Evidence for diurnal periodicity in human cholesterol synthesis

Peter J. H. Jones[1] **and Dale A. Schoeller**

Division of Human Nutrition, University of British Columbia, Vancouver, B. C., Canada, and Clinical Nutrition Research Unit, University of Chicago, Chicago, IL

Abstract Diurnal variation in human cholesterol synthesis in the rapidly exchangeable pool was studied in six healthy normolipidemic individuals by measurement of deuterium incorporation from body water into plasma cholesterol. After oral administration of D_2O, free and de-esterified plasma cholesterol and plasma water were sampled over 48 h, converted to hydrogen, and deuterium enrichment was determined by isotope ratio mass spectrometry. Deuterium enrichment changes over 4-h intervals were used to calculate fractional synthetic rate (FSR). No significant time effects were observed on plasma total cholesterol levels over the 48-h study. The highest rate of deuterium incorporation into free cholesterol occurred during early morning, whereas during afternoon and early evening incorporation rates were lower. Free cholesterol FSR values were lowest at 14:00 to 18:00 h and peaked at 06:00 h. The periodicity across timepoints was not significantly different from that of a derived sine function equation. For cholesteryl ester, FSR data showed less distinct variation over time, peaking during early morning, indicative of maximal efflux of free cholesterol to the ester pool during this period. These findings offer direct evidence for diurnal patterns in human cholesterol synthesis. —**Jones, P. J. H., and D. A. Schoeller.** Evidence for diurnal periodicity in human cholesterol synthesis. *J. Lipid Res.* 1990. **31:** 667–673.

Supplementary key words diurnal rhythm • free cholesterol • cholesteryl ester

The circadian nature of cholesterol synthesis has been well established in animals. In nocturnal species such as the rat, hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate limiting enzyme in the cholesterol synthesis pathway, peaks in activity at midnight and reaches a nadir at noon (1–3). In guinea pigs, which are diurnal, maximal enzyme activity occurs between 12:00 and 15:00 h (4). It has been suggested that these diurnal variations occur due to periodicity in synthesis or degradation of this regulatory enzyme (5).

In contrast to animals, direct study of such circadian rhythm in humans has been difficult to carry out largely due to lack of sensitivity of current methods for determining short term cholesterol synthesis. Miettinen (6) observed in healthy humans diurnal periodicity in plasma levels of cholesterol precursors squalene and lanosterol, with peak levels occurring at night while plasma LDL and HDL cholesterol values remained relatively constant over 24 h. Similarly, Parker et al. (7) showed circadian rhythmicity in plasma mevalonic acid levels which was abolished by fasting and suppressed by cholesterol feeding. As levels of cholesterol precursors in plasma have been demonstrated to correlate well with hepatic activity of HMG-CoA reductase (8) and synthesis, as determined by cholesterol balance studies (7), such periodicity suggests the possibility of a corresponding rhythm in human whole body sterol production.

Fluctuations in other pathways of cholesterol metabolism further suggest that cholesterol production may not be constant through the day. Diurnal variation in bile acid production has been observed in humans, with peak activity occurring in the early morning (9).

In this report we confirm the circadian periodicity in cholesterol synthesis previously reported (6, 7) using a nonhazardous direct method for determining the synthetic rate of cholesterol within the rapidly exchangeable central pool. This methodology, using the incorporation into newly synthesized cholesterol of deuterium from body water (10), was used to document short-term fluctuations in cholesterol synthesis and esterification.

METHODS

Subjects and screening procedures

Six male volunteers reporting no family history of lipid disorders or present use of medications were recruited for study. Initially, subjects provided a blood sample for

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HDL, high density lipoproteins; FSR, fractional synthetic rate; LDL, low density lipoproteins.

[1]To whom reprint requests should be addressed at: Division of Human Nutrition, 2205 East Mall, University of British Columbia, Vancouver, B. C., Canada V6T 1W5.

measurement of plasma cholesterol and triglyceride levels. Lipid levels were within normal ranges (total cholesterol, 2.6–5.4 mmol/l; total triglyceride, 0.11–2.1 mmol/l) in all individuals studied. Informed consent was obtained from each volunteer prior to investigation. The study protocol was approved by the Ethical Review Committee at the University of British Columbia.

