Incorporation of deuterium-labeled *trans*- and *cis*-13-octadecenoic acids in human plasma lipids[1]

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Abstract The absorption and distribution of deuterated *trans*- and *cis*-13-octadecenoic acid (13*t*-18:1 and 13*c*-18:1) in plasma lipids were compared to deuterated *cis*-9-octadecenoic acid (9*c*-18:1) in two young adult male subjects. A mixture of triglycerides was fed in a multiple-labeled experiment where each triglyceride contained a fatty acid labeled with a different number of deuterium atoms. Analysis of human plasma lipids by mass spectroscopy allowed the distribution of the two 13-octadecenoic acid isomers to be directly compared to *cis*-9-octadecenoic acid. Plasma lipids selectively excluded both the 13*t*-18:1 and 13*c*-18:1 isomers relative to 9*c*-18:1 in all neutral and phospholipid fractions. Discrimination against incorporation of the 13*t*-18:1 isomer into plasma cholesteryl ester and 2-acyl phosphatidylcholine was nearly absolute. The 1-acyl phosphatidylcholine fraction exhibited a large positive selectivity for the 13*t*-18:1 isomer. Differences in the relative distribution of the *trans* and *cis* 13-18:1 isomers vs. 9*c*-18:1 in the various lipoprotein lipid classes were found. Analysis of the chylomicron triglyceride component of the plasma lipids indicated all three fatty acids were equally well absorbed.—**Emken, E. A., R. O. Adlof, W. K. Rohwedder, and R. M. Gulley.** Incorporation of deuterium-labeled *trans*- and *cis*-13-octadecenoic acids in human plasma lipids. *J. Lipid Res.* 1983. **24:** 34–46.

Supplementary key words lipid metabolism • deuterium-labeled • isomeric fatty acids

Soybean oil is the most important source of visible dietary fat in the United States and is an excellent source of polyunsaturated fatty acids (1–3). About 75% of the edible soybean oil produced is partially hydrogenated during processing to improve flavor stability and to increase the melting point or solid fat index. Partial hydrogenation or hardening of soy oil allows it to be formulated into margarines and shortenings. Part of the increase in melting point is a result of the formation of positional and *trans* fatty acid isomers. Analysis of commercial salad oils, margarines, and shortenings has identified the 8 through 13 positional *cis* and *trans* isomers as the major monounsaturated fatty acids in hydrogenated soybean oil (4, 5). These fatty acid isomers are incorporated into animal (6–17) and human tissue lipids (18–23), and their metabolism and biochemistry have been the subject of recent reviews (24–26).

Little human data now exists on the metabolism of specific positional fatty acid isomers that can be used to determine the validity of animal and in vitro data for predicting human metabolic response to specific fatty acid isomers. To provide human data for evaluating possible nutritional and metabolic effects of fatty acid isomers, the absorption, distribution, and disappearance of deuterium labeled *cis*- and *trans*-13-octadecenoic acid compared to *cis*-9-octadecenoic acid in human plasma and lipoprotein lipid classes were determined using a triple-labeled experimental approach.

EXPERIMENTAL

Triple-labeled methodology

The triple-labeled experimental approach used in these experiments was similar to the methodology used in a previous study (23). This approach consists of feeding to each subject a mixture of three triglycerides that contain three different deuterated fatty acids. Each triglyceride contained one of the three deuterated fatty acids. The deuterium-labeled fatty acids contained 2, 4, or 6 deuterium atoms. The use of different numbers of labels for each fatty acid results in a different molecular weight, and thus each fatty acid in the fed mixture and the plasma samples can be quantitatively measured by gas–liquid chromatography–mass spectros-

Abbreviations: TG, triglyceride; FFA, free fatty acid; CE, cholesteryl ester; PE, phosphatidylethanolamine; PS, phosphatidylserine; PC, phosphatidylcholine; LPC, lysophosphatidylcholine; SM, sphingomyelin; Pc-1 or -2, 1- or 2-acyl phosphatidylcholine.

[1] The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

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TABLE 1. Deuterated fatty acids used in triglyceride (TG) mixtures[a]

Subject	Mixture Fed	Melting Point of TG	Total Weight	Ratio in Fed Mixture	
				13t/9c	13c/9c
		°C	g		
1	13t-18:1-17,18-d_2	46	30.9	1.08	1.06
	13c-18:1-17,17,18,18-d_4	17			
	9c-18:1-14,14,15,15,17,18-d_6	5			
2	13t-18:1-17,17,18,18-d_4	46	31.0	1.09	1.10
	13c-18:1-17,18-d_2	17			
	9c-18:1-14,14,15,15,17,18-d_6	5			

[a] Abbreviations: The first number indicates position of double bond, the *c* or *t* indicates *cis* or *trans*, and the 18:1 indicates octadecenoic acid. The remaining numbers indicate position of deuterium label and number of deuterium atoms/molecule of fatty acid.

copy (GLC–MS) without interference from nonlabeled fats (27).

