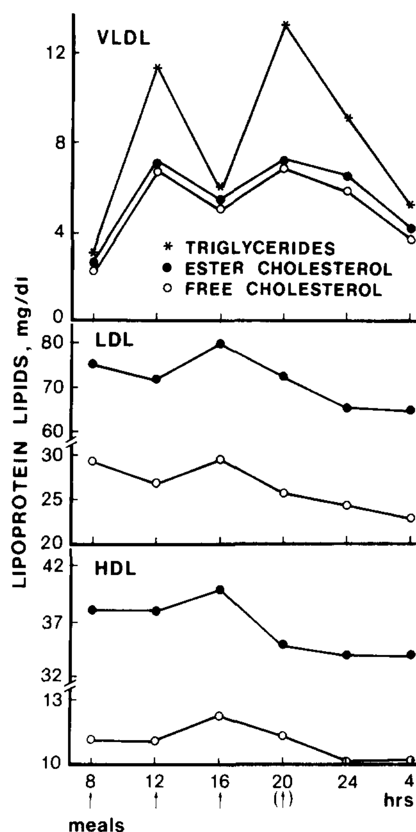

Fig. 1. Diurnal variation of triglycerides and cholesterol in plasma lipoproteins. Means are of the three subjects in whom LDL and HDL were separated. Meal intakes indicated by arrows; snack by an arrow in parentheses. Triglycerides (mg · 10^{-1}/dl) are shown for VLDL (density <1.006 g/ml) only.

Quantitation of squalene and methyl sterols was performed by GLC on a 1% neopentenyl glycolyl succinate column using trimethylsilyl derivatives for sterols and 5α-cholestane as the internal standard. On GLC the methyl sterol mixture gave five different peaks (8) designated as fractions I–V in increasing order of retention. Tentative GLC–mass spectrometric identifications were: I, Δ^8-methostenol and some dihydrolanosterol; II, monounsaturated monomethyl sterol (4,4 dimethyl Δ^8); III, methostenol; IV, lanosterol; V, diunsaturated dimethyl sterol (4,4 dimethyl $\Delta^{7,24}$). Triglycerides were quantitated with an automated method (13) and cholesterol with an enzymatic procedure (14). The analysis of variance was applied for the statistical treatment of the data, using logarithmic transformation when necessary.

RESULTS

VLDL

Triglyceride and cholesterol ***(Fig. 1)***. Postabsorption hyperlipidemia increased VLDL (including any chylo-

JOURNAL OF LIPID RESEARCH
ASBMB
Downloaded from www.jlr.org by guest, on June 19, 2012

microns present) triglycerides about threefold, and free and esterified VLDL-cholesterol twofold. The maximum values were found in the samples taken at 8 PM and the lowest levels at 8 AM. The respective values for total cholesterol in VLDL of the seven subjects were 12.5 ± 1.7 and 5.4 ± 0.8 mg/dl.

Squalene. The mean VLDL squalene concentration (**Table 1, Fig. 2**) was lowest at 8 AM. The concentration curve followed the pattern of major VLDL lipids during the daytime but not during the night. Thus, a low peak was found in many cases at noon, followed by an inconsistent drop, and the maximum value, about 3 times higher than at 8 AM, occurred at midnight. In individual cases the peak value was found between 8 PM and 4 AM. VLDL squalene (in terms of μg/mg of free cholesterol) was lowest during the daytime, indicating that the postprandial VLDL is poor in squalene (**Fig. 3**). This was followed by a continuous increase; VLDL richest in squalene was found at midnight and early in the morning. The peak values were 2.5 times higher than those at noon.

Free methyl sterols (Table 1, Figs. 2 and 3). Total unesterified VLDL methyl sterols exhibited the same pattern as squalene, viz. an inconsistent peak at noon and a consistent peak at 8 PM or at midnight. In fact, the squalene and methyl sterol concentrations correlated highly significantly with each other. The lowest mean value was found at 4 PM and the mean peak level, 2.9 times the lowest, at midnight. Methyl sterols (in terms of μg/mg free cholesterol) demonstrated a single peak at 4 AM. The most consistent changes were seen in fractions IV (lanosterol), V (diunsaturated dimethyl sterol), and III (methostenol). Despite marked changes in the total concentrations, the relative composition of the methyl sterol mixture was fairly constant throughout the 24-hr period.