Protocol

The study was conducted over 48 h in a Metabolic Research Unit. Subjects were instructed to abstain from alcohol and caffeine for 3 days prior to and during the study. Subjects were asked to self-select breakfast between 08:00 and 09:00 h, lunch between 12:00 and 13:00 h, and supper between 18:00 and 19:00 h. They were asked not to consume any foods except water after 19:00 h each study day. Subjects prepared their own meals within the Unit or obtained them elsewhere. After an overnight period in the Unit, subjects drank a priming dose of deuterium oxide (0.7 g D_2O/kg body water) (99.8 atom % excess (A%E), ICN Biomedicals, Montreal, Quebec) at 07:30 h on day 1 ($t = 0$). Deuterium-labeled drinking water was consumed over the following 48 h to maintain body water deuterium enrichment at a plateau. This water contained 1.4 g D_2O/kg body water to account for an estimated equivalent quantity of unlabeled water obtained by diet. Twenty-ml blood samples were collected at 07:00 and 08:00 h on day 1 and every 4 h thereafter for the following 48 h. Plasma was obtained immediately and frozen at −20°C.

Analytical

Deuterium enrichment was measured in plasma cholesterol, cholesteryl ester, and in plasma water. Four ml of plasma from each timepoint was combined with methanol and heated as described previously (10). Hexane–chloroform 4:1 (v/v) was added and the mixture was shaken mechanically for 20 min. Water was added, the sample was shaken again, and the upper solvent layer was removed after centrifugation at 1500 *g*. The extraction procedure was then repeated and solvent layers were combined. Solvent was removed with nitrogen and the residue was chromatographed on silica plates (Whatman Inc., Clifton, NJ). Plates were double-developed in petroleum ether–ethyl ether–acetic acid 135:15:1.5 and lipid bands were identified according to a corresponding co-chromatographed standard (Supelco, Bellefonte, PA). Free and esterified cholesterol bands were scraped from plates and extracted three times using hexane–chloroform–ether 5:2:1. Extractions were carried out by shaking for 10 min followed by centrifugation and removal of solvent from the silica pellet. Cholesteryl esters were saponified in 6 N KOH and the resultant free cholesterol was purified by thin-layer chromatography as described. Efficiencies of the entire purification processes for free and esterified cholesterol were determined as 86 ± 4 and 76 ± 6% (CV), respectively, using [^{14}C]cholesterol and [^{14}C]cholesteryl oleate standards. Dried cholesterol samples were transferred to 18-cm combustion tubes (Vycor, Corning Glass Works, Corning, NY) using three washes of chloroform. Cupric oxide (0.5 g) and a 2-cm length of silver wire were added and the tubes were sealed at less than 50 mtorr pressure.

Cholesterol samples were combusted for 2 h at 750°C and water produced was vacuum-distilled into Vycor tubes containing 80 mg zinc reagent (Biogeochemical Laboratories, Indiana University, Bloomington, IN). To measure the deuterium enrichment of body water, additional plasma samples from 12 and 36 h timepoints were diluted sixfold by 5% bovine serum albumin to reduce the deuterium enrichment to within the normal analytical range. Baseline samples were not diluted. Duplicate 2-μl respective samples were vacuum-distilled into zinc-containing Vycor tubes. Cholesterol and plasma water samples were reduced to hydrogen gas at 480°C for 30 min. The deuterium enrichment of samples was measured by differential isotope ratio mass spectrometry (Nuclide 3-60 H/D, MAAS, Bellefonte, PA) using a triple inlet system with electrical H_3^+ compensation (11).

Calculations

Daily fractional synthetic rate (FSR) was determined for each 4-h interval as the proportion of the rapidly exchangeable pool of cholesterol synthesized de novo during that interval, expressed per 24 h. The free cholesterol pool was selected as representing that into which newly formed sterol initially appears. Appearance of label in cholesteryl ester is considered to reflect newly synthesized cholesterol which has undergone esterification. Values for total cholesterol synthesis were obtained using weighted averages of free and ester data, assuming that one third of plasma cholesterol is in the free form. FSR values were calculated as cholesterol deuterium enrichment in relation to that of the precursor body water pool adjusted for the fraction of hydrogens of cholesterol derived from labeled substrate (12). The FSR values were derived using the following equation:

$$\text{FSR (day}^{-1}) = \frac{\delta \text{ cholesterol (o/oo)} \times 6}{\delta \text{ plasma (o/oo)} \times (22D/27C \times 27C/46H)} \quad \textit{Eq. 1)}$$

where δ cholesterol is deuterium enrichment over each 4-h period and δ plasma is the mean plasma deuterium enrichment over the entire 48-h test period. The multiplication factor of 6 converts the 4-h FSR value to that per day.