Deuterated fats fed

Synthesis of the deuterated fatty acids and their triglycerides has been described previously (28, 29). The amount and identity of the labeled fatty acids in the triglyceride mixture fed to each subject are summarized in **Table 1.** A different labeling pattern was used for the 13*t*- and 13*c*-18:1 fatty acids fed to each subject to reduce the possibility of systematic error and to detect possible isotope effects.

Subjects, sampling, and sample preparation

The subjects were two Caucasian males, ages 25 and 27, with no history of congenital ailments. They were in excellent health as judged by medical examinations and standard clinical blood profile analysis. Their weight, blood pressure, serum cholesterol (177 and 161 mg/dl), and fasting triglyceride levels (40 and 53 mg/dl) were normal and they were taking no medications. For 1 week before the feeding study, both subjects were placed on a 1800 kcal/day standard American Diabetic Association diet normally prescribed for diabetics, and no food was eaten for 10 hr before the feeding study. The diabetic diet was used in an attempt to achieve a reasonable consistency in the percent calorie intake from fat (40%), carbohydrate (40%), and protein (20%). No weight change was noted for either subject while on the diabetic diet. The subjects remained on this diet for the course of the experiment. Dietary histories were typical of a U.S. diet. Both subjects were nonsmokers; however, the first subject was a heavy coffee drinker (8–10 cups per day) and the second subject a light coffee drinker (1–2 cups per day).

The deuterated triglyceride mixture (ca 31 grams) was emulsified with 30 grams of calcium caseinate (Savortone® 100, Western Dairy Products, San Francisco, CA), 30 grams of dextrose, 15 grams of sucrose, and 200 ml of water. The labeled fats were fed at 8:00 AM, and the subjects were allowed to eat a light lunch at 1:00 PM and an evening meal. The mixture of deuterated triglycerides was fed in place of the subjects' normal breakfast and immediately after a 0-hr blood sample was drawn.

Blood samples (30 ml) for plasma lipid class analyses were drawn at 0, 2, 4, 6, 8, 12, 15, 24, and 48 hr. Blood samples (40 ml) for lipoprotein lipid analysis were collected at 2, 4, 6, 8, 12, 15, and 24 hr from subject 2, but a 2-hr and 24-hr sample were not obtained from subject 1. Platelets were isolated from one blood sample (50 ml) from each subject drawn 24 hr after the deuterium-labeled fats were fed. Other details of the feeding and sampling procedures have been described previously (21–23).

Analysis of blood lipids

Isolation, separation, derivatization and GLC–MS analysis by selective ion monitoring of plasma, lipoprotein, red blood cells, and platelet triglycerides (TG), free fatty acid (FFA), cholesteryl ester (CE), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylcholine (PC), lysophosphatidylcholine (LPC), sphingomyelin (SM), 1-acyl phosphatidylcholine (PC-1), and 2-acyl phosphatidylcholine (PC-2) samples have been described previously (21–23). The accuracy of GLC–MS analysis was estimated to be ±0.3% from analysis of weighed standard mixtures of 9*c*-18:1-d_0, 9*c*-18:1-d_2, 9*t*-18:1-d_4, and 9*c*-18:1-d_6 (27).

Fatty acid composition of plasma lipid samples was determined by analysis with a Packard 7400 series gas chromatograph equipped with dual flame ionization detectors and a 20 ft × 4 mm glass column packed with 15% OV 275 on 100/120 mesh chromasorb P. Methyl ester peaks were identified by comparison of retention times to authentic standards purchased from Applied

Science and Nu-Chek Prep. Inc. Peak areas were corrected for number of ionizable carbons and quantitation was confirmed by analysis of weighed standard mixtures.

Calculation of selectivity values

Selectivity values were calculated for plasma lipid samples to compare the relative incorporation of 13*t*-18:1 and 13*c*-18:1 to 9*c*-18:1. The selectivity for 13*t*-18:1 was obtained by determining the logarithm of the 13*t*-18:1/9*c*-18:1 experimental ratio found in the lipid fraction divided by the ratio in the fed mixture. Logarithms were used to obtain positive and negative selectivity values of comparable magnitude. Selectivities for 13*c*-18:1 were calculated in the same way except the 13*c*-18:1/9*c*-18:1 ratios were used. Total area selectivities were calculated by the same procedure, except the 13-18:1 isomer/9*c*-18:1 experimental ratios were based on the areas of the curves obtained by plotting percent deuterated fatty acid vs. sampling time (Figs. 1–4). Area selectivity values agreed closely with selectivity values for individual samples, which contained the maximum percentage of deuterated fatty acids. Area selectivities were considered to better represent those curves that contain more than one peak or where selectivity values varied between individual samples. Based on the standard deviation obtained from a series of standard mixtures, the accuracy of the selectivity values is better than ±0.02.

Positive selectivity values indicate a preferential incorporation of the 13-18:1 isomer and negative values indicate a preferential exclusion of the 13-18:1 isomer compared to 9*c*-18:1.

