Dietary Fat Saturation, But Not the Feeding State, Modulates Rates of Cholesterol Esterification in Normolipidemic Men

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To determine whether the rates of cholesterol esterification in normal individuals are affected by diets differing in fats, nine men were randomly assigned to three groups receiving a diet rich in monounsaturated (MONO), polyunsaturated (POLY), or saturated (SAT) fat for 2 weeks using a crossover design. Subjects drank a dose of deuterium oxide, and the fractional esterification rate (FER) was calculated during fed and unfed periods. Total esterified cholesterol was calculated as the product of the FER and pool size, the latter obtained from a decay curve following injection of [4-^{14}C]-cholesterol. The POLY diet produced the lowest serum cholesterol concentration and the SAT diet the highest ($P < .001$). For cholesterol ester (CE) deuterium enrichment, an interaction was noted between diet and time ($P < .01$). The FER was greater ($P < .003$) in subjects fed the POLY diet versus either of the other diets, although the amount of esterified cholesterol produced, expressed as either milligrams per day ($P < .103$) or milligrams per kilogram of body weight per day ($P < .100$), did not differ among groups. No effect of the feeding state was found for either the FER ($P < .187$) or total esterified cholesterol expressed as milligrams per day ($P < .146$) or milligrams per kilogram of body weight per day ($P < .128$). The results suggest that the diet fat type, but not the feeding state, may be responsible for serum esterified cholesterol concentrations.

IN HUMANS, high levels of total serum cholesterol are viewed as a risk factor for coronary heart disease.[1] Consumption of monounsaturated (MONO) and polyunsaturated (POLY) fat is considered prudent because these fats reduce cholesterol concentrations, while saturated (SAT) fat has the opposite effect.[2] Several mechanisms are thought to be responsible for the changes in serum cholesterol induced by dietary fat.[2-6]

Of particular interest is whether cholesterol synthesis rates respond to shifts in dietary fat saturation. Until recently, no simple techniques have been available for monitoring short-term changes in cholesterol synthesis in humans. However, the deuterium oxide incorporation method[7] is a safe and practical tool for the investigation of human lipid metabolism. Its diagnostic accuracy has been validated against several methods, including sterol balance techniques.[8-12] Using deuterium, it has been shown that the rate of free cholesterol synthesis is greater in subjects fed diets high in POLY versus SAT fats, whether they are normal or hyperlipidemic[9-10,13]; the rates on MONO diets are intermediate. Additionally, free cholesterol synthesis is greater when subjects are fed versus unfed.[13]

Researchers have also been interested in factors that influence cholesterol esterification rates. Two thirds of serum cholesterol is esterified in normolipidemic individuals, and the proportion increases in hypercholesterolemic, hypertensive, and hypoalphalipoproteinemic humans.[14-15] Most cholesterol esterification occurs in high-density lipoprotein (HDL)-cholesterol proportionally to the activity of lecithin:cholesterol acyltransferase (LCAT); this in turn is closely correlated with plasma triglyceride (TG) and HDL-cholesterol concentrations.[16] An increased reactivity to LCAT has been reported in patients with coronary heart disease.[14] The question was posed as to whether the esterification rate in normolipidemic individuals is altered by consumption of diets rich in MONO, POLY, or SAT fats. Recent study also suggests that free cholesterol synthesis is subject to the feeding state.[13] It is of interest to determine if such a pattern is evident for cholesterol esterification rates.

The primary aim of this study was thus to determine whether cholesterol esterification rates assessed using deuterium incorporation are sensitive to the type of fat consumed, either MONO, POLY, or SAT. The secondary objective was to establish whether these rates are affected by the feeding state of the subjects. It was hypothesized that the rates of esterification in humans are directly correlated with both dietary fat saturation and the feeding state.

SUBJECTS AND METHODS

Subjects

Nine men (aged 25.6 ± 2.8 years) with a serum cholesterol concentration between 3.4 and 5.7 mmol/L were studied. Participants reported light daily physical activity, no drug intake, and no medical problems. Body fat was between 10% and 15%.[17] The body mass index was 20.9 ± 4.7 kg/m^2. The protocol was approved by the Clinical Screening Committee for Human and Other Studies Involving Human Subjects, University of British Columbia. The subjects signed informed-consent forms.