Statistical analyses

Two-factor ANOVA was used to examine data for effects of time and subject on plasma cholesterol levels. Plasma deuterium enrichments at different time points

were compared using a paired Student's *t*-test. For FSR data, shifted sine wave functions were generated to define the periodicity of circadian trends, and linear regression analysis was used to examine the significance of such trends. The sine wave form was chosen as a reasonable choice for modelling the major deviations from randomness, thus allowing for a statistically more powerful test than a conventional ANOVA using time as a factor. Subsequently, ANOVA was used to investigate the variance associated with the sine wave compared to total variance. A level of significance of 0.05 was used.

RESULTS

Ages, body weights and heights, and screening plasma cholesterol and triglyceride concentrations are reported in **Table 1**. All individuals studied were within 15% ideal weight for height. Plasma lipid levels were within normal ranges in all subjects. Plasma cholesterol values at each 4-h timepoint study are shown for subjects in **Fig. 1**. No significant time effects were observed on plasma cholesterol levels over the 48-h study. Two subjects showed cholesterol levels that were significantly different ($P<0.05$) from those of others within the group.

Mean deuterium levels in plasma water were not different measured at 12 (3853 ± 162 o/oo) (mean ± SEM) and 36 (3753 ± 99 o/oo) h.

Fig. 2 shows the rate of incorporation of deuterium into plasma compartment free cholesterol over the 48-h period. Highest rates of deuterium incorporation occurred between 08:00 and 12:00 h, whereas, during afternoon and early evening, enrichment values were low or slightly negative. Negative enrichment values indicate entry of free cholesterol into the rapidly exchangeable pool at a rate that exceeds that of de novo synthesis.

When enrichment data are normalized for the level of deuterium in the precursor body water compartment, FSR measures are obtained as shown in Figs. 3–6 and Table 2. Free cholesterol data represent the fraction of the rapidly exchangeable pool that has been synthesized in the period measured, expressed per 24 h. Fractional synthetic rate was lowest at 14:00 and 18:00 h, peaking at 06:00 h. The sine wave amplitude was significantly ($P<0.001$) different from zero as expressed by the equation (**Fig. 3**):

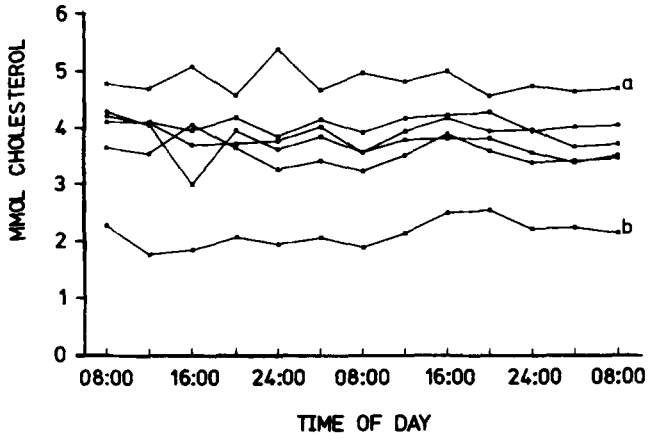

Fig. 1. Plasma cholesterol levels of subjects over the study period; a, significantly higher than other subjects ($P<0.05$); b, significantly lower than other subjects ($P<0.05$).

$$\text{FSR (day}^{-1}) = 0.0585 + 0.0484 \cdot \sin [0.262 \cdot (h + 0.415)] \quad \textit{Eq. 2)}$$

where h represents the hour of day after D_2O administration. There was no significant systematic deviation with time of the points from the fitted sine wave, as determined by ANOVA.

Cholesteryl ester FSR over time (**Table 2**) showed poorer diurnal periodicity ($P = 0.068$) compared with the fitted equation (**Fig. 4**):

$$\text{FSR (day}^{-1}) = 0.0341 + 0.0339 \cdot \sin [0.262 \cdot (h - 1.624)] \quad \textit{Eq. 3)}$$

Ester values peaked at 06:00 h and reached a nadir during the evening period. There were significant deviations ($P<0.05$) with time from the fitted sine wave indicating second order deviations from this wave form.

Weighted FSR values of free and esterified cholesterol are shown in Table 2. A nadir in synthesis between 14:00 and 18:00 h was followed by a maximum between 06:00

TABLE 1. Physical parameters and lipid concentrations

Subject	Age	Weight	Height	Screening Plasma Levels	
				Cholesterol	Triglycerides
	yr	*kg*	*cm*	*mmol/l*	
1	29	67.3	185	4.78	1.10
2	24	73.2	183	2.28	0.85
3	30	62.3	172	4.22	1.10
4	27	67.3	150	4.29	0.93
5	21	62.3	183	3.65	0.25
6	29	86.4	183	4.11	1.02
Mean ± SEM	26.7 ± 1.4	69.8 ± 3.7	176 ± 5.6	3.89 ± 0.35	0.88 ± 0.13