Esterified methyl sterols (Table 1). The concentration of total esterified methyl sterols showed a relatively weak diurnal variation in VLDL. Methostenol (fraction III) was the major component and it also showed the most consistent diurnal rhythm with a peak at 8 PM (**Fig. 4**). Only traces of esterified fraction V were detectable and lanosterol was esterified only to a small extent.

LDL and HDL

Triglyceride and cholesterol. The triglyceride concentration showed little variation even though the pattern followed that of cholesterol especially in LDL (data not shown). The free and esterified cholesterol levels were 10–20% lower at midnight and early in the morning than at any other time. This was seen in both LDL and HDL (Fig. 1).

Squalene (Table 1, Figs. 2 and 3). The squalene content of the combined LDL and HDL fraction was lowest at noon, and the midnight values were almost twice as high. In LDL the diurnal rhythm of squalene resembled that in VLDL. In HDL the pattern was different; a peak at 4 PM was detectable in all the cases, and a less marked increase occurred during the night.

Free methyl sterols (Table 1, Figs. 2 and 3). The lowest mean methyl sterol values were found from noon to 8 PM in LDL and HDL, and the peaks were at 4 AM. The correlation between the squalene and methyl sterol values was less consistent, especially in HDL, than in VLDL and the methyl sterol peaks occurred later than those of squalene (Fig. 2). Fractions I and II were relatively small in HDL. The relative distribution of HDL methyl sterols resembled more that in VLDL than in LDL. The peaks tended to occur earlier in HDL (clear for methostenol) than in LDL, causing a substantial decrease in LDL methyl sterols between 8 AM and noon. The highest absolute variation was found for fractions IV and V both in HDL and LDL. The mean amplitude of diurnal variation was higher in LDL (3.5 to 6.9-fold for different methyl sterols) than in HDL (2.0 to 4.5-fold) or in VLDL (2.6 to 3.6-fold). Fractions II and III of LDL and fraction I of HDL exhibited the highest mean relative amplitude (6.9-, 6.5-, and 4.5-fold, respectively). The concentration peaks (at 4 AM) of LDL and HDL methyl sterols occurred later than those in VLDL (Fig. 2). However, the curves, in terms of μg/mg free cholesterol, showed maxima at the same time, indicating that all the lipoproteins were richest in methyl sterols at 4 AM (Fig. 3).

Esterified methyl sterols (Table 1, Fig. 4). Total esterified methyl sterols of the combined LDL and HDL fraction showed virtually no diurnal fluctuation. Methostenol tended to increase at 8 PM, mainly in LDL. The esterified methyl sterol pattern of HDL resembled more that of VLDL than of LDL. Thus, at different time points, methostenol comprised 48–55% and fraction II 14–18% of total esterified methyl sterols in HDL. The respective values were 58–64% and 7–14% in VLDL, and 17–34% and 35–45% in LDL.

Transport of precursors in different lipoproteins

During the 24-hr period, 30 to 50% of squalene was transported by VLDL, 35 to 45% by LDL, and 15 to 30% by HDL. This agrees with earlier observations on fasting samples (15, 16). Unesterified methyl sterols were found mainly in LDL, only 5–15% in VLDL, and 20–40% in HDL. LDL contained about 50% of esterified methyl sterols whereas HDL contained 35–45% and VLDL only 5–10%.

The esterification percentage

The percentage of esterified cholesterol varied little throughout the day in each lipoprotein. With the excep-

Downloaded from www.jlr.org by guest, on June 19, 2012

JOURNAL OF LIPID RESEARCH ASBMB

TABLE 1. Diurnal variation of squalene and free and esterified total methyl sterols in plasma lipoproteins of normolipemic human subjects

