Quantification of menstrual and diurnal periodicities in rates of cholesterol and fat synthesis in humans

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Abstract The mass isotopomer distribution analysis (MIDA) technique is applied here in men and menstruating women to quantify periodicities in the biosynthesis of serum cholesterol and very low density lipoprotein (VLDL)-palmitate. The isotopic enrichment of the true biosynthetic precursor (intracellular acetyl-CoA) during oral or intravenous administration of sodium[1-^{13}C]- or [2-^{13}C]acetate was calculated from mass isotopomer fractional abundances in free cholesterol and VLDL-palmitate, determined by gas chromatography–mass spectrometry (GC–MS). To convert fractional into absolute cholesterol synthesis rates, decay rate constants of plasma cholesterol were determined from the die-away curves of endogenously labeled high-mass isotopomers. Oral [^{13}C]acetate was a 3–4 times more efficient means of labeling the precursor pool for VLDL-palmitate than was intravenous [^{13}C]acetate, consistent with a splanchnic site of VLDL-fatty acid synthesis, whereas the precursor for free cholesterol had an intermediate enrichment, suggesting a contribution from extra-splanchnic tissues as well. Endogenous synthesis of serum cholesterol was 8–11 mg/kg per day (an estimated 65–75% of input into serum cholesterol); it was 1.5- to 3-fold higher at night than during the day (37–49 mg/h at night compared to 9–23 mg/h during the day) and did not vary over the menstrual cycle (608–697 mg/day). In contrast, endogenous synthesis of fatty acids made a relatively minor contribution to body fat pools (1/10–1/20 of input into VLDL-palmitate) compared to dietary fat intake; it was greater in the day-time, and was influenced by menstrual cycle (3-fold elevated in the follicular phase compared to the luteal phase), and body composition (higher in obese men than normal weight men, $r^2 = 0.59$ for lipogenesis vs. body mass index). Factors responsible for periodicities in endogenous lipid synthesis can be studied in humans using this approach.—**Faix, D., R. Neese, C. Kletke, S. Wolden, D. Cesar, M. Coutlangus, C. H. L. Shackleton, and M. K. Hellerstein.** Quantification of menstrual and diurnal periodicities in rates of cholesterol and fat synthesis in humans. *J. Lipid Res.* 1993. **34:** 2063–2075.

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Enzymatic pathways exist for the endogenous synthesis of cholesterol and nonessential fatty acids from 2-carbon precursor subunits in most organisms, including humans. Diet also contributes to body pools of fat and cholesterol. The relative quantitative importance of dietary versus endogenous sources of these lipids is a metabolic question with broad significance for metabolic regulation, clinical nutrition, and public health, with implications for dietary practices, for our understanding of susceptibility to common metabolic disorders influenced by diet (including hypercholesterolemia, hypertriglyceridemia, and obesity), for identification of genes involved in regulation of lipid metabolism, and for the development of rational metabolic therapeutics. Previous estimates of the synthetic rates of cholesterol and fatty acids in humans have been based on methods that are indirect and generally nonquantitative, however. Endogenous cholesterogenesis has been estimated by the sterol balance technique (1, 2) which is labor-intensive, requires long-term metabolic ward incarceration, and cannot address diurnality or other short-term modulations in synthesis rates. Most other techniques are also indirect, such as measurement of plasma mevalonate (3) or lathosterol (4) concentrations, and cholesterol synthesis from labeled acetate by peripheral blood cells in vitro (5) or 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase activity from biopsy specimens (6). De novo lipogenesis has generally been estimated from gas exchange (indirect calorimetric) measurements. This is not a direct measurement method, and is based on a number of assumptions (7); at best it can only estimate net fat synthesis in the whole body, rather than unidirectional lipogenic rates (8).

More recently, two isotope incorporation techniques have been developed for measuring directly the synthesis of cholesterol and fats in humans. Investigators have used

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; MIDA, mass isotopomer distribution analysis; GC–MS, gas chromatography–mass spectrometry; FAME, fatty acid methyl esters; VLDL, very low density lipoprotein; FC, free cholesterol.

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^2H_2O incorporation to determine the fractional synthesis of cholesterol (9, 10) and fat (11, 12) in normal subjects. Water incorporation techniques have an important advantage over most other isotopic techniques in that they provide an accurate estimate of the true precursor enrichment for lipogenesis or cholesterogenesis, as water can be sampled in vivo and is in relatively rapid equilibrium across all cells and compartments of the body. This technique has some uncertainties and limitations, however, as we have discussed elsewhere (13). Our laboratory (13–22) recently developed a stable isotopic technique for measuring synthesis rates of biological polymers, including cholesterol and fatty acids. Using this technique, which we have termed MIDA, one can calculate the isotopic enrichment of precursor acetyl-CoA subunits that entered newly synthesized cholesterol or fatty acids during the experimental administration of acetate enriched in ^{13}C. The fractional synthesis rate (f, the fraction of polymer derived from endogenous synthesis during the labeling experiment) is then calculated by use of the precursor–product relationship (13, 15, 17, 23). After discontinuing labeled precursor administration, the die-away curve of endogenous multiply-labeled polymers (high-mass isotopomers, whose decay curves conform to the assumption of flash-labeling, ref. 13) is followed to establish the rate constant of labeled polymer removal from the sampled compartment (serum). By combining label incorporation data with label exit data and an estimate of pool size, the absolute synthesis rate of the polymer can be calculated (13).

To date, MIDA has been used by our laboratory to estimate acetyl-CoA enrichments and endogenous synthesis rates of fatty acids in animals (15, 18) and humans (16, 17, 21), hepatic triose-phosphate enrichments and gluconeogenic rates in animals (13, 20), and to quantify cholesterol synthesis rates in women during one phase of their menstrual cycle (22). Lee, Bergner, and Guo (23) have also validated a similar approach in vitro using the synthesis of acetate polymers from ^{13}C-labeled acetic anhydride. Here, we use MIDA to measure endogenous cholesterol and fat synthesis in reproductive-age women and in men, with the primary objective being to quantify menstrual and diurnal periodicities of lipid synthesis in humans.

METHODS

Human subjects and experimental protocols

Subjects were recruited by advertisement. Normal men and women were <120% ideal body weight, had no history of significant medical or metabolic diseases, were taking no medications, and had no abnormalities of screening blood chemistries or hematologic parameters. Women were studied twice, in the first 2 weeks after onset of menses (follicular phase) and in the 2 weeks prior to menses (luteal phase), based on the subjects' report of dates. Elevated serum progesterone concentrations were used to confirm the luteal phase. Results from five of the six follicular-phase women have been presented previously (22). Obese men were >150% ideal body weight and >35% body fat and were selected on the basis of serum cholesterol concentrations <200 mg/dl and triglycerides <150 mg/dl. All subjects habitually consumed standard, non-vegetarian diets and performed either 7-day weighed food records as outpatients or 3-day diet recalls to estimate dietary cholesterol intake, which was <400 mg/day in all subjects. All subjects reported a constant day:night, wake:sleep cycle, without shift-work or travel within the preceding 6 weeks. Subject characteristics are shown in **Table 1.**

Studies had the prior approval of the University of California at San Francisco Committee on Human Research, and subjects gave written informed consent before enrollment. All subjects were tested for human immunodeficiency virus (HIV) serologic status because we have found that asymptomatic HIV-seropositivity has an effect on de novo lipogenesis (21). All subjects included here were HIV sero-negative, had fasting triglyceride concentrations less than 150 mg/dl, and fasting cholesterol concentrations <200 mg/dl. Measurement of de novo

TABLE 1. Subject characteristics

Group	n	Age	Height	Weight	Body Mass Index
		yr	*cm*	*kg*	*kg/m²*
Women					
Follicular phase	6	31 ± 2	162.2 ± 3.0	65.0 ± 5.7	24.5 ± 2.0
Luteal phase	5	34 ± 4	164.1 ± 3.4	69.5 ± 5.9	25.6 ± 2.5
Normal men	8	27 ± 3	180.0 ± 3.0	74.1 ± 2.8	19.2 ± 3.6
Obese men	4	35 ± 1	178.0 ± 1.0	110.6 ± 7.7	34.8 ± 3.0

Values given as means ± SE.