Protocol

The cholesterol pool size and rates of synthesis and turnover were determined on each subject as described previously.[13] Briefly, M(1) pool size was determined in each subject by taking a serum sample, combining it with 925 kBq [4-^{14}C]-cholesterol (New England Nuclear–Du Pont, Markham, Ontario, Canada) dissolved in ethanol, and reinjecting it into an antecubital vein.[18] Blood samples were drawn over a 9-month period, and duplicate 200-μl serum aliquots were counted for 10 minutes each using a liquid scintillation system. Data were converted to dpm, plotted as dpm per milligram of serum cholesterol versus time (days), and fitted to multiexponential equations using **CON**versational **S**imulation, **A**nalysis and **M**odeling (CONSAM).[19] Of particular interest was the size of each individual's M(1), or rapid turnover, cholesterol pool; it is the site of de novo cholesterol synthesis measured with

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deuterium.[20] Theoretically, changes in serum cholesterol concentrations are reflected in changes in the size of the M(1) pool.

Subjects were assigned to three groups using a randomized crossover design, and they ate all meals under supervision for 13 days. Subjects reported to the metabolic research unit three times daily to be fed, and were weighed periodically to ensure adequate consumption. On day 13, baseline blood samples were drawn, followed by prebreakfast administration of an oral dose of deuterium oxide (99.8 atom percent excess; ICN Biomedicals, Montreal, Quebec, Canada) at 0.7 g deuterium oxide/kg estimated body water. Using bioelectrical impedance analysis, body water was estimated as 73% of the fat-free mass.[17] Blood was drawn at 12-hour intervals for 48 hours. Day 13 was a feeding day, when three meals were consumed, whereas on day 14 no meals were eaten. Body water deuterium enrichment was maintained by consumption of lightly labeled water (1.4 g deuterium oxide/kg H_2O). Diet periods were separated by 8-week intervals, allowing body deuterium levels to stabilize. The three diet periods were embedded in the 9-month ^{14}C-cholesterol decay study.

Diets

The subjects' resting energy requirements and total energy needs were calculated using the Mifflin predictive equation[21] with an activity factor of 1.7.[22] Percent body fat was determined using a bioelectrical impedance analyzer (model 101; RJL Systems, Detroit, MI). Diet energy content was verified with isothermal bomb calorimetry (LECO AC300; LECO, St. Joseph, MO) using benzoic acid as a combustion standard.

The composition of the three test diets, MONO, POLY, and SAT, was based on Canadian Nutrient File data.[23] The composition of the diets is provided in Table 1. Each diet was formulated to contain 40% of energy as fat, 45% as carbohydrate, and 15% as protein. The main fats in the MONO, POLY, and SAT diets were olive oil, soft safflower margarine, and butter, respectively. Because these were the main fat sources in each diet, it was expected that each diet's individual fatty acid content would reflect what is customarily found in these fats. The POLY to SAT fatty acid ratio for the SAT diet was 0.5 and for the POLY diet 1.6. Meals were made using fresh, frozen, and canned ingredients weighed to the nearest 0.5 g. Fatty acid composition was assessed by gas-liquid chromatography (model 5890 Series II; Hewlett-Packard [HP], Palo Alto, CA) using flame ionization and a HP-5 capillary column (25-m length, 0.20-mm diameter, and 0.33-μm thickness).[24-25] Identification of fatty acids was performed by comparison of the retention times to those of authentic standards (Supelco, Bellefonte, PA). Column operating conditions during testing were as follows: split ratio 100:1, initial temperature 180°C, ramp 1°C/min to 210°C, hold 30 minutes, injector temperature 300°C, detector temperature 320°C, column flow rate 1 mL/min, and purge vent flow rate 5 mL/min. The carrier gas was helium, and nitrogen was a makeup gas.