	Squalene, μg/100 ml						Total Free Methyl Sterols						Esterified Total Methyl Sterols					
Case	8	12	16	20	24	4 (hr)	8	12	16	20	24	4 (hr)	8	12	16	20	24	4 (hr)
Very low density lipoproteins, μg/dl																		
1	4.7	3.6	4.6	14.5	22.4	5.0	2.0	2.5	3.0	5.8	4.5	3.0	0.5	0.8	1.1	1.7	1.6	1.0
2	2.7	2.2	3.5	11.6	3.9	3.1	1.7	1.1	1.6	3.4	2.4	1.8	0.2	0.3	0.7	1.4	0.4	0.2
3	4.7	3.1	6.3	15.8	19.2	16.9	1.1	0.6	1.0	2.7	4.2	2.8	1.3	0.7	1.4	1.4	1.5	1.4
4	3.2	4.0	8.5	7.2	12.2	4.5	0.6	0.8	0.8	0.7	1.0	0.8	0.3	1.0	0.6	0.7	0.7	0.5
5	7.7	5.6	4.8	13.5	12.1	17.8	1.2	2.4	1.4	2.0	3.3	3.6	0.9	1.1	0.8	1.3	1.4	1.1
6	8.5	10.7	8.3	12.3	17.5	14.7	3.0	3.7	2.3	3.4	8.9	5.9	1.7	1.5	1.1	1.5	1.3	2.3
7	4.4	18.7	11.2	26.4	22.7	16.0	2.6	6.5	3.2	5.5	7.7	5.7	1.0	1.4	1.1	1.4	1.2	1.7
Mean	5.1	6.8	6.7	14.5	15.7	11.1	1.7	2.5	1.9	3.4	4.6	3.4	0.8	1.0	1.0	1.4	1.2	1.2
± SE	± 0.8	± 2.2	± 1.0	± 2.2	± 2.6	± 2.5	± 0.3	± 0.8	± 0.4	± 0.7	± 1.1	± 0.7	± 0.2	± 0.2	± 0.1	± 0.1	± 0.2	± 0.3
Low and high density lipoproteins, μg/dl																		
1	1.5	2.8	4.0	2.5	7.0	2.7	45.8	39.7	57.7	44.5	69.2	58.6	27.2	36.6	33.2	29.4	35.6	28.5
2	4.1	4.0	5.2	5.3	5.5	5.5	50.7	64.2	56.6	47.5	43.3	55.7	25.0	21.6	23.3	29.4	23.7	27.3
3	7.4	7.8	8.8	11.5	13.2	17.9	22.7	22.5	19.6	31.0	44.1	48.1	38.2	37.1	30.1	36.9	35.7	33.3
4	3.2	1.0	3.3	5.6	4.6	4.3	24.1	12.4	15.0	17.1	18.1	19.4	16.9	20.9	22.1	18.8	12.8	15.2
5 LDL	18.4	16.9	15.7	17.8	21.7	21.7	22.6	9.5	10.8	11.8	26.2	28.1	9.2	8.1	8.3	8.8	7.0	8.4
HDL	4.8	4.3	6.2	3.9	5.8	5.7	7.4	6.9	5.9	4.7	7.4	9.9	5.8	6.1	6.2	5.6	5.2	4.6
6 LDL	7.9	9.7	7.7	11.7	11.3	9.0	58.4	18.6	12.4	16.7	44.1	66.0	11.6	7.0	9.3	10.7	9.5	9.0
HDL	6.1	7.0	8.2	6.6	7.8	7.2	13.1	9.0	10.0	7.4	18.4	25.6	10.6	7.3	10.6	8.5	8.6	9.2
7 LDL	3.9	4.0	0.2	10.3	12.9	15.1	22.8	9.8	8.6	9.5	14.4	36.6	2.7	6.6	6.0	4.5	5.1	6.4
HDL	6.0	2.7	9.7	6.2	5.7	5.6	11.2	6.6	7.2	7.5	10.7	17.8	5.5	4.2	5.2	4.4	4.1	4.1
Mean	9	9	10	12	14	14	40	28	29	28	42	52	22	22	22	22	21	21
± SE	± 3	± 3	± 3	± 3	± 4	± 4	± 7	± 7	± 7	± 5	± 7	± 8	± 4	± 4	± 3	± 4	± 4	± 3

LDL and HDl were quantitated separately in cases 5–7 only; the combined values were used for the calculations. Analysis of variance showed a significant ($P < 0.05$ or less) diurnal rhythm for squalene and free methyl sterols in VLDL (density <1.006 g/ml) and LDL + HDL. Logarithmic transformation used when necessary.