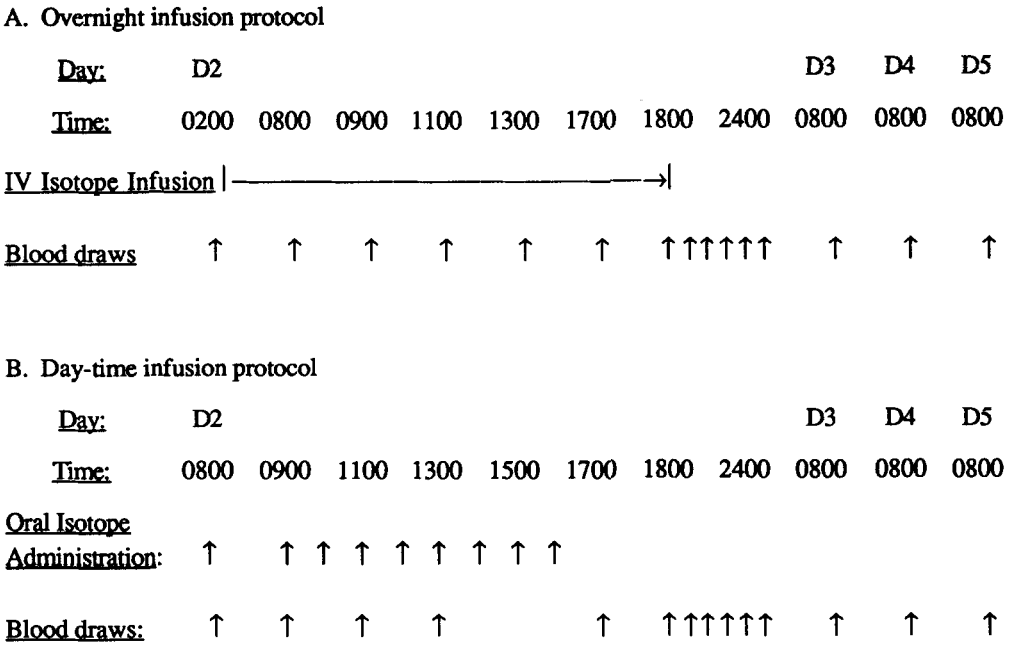

Fig. 1. Experimental protocols.

hepatic lipogenesis and endogenous synthesis of serum cholesterol was carried out as described previously (13, 16, 21, 22). In brief, subjects were admitted to the General Clinical Research Center (GCRC) of the SF General Hospital for 48 h (**Fig. 1**). Intravenous (IV) infusion of sodium [2-^{13}C]- or [1-^{13}C]acetate began at 0200 on day 2, at a rate of 0.050–0.080 mmol/kg per h (roughly 400 mg/h). Diet was ad libitum until 11:00 PM on day 1, after which time no food was allowed until 0900 the next morning, when oral administration of a low-cholesterol liquid formula diet (Ensure®) was begun, as described previously (16, 21, 22). The liquid formula was given hourly, from 0900 to 1600, to provide the equivalent of 7 mg carbohydrate/kg per min (ca. 30 g/h) while providing $<$20 mg cholesterol over the 9-h feeding period. We have used this feeding regimen previously for studies of fat and cholesterol synthesis (16, 21, 22). It has the advantage of maintaining fairly constant isotopic and metabolic conditions in the fed state. At 1800 of day 2, IV [^{13}C]acetate infusions were discontinued. For oral administration protocols, [^{13}C]acetate was begun orally at 0800 and given as hourly doses (400–500 mg/h) until 1600, for a total of nine doses. Blood samples were drawn prior to isotope infusion (baseline) and then at 0800, 0900, 1300, 1700, and 1800 during the incorporation phase and at 2000, 2200, 2400, 0800 (day 3), and 0800 (days 4 and 5) during the decay phase. Indirect calorimetry was performed using a DeltaTrac Metabolic Cart (Sensormedix Corp., Yorba Linda, CA) during fasting and fed states. Body composition was measured by anthropometrics and bio-electrical impedance analysis (BIA, Model #1990B, Valhalla Scientific Inc., San Diego, CA). Body fat content was calculated according to the BIA instrument-manufacturer's equations.

Isotopes and chemicals

Sodium [1-^{13}C]- and [2-^{13}C]acetate were purchased from Isotec, Inc. (Miamisburgh, OH) and were chromatographically $>$99% pure and $>$98% enriched. Chemicals were reagent grade.

Metabolite isolation and measurement

VLDL was isolated from plasma by ultracentrifugation. Fatty acids were transmethylated to fatty acid methyl esters (FAME) as described previously (15, 16). We have compared labeling in VLDL triglyceride-palmitate, VLDL phospholipid-palmitate, and VLDL cholesteryl ester palmitate, separated by thin-layer chromatography (Hudgins, L., R. Neese, J. Hirsch, and M. K. Hellerstein, unpublished observations). The phospholipid and cholesteryl ester palmitate represented $<$15% of the total, an even smaller percent of the de novo palmitate synthesis

(reflecting slower turnover of phospholipids and cholesteryl esters) and had similar precursor enrichments. The vast majority (>90%) of de novo palmitate synthesis in VLDL could therefore be attributed to triglycerides and it was not necessary to separate these subfractions for accurate estimates.

Serum free cholesterol (FC) was extracted from 100 μl serum with 3 ml 95% ethanol–acetone 1:1 (v/v). The trimethylsilyl (TMS) derivative of FC was formed using N,O-bis-(TMS)-trifluoroacetamide with 1% trimethylchlorosilane. Cholesteryl ester (CE) is not derivatized by this technique. HIV serology was performed by ELISA (San Francisco General Hospital). Serum estradiol and progesterone were measured by radioimmunoassay on morning and evening blood specimens. Serum triglycerides and cholesterol were measured by standard techniques.

Mass spectrometry

GC–MS was performed with an HP model 5970 GC/MS (Hewlett-Packard Co., Palo Alto, CA) using electron-impact ionization. FAME were analyzed using a 15-meter DB-1 column isothermally at 200°C (16). For methyl palmitate, the molecular anion and its isotopes (*m/z* 270–274) were quantified under the selected ion monitoring mode. For TMS-cholesterol analysis, conditions were isothermal at 270°C using split injection with the same DB-1 column. The parent M_O fragment (*m/z* 368), representing underivatized FC minus [OH], was monitored as were M_1–M_4 (*m/z* 369–372).