Serum Lipid Determinations

Blood samples were taken at 12-hour intervals during each 48-hour test period. Serum total (Diagnostic Chemicals, Charlottetown, Prince Edward Island, Canada) and unesterified (Boehringer Mannheim Canada, Dorval, Quebec) cholesterol levels were measured in duplicate at 510 nm (model 111-050; Coleman Hitachi, Maywood, IL) using enzymatic techniques and certified standards.[26-27] Cholesterol ester (CE) levels were calculated as the difference between total and free cholesterol values. The interassay coefficient of variation (CV) for total cholesterol was 2.2% and the intraassay CV 0.9%; for unesterified cholesterol, the intraassay CV was less than 0.5%. Serum TG and HDL-cholesterol levels were assayed enzymatically.[28-29]

Table 1. Composition of Diets Consumed by Subjects During Each 2-Week Diet Period

Component	SAT	MONO	POLY
Protein (g/MJ)	9.4 ± 0.3	9.3 ± 0.3	9.4 ± 0.4
Carbohydrate (g/MJ)	27.5 ± 0.7	27.3 ± 0.5	27.3 ± 0.7
Fat (g/MJ)	10.8 ± 0.2	11.0 ± 0.1	10.7 ± 0.1
Fatty acid (%)			
C8:0	1.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
C10:0	3.1 ± 0.4	0.0 ± 0.0	3.5 ± 0.4
C14:0	7.2 ± 1.8	3.2 ± 0.6	2.5 ± 0.2
C16:0	24.1 ± 4.2	18.6 ± 1.8	13.3 ± 0.6
C18:0	10.0 ± 1.2	6.4 ± 0.6	9.3 ± 0.8
C18:1	32.5 ± 4.4	61.4 ± 1.9	24.2 ± 4.6
C18:2	21.7 ± 5.6	10.4 ± 1.3	47.1 ± 2.8

NOTE. Data are the mean ± SD (n = 6 replicate meals). Percentage (%) values for individual fatty acids are the mass relative to the total fatty acids identified.

Cholesterol Synthesis and Esterification Rate Determinations

The determination of free cholesterol synthesis rates using deuterium incorporation has been described.[13] Serum samples obtained at 24-hour intervals during each test period were used for measurement of cholesterol esterification.[7,30] Briefly, lipids were extracted from 3 to 4 mL serum and separated using thin-layer chromatography (TLC). Silica bands of esterified and unesterified cholesterol were scraped into separate tubes. Tubes containing esterified cholesterol were stored at −80°C until they could be saponified, at which point the silica was combined with 5 mL methanolic KOH, capped tightly, and boiled (100°C) for 30 to 40 minutes. After cooling, 3 mL H_2O and 13 mL petroleum ether were added per tube, which was shaken for 5 minutes and then centrifuged at 1,500 *g* for 5 minutes. The ether phase was removed and retained, and the extraction was repeated. Combined ether phases were dried under nitrogen and then plated on activated TLC to separate the cholesterol from the free fatty acids. Cholesterol bands were transferred to pre-annealed Pyrex (Corning Glassworks, Corning, NY) combustion tubes (18 cm × 6 mm) containing 500 mg cupric oxide (BDH Chemicals, Toronto, Ontario, Canada) and 2 cm of 1-mm diameter silver wire. The tubes were sealed under vacuum and heated at 520°C for 4 hours. The resulting combustion water was vacuum-distilled into pre-annealed Pyrex tubes containing 60 ± 5 mg zinc (Biogeochemical Laboratories, Indiana University, Bloomington, IN). The tubes were sealed and heated at 520°C for 30 minutes to reduce combustion water to hydrogen/deuterium gas. Plasma water deuterium oxide–enriched samples were diluted sevenfold to reduce the enrichment to the range of working standards. Microcapillary tubes with a 2-μL sample were distilled into Pyrex tubing sections containing zinc and heated at 520°C for 30 minutes. Enrichment of the hydrogen gas obtained from samples was determined by isotope ratio mass spectrometry (903D; Isomass, Cheshire, England), with an internal analytical error of 0.17 parts per thousand (‰) relative to standard mean ocean water (SMOW). The mass spectrometer was calibrated daily using SMOW, standard light Antarctic precipitation (SLAP), and Greenland ice sheet precipitation (GISP). Samples were analyzed in duplicate. The average SD of replicate deuterium enrichment measurements for body water and serum esterified cholesterol was 1.7 and 6.2 ‰, respectively, relative to SMOW.