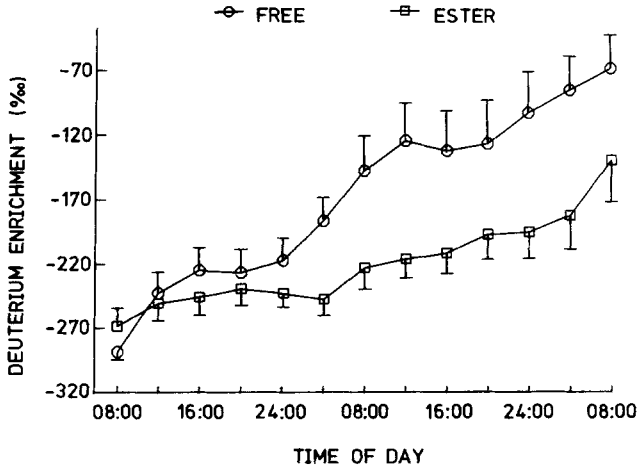

Fig. 2. Deuterium incorporation into plasma free and esterified cholesterol of subjects over the study period (means ± SEM).

and 10:00 h. The amplitude of the sine wave was not significantly different ($P<0.01$) from zero as expressed in the equation (**Fig. 5**):

$$\text{FSR (day}^{-1}) = 0.0424 + 0.0373 \cdot \sin [0.262 \cdot (h - 0.883)] \quad \textit{Eq. 4)}$$

There were significant deviations ($P<0.05$) with time from the fitted sine wave indicating second order deviations from this wave form.

Fig. 6 compares the predicted-fit equations for free, esterified, and total cholesterol FSR. Periodicity of incorporation of deuterium into cholesteryl ester appears to be phase-shifted about 2 h behind deuterium incorporation into free sterol.

Calculation of mean synthesis rates for study subjects requires the assumption that the rapidly exchangeable pool contains 24 g of cholesterol (13) and that 50% of this pool is unesterified. The FSR mean value for free cholesterol of 0.0585 day^{-1} thus corresponds to a net formation rate of 0.71 g day^{-1}.

DISCUSSION

Diurnal variation in the rate of human whole body cholesterol formation has been suggested through direct evidence from animal studies (1-4) and indirect data in humans (6-10). The present findings indicate that whole body, free, and total cholesterol syntheses oscillate periodically and predictably. Furthermore, movement of free cholesterol into the ester pool occurs at a rate dependent on, or concomitant with, free cholesterol synthesis. Similar patterns of cycling of plasma sterol precursor levels have been reported (6, 7); however, phase-shift differences were observed with peaks in precursor levels occurring 2-4 h before those of cholesterol FSR observed in our study. A lag period between the synthesis of precursors and the final sterol molecule, or inter-study differences in meal or sleep cycling, may explain this discrepancy.

The deuterium uptake method for determining cholesterol FSR relies on at least three modeling assumptions. These include *i)* the reliability of plasma cholesterol turnover to indicate that of the total mass of rapidly exchangeable cholesterol; *ii)* reliability of changes in FSR to reflect changes in actual formation rates; and *iii)* the assumption that flux of sterol between the rapidly exchangeable and other pools is small during the interval of measurement.

First, free cholesterol migrates rapidly between lipoproteins, erythrocytes, and other cellular structures (14, 15). Thus, the deuterium enrichment of free cholesterol obtained from plasma should represent that of free sterol within the rapid exchangeable (M_1) pool found in liver, intestine, and the circulation. Turnover rates for ester are slower (14, 15), thus present data likely reflect events occurring within the plasma, more so than the overall, ester pool within the M_1 compartment.

Second, changes observed in FSR can be interpreted as shifts in either absolute synthetic rate or size of the M_1 cholesterol pool. Plasma total cholesterol levels, which represent a significant proportion of the total rapidly exchangeable pool, remain fairly constant over 24 h (6). Moreover, variations in daily production of 1 g cholesterol per day would impact minimally on the average 24 g M_1 pool. Fluctuations in FSR thus likely reflect changes in absolute synthetic rate and not M_1 pool size.