MIDA model and calculations

The MIDA technique has been described in detail previously (13–17, 22). Briefly, MIDA is a method for calculating the biosynthetic rate of polymers and the isotope enrichments of their monomeric precursor subunits during a stable isotope labeling experiment. By applying probability logic to analyze the change from baseline in the pattern of fractional abundances of mass isotopomers, one can deduce the kinetic parameters necessary for calculating fractional and absolute biosynthetic rates of the polymer. The mathematical elements of the MIDA technique have been described in detail elsewhere (13–17) but will be reviewed here.

The central principle of MIDA is that the mathematics of combinatorial probabilities can be applied to aggregates of monomers (polymers) in order to infer parameters relating to their assembly and disassembly. The long-standing problem of how to determine the isotope content of the actual monomeric subunits that entered a polymer of interest in vivo (the "true precursor") is resolved, without the need for isolation of or measurements on monomeric subunits putatively representing the true precursor pool. Rather, mass spectrometry is used to establish the fractional abundances of mass isotopomers[2] in a polymer of interest at natural isotope abundance and after introduction of an isotopically labeled precursor. From the perturbed pattern or distribution of these mass isotopomeric abundances, the proportion of isotopically labeled subunits in the true biosynthetic precursor pool is inferred. The proportion and rate of endogenous synthesis of the polymer are then easily calculated, by use of precursor–product relationships (13, 15, 16). Operationally, the central implication of MIDA is that measurements must be made on aggregated monomers because the probability information is only available if two or more monomeric subunits can combine, and is lost if the monomers are analyzed in their nonaggregated (isolated) form. Thus, hydrolysis of a polymer to its subunit form for analysis (e.g., proteins to amino acids; fatty acids or cholesterol to acetate, etc.) is not compatible with MIDA.

For the case of serum cholesterol synthesis, the method requires experimental administration of an isotopically labeled precursor that can enrich cellular cholesterogenic acetyl-CoA pools. Sodium [1-^{13}C]- or [2-^{13}C]acetate are convenient and relatively inexpensive labels for this purpose. Cholesterol is isolated from the site of interest (blood) and analyzed in its aggregated form by mass spectrometry (see below). The fractional abundances of the parent (unlabeled), single-, double-, triple-, and quadruple-labeled mass isotopomers are determined at various times after administration of [^{13}C]acetate. After correction for natural fractional abundances of these mass isotopomers, the MIDA calculation consists of three steps.

1) Calculation of the proportion of isotopically labeled monomeric precursor subunits (p) that entered newly synthesized polymers during the labeling experiment. Because the actual precursor subunits that entered the polymer are used for calculating p, there can be no question

[2]Terms are defined as in previous publications (13, 22). Mass isotopomers, molecules of identical elemental composition and chemical structure, differing only in mass and able to be resolved by mass spectrometry.

$$\text{Fractional abundance} = \frac{\text{Abundance } M_x}{\sum_{i=0}^{n} \text{Abundance } M_i}$$

p = proportion of isotopically labeled monomeric precursor subunits in the subunits that entered the polymer, after subtraction of natural abundance proportion (= true precursor isotope enrichment). EM_x = excess fractional abundance of mass isotopomer M_x = $(\text{fractional abundance})_e - (\text{fractional abundance})_b$, where e = enriched and b = baseline. A_x^* = asymptotic or expected value for any EM_x if 100% of the polymer molecules present derived from endogenous synthesis during the labeling period. f = fractional endogenous synthesis = EM_x/A_x^*. k = rate constant(s) of labeled polymer removal from the polymer pool (decay rate, time^{-1}). R = ratios among EM_x, characteristic for a value of p and A_x^*. K = absolute synthesis rate of polymer (mass/time).

regarding compartmentalized precursor pools or other local subcellular inhomogeneities (i.e., whether it is the "true precursor" pool). All measurements are made on the secreted polymer itself; thus, there is no need for physical access to the intracellular biosynthetic site(s). It can be shown (13, 15) that the pattern among excess fractional abundances (EM_x) is independent of the mixture of natural abundance and enriched polymers. Accordingly, p can be inferred from the measured pattern of mass isotopomer excesses in any polymeric mixture, regardless of the replacement rate during the labeling period.

2) Comparison of measured EM_x for any convenient mass isotopomer to the excess fractional abundances that would be present (A_x^*, the asymptotic or expected value) if 100% of the polymer molecules present came from endogenous synthesis during the labeling period. The fraction (f) from endogenous synthesis during a time interval of labeling is calculated as EM_x/A_x^*.

3) Determination of the rate constant(s) of labeled polymer removal (k) from the pool, in order to transform fractional into absolute synthesis rates. The mathematics of high-mass isotopomers are useful for this purpose (13). High-mass isotopomers much more closely fulfill the assumption of flash-labeling (that is, cessation of label incorporation almost immediately after discontinuing label administration), since the frequency of an M_3 or M_4 isotopomer, for example, is roughly a function of p to the 3rd or 4th power. At low values of p, the frequency of newly produced M_3 or M_4 isotopomers, therefore, falls off rapidly toward zero, and true rate constants of decay can be determined for these masses, uninfluenced by persistent label incorporation (13). Having established k, absolute synthesis rates can be calculated by using standard equations for the nonsteady state (13, 24) and estimates or measurements of the pool size.

A sample calculation is presented (**Table 2**), wherein mass spectrometric data are converted into an estimate of cholesterol synthesis rates. First, fractional abundances for mass isotopomers M_0–M_4 (*m/z* 368–372, ref. 22), are calculated. Then baseline fractional abundances are sub-

TABLE 2. Conversion of MS data into cholesterol synthesis rates

		Time							
		Incorporation Phase			Decay Phase				
Parameter	Baseline	0900	1300	1700	2000	2200	2400	0800 D3	0800 D4
1) MS abundances									
m/z 368	1.000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
m/z 369	0.3187	0.3351	0.3348	0.3345	0.3324	0.3308	0.3286	0.3270	0.3206
m/z 370	0.0534	0.0664	0.0653	0.0653	0.0632	0.0616	0.0616	0.0589	0.0548
m/z 371	0.0075	0.0129	0.0124	0.0123	0.0116	0.0109	0.0107	0.0099	0.0084
m/z 372	0.0025	0.0039	0.0038	0.0039	0.0036	0.0036	0.0033	0.0029	0.0025
2) Fractional abundances (M_x)									
M_1 (369)	0.2306	0.2363	0.2364	0.2362	0.2356	0.2351	0.2340	0.2338	0.2313
M_2 (370)	0.0386	0.0468	0.0461	0.0461	0.0448	0.0438	0.0439	0.0421	0.0395
M_3 (371)	0.0054	0.0091	0.0088	0.0087	0.0082	0.0077	0.0076	0.0071	0.0061
M_4 (372)	0.0018	0.0027	0.0027	0.0028	0.0026	0.0026	0.0024	0.0021	0.0018
3) Excess fractional abundances (EM_x)									
EM_1		0.0057	0.0058	0.0056	0.0050	0.0045	0.0034	0.0032	0.0007
EM_2		0.0082	0.0075	0.0075	0.0062	0.0051	0.0052	0.0035	0.0009
EM_3		0.0037	0.0033	0.0033	0.0028	0.0023	0.0022	0.0017	0.0006
EM_4		0.0009	0.0009	0.0009	0.0007	0.0007	0.0005	0.0003	0.0000
4) p									
From EM_1/all		0.0832	0.0773	0.0789	0.0752	0.0718	0.0849		
From EM_3/all		0.0832	0.0795	0.0785	0.0793	0.0757	0.0805		
From EM_4/all		0.0787	0.0779	0.0817	0.0783	0.0849	0.0757		
Average EM_1/all + EM_3/all		0.0832	0.0784	0.0787	0.0773	0.0738	0.0827		
5) A_1^*		0.1426	0.1473	0.1471					
6) f (%)		3.98	3.94	3.83					
7) f (Fed-corrected) (%)				1.17					