The practional synthesis rate (FSR), defined as the proportion of the central, or M(1), pool replaced daily by newly synthesized cholesterol, and the fractional esterification rate (FER), defined as the proportion of newly synthesized cholesterol of the central, or M(1), pool esterified daily, were calculated as the change in product deuterium enrichment over time divided by the maximum possible enrichment, based on a linear rate of uptake of label into cholesterol.[7,8] The FSR was calculated

for each 12-hour period,[13] and the FER was calculated over each 24-hour period to allow the accumulation of newly formed free cholesterol for esterification. The equation calculates differences in deuterium enrichment of each tissue expressed as parts per thousand versus SMOW, while correcting for the absolute ratio of carbon to hydrogen atoms within the cholesterol molecule.

Deuterated water enters cells and equilibrates quickly with intracellular water. Little unlabeled water is generated intracellularly, allowing the cell precursor pool enrichment to equal that of the plasma.[8] The calculated rates of cholesterol synthesis depend on the rate of label incorporation per molecule of cholesterol. During the synthesis of cholesterol, hydrogen atoms from water are incorporated into the sterol molecule in three different ways. Seven atoms of hydrogen are incorporated directly from water and 15 atoms from NADPH, and eventually, hydrogen atoms from water are incorporated into the acetyl coenzyme A (CoA) pool, which can be used as a cholesterol precursor. If it is assumed that over a 48-hour period there is complete equilibration of deuterated water with plasma water and with NADPH but that the acetyl coA pool is not yet labeled, then 81% of the hydrogen atoms per cholesterol carbon atoms, ie, the H/C ratio, will be incorporated into the molecule from D.[5,8] Other assumptions that must be incorporated if this method is to be used include (1) the amount of recycling of label into other pools, such as acetate, during the time of interest; (2) the form of the mathematical equation, usually accepted as monoexponential over short periods, of D incorporation over time; and (3) the theoretical and actual maximum plasma cholesterol enrichment.[7]

Each subject's M(1) pool size was calculated using SAAM/CONSAM. The M(1) mass was then multiplied by the FER values to yield the absolute rate of esterification (ARE) of the free cholesterol produced daily. Total daily de novo esterified cholesterol synthesis was calculated as the product of the FER and the mass of the M(1) pool that is esterified. The latter was defined as two thirds of the total M(1) pool,[18] and was verified by measuring the proportion of serum cholesterol that was esterified and nonesterified.

Statistics

All data are expressed as the mean ± SD. Analyses were performed using SAS Version 6.04 (SAS Institute, Cary, NC); statistical significance was set at a P level of less than .05. Data were first tested for normality with the Kolmogorov-Smirnov D statistic.[31] All data sets were normally distributed except for serum TG and HDL-cholesterol concentrations. These were ranked and analyzed nonparametrically. All other data were analyzed parametrically. Diet trial data were tested with ANOVA for a factorial experiment, with diet and time as the factors. Analysis of covariance was used to compare the subjects' final body weight, with initial body weight as the covariate. Tukey's test was used for multiple comparison testing.

RESULTS

Subjects tolerated the test diets well with no complaints of gastrointestinal disorders. Neither initial nor final body weight varied among the three diet periods, nor did body weight differ from the beginning to the end of each diet period, as we reported previously.[13] Energy intake also did not differ among diets.[13]

The meals did not differ in protein, fat, or carbohydrate content. The SAT diet contained high concentrations of C8:0, C10:0, C14:0, and C18:0, the POLY diet was rich in C18:2, and the MONO diet was rich in C18:1.[13] The dietary fat content reflected the fatty acid profile of the main fats used in each of the different diets; as such, some fatty acids are more prevalent.