Third, changes in apparent FSR are assumed to represent new synthesis and not result from variations in flux of M_1 cholesterol with other pools. A potential efflux route for cholesterol is conversion to bile acids from a specific

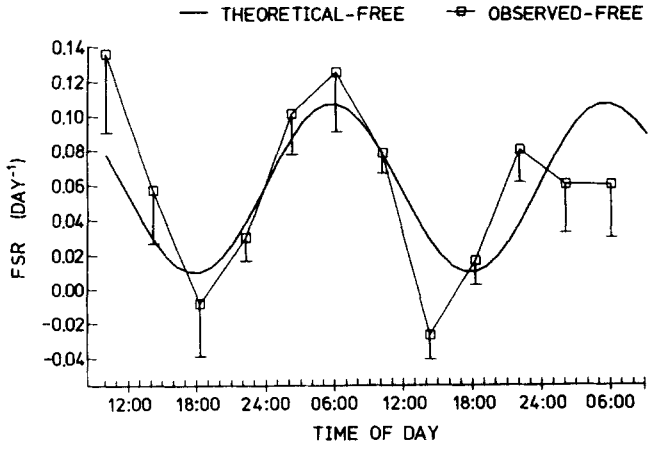

Fig. 3. Fractional synthetic rate (FSR) for free cholesterol as measured experimentally and using the regression fit equation in subjects over the study period (means ± SEM). Mean of data points for subjects is not different from fitted sine wave ($P<0.05$).

TABLE 2. Fractional synthetic rates for free, esterified, and total rapidly exchangeable cholesterol of subjects

Time of Day	Subject 1	2	3	4	5	6	Mean ± SEM
h	Free Cholesterol						
10:00	0.1459	0.3216	0.0821	0.1104	0.0209	na	0.1362 ± 0.0453
14:00	0.0468	−0.0394	0.0142	0.1622	0.0341	0.1272	0.0575 ± 0.0304
18:00	0.0130	0.0307	0.0528	−0.1807	0.0122	0.0242	−0.0080 ± 0.0351
22:00	−0.0125	0.0326	0.0317	0.0288	0.0869	0.0156	0.0305 ± 0.0132
02:00	0.1078	0.0941	0.2011	0.0262	0.0802	0.0974	0.1011 ± 0.0232
06:00	0.0857	0.2693	0.0209	0.1298	0.0917	0.1534	0.1251 ± 0.0342
10:00	0.0857	0.1051	0.0648	0.1015	0.0300	0.0833	0.0784 ± 0.0113
14:00	−0.0271	−0.0574	−0.0552	−0.0216	−0.0343	0.0360	−0.0266 ± 0.0139
18:00	0.0641	0.0156	−0.0216	0.0014	−0.0120	0.0538	0.0169 ± 0.0143
22:00	0.0869	0.0218	0.0408	0.1493	0.1030	0.0787	0.0801 ± 0.0185
02:00	0.0857	−0.0442	0.1586	0.0451	0.0842	0.0331	0.0604 ± 0.0276
06:00	0.1975	0.0574	0.0739	0.0396	−0.0053	−0.0014	0.0603 ± 0.0303
	Cholesteryl Ester						
10:00	0.0278	−0.0082	0.2179	0.1906	−0.0470	0.0005	0.0636 ± 0.0457
14:00	0.0278	0.0308	0.0326	−0.0034	0.0096	0.0005	0.0163 ± 0.0066
18:00	0.0279	0.1949	−0.1126	−0.0034	0.0127	0.0005	0.0200 ± 0.0405
22:00	0.0695	−0.1320	0.0353	−0.0900	0.0499	0.0005	−0.0111 ± 0.0333
02:00	−0.0473	0.0397	0.0353	−0.0900	−0.0286	−0.0113	−0.0170 ± 0.0203
06:00	0.0452	0.2498	0.0254	0.0602	0.0586	0.0425	0.0803 ± 0.0343
10:00	0.0158	−0.0590	0.1198	−0.0161	0.0514	0.0245	0.0227 ± 0.0248
14:00	−0.0343	0.0873	−0.1116	0.0324	0.0427	0.0612	0.0129 ± 0.0299
18:00	0.1198	0.0368	0.0024	0.0324	0.0427	0.0516	0.0476 ± 0.0160
22:00	−0.0374	−0.0031	0.0446	−0.0379	0.0178	0.0401	0.0040 ± 0.0149
02:00	0.0603	0.0422	−0.0098	0.0540	0.0826	0.0286	0.0430 ± 0.0129
06:00	na	0.3396	0.0463	0.1670	0.0826	0.0636	0.1398 ± 0.0484
	Total Cholesterol						
10:00	0.0672	0.1018	0.1728	0.1639	−0.0242	na	0.0963 ± 0.0322
14:00	0.0341	0.0074	0.0264	0.0518	0.0178	0.0427	0.0300 ± 0.0067
18:00	0.0228	0.1402	−0.0574	−0.0624	0.0215	0.0084	0.0107 ± 0.0299
22:00	0.0422	−0.0770	0.0341	−0.0504	0.0624	0.0055	0.0028 ± 0.0226
02:00	0.0043	0.0578	0.0907	−0.0514	0.0077	0.0250	0.0224 ± 0.0199
06:00	0.0588	0.2563	0.0240	0.0835	0.0696	0.0794	0.0953 ± 0.0334
10:00	0.0391	−0.0043	0.1015	0.0230	0.0442	0.0439	0.0412 ± 0.0142
14:00	−0.0322	0.0391	−0.0929	0.0144	0.0170	0.0528	−0.0003 ± 0.0220
18:00	0.1013	0.0298	−0.0055	0.0221	0.0245	0.0523	0.0374 ± 0.0148
22:00	0.0038	0.0053	0.0434	0.0245	0.0461	0.0530	0.0294 ± 0.0087
02:00	0.0686	0.0134	0.0463	0.0511	0.0830	0.0300	0.0488 ± 0.0103
06:00	na	0.2455	0.0554	0.1246	0.0533	0.0420	0.1042 ± 0.0342