RESULTS

Absorption of 13*t*- and 13*c*-18:1 isomers from the intestinal tract

The percent incorporation of deuterium labeled 13*t*-, 13*c*-, and 9*c*-18:1 into the octadecenoic acid fraction of plasma chylomicron-TG samples is shown in **Fig. 1.** Differences in curve shapes and in the maximum level of deuterated fatty acids incorporated reflected differences in absorption of the labeled fatty acid for each subject. The maximum level of total labeled fat incorporated was about 25% higher for the second subject. For subject 1, absorption of the fed mixture appeared to have occurred in two stages as indicated by appearance of a major peak at 4 hr and a shoulder or second peak at 12 hr. This difference between subjects for absorption of the labeled fatty acid was reflected in the shape of the plasma-FFA curve and the broadened plasma-TG curve (**Fig. 2**) for subject 1. Unfortunately the plasma-TG 8-hr sample from subject 1 was lost and is not available to indicate whether a double peak similar to the chylomicron-TG and plasma-FFA curves may have been present in the plasma-TG curve from subject 1.

The chylomicron-TG curves for 13*t*-, 13*c*-, and 9*c*-

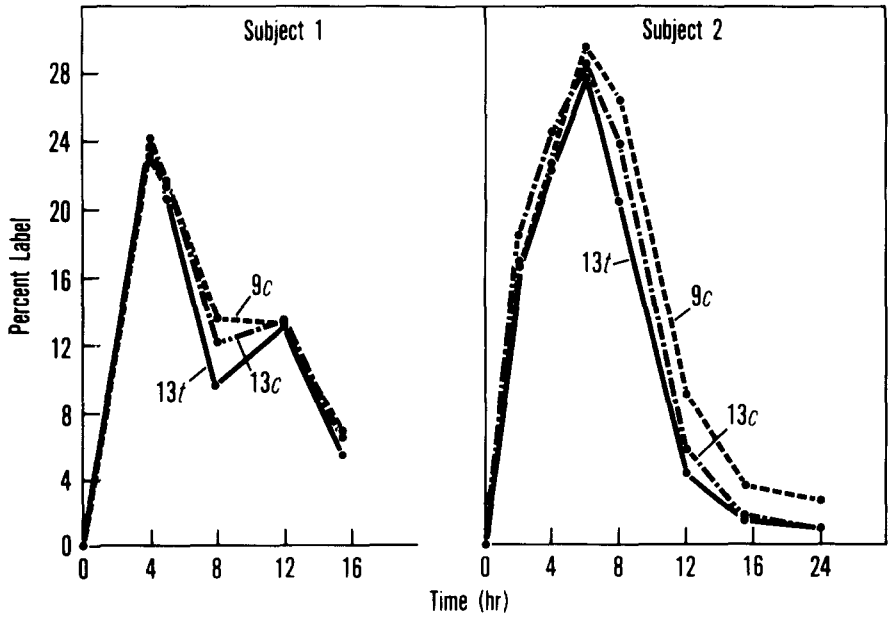

Fig. 1. Uptake and disappearance of deuterium-labeled 13*t*-, 13*c*-, and 9*c*-octadecenoic acids in human chylomicron triglycerides from two subjects. Percent label represents percentage of labeled fatty acid in the octadecenoic acid fraction of the chylomicron triglycerides.