The value for p is calculated from cholesterol reference tables (22) for [1-^{13}C]acetate by using the ratios of $EM_x / \sum_{i=0}^{n} EM_i$. A_1^* is calculated from the same reference tables. Pool size of free cholesterol is estimated to be 9 g (see text). f = EM_1/A_1^*. Corrected f is calculated as the difference from expected f if no interval synthesis had occurred. The decay rate constant (k) is calculated from the best fit exponential for the decay time points by using the average of M_2–M_4 mass isotopomers. $K = f \cdot \text{pool size} \cdot [k/(1 - e^{-kt})]$. Calculated parameters in this subject were $k = 0.0536\ h^{-1}$; K = 60.7 mg/h (night-time), K = 15.3 mg/h (day-time), K = 822 mg/day.

tracted, leaving excess fractional abundances (EM_x). The distribution or pattern of EM_x is characterized by ratios (R) among EM_x. As each value of R is uniquely associated with a value for p and for A_x* (13, 15), p and A_x* can be determined from a reference table (22) or best fit equations. Fractional decay rate (k) is calculated by best fit to the average of the EM_2 through EM_4 decay curves after discontinuing label administration. Absolute cholesterol synthesis rate (K) is then calculated from f, k, and estimated pool size according to the equations described (Table 2 legend).

Analytic considerations

Several features of this technique allow for a high degree of reproducibility for the biosynthesis estimates, even at low product enrichments. First, using quadruplicate analysis, the standard error of GC–MS measurements in our hands is less than 0.03 molar percent excess (MPE) for cholesterol and for FAME (14, 16, 17, 22). Also, fasted and fed measurements in the present studies were based on analyses of two to three time points each, and the derived estimates of p exhibit less than a 15% variation between time points. In addition, p for cholesterol was based on the average of two independent measurements ($EM_1/\Sigma EM_{1-4}$ and $EM_3/\Sigma EM_{1-4}$), which correlate extremely closely (r^2 = 0.89, ref. 22). This serves as an internal replicate for calculating p and A_1*. Finally, an additional analytic advantage is provided by a unique feature of the MIDA technique, which is particularly useful for cholesterol synthesis. The relationship between p and A_1* can act as a buffer against experimental error if there is a relatively flat portion of the curve around the value for p that is achieved under a particular labeling protocol (13, 22). The possibility of asymptotic product enrichments that are essentially independent of precursor enrichments over a discrete range represents a unique feature of mass isotopomers for which there is no analogy using radioisotopes, or stable isotopes analyzed as monomeric subunits, as we have discussed in detail previously (13). In the case of cholesterol, a relative plateau of this type exists in the graph of p versus cholesterol-EM_1, over the range of about p = 0.035 to p = 0.090 (see Fig. 5A, ref. 13 or Table 1, ref. 22). This is the range of p attained in human subjects at the [^{13}C]acetate infusion rates that we used here (see below). The effect of this feature of MIDA is that analytic imprecision resulting in an error of 100% in the estimate of p (p doubling from 0.04 to 0.08, for example) results in a variation in A_1* in the calculated fractional synthesis rate of only 8% (13), when operating in this range. As a consequence of these considerations, estimates of p and fractional synthesis are highly reproducible even at low product enrichments.

Whole body cholesterol model

The whole body cholesterol model that we used has been described in detail previously (22). The discussion of cholesterol pool size estimates is reviewed here. The key point is that cholesterol pools for isotopic studies are functional or mathematical constructs and the appropriate pool size to use depends upon the isotopic experiment performed. Goodman and colleagues (25) have developed equations for estimating total body cholesterol pool size in long-term (10 week–50 week) studies of plasma decay curves after [^{14}C]cholesterol injection. Their central, exchangeable pool of cholesterol (pool 1) includes cholesteryl esters as well as free cholesterol in plasma, blood cells, liver, intestines, and other pools in "fairly rapid equilibrium with plasma cholesterol" (25) and is estimated to be ~25 g. This value is far too high an estimate of FC for short-term endogenous labeling studies, such as ours. The best estimates of functional pool size for such short-term experiments derive from earlier studies using [^{14}C]acetate, such as performed by Gould et al. (26) or Hellman, Rosenfeld, and Gallagher (27). Since we measured incorporation of label into FC only and treated conversion into labeled cholesteryl ester as a route of exit (removal or decay), the most appropriate pool size is, roughly speaking, FC in liver, plasma and red cells, as these FC pools are in rapid equilibrium in humans (26–28) as well as animals. This FC pool has been estimated at ca. 9.0 g (3.8–4.3 g liver, 1.75 g plasma, 3.0 g red cells, refs. 26, 27). Hypercholesterolemia increases this pool size slightly, by increasing the plasma pool size, but none of our subjects were hypercholesterolemic. The effect of obesity per se on this pool is hard to estimate but the correlation equations of Goodman et al. (25) between obesity and their central cholesterol pool for long-term decay studies certainly cannot be applied (these pool sizes ranged from 25 to 35 g, depending upon body weight). For simplicity, we chose a 9-g pool size for all subjects, although slightly larger liver and blood volumes could be factored in for obese subjects. Choice of larger pool sizes would lead to proportionally higher estimates of cholesterol synthesis but should not alter estimates of periodicities within individuals.

Thus, it is important to state explicitly what is measured with the short-term isotope incorporation method used here. We measure here the amount of cholesterol in serum (really in liver, red cell, and serum) that derived from endogenous synthesis over the course of 18 h. We do not measure total body cholesterol synthesis, because tissue cholesterol synthesis that does not leave the cell or equilibrate with the serum compartment is not measured. Although absolute quantitative certainty may be difficult to achieve for cholesterogenesis by any method, due to the complexity of whole body pools and uncertainties regard-

ing their sizes and metabolic interactions (25), this method is especially well suited for longitudinal studies.