na, Not available.

hepatic pool (14, 16). The cyclic pattern in cholesterol FSR resembles the circadian rhythm established in bile acid synthesis in healthy humans where a peak occurred in early morning with lower rates of synthesis during the afternoon (9). Thus, the close parallelism in the persistent fluctuations of cholesterol and bile acid synthesis suggests that the reduction in cholesterol formation seen during the daylight period represents lowered de novo production, not enhanced diversion of sterol to bile acid production.

In contrast, gradual turnover of cholesterol between rapidly exchangeable and slower turnover pools, as does occur (13), would be expected to alter apparent FSR through input of unlabeled cholesterol into M_1. Indeed, apparent negative FSR values for free cholesterol during afternoon periods during the study likely reflect an input route of unlabeled cholesterol that is greater than that provided by synthesis. Also, some exit of sterol from the free pool is indicated from the appearance of label in ester. It remains unclear whether sterol input into M_1 during periods of apparent negative FSR represents exogenous absorbed cholesterol or endogenous sterol entering from other pools or through de-esterification. Previous findings of apparent negative FSR values in healthy subjects during a period of fasting suggest that the input is not solely dietary in origin (10).

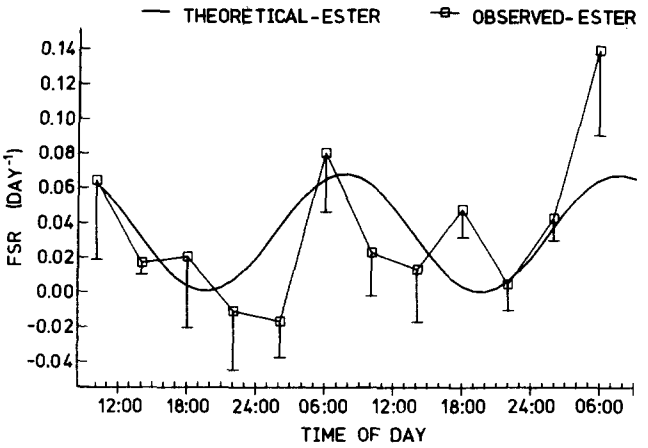

Fig. 4. Fractional synthetic rate (FSR) for cholesteryl ester as measured experimentally and using the regression fit equation in subjects over the study period (means ± SEM).

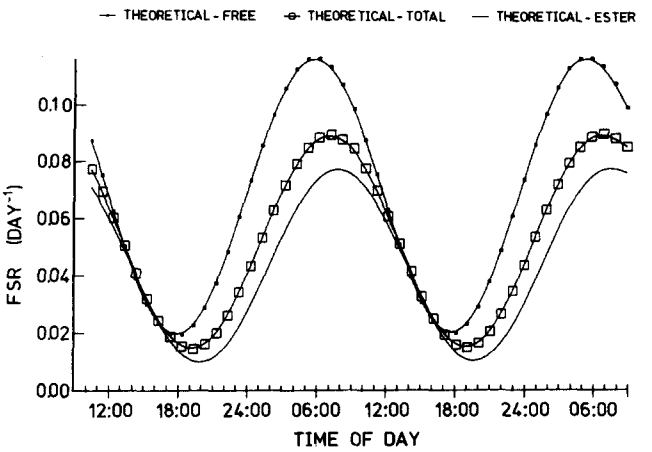

Fig. 6. Comparison of predicted fit equations for free, esterified, and total cholesterol in subjects over the study period.

Cholesterol synthesis also occurs outside the rapidly exchangeable sterol pool. Any such synthesis would not be detected in our study due to the slow inter-pool cholesterol exchange rate. However, formation rates outside of the M_1 pool are low. Dell et al. (17) found that in baboons only 25% of total body cholesterol synthesis occurred outside the M_1 pool. Whether similar side pool synthesis occurs in humans is unknown.