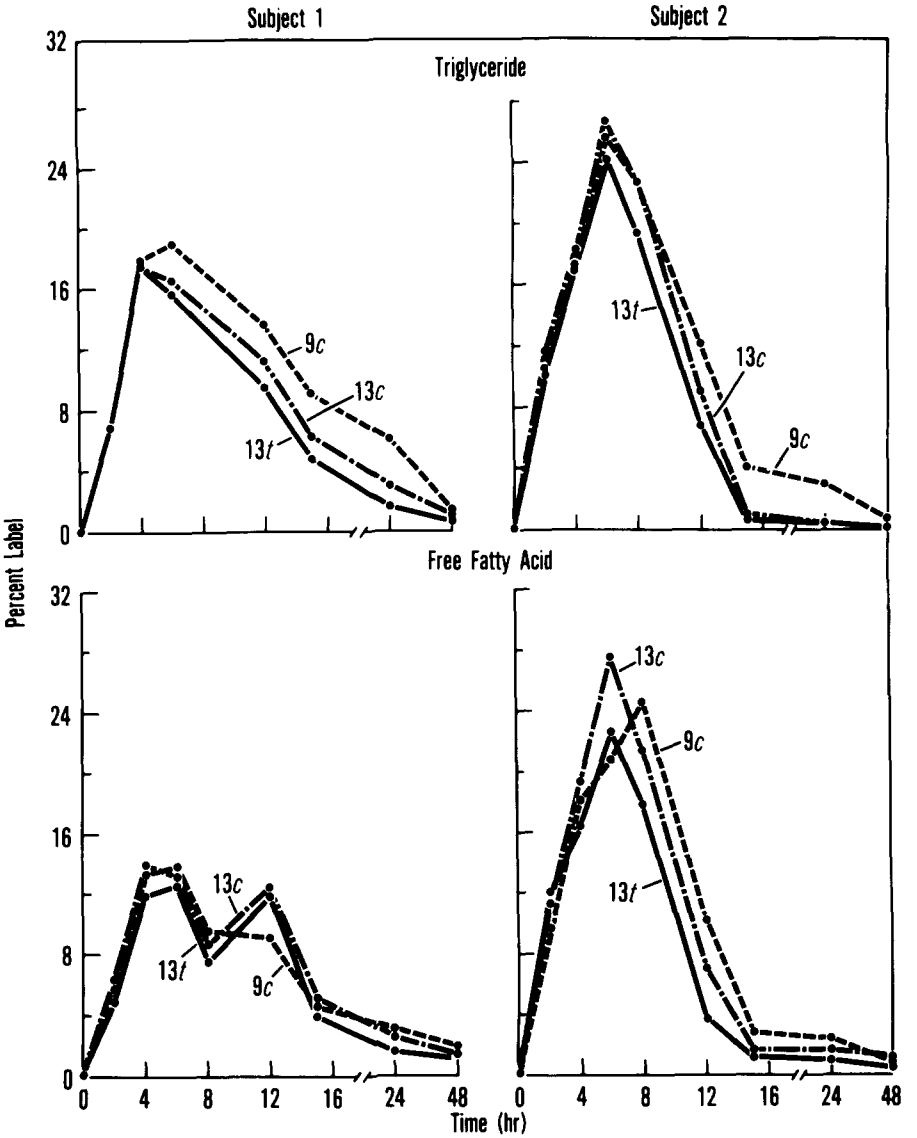

Fig. 2. Uptake and disappearance of deuterium-labeled 13*t*-, 13*c*-, and 9*c*-octadecenoic acids in human plasma triglycerides and free fatty acids from two subjects. Percent label represents percentage of labeled fatty acid in the octadecenoic acid component of the samples.

18:1 from an individual subject had the same shape, and the percentages of labeled fatty acids incorporated were similar. These data and the area selectivity values for 13*t*- and 13*c*-18:1 isomers (−0.07 and −0.04) indicated all three fats were equally well absorbed. Small negative selectivity values for individual samples were probably the result of selective hydrolysis of chylomicron-TG which contained 13*t*- and 13*c*-18:1 isomers. This selectivity is small and is apparent in the second half of the curve for subject 2 (Fig. 1).

Incorporation of 13*t*- and 13*c*-18:1 isomers into plasma lipids

The percentages of deuterated 13*t*, 13*c*-, and 9*c*-18:1 in the octadecenoic acid fraction of various plasma lipid samples are plotted in Figs. 2, 3, and 4. For plasma TG and FFA (Fig. 2) there was a slight discrimination against 13*t*- and 13*c*-18:1 compared to 9*c*-18:1. Obvious differences in incorporation of 13*t*-, 13*c*-, and 9*c*-18:1 were evident for the plasma-PC-1, LPC, CE, SM, and PE samples (**Figs. 3 and 4**). For all plasma lipid samples, the relative uptake of 13*t*- and 13*c*-18:1 was never significantly greater than 9*c*-18:1 except in the PC-1 samples. In the PC-1 samples (Fig. 3), the total area for the 13*t*-18:1 curve was 5.7-fold greater than for total area of the curve 9*c*-18:1, and the area of the 13*c*-18:1 curve was about 3-fold greater than for the area of the 9*c*-18:1 curve.

Removal or turnover of the 13-18:1 isomers in the plasma lipid classes (see Figs. 2–4) decreased to 5% or