RESULTS

Comparison of precursor pools for cholesterogenesis and lipogenesis

The acetyl-CoA pool for cholesterogenesis was compared to the pool for lipogenesis in subjects given intravenous and oral [^{13}C]acetate. There were no consistent differences between calculated cholesterol and VLDL-palmitate precursor acetyl-CoA enrichments in subjects given intravenous [^{13}C]acetate (**Fig. 2**). In contrast, oral [^{13}C]acetate administration resulted in a higher calculated precursor subunit enrichment for VLDL-palmitate than for cholesterol in every subject studied. The precursor enrichment for VLDL-palmitate was 16.7 ± 0.8 MPE while for cholesterol it was 13.1 ± 0.93 MPE ($P < 0.05$ by matched-paired *t*-test). These results suggest a splanchnic site for synthesis of VLDL-palmitate in humans with a contribution from extrasplanchnic tissues to the synthesis of serum cholesterol.

Endogenous cholesterol synthesis

The absolute rate of endogenous synthesis of serum cholesterol was measured by combining measurements of fractional incorporation with decay rate-constants (Table 2 and **Table 3**). Conversion of primary GC-MS data into fractional and absolute rates of cholesterol synthesis is shown in Table 2. Absolute cholesterol synthesis rates tended to be higher during the overnight fasting period (2:00 AM–9:00 AM) than during the day-time feeding period (9:00 AM–6:00 PM). In luteal-phase women (n = 5), the rates of day-time and night-time cholesterogenesis were 40.7 ± 10.3 and 14.4 ± 3.9 mg/h, respectively ($P < 0.01$). In the follicular-phase (n = 6), rates were 38.9 ± 6.2 and 15.8 ± 4.3 mg/h ($P < 0.01$), respectively, as reported previously (22). Night-time cholesterogenesis was 37.5 ± 5.8 mg/h (mean ± SE, n = 8) in normal men, while during the day-time it was 23.0 ± 3.0 mg/h (NS by paired *t*-test). Assuming a typical 10-h overnight fast and 14-h postprandial state, daily endogenous cholesterogenesis in luteal-phase women was estimated to be 608 ± 146 mg/d (9.9 ± 3.6 mg/kg/day) and the rate in the follicular phase was not significantly different (610 ± 62 mg/day, 9.8 ± 1.5 mg/kg/day, NS by paired *t*-test). In normal-weight men daily cholesterogenesis was 697 ± 55 mg/day (9.5 ± 0.8 mg/kg/day). In obese men, daily cholesterol synthesis was similar (621 ± 111 mg/day, 6.0 ± 1.1 mg/kg/day). It should be emphasized that these were all free-living subjects not on controlled diets, although dietary cholesterol intakes estimated from 7-day weighed food records were <400 mg/day. The lack of control over diet and the selection of normolipidemic obese subjects precludes group comparisons for determining the effect of obesity on cholesterol synthesis.

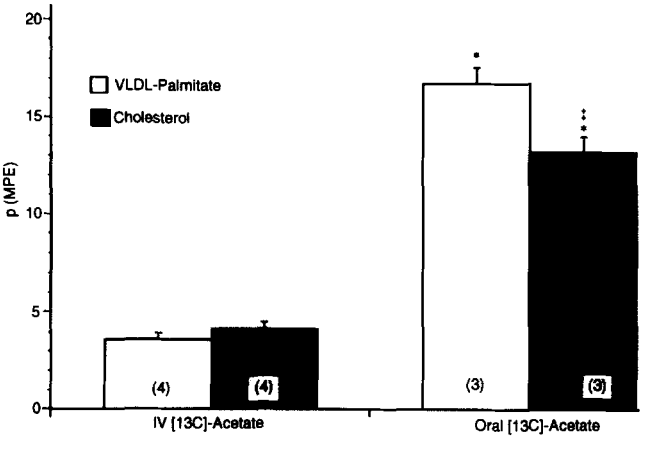

Fig. 2. Comparison of p values for free cholesterol and VLDL-palmitate in blood from human subjects given [^{13}C]acetate, once by the oral route and once by the IV route. See text for details. Number of subjects is indicated in parentheses. Data shown are mean ± SE. *$P < 0.01$ by matched paired *t*-test versus IV acetate. ‡$P < 0.05$ by matched paired *t*-test versus VLDL-palmitate.

De novo hepatic lipogenesis

The fraction of circulating VLDL-palmitate synthesized endogenously during overnight fasted and fed states was also determined in normal women (luteal and follicular menstrual phases), in normal men, and in obese men. The enrichments of hepatic acetyl-CoA (p) and the fractional contribution (f) to VLDL-palmitate from de novo lipogenesis are shown (**Table 4**). The values for de novo hepatic lipogenesis observed in normal men are similar to those we have reported previously (16). Women in the luteal menstrual phase had rates of de novo lipogenesis similar to those of men, but follicular phase lipogenesis was significantly higher in both fasted and fed states (Table 4). The higher follicular lipogenesis was partially but not completely explained by levels of circulating estradiol ($r^2 = 0.39$, $P < 0.05$) measured on the morning and evening of the study. Serum progesterone concentrations did not exhibit a significant correlation with fractional de novo lipogenesis ($r^2 < 0.02$). There were no differences in whole-body substrate oxidation rates calculated by indirect calorimetry between the menstrual phases in women, and non-protein RQ was <1.0 in all subjects, indicating that net lipogenesis was not occurring. Obese men exhibited significantly higher de novo lipogenesis than lean men, and there was a strong positive correlation ($r^2 = 0.59$, $P < 0.01$) between lipogenesis and body mass index for men (**Fig. 3**).