Serum cholesterol concentrations varied with the diet consumed ($P < .001$); the highest values were found for the SAT diet and the lowest values for the POLY diet.[13] Serum HDL-cholesterol concentrations also varied significantly with the diet consumed ($P < .05$), with the highest values for the MONO and POLY diets and the lowest values for the SAT diet.[13] The concentration of free and esterified serum cholesterol and the ratio of esterified cholesterol to total serum cholesterol are listed in Table 2. Differences were found among the three diets for the concentration of esterified cholesterol, with the level on the SAT diet being higher than the level on the PUFA diet ($P < .008$). Neither the free cholesterol level ($P < .74$) nor the ratio of esterified cholesterol to total serum cholesterol ($P < .17$) differed among the diets. Serum TG concentrations showed an effect of the time of sampling ($P < .001$). On all three diets, TGs were highest at the 12-hour sampling, 3 hours after a meal, and lowest at the 48-hour sampling, after the subjects fasted for 24 hours.[13] The fat diet consumed also had a significant effect on serum TG concentrations ($P < .03$); the highest values were found on the SAT diet and the lowest values on the POLY diet.[13]

Total body water deuterium enrichment remained at a constant level throughout each 48-hour test period. No effect of either diet or time was noted.[13] For CE deuterium enrichment (Fig 1), a significant interaction between diet and time was found ($P < .01$), and consequently, each diet type and sampling time was examined separately. On the MONO diet, the levels at 0 and 12 hours were lower than at all other times, while the levels at 24 hours were lower than those at 36 and 48 hours ($P < .001$). On the POLY diet, the levels at 0 and 12 hours were lower than those at 24, 36, or 48 hours ($P < .001$). On the SAT diet, the levels at 12, 24, 36, and 48 hours were higher versus the baseline and the levels at 48 hours were higher than at 12 hours ($P < .001$). No diet effects were found at either 12 hours ($P < .63$) or 24 hours ($P < .14$). At 36 hours, the levels on the SAT diet were lower than the levels on either the MONO or POLY diets ($P < .001$). At 48 hours, this pattern was repeated ($P < .005$).

FERs were higher when subjects consumed the POLY diet versus either the MONO or SAT diets ($P < .003$). The rates on the MONO versus SAT diet did not differ significantly. Esterification rates on all diets were not significantly affected by the feeding state ($P < .187$) (Fig 2). AREs did not differ among diets whether expressed as milligrams per day ($P < .103$) or as milligrams per kilogram of body weight per day ($P < .100$). AREs also did not differ by time whether expressed as milligrams per day ($P < .146$) or as milligrams per kilogram of body weight per day ($P < .128$) (Table 3).

DISCUSSION

In this study, the deuterium incorporation method showed differences in the FER among diets, with the FER being greater on the POLY diet versus either of the other diets. Other

Table 2. Serum Free and Esterified Cholesterol Concentrations in Subjects During Each 2-Week Diet Period

Diet	Free Cholesterol (mmol/L)	Esterified Cholesterol (mmol/L)	Ratio (esterified/total)
MONO	1.17 ± 0.36	2.84 ± 0.34†	0.71 ± 0.08
POLY	1.23 ± 0.40	2.18 ± 0.86*	0.62 ± 0.15
SAT	1.32 ± 0.40	3.16 ± 0.56†	0.70 ± 0.09

NOTE. Data are the mean ± SD (n = 9). Mean values in a column with different superscripts are significantly different ($P \leq .05$).