Although the rhythmicity is well defined, the timing of the peak and nadir of human cholesterol synthesis observed here is inconsistent with findings from animal studies where maximum formation occurs during periods of food intake and activity. This disparity suggests that mechanisms that coordinate synthesis regulation may differ between species. In animals, circadian rhythms for cholesterol formation are more closely related to feeding schedule than to light periods (18, 19). In groups of hamsters exposed to the identical photoperiod, but fed at different times of day, peak synthesis in liver and intestine occurred 4 to 6 h after feeding, independent of light cycling (19). In contrast to animal data, present findings show peak production lagging almost almost 12 h after the last meal in humans.

Enhancement of sterol synthesis secondary to feeding seen in animals may occur due to increased bile acid synthesis (20) as demonstrated by a correlation between activities of HMG-CoA reductase and 7α-hydroxylase enzymes in rat liver (21). In humans, bile acid formation also peaks simultaneously with cholesterol synthesis (9); however, in relation to the feeding cycle, the periodicity is distinctly phase-shifted. Direct comparison between the two species is difficult because of the absence of a gallbladder in the rat which likely results in bile acid enterohepatic cycling and synthesis regulation that differs from that in humans.

In contrast, animals (14, 20, 22–24) and humans (7, 10) do respond similarly to total food restriction by markedly reducing cholesterol synthesis. Whether absorptive state-dependent changes in metabolite or hormone levels regulate sterol synthesis remains to be determined; however, the persistence of diurnal rhythmicity in fasted rats (25) suggests additional control mechanisms. Thus, while in animals sterol synthesis peaks are coincident with periods of feeding, why maximal cholesterol production lags well behind meal intake in humans is unknown. In animals (5, 22, 24, 25) and humans (7, 26) cholesterol feeding also results in reduced sterol synthesis. Perhaps in humans the peak in synthetic activity is delayed because synthesis inhibition by incoming dietary cholesterol is as important a control element as is stimulation of synthesis by elevated precursor availability post-meal. Such feed-

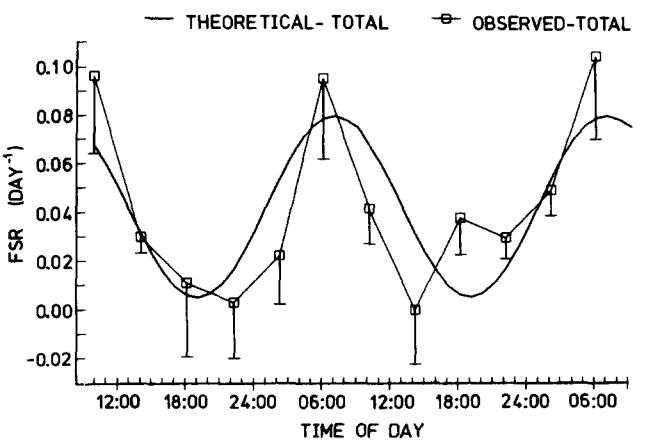

Fig. 5. Total cholesterol fractional synthetic rate (FSR) expressed as experimentally derived from average of free and esterified values and as predicted in subjects over the study period (means ± SEM). Mean of data points for subjects is not different from fitted sine wave ($P<0.05$).

back inhibition of synthesis to incoming dietary cholesterol would result in reduced sterol synthesis during times of feeding, with subsequent synthetic rate increases as hepatic cholesterol influx diminishes while energy substrate availability remains relatively high.

In summary, although regulatory mechanisms responsible remain to be characterized, daily periodicity in rates of free and total cholesterol formation is indicated in humans. In particular, a pronounced depression of synthesis is observed during the daytime period. As cholesterol synthesis accounts for a significant proportion of total daily production (26), level of synthesis may impact on long term cholesterol accretion in vivo. Thus, the efficacy of specific dietary or pharmacological factors aimed at decreasing atherosclerotic disease risk by reducing sterol synthesis will likely exhibit time-of-day variation.