TABLE 3. Endogenous cholesterol synthesis in men and women

Group/Subject No.	Cholesterol Enrichments (M.P.E.) EM_1	EM_2	EM_3	EM_4	p (M.P.E.)	A_1* (M.P.E.)	f	k	K	K	K
							%	h^{-1}	mg/h	mg/day	mg/kg/day
Men											
Normal (n = 8)											
1. Fasted	0.43	0.64	0.29	0.11	6.73	14.20	3.05		45.4		
Refed	0.68	0.82	0.40	0.14	6.29	14.73	4.65	0.0427		887	11.2
Refed/corrected							2.57		31.0		
2. Fasted	0.44	0.52	0.13	0.00	4.27	15.32	2.90		41.7		
Refed	0.55	0.67	0.24	0.02	5.31	15.45	3.57	0.0326		645	8.4
Refed/corrected							1.41		16.3		
3. Fasted	0.26	0.39	0.15	0.02	6.21	14.81	1.73		25.3		
Refed	0.68	0.78	0.30	0.07	5.34	15.43	4.40	0.0382		777	10.0
Refed/corrected							3.17		37.5		
4. Fasted	0.15	0.23	0.11	0.06	7.04	13.77	1.11		16.8		
Refed	0.45	0.53	0.19	0.06	5.31	15.45	2.94	0.0486		584	7.5
Refed/corrected							2.19		29.7		
5. Fasted	0.37	0.22	0.09	0.00	3.35	14.30	2.61		36.4		
Refed	0.41	0.23	0.07	0.02	2.39	12.10	3.42	0.02334		566	7.2
Refed/corrected							1.30		14.4		
6. Fasted	0.28	0.19	0.07	0.03	3.41	14.36	1.97		30.0		
Refed	0.44	0.40	0.17	0.03	4.93	15.52	2.81	0.04947		582	8.3
Refed/corrected							1.48		20.2		
7. Fasted	0.75	0.55	0.18	0.40	3.59	14.65	5.11		71.2		
Refed	0.83	0.61	0.20	0.04	3.48	14.51	5.73	0.02328		958	13.6
Refed/corrected							1.59		17.6		
8. Fasted	0.33	0.43	0.16	0.04	5.72	15.23	2.16		33.5		
Refed	0.41	0.47	0.21	0.06	5.89	15.10	2.69	0.05542		580	13.6
Refed/corrected							1.38		17.5		
Mean ± SE											
Fasted					5.04 ± .55		2.58 ± .43	0.0392	37.5 ± 5.8	697 ± 55	9.5 ± 0.8
Refed/corrected					4.87 ± .46		1.89 ± .24	± 0.0043	23.0 ± 3.0		
Obese (n = 4)											
1. Fasted	0.50	0.36	0.15	0.04	4.18	15.27	3.25		46.4		
Refed	0.69	0.63	0.26	0.07	4.88	15.52	4.43	0.0352		771	7.4
Refed/corrected							2.22		21.9		
2. Fasted	0.35	0.26	0.07	0.03	3.09	13.82	2.53		34.5		
Refed	0.24	0.23	0.07	0.02	4.27	15.30	1.58	0.0173		345	3.3
Refed/corrected							0.00		0		
3. Fasted	0.42	0.37	0.10	0.01	4.69	15.08	2.76		39.7		
Refed	0.41	0.29	0.08	0.02	3.84	14.02	2.95	0.0324		541	5.3
Refed/corrected							0.89		10.3		
4. Fasted	0.80	0.61	0.22	0.04	3.81	14.93	5.37		74.8		
Refed	0.72	0.55	0.18	0.04	3.65	14.75	4.87	0.0232		828	8.1
Refed/corrected							0.51		5.7		
Mean ± SE											
Fasted					3.94 ± .34		3.47 ± .65	0.0270	48.9 ± .90	621 ± 111	6.0 ± 1.1
Refed/corrected					4.16 ± .27		0.91 ± .47	± .0041	9.4 ± 4.6[a]		
Women											
Luteal phase (n = 5)											
1. Fasted	0.34	0.28	0.11	0.01	3.88	15.39	2.23		32.9		
Refed	0.47	0.62	0.32	0.08	6.75	14.18	3.34	0.04047		635	7.0
Refed/corrected							1.66		21.9		
2. Fasted	0.80	0.64	0.19	0.04	3.44	14.58	5.50		81.5		
Refed	0.87	0.74	0.27	0.05	3.50	15.30	5.69	0.04171		1,139	23.9
Refed/corrected							1.75		23.2		
3. Fasted	0.35	0.49	0.22	0.07	6.98	14.55	2.42		33.7		
Refed	0.51	0.57	0.24	0.06	5.37	15.28	3.32	0.02345		556	8.7
Refed/corrected							1.27		15.7		
4. Fasted	0.33	0.31	0.12	0.03	6.06	15.63	2.08		24.8		
Refed	0.60	0.38	0.16	0.06	4.76	15.13	2.03	0.01505		282	3.8
Refed/corrected							0.21		2.5		
5. Fasted	0.27	0.51	0.28	0.10	8.15	11.88	2.24		30.7		
Refed	0.33	0.58	0.30	0.10	7.77	12.57	2.64	0.01840		427	6.0
Refed/corrected							0.71		8.6		

TABLE 3. Continued

Mean ± SE						
Fasted	5.70 ± .90	2.89 ± 0.65	0.2782	40.7 ± 10.3	608 ± 146	9.9 ± 3.6
Refed/corrected	5.63 ± .75	1.12 ± 0.29	± 0.00558	14.4 ± 3.9[a]		
Follicular phase (n = 6)[b]						
Mean ± SE						
Fasted	6.53 ± 0.51	2.73 ± 0.41	0.02915	38.9 ± 6.2	610 ± 62	9.8 ± 1.5
Refed/corrected	6.47 ± 0.35	1.25 ± 0.34	± 0.00489	15.8 ± 4.3[a]		

Infusions of [^{13}C]acetate and analysis of plasma free cholesterol by GC–MS were performed as described in the text and elsewhere (22). Abbreviations used: M.P.E., molar percent excess; p, isotopic enrichment of precursor; A_1*, asymptotic enrichment of M_1-cholesterol isotopomer if 100% of cholesterol were newly synthesized from acetyl-CoA units at enrichment p; f, fraction of cholesterol derived from endogenous synthesis; k, rate constant for exit or labeled cholesterol from plasma, based on decay curves of higher mass isotopomers (22); K, absolute synthesis rate of plasma cholesterol. Fasted refers to value after 7 h of [^{13}C]acetate infusion during an overnight fast; refed refers to value after 9 h of continued [^{13}C]acetate infusion during day-time refeeding with liquid mixed meal formula (Ensure®), given hourly for 9 h (16, 22); refed, corrected refers to calculated fractional synthesis (k) and absolute synthesis (K) rates during the day-time refeeding period, after correction for decay of labeled cholesterol synthesized during the overnight fasting period (22).

[a] $P < 0.05$ versus overnight fasted values.

[b] Individual data from five of the six follicular-phase women have been reported previously (22), so only mean ± SE results are included here.

Obese men were studied twice, in order to compare the effect of IV and oral [^{13}C]acetate administration routes. The two studies in obese subjects were performed in randomized sequence, 2 weeks apart: once with IV [^{13}C]acetate at 0.070 mmol/kg/h and once with oral [^{13}C]acetate at 0.086 mmol/kg/h. Oral administration of labeled acetate resulted in markedly higher hepatic enrichments for acetyl-CoA entering VLDL-palmitate (p = 15.7 ± 1.7 MPE, mean ± SE, Table 4) than was observed with IV administration of labeled acetate (p = 3.58 ± 0.34 MPE) but calculated lipogenesis was similar in the two studies for three of the four subjects (12.2 ± 0.7% from IV [^{13}C]acetate vs. 11.2 ± 0.8% from oral [^{13}C]acetate, NS). The fourth subject had markedly higher lipogenic rates during the IV [^{13}C]acetate study (29.4% vs. 10.0%), but the group differences were still not significantly different (16.5 ± 4.3% vs. 10.8 ± 0.8%, NS). These observations confirm the importance of measuring precursor acetyl-CoA enrichment if lipogenesis is to be measured accurately. That raw isotope incorporation data can be misleading, with regard to true lipogenesis, can be seen in **Fig. 4.** Oral [^{13}C]acetate resulted in much higher enrichments of EM_1 and EM_2-palmitate, but the higher relative

TABLE 4. De novo hepatic lipogenesis in women, normal weight men, and obese men

Group	P (MPE)	f (%)
Women		
Follicular phase (n = 7)		
ON fasted	5.09 ± 0.36	4.2 ± 1.4[a]
Refed	5.03 ± 0.27	9.7 ± 1.6[a,b]
Luteal phase (n = 6)		
ON fasted	5.97 ± 0.63	1.5 ± 0.6
Refed	6.35 ± 0.67	3.5 ± 0.7[b]
Normal weight men (n = 7)		
ON fasted	4.41 ± 0.57	0.86 ± 0.21
Refed	4.27 ± 0.27	4.94 ± 1.93[b]
Obese men (n = 4)		
Intravenous [^{13}C]acetate		
ON fasted	3.75 ± 0.43	6.9 ± 2.8[a]
Refed	3.35 ± 0.45	16.5 ± 4.3[a,b]
Oral [^{13}C]acetate		
Refed	15.7 ± 1.7	10.8 ± 0.8[a]

Stable isotope infusions and GC–MS analysis of palmitate-methyl ester from plasma VLDL were performed as described in the text. Abbreviations: ON, overnight; p, enrichment of precursor acetyl-CoA subunits that entered newly synthesized VLDL-palmitate molecules; f, fraction of circulating VLDL-palmitate derived from endogenous synthesis. Data shown are mean ± SE.