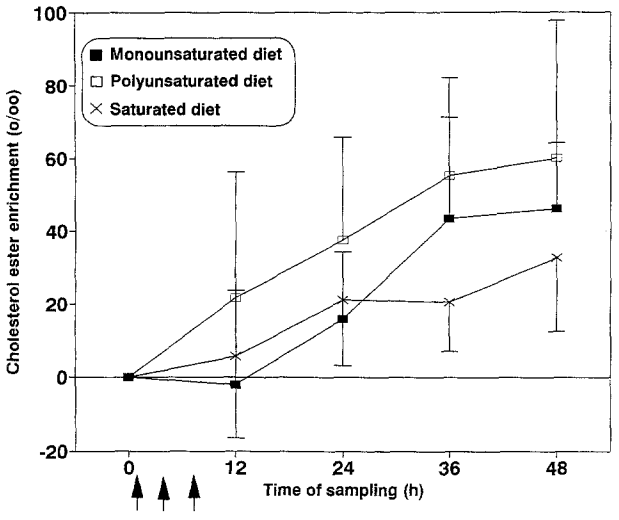

Fig 1. Serum esterified cholesterol deuterium enrichment relative to SMOW during 2-day test periods on each diet, MONO, POLY, and SAT. Data are the mean ± SD (n = 9). The baseline value was either added to or subtracted from each value of a set to allow comparison of relative enrichment among subjects and diets. Arrows indicate meals. A significant interaction between diet and time was found ($P < .01$), and consequently, each diet type and time of sampling was examined separately. On the MONO diet, levels at 0 and 12 hours were lower than all other times, while levels at 24 hours were lower *v* 36 and 48 hours ($P < .001$). On the POLY diet, levels at 0 and 12 hours were lower versus 24, 36, or 48 hours ($P < .001$). On the SAT diet, levels at 12, 24, 36, and 48 hours were higher *v* baseline, and levels at 48 hours were higher *v* 12 hours ($P < .001$). No diet effects were found at either 12 hours ($P < .63$) or 24 hours ($P < .14$). At 36 hours, levels were lower on the SAT diet *v* either the MONO or POLY diets ($P < .001$). At 48 hours, this pattern was repeated ($P < .005$).

researchers using different methods of investigation have also shown that less cholesterol esterification occurs during periods of high SAT fat intake; esterifying enzymes seem to have a marked preference for POLY and MONO fatty acids.[32] This may result in an expanded hepatic pool of unesterified cholesterol when SAT fat is consumed, which is thought to contribute

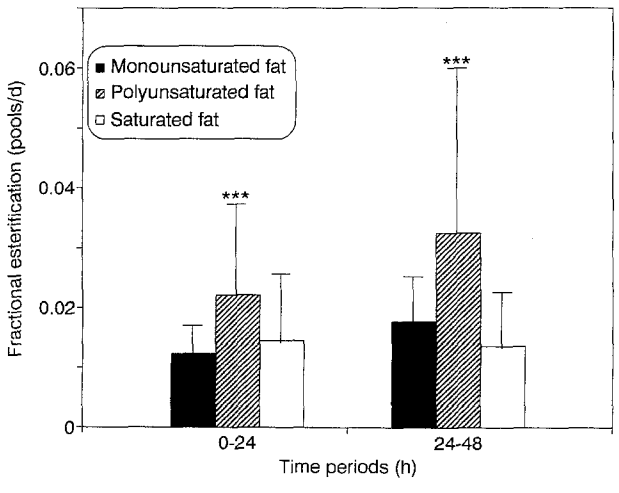

Fig 2. FERs expressed as pools/d obtained from subjects during each 12-hour dietary period when short-term synthesis was measured. Data are the mean ± SD (n = 9). ***$P < .003$.

Table 3. ARES Obtained From Subjects During Each 24-Hour Dietary Period When Short-Term Synthesis Was Measured

	ARE (mg/d)		ARE (mg/kg · d)	
Diet	0-24 h	24-48 h	0-24 h	24-48 h
MONO	151.9 ± 93.7	213.6 ± 123.5	2.2 ± 1.3	3.2 ± 2.0
POLY	218.8 ± 162.7	451.7 ± 491.1	3.3 ± 2.5	7.1 ± 8.2
SAT	207.1 ± 137.6	178.3 ± 162.2	3.1 ± 2.0	2.7 ± 2.5

NOTE. Data are the mean ± SD (n = 9). Values were calculated by multiplying FERs by the size of the esterified cholesterol M(1) pool to obtain mg produced per day; this was divided by the body weight in kilograms to obtain mg/kg · d.

to the downregulation of low-density lipoprotein (LDL) receptors and an increase in circulatory LDL levels.[33] Although the ratio of serum unesterified cholesterol to CE was not affected by diet, subjects did display elevated serum cholesterol levels while consuming the SAT diet compared with the other two diets.[13] This finding suggests that an increase in serum cholesterol may reflect equal increases in the total of both esterified and unesterified cholesterol.

Several human studies support the view, which our D_2O incorporation study reiterated, that when dietary TGs are saturated, the rate of esterification decreases and LDL-cholesterol production simultaneously increases[33]; decreases in hepatic LDL-cholesterol receptor mRNA are also found.[34] The converse is true when dietary fats are unsaturated. Additionally, most of the cholesterol esterification in plasma occurs in HDL-cholesterol.[14] HDL_3 acts as a substrate for LCAT esterification, while HDL_2 inhibits this process.[35-36] Finally, it has been demonstrated that the ratio of HDL_2 to HDL_3 decreases 28% in normal men consuming a POLY diet as compared with a SAT diet.[37] In our study, the relative rates of appearance of CE during both the SAT and POLY diet periods concur with the relevant literature,[37] although we cannot correlate CE appearance with the concentration of different HDL-cholesterol fractions.