Downloaded from www.jlr.org by guest, on June 19, 2012

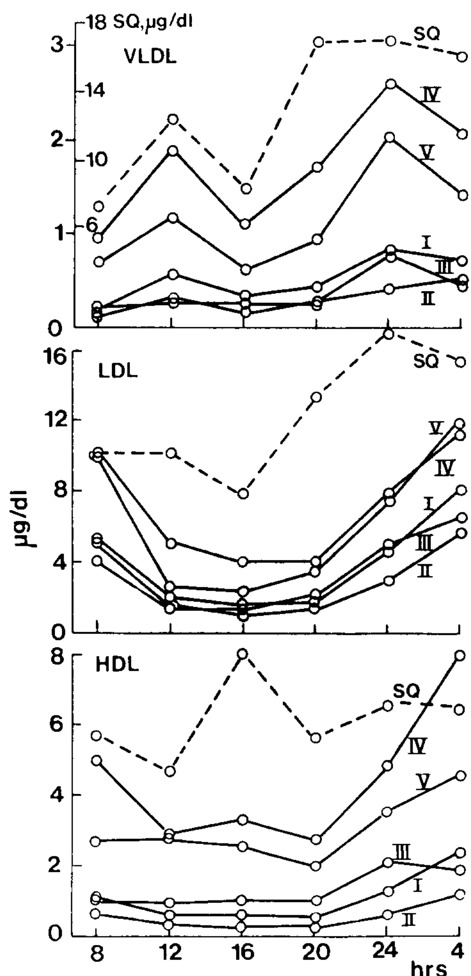

Fig. 2. Diurnal variation of free methyl sterol (I–V) and squalene (SQ) concentrations in plasma lipoproteins. Means are of the three subjects in whom LDL and HDL were analyzed separately. Tentative identification of each GLC fraction in increasing order of retention times: I, Δ^8-methostenol (some dihydrolanosterol); II, dimethyl sterol (4,4 dimethyl Δ^8); III, methostenol; IV, lanosterol; and V, dimethyl sterol (4,4 dimethyl $\Delta^{8,24}$).

tion of VLDL methostenol, the percentage of esterified methyl sterols was lower than that of cholesterol and it was highest in HDL and lowest in VLDL. Even within the lipoprotein, the esterification percentage varied markedly from one methyl sterol to another. Fraction V was practically unesterified in all lipoprotein fractions, only 10–30% of lanosterol being esterified. Methostenol exhibited the highest esterification percentage in VLDL (up to 70%) and HDL (up to 80%), whereas in LDL the percentage was highest (75%) for fraction II.

Since esterified methyl sterols exhibited a relatively low amplitude of diurnal variation, especially in LDL and HDL, the esterification percentage showed a variation opposite to that of the unesterified methyl sterol concentration. Thus, the highest percentage occurred at 4 PM and the lowest 12 hr later at 4 AM. In VLDL this pattern was less consistent.

DISCUSSION

The 24-hr profile of the lipoprotein lipids has been studied very little, especially for normal dietary habits. With the exception of chylomicrons and VLDL, the lipids of other lipoproteins show quite small fluctuations after food intake unless a large fatty meal is consumed (17–21). The present results confirm the existence of postprandial hyperlipidemia and show that, after normal daily consumption of mixed food meals, VLDL triglycerides (including chylomicrons) and free and esterified cholesterol reach a peak late in the evening. In addition, in agreement with an earlier article (22), nocturnal free and esterified cholesterol of LDL and HDL were 10–20% lower than at noon or 4 PM. The reason for the significant increase in the free and esterified cholesterol in LDL and HDL before breakfast is not known. The squalene and methylene sterol contents in LDL and HDL exhibited, however, a reciprocal pattern to those of free and esterified cholesterol.