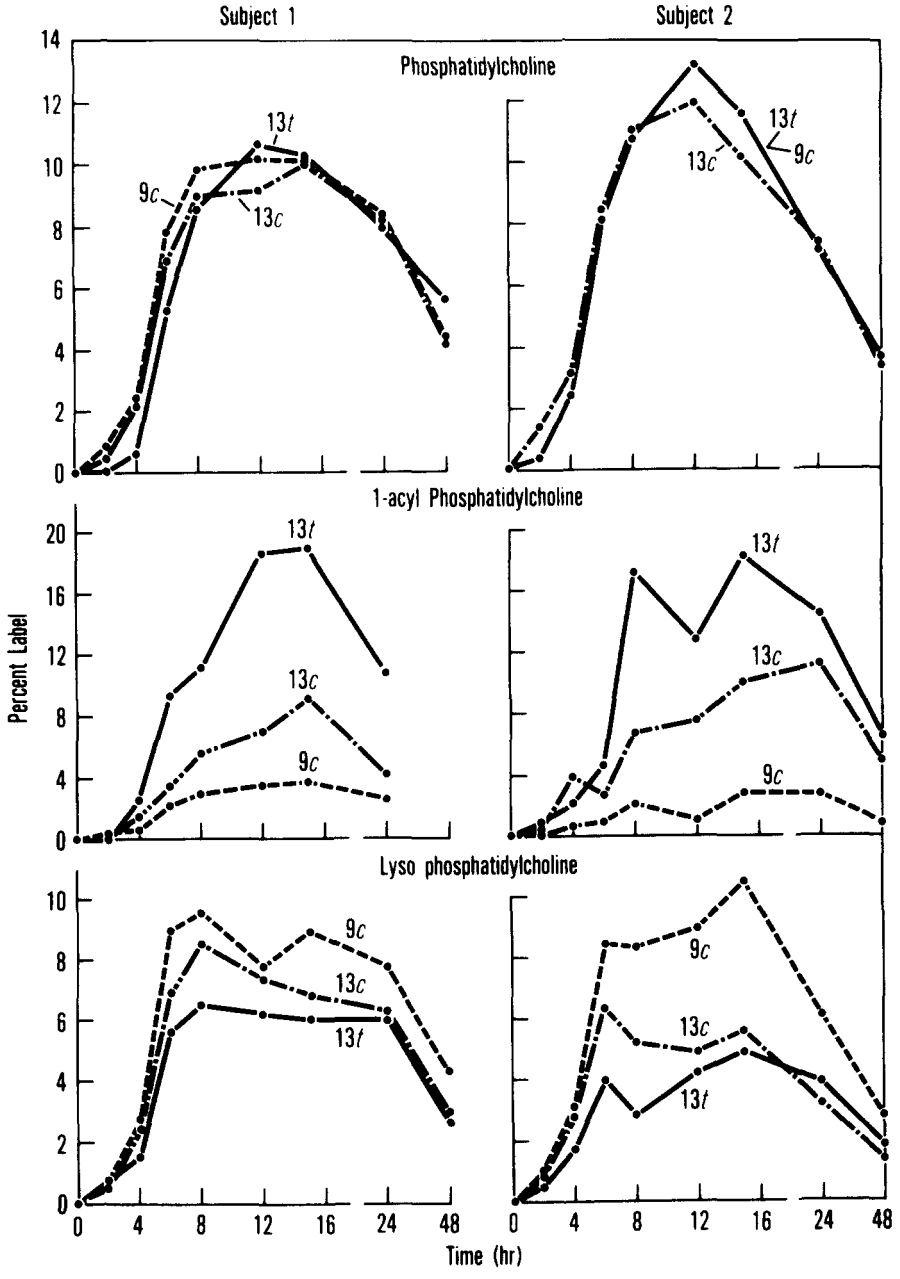

Fig. 3. Uptake and disappearance of deuterium-labeled 13*t*-, 13*c*-, and 9*c*-octadecenoic acids in human plasma phosphatidylcholine, 1-acyl phosphatidylcholine, and lysophosphatidylcholine from two subjects. Percent label represents percentage of each labeled fatty acid in the octadecenoic acid component of the samples.

less after 48 hr. Based on the rates of removal relative to 9*c*-18:1, 13*t*- and 13*c*-18:1 isomers would not be expected to accumulate in the plasma lipids. Incorporation and turnover of 9*c*-18:1 into plasma cholesteryl ester samples were significantly slower than for other plasma lipid fractions, and a strong selective exclusion of 13*t*- and 13*c*-18:1 compared to 9*c*-18:1 was apparent.

Small but definitely measurable amounts of labeled fatty acids were present in the 2-hr neutral lipid and phospholipid samples (Figs. 2–4). The appearance of labeled fats in the 2-hr phospholipid samples was unexpected since 2–4 hr are required for the lymphatic system to transport adsorbed dietary fat to the circulatory system (30). A small shoulder at the front of the phospholipid curves was apparent for the plasma-PC, LPC, and SM fractions. This shoulder suggests small amounts of labeled fatty acids were incorporated into the phospholipids before the main body of labeled fatty acids entered the circulatory system via the lymphatic system.