[a] $P < 0.05$ versus normal weight men.

[b] $P < 0.05$ versus ON fasted

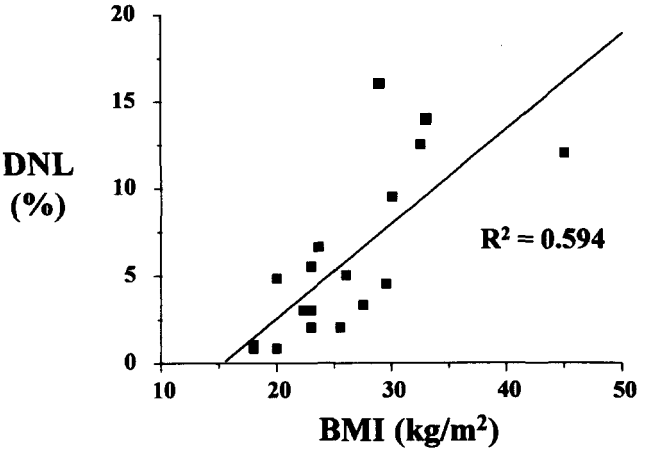

Fig. 3. Relationship between body mass index (kg/m²) and de novo hepatic lipogenesis in men.

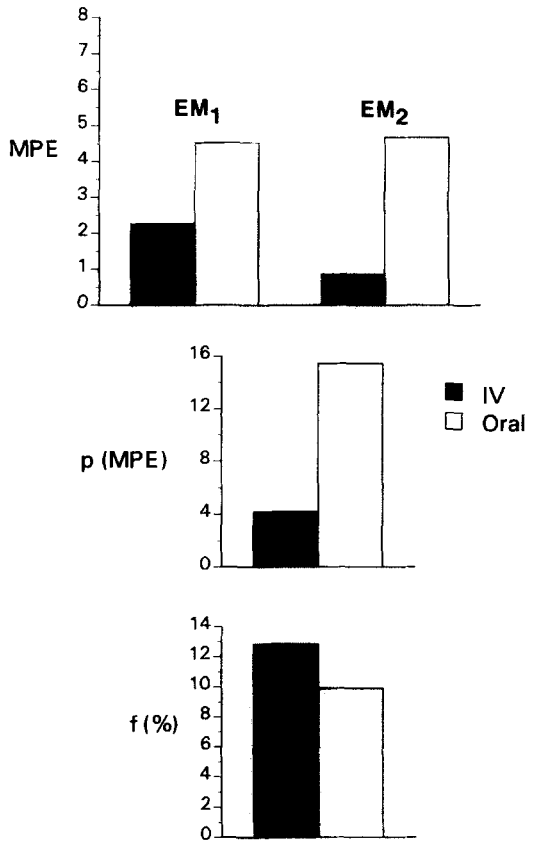

Fig. 4. Comparison of IV and oral administration routes of [^{13}C]acetate in an obese male subject. Precursor enrichments (p) for VLDL-palmitate and calculated fractional synthesis rates (f) are shown.

proportion of EM_2 to EM_1 indicated a more highly enriched acetyl-CoA pool, so that lipogenesis was actually slightly lower during the oral labeling study. A second implication of these results is that de novo lipogenesis did not appear to vary over time in most obese men, in contrast to women (Table 4). Finally, the absence of increased lipogenesis in the presence of higher precursor enrichments from the oral labeling protocol makes it unlikely that the experimental introduction of 5–15% [^{13}C]acetate in the precursor pool itself stimulates lipogenesis.

DISCUSSION

We recently reported a diurnal variation in cholesterogenesis in follicular-phase women (22). Here, we extend these findings to luteal-phase women and to men. Measurements of fractional synthesis using ^2H_2O incorporation (9), as well as several indirect methods (3–6), have indicated qualitatively that cholesterol synthesis exhibited diurnality, although none of these methods were able to generate a quantitative estimate of the variation. Incorporation of ^2H_2O into cholesterol has revealed fractional synthesis from the ratio of ^2H in cholesterol to ^2H in body water (9), equivalent to our parameter f (Table 2). This parameter underestimates true fractional synthesis to the extent that newly synthesized FC exchanges with unlabeled cholesterol (e.g., in circulating cholesteryl esters, membrane cholesterol, FC in other cells, etc.). The exit rate of labeled cholesterol from the FC pool is quite high, as evidenced by the nearly equal net entry of label into circulating cholesteryl esters compared to FC from either labeled acetate (25, 26) or water (9, 10). This isotope exchange is why we measured the decay rate constant (k) from the die-away curve of high-mass isotopomers of FC, so that absolute synthesis rates could be calculated (13). Decay rate constants cannot be readily calculated after stopping ^2H_2O administration, because of the extremely slow turnover of the precursor (the body water pool). It is interesting to compare the estimates of f (uncorrected fractional synthesis) using MIDA to those from ^2H_2O incorporation. Jones and Schoeller (9) reported f $\cong$ 0.05 d^{-1}, which is essentially identical to our results (ca. 0.03–0.04 after 17 h). These workers also reported that almost as much labeled cholesterol appeared in the total circulating cholesteryl ester pool as in FC. Our estimates of the rate constant for isotopic FC exit (k, Table 2) were ca. 0.03–0.04 h^{-1}, or $t_{1/2} \cong$ 17–23 h. A 17-h study, therefore, represents almost one half-life, so that nearly as much labeled cholesterol should be present in other pools as in FC, which fits nicely with what Jones and Schoeller (9) and others (26, 27) have reported previously.

Absolute cholesterol synthesis rates estimated using MIDA were also similar to previous estimates of 8–11 mg/kg/day, mostly performed in men, using the sterol balance technique (1, 2). Although we did not measure cholesterol absorption from the diet, this is likely to have been in the range of 200 mg/day in those subjects, based on their estimated dietary intakes. At least 2/3–3/4 of circulating cholesterol must therefore derive from endogenous synthesis in men and women. The isotopic evidence and previous work in animals as well as humans (2, 28, 29) suggest that extrasplanchnic tissues contribute to endogenous cholesterogenesis, although their relative contributions remain uncertain. The quantitative contribution by the liver might be directly evaluated in humans through techniques used in animals for manipulating hepatic cholesterogenesis (2, 29), e.g., feeding cholesterol or administration of bile acid-binding resins, in combination with MIDA.