Correlations between low levels of plasma CE and ingestion of MONO diets have been shown in humans.[38] In this investigation, CE rates of appearance did not differ significantly during the MONO diet versus either of the other diets. In addition, serum HDL-cholesterol concentrations were very high in subjects consuming the MONO diet. It is possible that a correlation between the rate of esterification and the concentration of HDL-cholesterol was not found because we used a small sample size. One study has shown that HDL_2 levels are higher and HDL_3 levels are lower on safflower and corn oil diets compared with peanut and olive oil diets.[39] The opposite effect was found with diets supplemented with either sunflower oil or rapeseed oil.[40] The explanation given by Valsta et al[40] for the contradictory effects is that the rapeseed oil they used is considerably higher in n-3 α-linolenic acid than the olive oil used predominantly by researchers in similar studies. This may alter the rate of synthesis and use of the two different HDL fractions. It should be noted that neither of the latter two studies showed an improvement in total HDL-cholesterol concentrations on either the MONO or the POLY diet; improvement is usually noted for comparisons to results from a SAT fat diet. Since HDL-cholesterol fractions were not measured in our study, it cannot be concluded with certainty that the rates of

cholesterol esterification are affected by the inhibitory effect of a high percentage of HDL_2.

The statistical analysis in this study did not show an effect of the study period; ie, FERs were not higher when subjects were unfed compared with fed. In studies using baboons as a model of human cholesterol metabolism, animals fed a high-fat diet had greatly increased fasting levels of LCAT activity compared with the fed period.[41] There is some question as to whether the baboon is a good model for humans. In one research project, an increased esterification rate was observed after chronic SAT feeding in baboons,[42] counter to what has been reported in humans, as well as the present investigation. Others measured LCAT activity following ingestion of a high-fat meal and noted that LCAT activity closely paralleled the serum TG concentration, peaking 5 hours following consumption of a high-fat liquid meal and remaining elevated for over 7 hours, with a mean increase of 37.2% over baseline levels.[43-44] This increase was suppressed over 98% in the presence of a LCAT inhibitor, suggesting that acyl-coA:cholesterol acyltransferase (ACAT) plays a minor role as a source of plasma CE during fed periods.[45] Unfortunately, in this study, LCAT activity was not assayed; it is therefore difficult to make any conclusions concerning its activity under different conditions. A more simple explanation may be that the rates of esterification cannot be compared from one period to another in this study because the labeled precursor pool size of unesterified cholesterol varied enormously—the percentage of labeled unesterified cholesterol would determine how much newly esterified CE would be labeled. This was not determined in the present study. It would be of interest to compare cholesterol esterification rates, as determined using deuterium oxide incorporation, with other indices such as LCAT or ACAT activity.

The deuterium oxide incorporation method has been criticized for two reasons. Essentially, there is no physiological basis for a model based on linear regression; in addition, occasionally negative FSRs are obtained.[46] A negative FER is considered to be an indication that the rates are lower versus the other periods being compared, but the effects of substrate recycling or incoming cholesterol from other pools cannot be assessed in the present model. Although a physiological basis for the linear regression model may be unclear, the short-term deuterium oxide incorporation rate is linear.[7-8] Because this rate is unaffected by flux rates of other, unlabeled material into the system, the short-term linear uptake rate can be used to represent a direct measure of synthesis independent of the total whole-body production rate.[9-10] The conclusion from this study, therefore, is that in a simple and short-term one-pool model, where the entry of one tracer into one pool is examined and points are spaced at 12-hour intervals, the deuterium oxide incorporation method can yield reasonable estimates for the rates of cholesterol synthesis and esterification.

In summary, in this study, FERs were shown to be influenced by the type of fat consumed, being greater when subjects were fed the POLY diet versus either the MONO or SAT diets. However, the rates were not linked to the feeding state (ie, fed or unfed).

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