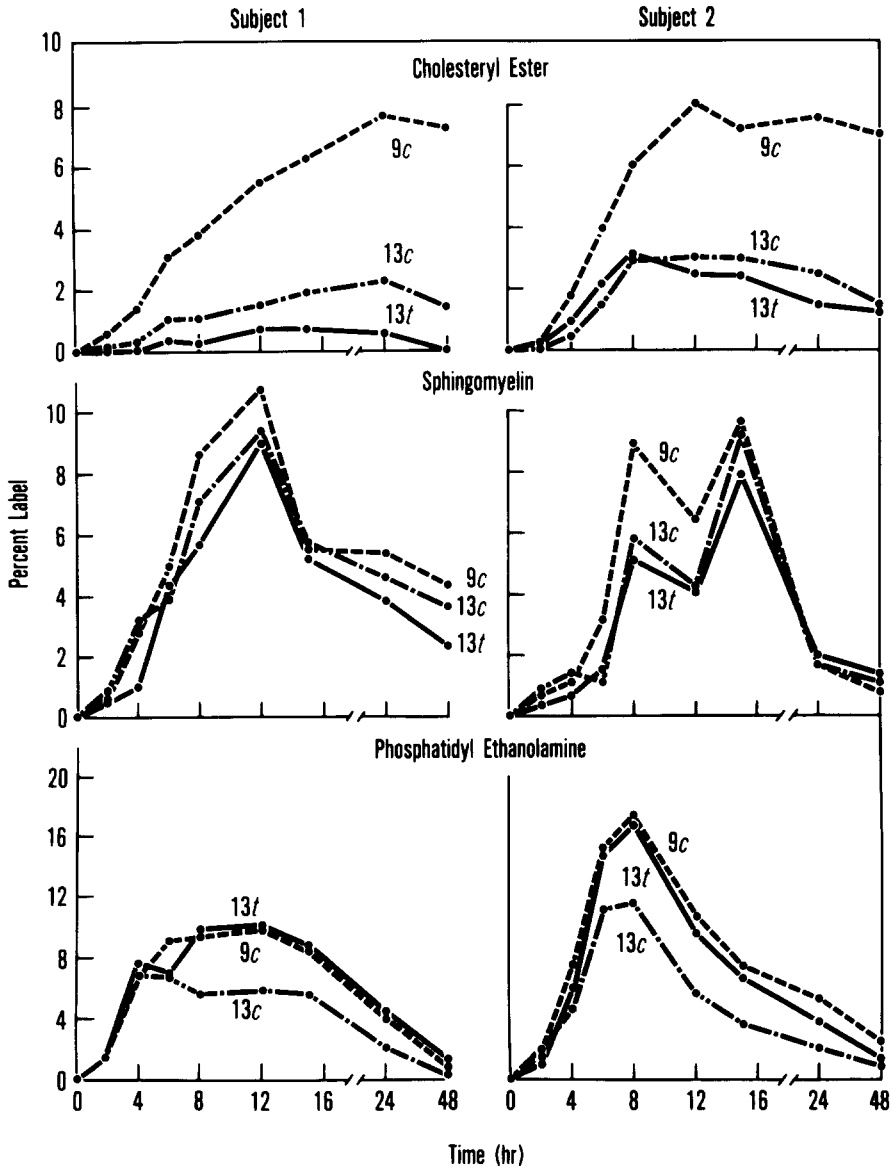

Fig. 4. Uptake and disappearance of deuterium-labeled 13*t*-, 13*c*-, and 9*c*-octadecenoic acids in human plasma cholesteryl ester, sphingomyelin, and phosphatidylethanolamine from two subjects. Percent label represents percentage of labeled fatty acid in the octadecenoic acid component of the samples.

Incorporation of 13*t*- and 13*c*-18:1 isomers into lipoprotein lipids

The percent deuterium-labeled 9*c*-, 13*t*-, and 13*c*-18:1 incorporated into the 18:1 fatty acid fraction of chylomicron (Chylo), very low density (VLDL), low density (LDL), and high density lipoprotein (HDL) lipid classes are summarized in **Table 2.** The data are presented for those lipoprotein samples that contained the maximum level of labeled fatty acids. The rates of incorporation and disappearance of deuterated fatty acids associated with the various lipoprotein lipid classes were dependent on the lipoprotein fraction with which the lipid class was associated. The main differences were in the percentage and time of maximal incorporation of the labeled fats.

Plasma lipid selectivity values

The average area selectivity values calculated for plasma neutral and phospholipid samples are plotted in **Fig. 5.** All selectivity values for the 13*c*- and 13*t*-18:1 isomers were negative, which indicates a general discrimination against their incorporation. Selectivity values from previous human studies (21–23) with 9*t*-, 12*t*-, and 12*c*-18:1 are included in Fig. 5 for comparison. Selectivity values for 13*t*- and 13*c*-18:1 are dependent on the specific plasma lipid and on the configuration of

TABLE 2. Summary of neutral lipid and phospholipid lipoprotein data[a]

Lipid Class	Time[b] Subject 1	Time[b] Subject 2	Distribution of Label 13t-18:1	Distribution of Label 13c-18:1	Distribution of Label 9c-18:1	Selectivity 13t-18:1/9c-18:1	Selectivity 13c-18:1/9c-18:1
	hr			%			
Triglyceride							
Chylo	4	6	25.9	26.3	26.4	−0.02	−0.02
VLDL	8	8	9.6	14.8	18.6	−0.32	−0.13
LDL	12	8	7.3	9.4	19.2	−0.44	−0.34
HDL	6	8	9.3	10.5	20.3	−0.36	−0.32
Free fatty acid							
Chylo	4	6	14.6	16.6	15.9	−0.07	−0.03
VLDL	15	12	5.8	6.8	9.6	−0.23	−0.17
LDL[c]	15		3.7	4.1	8.3	−0.38	−0.33
HDL	6	8	10.2	10.5	11.2	−0.06	−0.05
Cholesteryl ester							
Chylo	4	8	3.5	5.4	8.0	−0.60	−0.24
VLDL	12	6	2.7	4.0	7.0	−0.47	−0.30
LDL	8	15	3.1	3.2	8.0	−0.41	−0.42
HDL	15	15	1.8	4.2	9.1	−0.75	−0.38
Phosphatidylethanolamine							
Chylo	6	6	9.5	7.9	10.9	−0.11	−0.22
VLDL[c]		6	1.8	1.2	3.1	−0.23	−0.43
LDL	8	8	3.0	3.4	4.7	−0.21	−0.17
HDL	6	12	9.6	8.1	10.4	−0.06	−0.14
Sphingomyelin							
Chylo	6	8	3.5	5.5	5.4	−0.36	−0.12
VLDL							
LDL[c]		12	1.9	2.1	2.7	−0.16	−0.13
HDL	6	8	4.1	4.3	3.6	+0.07	+0.05
Phosphatidylcholine							
Chylo	4	12	6.9	6.7	8.5	−0.14	−0.07
VLDL	12	12	8.7	7.1	8.6	−0.04	−0.11
LDL	12	12	10.2	11.2	8.8	+0.03	+0.07
HDL	12	12	11.8	11.5	10.0	+0.05	+0.03
Lysophosphatidylcholine							
Chylo[c]	6		1.2	1.5	2.8	−0.40	−0.35
VLDL							
LDL	8	6	3.3	4.4	5.0	−0.23	−0.22
HDL	6	8	4.2	3.5	4.9	−0.22	−0.20