The present study shows for the first time that the

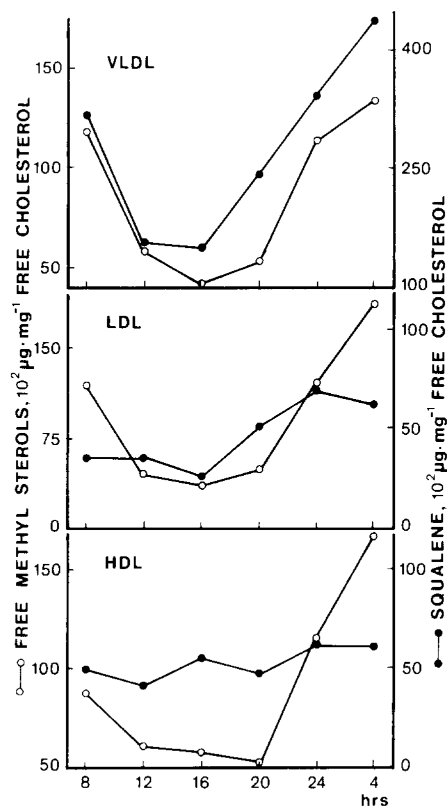

Fig. 3. Diurnal variations of squalene and free methyl sterols (sum of fractions I–V) contents in different plasma lipoproteins. Means are in terms of μg/mg of free cholesterol of the three subjects in whom LDL and HDL were analyzed separately. Meals as indicated in Fig. 1. Analysis of variance (calculated for VLDL and LDL + HDL of the seven subjects) revealed a significant ($P < 0.05$ or less) diurnal variation for squalene and free methyl sterols in VLDL and free methyl sterols in LDL + HDL.

Downloaded from www.jlr.org by guest, on June 19, 2012

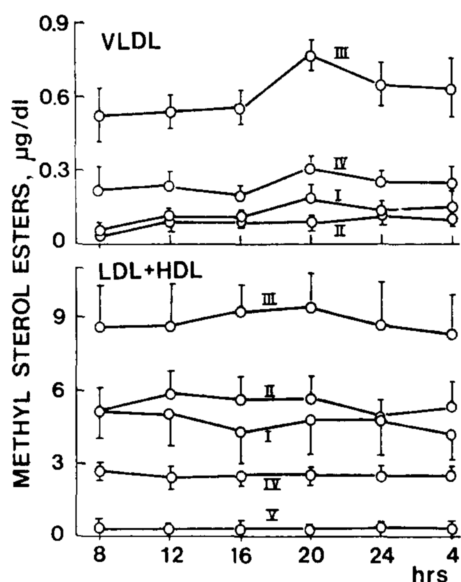

Fig. 4. Diurnal variation of esterified methyl sterol concentrations in different lipoproteins. Mean ± SE (vertical lines) of seven subjects. Fraction V was not measurable in VLDL. Analysis of variance showed a significant ($P < 0.05$) diurnal rhythm for VLDL III.

cholesterol precursor levels exhibit a marked diurnal variation in human plasma lipoproteins, the nocturnal values being several times higher than the daytime levels. Saudek, Frier, and Liu (16) followed the plasma squalene level after two 500-calorie meals for 7 hr. Triglycerides increased significantly at 2 hr, and squalene first at 6 hr. The night levels were not measured. Plasma squalene (15, 16, 23, 24) and methyl sterols (8, 15) are rapidly labeled after an intravenous administration of labeled mevalonate, suggesting that they undergo a rapid equilibrium with the sterols at their site of synthesis. This site has been suggested to be the liver (15), where in fact an increase in cholesterol synthesis caused by cholestyramine treatment increases both squalene and methyl sterol contents to about the same extent as in the plasma of the rat (9). In man, a stimulation of cholesterol synthesis by cholestyramine or ileal bypass operation increases serum methyl sterols, and, less consistently, squalene (8, 25, 26), usually in proportion to increased cholesterol elimination (8, 9, 12). On the other hand, reduced cholesterol synthesis by fasting lowers methyl sterols (8, 12). Though no direct evidence is available, these observations suggest that changes in cholesterol synthesis are associated with corresponding changes in the precursor production and in their subsequent release into the blood stream. Thus, the diurnal variation in the lipoprotein precursor levels might ultimately be a sign of diurnal rhythm in human cholesterol synthesis. The exact site of origin and the fate of the precursors in different lipoproteins can only be hypothesized at the moment.