Some caveats are in order regarding the quantitative values presented. Calculations of diurnality for serum cholesterol synthesis by isotope incorporation are by their nature somewhat uncertain because it is not possible to know exactly when the labeled cholesterol appearing in the serum was synthesized. We observed labeled cholesterol appearing in serum within about 2–3 h after initiating infusions of [^{13}C]acetate (not shown), so the time lag

between synthesis and serum appearance is probably of this general magnitude or less. Accordingly, we considered the 0900 and 1100 time points as representing cholesterol synthesis that occurred overnight (the start of isotope administration being 0200) and time points thereafter as representing synthesis during the fed state. This is a somewhat arbitrary distinction and may have blurred diurnal differences slightly. Even so, diurnal variation was observed (Fig. 1, Table 3). It should be emphasized that the decision to divide synthesis into two time intervals (overnight/fasted and day-time/fed) requires no kinetic assumptions regarding the two periods, i.e., there does not need to be a discrete, step-wise change in synthetic rates. Rather, our precursor incorporation method quantifies the fraction of newly synthesized cholesterol molecules accumulating in the serum between 0200 and 0900 then between 0900 and 1800. All else being equal (i.e., relatively constant p and k), an average value for f will be reflected in the interval value, even if diurnal changes were gradual and were not bracketed exactly by the experimental protocol. We documented that p is stable and it seems reasonable to assume k to be fairly constant, since k mostly reflects exchange and distribution processes between cholesterol in serum and other pools, rather than receptor-mediated irreversible uptake or other regulated processes (whose k is much lower than the isotopic k that we measured).

Other caveats regarding quantitative accuracy of absolute cholesterol synthesis measurements derive from two areas of uncertainty. First is the inherent imprecision of fitting exponentials to decay curves, which was required for estimating k. Calculated decay rate constants may vary according to the curve-fitting program used and tend to be sensitive to experimental error in the tail-end of the decay curve (13). An alternative method for estimating k (turnover of the serum FC pool) is available with MIDA, namely calculating the rate constant for the rise in FC labeling toward its plateau value (13). This approach would require a different blood sampling protocol but is otherwise identical to the method described here and has been used for other products with MIDA (e.g., VLDL-triglyceride turnover, Schwarz, J-M., R. Neese, M. K. Hellerstein, unpublished observations). The second area of uncertainty derives from estimates of total body cholesterol pool size, as discussed above. The relevant body pool of FC for short-term isotope incorporation studies is relatively small (reflecting plasma, liver, and red blood cells) compared to published pool sizes from long-term labeled cholesterol decay studies (25–27) and is not markedly affected by changes in serum cholesterol concentrations or body cell mass. However, any uncertainties due to variability in pool sizes would have far more important effects on comparisons between groups than on sequential changes within individual subjects.

Diurnality for cholesterogenesis may have therapeutic implications. These results and the data using ^2H_2O incorporation (9, 10) support the concept that use of HMG-CoA reductase inhibitors as hypocholesterolemic agents should be directed against night-time cholesterol synthesis. This will need to be confirmed experimentally, however, and reconciled with the uncertainty whether the mechanism of action of HMG-CoA reductase inhibitors is through reduced cholesterol synthesis or increased receptor-mediated clearance (30, 31). Also, it is interesting that not every subject exhibited greater cholesterogenesis at night than during the day-time (Table 1). A reversed pattern was observed in 2 of 8 normal men, 0 of 5 luteal-phase women, 1 of 6 follicular-phase women, and 0 of 4 obese men, for a total of 3 subjects with reversed diurnal pattern out of 23 subjects studied. More intensive study of these anomalous subjects may prove rewarding.

The menstrual effect on de novo hepatic lipogenesis is interesting for a number of reasons. First, it affirms the importance of establishing menstrual phase for future studies of lipogenesis in women. Second, the correlation with circulating estrogen concentrations suggests a hormonal effect. Previous studies in animals have indicated that sex steroids influence lipid metabolism, but reports have not all been consistent (32–34). Another possibility would be changes in diet over the course of the monthly cycle in these free-living subjects. It will be of interest to study menstruating women under metabolic ward conditions while maintained on a constant diet (Hellerstein, M. K., R. Neese, J-M. Schwarz, unpublished observations). Finally, other possible modulators could be involved which we did not measure. De novo hepatic lipogenesis might represent a useful integrative metabolic index of the hormonal and/or dietary milieu in menstruating women.

The menstrual periodicity observed for de novo lipogenesis has somewhat ambiguous nutritional implications, however. The absolute rate of lipogenesis observed was still a low value, even in follicular-phase women or obese men (approximately 2–4 g/day based on an assumed VLDL-triglyceride turnover rate of 20–25 g/day, as discussed in ref. 16), in comparison to the fat content of Western diets. One can calculate that the "fat burden" placed on women over the course of a year would be in the range of 1 or 2 extra pounds which would have to be oxidized in addition to dietary fat to avoid alteration in body composition. This is a small fraction of the yearly fat intake.

Comparison between cholesterol and VLDL-palmitate precursor enrichments was also revealing. The 3- to 4-fold higher efficiency of oral [^{13}C]acetate than IV [^{13}C]acetate in labeling the VLDL-fatty acid precursor pool (Fig. 2) presumably reflects first-pass splanchnic uptake of labeled acetate, which otherwise is predominantly used by extrahepatic tissues (35–37). The significantly lower enrichment of acetyl-CoA units entering plasma FC than those entering VLDL-palmitate during oral [^{13}C]acetate ad-

ministration (Fig. 2) must reflect cholesterogenesis in tissues with a lower precursor enrichment (e.g., extrasplanchnic). Although this is only qualitative evidence, it supports the view that, in humans, de novo lipogenesis occurs mostly in the splanchnic bed (e.g., the liver) whereas endogenous cholesterol synthesis is both splanchnic and extrasplanchnic (26–28, 38–41).

We have emphasized comparisons within subjects over time (periodicities) here rather than comparisons between groups because these studies were not designed to compare groups. Dietary factors were not controlled or standardized between groups, subjects were not matched for serum lipid parameters, and the number of subjects was also low. Power calculations can be carried out to estimate the differences that would have been detectable with the variability of our current data set. Setting power at 0.80 (β = 0.20) and α = 0.05, 13 subjects/group would have been necessary to detect a 25% difference in cholesterol synthesis rates (i.e., 625 vs. 500 mg/day) or 8 subjects/group to detect a 33% difference. For men versus follicular-phase women (unpaired data), 14 subjects would have been necessary to detect group differences of 33%. At the current number of subjects (n = 6–8/group), group differences of 168 mg/day ($\cong$25% difference) would have been of borderline significance. Thus, it is not justified to conclude, with the current sample size and lack of dietary standardization, that obese men have similar cholesterol synthesis rates as nonobese men, in contrast to previous reports (25), or that men and women have the same cholesterol synthesis rates. Future studies using MIDA could address these questions, however, if used in properly designed experiments.

In summary, endogenous synthesis of fat is influenced by body composition, menstrual cycle, and dietary state. Endogenous cholesterol synthesis also exhibits a diurnal rhythm (more than twice as great at night) but differs from endogenous fat synthesis in several important ways—quantitatively much more important than the dietary contribution, lack of apparent menstrual effect, opposite diurnal rhythm, and a contribution from extrahepatic tissues. Many questions remain unanswered regarding the physiologic regulation of these two biosynthetic pathways and their contribution to gender differences and to human diseases.

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