[a] Abbreviations: chylomicron (Chylo), very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL). For others, see Table 1. Average of data from two subjects except where noted.
[b] Hours after isomers fed at which maximum incorporation of deuterium labeled fatty acids occurred.
[c] Data from one subject.

the double bond. Comparison of selectivity values for 13-18:1 isomers to values for 9*t*-, 12*t*-, and 12*c*-18:1 isomers indicates the selectivities were dependent on double bond position.

Selectivity values calculated for the 1-acyl and 2-acyl position of phosphatidylcholine are tabulated in **Table 3.** Data from similar human studies with 9*t*-, 12*t*-, and 12*c*-18:1 are included for comparison (21–23). The large positive selectivity for 13*t*-18:1 incorporation into PC-1 is of the same magnitude as previously obtained for 12*t*-18:1. A much larger negative value for 13*t*-18:1 was found in PC-2 than with 12*t*-18:1. A negative selectivity for PC-2 was calculated for 13*c*-18:1 in contrast to a large positive value obtained when 12*c*-18:1 was fed. These data indicate that incorporation of these monounsaturated fatty acid isomers is dependent on the phospholipid acyl position, double bond configuration and position and specific plasma lipid class.

Platelet lipid selectivity values

The 18:1 fatty acids in the 24-hr platelet triglyceride, cholesteryl ester, and total phospholipid (PL) samples contained 9.75%, 3.6%, and 7.7% total deuterated fatty acids, respectively. Average (two subjects) selectivity values for 13*t*-18:1 were +0.39 (TG), −0.73 (CE), and −0.50 (PL) and for 13*c*-18:1 selectivities were +0.28 (TG), −0.48 (CE), and −0.26 (PL). Selectivity values for CE and PL were consistent with plasma lipid values,

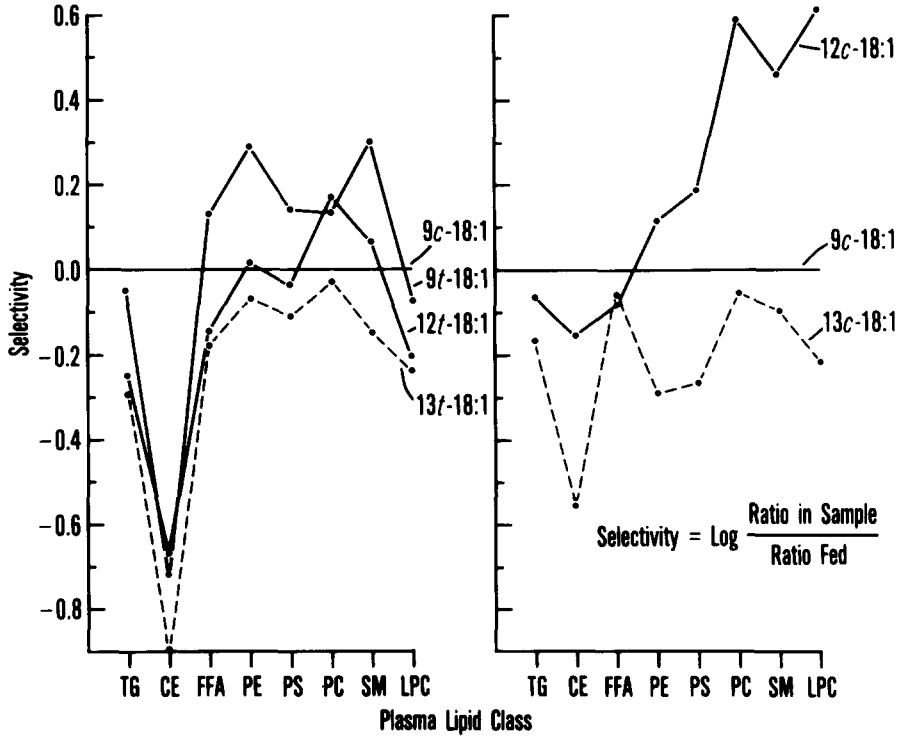

Fig. 5. Selectivity values for plasma triglyceride (TG), cholesteryl ester (CE), free fatty acid (FFA), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylcholine (PC), sphingomyelin (SM), and lysophosphatidylcholine (LPC). See Table 1 for fatty acid abbreviations. Each point is the average area selectivity value for two subjects. Selectivity values for 9*t*-, 12*t*-, and 12*c*-18:1 from references 21–23.

but the large difference between platelet and plasma lipid TG values was not anticipated.