The postprandial increase in VLDL seen in the present study (Fig. 1, VLDL includes chylomicrons) originates mainly from the intestine transporting absorbed dietary lipids. The inconsistent increase in the VLDL squalene and methyl sterols at noon (after breakfast) indicates that the intestinal chylomicron and VLDL production and the intestinal mucosa as a whole might not be important sources of the cholesterol precursors in serum. Increased intestinal mucosal synthesis and absorption of dietary and biliary precursors could enhance precursor production, but the quantitative role of the two sources is unknown. Human bile contains these precursors (26, 27), and large amounts of dietary squalene are known to increase plasma squalene (26).

VLDL (and chylomicron) particles of intestinal origin were actually relatively low in the cholesterol precursors because, in terms of µg/mg of cholesterol (or triglycerides), the contents were lowest during the afternoon and highest during the night (Fig. 3). The nocturnal VLDL, which was rich in precursors, may originate mainly from the liver and to a lesser extent from the intestine. Since newly synthesized hepatic lipids are released in VLDL into the circulation, the nocturnal VLDL peak of the precursors might originate mainly from the liver, reflecting a high rate of nocturnal hepatic cholesterol synthesis. The composition of the free methyl sterol mixture was fairly constant in VLDL throughout the 24-hr period, suggesting that the site(s) of origin remained unchanged. The mucosal and hepatic methyl sterol patterns appear to differ from each other in postmortem human specimens (28), but they are both different from that in VLDL. Methyl sterol patterns in intestinal mucosal biopsies[2] and biliary samples (27) were also different from that in VLDL. Thus, the mucosal fraction I and lanosterol each included about 40% of the methyl sterols, whereas in the bile almost 60% were lanosterol.

Alternatively, the precursors might have been transferred from the cells in the circulating VLDL or other lipoproteins. Removal of the precursors from the VLDL may take place by their transfer to LDL and HDL as VLDL is catabolized, or by hepatic uptake from VLDL or its remnants. In fact, apoproteins and some lipids of VLDL and/or chylomicrons can be transferred to both LDL and HDL as VLDL is catabolized (29, 30). Thus, precursors may also have been transferred. The rate of diurnal change in the cholesterol precursor concentrations of VLDL agrees with the established half-life of VLDL triglycerides (31), but the rate of change in the LDL and HDL precursor levels is clearly faster than the catabolism of these lipoproteins. The findings do not exclude the possibility that the cholesterol precursors in VLDL (and chylomicron) are metabolized with the li-

[2] Miettinen, T. A. Unpublished observations.

Downloaded from www.jlr.org by guest, on June 19, 2012
ASBMB JOURNAL OF LIPID RESEARCH

poprotein itself. However, the precursors appear to equilibrate on and off LDL and HDL.

The intravenous administration of labeled mevalonate by Saudek et al. (16) was followed by the appearance of labeled squalene in all lipoprotein fractions without complete equilibration. The subsequent turnover rates of VLDL and LDL + HDL squalene suggested that newly synthesized squalene was equilibrated on and off all lipoproteins. The half-life of plasma total squalene is about 2 hr (23, 24) and that of the free methyl sterol fraction is about 5 hr (8).

Esterified methyl sterols exhibited a markedly different pattern from that of free sterols and showed a quite negligible circadian rhythm seen in VLDL methostenol only. Furthermore, despite marked changes in the levels of free lanosterol and diunsaturated dimethyl sterol (fraction V), their ester concentrations remained low throughout the day. These observations are explainable by a finding that methyl sterols are not esterified in serum by lecithin:cholesterol acyltransferase (32). Thus, the serum methyl sterol esters apparently originate from tissue(s) where sterol esters are known to be formed by acyl coenzyme A cholesterol-acyltransferase from newly synthesized methyl sterols (33). Esterification of methostenol predominates, but the reason for the low ester content of lanosterol and dimethyl sterol (fraction V) is not known. The esterified methyl sterol pattern of LDL resembles those found in postmortem specimens of human liver and intestine (28) in that fraction II is predominant. After an intravenous injection of labeled mevalonate, a small amount of label appears in the esterified methyl sterol fraction in serum (8). However, acute diurnal changes in free methyl sterol production have little effect on the level of esterified methyl sterols (Fig. 4), whereas a cholestyramine-stimulated increase in cholesterol synthesis also augments esterified serum methyl sterols significantly (8). It can be inferred that methyl sterol esters, being comparatively nonpolar lipids, enter the circulation with newly formed VLDL and possibly also with chylomicrons. As in the case of free methyl sterols, the methyl sterol pattern of HDL resembles that of VLDL, and to a lesser extent that of LDL. It can be hypothesized that methyl sterol esters are transferred to HDL as VLDL is catabolized.