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REFERENCES

1. 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.
2. 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.
3. Hamprecht, B., C. Nussler, and F. Lynen. 1969. Rhythmic changes of hydroxy-methyl-glutaryl coenzyme A reductase activity in livers of fed and fasted rats. *FEBS Lett.* **4:** 117–121.
4. Alede, I. 1980. Studies on the effects of supraphysiological levels of dietary vitamin A and ascorbic acid on cholesterol metabolism in the guinea pig. Ph.D. Thesis. Rutgers University, New Brunswick, NJ.
5. Ott, D. B., and P. A. Lachance. 1981. Biochemical controls of liver cholesterol biosynthesis. *Am. J. Clin. Nutr.* **34:** 2295–2306.
6. Miettinen, T. A. 1982. Diurnal variation of cholesterol precursors squalene and methyl sterols in human plasma lipoproteins. *J. Lipid Res.* **23:** 466–473.
7. Parker, T. S., D. J. McNamara, C. Brown, O. Garrigan, R. Kolb, H. Batwin, and E. H. Ahrens. 1982. Mevalonic acid in human plasma: relationship of concentration and circadian rhythm to cholesterol synthesis rates in man. *Proc. Natl. Acad. Sci. USA.* **79:** 3037–3041.
8. 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.
9. Pooler, P. A., and W. C. Duane. 1988. Effects of bile acid administration on bile acid synthesis and its circadian rhythm in man. *Hepatology.* **8:** 1140–1146.
10. Jones, P. J. H., A. M. Scanu, and D. A. Schoeller. 1988. Plasma cholesterol synthesis using deuterated water in humans: effect of short term food restriction. *J. Lab. Clin. Med.* **111:** 627–633.
11. Schoeller, D. A., D. W. Peterson, and J. M. Hayes. 1983. Double comparison method for mass spectrometric determination of hydrogen isotopic abundances. *Anal. Chem.* **55:** 827–832.
12. Dietschy, J. M., and D. K. Spady. 1984. Measurement of rates of cholesterol synthesis using tritiated water. *J. Lipid Res.* **25:** 1469–1476.
13. Green, C. 1983. Sterol biosynthesis and function. *Biochem. Soc. Trans.* **11:** 637–639.
14. Norum, K. R., T. Berg, P. Helgerud, and C. A. Drevon. 1983. Transport of cholesterol. *Physiol. Rev.* **63:** 1343–1419.
15. Robins, S. J., and H. Brunengraber. 1982. Origin of biliary cholesterol and lecithin in the rat: contribution of new synthesis and preformed hepatic stores. *J. Lipid Res.* **23:** 604–608.
16. Goodman, D. S., F. R. Smith, A. H. Seplowitz, R. Ramakrishnan, and R. B. Dell. 1980. Prediction of the parameters of whole body cholesterol metabolism in humans. *J. Lipid Res.* **21:** 699–713.
17. Dell, B., G. E. Mott, E. M. Jackson, R. Ramakrishnan, K. D. Carey, H. C. McGill, and D. S. Goodman. 1985. Whole body and tissue cholesterol turnover in the baboon. *J. Lipid Res.* **26:** 327–337.
18. Dugan, R. E., L. L. Slakey, and A. V. Briedis. 1972. Factors affecting the diurnal variation in the level of β-hydroxy-β-methylglutaryl coenzyme A reductase and cholesterol synthesizing activity in rat liver. *Arch. Biochem. Biophys.* **152:** 21–27.
19. Ho, K-J. 1979. Circadian rhythm of cholesterol biosynthesis: dietary regulation in the liver and small intestine of hamsters. *Int. J. Chronobiol.* **6:** 39–50.
20. Hassan, A. S. 1986. Feeding-induced regulation of cholesterol metabolism: a unified proposal. *Proc. Soc. Exp. Biol. Med.* **182:** 143–150.
21. Björkhem, I., and J-E. Åkerlund. 1988. Studies on the link between HMG-CoA reductase and cholesterol 7α-hydroxylase in rat liver. *J. Lipid Res.* **29:** 136–143.
22. Jeske, D. J., and J. M. Dietschy. 1980. Regulation of rates of cholesterol synthesis in vivo in the liver and carcass of the rat measured using [^{3}H]water. *J. Lipid Res.* **21:** 364–376.
23. Ide, T., H. Okamatsu, and M. Sugano. 1978. Effects of dietary fats on the activity of 3-hydroxymethylglutaryl-CoA reductase and sterol synthesis in the liver of fasted-refed rats. *J. Nutr. Sci. Vitaminol.* **24:** 535–546.
24. Kelley, M. J., and J. A. Story. 1985. Effect of type of diet and feeding status on modulation of hepatic HMG-CoA reductase in rats. *Lipids.* **20:** 53–55.
25. Shapiro, D. J., and V. W. Rodwell. 1972. Fine structure of the cyclic rhythm of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Differential effects of cholesterol feeding and fasting. *Biochemistry.* **11:** 1042–1045.
26. Dietschy, J. M. 1984. Regulation of cholesterol metabolism in man and other species. *Klin. Wochenshr.* **62:** 338–345.