Supported by the National Council for Medical Research, Finland. Ritva Nissilä, Eeva Gustafsson, and Marja Aarnio provided technical and secretarial assistance.

Manuscript received 26 February 1981, in revised form 10 August 1981, and in re-revised form 17 November 1981.

REFERENCES

1. Kandutsch, A. A., and S. E. Saucier. 1969. Prevention of cyclic and Triton-induced increases in hydroxymethylglutaryl coenzyme A reductase and sterol synthesis by puromycin. *J. Biol. Chem.* **244:** 2299–2305.
2. Back, P., B. Hamprecht, and F. Lynen. 1969. Regulation of cholesterol biosynthesis in rat liver: diurnal changes in activity and influence of bile acids. *Arch. Biochem. Biophys.* **133:** 11–21.
3. Edwards, P. A., H. Muroya, and R. G. Gould. 1972. In vivo demonstration of the circadian rhythm of cholesterol biosynthesis in the liver and intestine of the rat. *J. Lipid Res.* **13:** 396–401.
4. Shefer, S., S. Hauser, V. Lapar, and E. H. Mosbach. 1972. Diurnal variation of HMG CoA reductase activity in rat intestine. *J. Lipid Res.* **13:** 571–573.
5. Goldfarb, S., and H. C. Pitot. 1972. Stimulatory effect of dietary lipid and cholestyramine on hepatic HMG CoA reductase. *J. Lipid Res.* **13:** 797–801.
6. Hamprecht, B. C., C. Nüssler, and F. Lynen. 1969. Rhythmic changes of hydroxymethylglutaryl coenzyme A reductase activity in livers of fed and fasted rats. *FEBS Lett.* **4:** 117–121.
7. Shapiro, D. J., and V. W. Rodwell. 1969. Diurnal variation and cholesterol regulation of hepatic HMG-CoA reductase activity. *Biochem. Biophys. Res. Commun.* **37:** 867–872.
8. Miettinen, T. A. 1970. Detection of changes in human cholesterol metabolism. *Ann. Clin. Res.* **2:** 300–320.
9. Miettinen, T. A. 1969. Serum squalene and methyl sterols as indicators of cholesterol synthesis in vivo. *Life Sci.* **8:** 713–721.
10. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
11. Burstein, M., and J. Samaille. 1959. Nouvelle methode de separation et de dosage lipoprotein de faible densité. *Ann. Biol. Clin.* **17:** 23–24.
12. Miettinen, T. A. 1971. Serum methyl sterols and their distribution between major lipoprotein fractions in different clinical conditions. *Ann. Clin. Res.* **3:** 264–271.
13. Kessler, G., and H. Lederer. 1966. Fluorometric measurement of triglycerides. *In* Automation in Analytical Chemistry. L. T. Skeggs, editor. Mediad, New York. 341.
14. Röschlau, P., E. Bernt, and W. Gruber. 1974. Enzymatische Bestimmung des Gesamt-Cholesterins im Serum. *Z. Klin. Chem. Klin. Biochem.* **12:** 403–407.
15. Goodman, D. S. 1964. Squalene in human and rat blood plasma. *J. Clin. Invest.* **43:** 1480–1485.
16. Saudek, C. D., B. M. Frier, and G. C. K. Liu. 1978. Plasma squalene: lipoprotein distribution and kinetic analysis. *J. Lipid Res.* **19:** 827–835.
17. Havel, R. J. 1957. Early effects of fat ingestion on lipids and lipoproteins of serum in man. *J. Clin. Invest.* **36:** 848–854.
18. Schlierf, G., S. Jessel, J. Ohm, C. C. Heuck, G. Klose, P. Oster, B. Schellenberg, and A. Weizel. 1979. Acute dietary effects on plasma lipids, lipoproteins and lipolytic enzymes in healthy normal males. *Eur. J. Clin. Invest.* **9:** 319–325.
19. Havel, R. J., J. P. Kane, and M. L. Kashyap. 1973. Interchange of apolipoproteins between chylomicrons and high density lipoproteins during alimentary lipemia in man. *J. Clin. Invest.* **52:** 32–38.
20. Terpstra, J., L. W. Hessel, J. Seepers, and C. M. van Gent. 1978. The influence of meal frequency on diurnal lipid, glucose and cortisol levels in normal subjects. *Eur. J. Clin. Invest.* **8:** 61–66.

ASBMB
JOURNAL OF LIPID RESEARCH
Downloaded from www.jlr.org by guest, on June 19, 2012

21. van Gent, C. M., C. Pagano Mirani-Oostdijk, P. H. van Reine, M. Fröhlich, L. W. Hessel, and J. Terpstra. 1979. Influence of meal frequency on diurnal lipid, glucose and insulin levels in normal subjects on a high fat diet; comparison with data obtained on a high carbohydrate diet. *Eur. J. Clin. Invest.* **9:** 443–446.
22. Miettinen, T. A. 1980. Diurnal variation in LDL and HDL cholesterol. *Ann. Clin. Res.* **12:** 295–298.
23. Liu, G. C. K., E. H. Ahrens Jr., P. H. Schreibman, P. Samuel, D. J. McNamara, and J. R. Crouse. 1975. Measurement of cholesterol synthesis in man by isotope kinetics of squalene. *Proc. Natl. Acad. Sci. USA.* **72:** 4612–4616.
24. Frier, B. M., and C. D. Saudek. 1979. Cholesterol metabolism in diabetes: the effect of insulin on the kinetics of plasma squalene. *J. Clin. Endocrinol. Metab.* **49:** 824–829.
25. Nestel, P. J., and B. Kudchodkar. 1975. Plasma squalene as an index of cholesterol synthesis. *Clin. Sci. Mol. Med.* **49:** 621–624.
26. Liu, G. C. K., E. H. Ahrens Jr., P. H. Schreibman, and J. R. Crouse. 1976. Measurement of squalene in human tissue and plasma: validation and application. *J. Lipid Res.* **17:** 38–45.
27. Miettinen, T. A. 1981. Effects of bile acid feeding and depletion on plasma and biliary squalene, methyl sterols and lathosterol. *In* Bile Acids and Lipids. G. Paumgartner, A. Stiehl, and W. Gerok, editors. MTP Press Limited, Lancaster. 255–262.
28. Tilvis, R., and T. A. Miettinen. 1980. Squalene, methyl sterol and cholesterol levels in human organs. *Arch. Pathol. Lab. Med.* **104:** 35–40.
29. Tall, A. R., and D. M. Small. 1980. Body cholesterol removal: role of plasma high-density lipoproteins. *Adv. Lipid Res.* **17:** 1–51.
30. Nicoll, A., N. E. Miller, and B. Lewis. 1980. High-density lipoprotein metabolism. *Adv. Lipid Res.* **17:** 53–106.
31. Farquhar, J. W., R. C. Gross, R. M. Wagner, and G. M. Reaven. 1965. Validation of an incompletely coupled two-compartment nonrecycling catenary model for turnover of liver and plasma triglyceride in man. *J. Lipid Res.* **6:** 119–134.
32. Tilvis, R., and T. A. Miettinen. 1980. A lack of esterification of lanosterol and other methyl sterols in human serum in vitro. *Scand. J. Clin. Lab. Invest.* **40:** 671–674.
33. Brady, D. R., and J. L. Gaylor. 1971. Enzymic formation of esters of methyl sterol precursors of cholesterol. *J. Lipid Res.* **12:** 270–276.

Downloaded from www.jlr.org by guest, on June 19, 2012