Plasma lipid fatty acid composition

The fatty acid composition for plasma lipid samples that contained the maximum total percent deuterated 13-18:1 isomers is tabulated in **Table 4.** Fatty acid composition of the 0-hr samples is included for comparison. Total deuterated fatty acid incorporated ranged from 64.7% (TG) to 2.2% (CE). As expected, incorporation of deuterated fatty acids increased the total percent 18:1 content of the samples and decreased the percentage of other fatty acids in the TG samples. In the TG samples, this decrease appeared to result from a simple dilution of the endogenous fatty acids due to the high level of deuterated 18:1 fatty acids in this fraction. In the FFA fraction, the percent 18:2 was decreased by almost 50%, which is more than expected based on the deuterated fatty acid content. Incorporation of deuterated fats appeared to selectively alter specific fatty acid percentages other than 18:1 in the LPC and PE samples but not in PC. Specifically, the percent 16:0 and 16:1 decreased and 18:2 increased in the PE and LPC samples. The 18:1 content of both samples increased more than expected based on the total deuterated fatty acid content of these samples. The deuterated fatty acids had very little effect on the CE fatty acid composition, presumably due to the very low level of labeled fatty acids incorporated.

DISCUSSION

Multiple-labeled technique

The advantages of simultaneously feeding differently labeled compounds were first demonstrated in dual-labeled studies that used carbon-14 and tritium compounds. The multiple deuterium-labeled approach used in this study follows the same rationale as earlier radioisotope experiments, except that two experimental fatty

TABLE 3. Selectivity values for incorporation of 18:1 isomers into the 1- and 2-acyl position of phosphatidylcholine

Isomer	Selectivity Value[a]		
	Total PC	1-acyl PC	2-acyl PC
9*t*-18:1[b]	+0.02	+0.56	−0.21
12*t*-18:1[b]	+0.17	+0.73	−0.90
12*c*-18:1[b]	+0.58	+0.51	+0.65
13*t*-18:1	−0.03	+0.70	−1.60
13*c*-18:1	−0.06	+0.43	−0.20

[a] Average for data from two subjects.
[b] Data from previous studies (21–23).

TABLE 4. Effect of 13-18:1 isomers on composition of human plasma lipids

Subject	Plasma Lipid Class	Sample	Total Label[a]	Fatty Acid								P/S Ratio
				14:0	16:0	16:1	18:0	*t*-18:1	*c*-18:1	18:2	20:4	Other	
		hr	%					%					
1	TG	0	0	4.4	24.6	5.0	4.0	3.1	33.8	23.1	1.0	1.0	0.73
1	TG	4	17.7	3.3	21.4	4.3	3.3	9.2	41.3	15.0	0.8	1.4	0.56
2	TG	0	0	5.7	28.2	3.3	5.9	2.7	34.0	18.1	1.1	1.1	0.51
2	TG	6	36.2	1.4	11.4	2.2	2.8	15.2	56.0	9.9	0.8	0.3	0.69
1	CE	0	0	4.2	12.8	3.1	1.3	0	15.6	55.7	6.2	1.1	3.38
1	CE	24	0.5	3.3	13.7	2.5	1.5	0.4	17.3	53.2	6.4	1.7	3.22
2	CE	0	0	1.9	13.0	3.6	1.3	0.2	14.6	60.6	4.8	0	4.04
2	CE	12	1.0	1.2	12.8	2.1	1.4	0.2	19.0	55.2	6.5	1.6	4.27
1	FFA	0	0	10.6	30.5	7.2	11.5	3.6	23.8	11.3	0.7	0.8	0.23
1	FFA	12	7.6	10.5	31.9	7.1	11.2	4.4	26.6	5.2	1.5	0.0	0.13
2	FFA	0	0	6.9	31.6	6.0	10.8	3.2	26.8	12.4	1.3	1.0	0.28
2	FFA	6	17.3	6.2	27.0	3.7	8.2	8.7	36.1	7.5	2.7	0.0	0.25
1	PC	0	0	1.1	34.2	2.1	14.0	2.6	10.7	23.7	9.1	2.5	0.67
1	PC	12	3.5	1.5	28.7	1.6	13.2	3.0	14.6	23.2	11.2	3.0	0.79
2	PC	0	0	0.8	27.9	1.7	13.8	2.5	10.8	29.2	11.4	1.9	0.96
2	PC	12	4.9	0.9	24.1	0.9	12.9	4.3	15.1	27.1	12.3	2.5	1.04
1	LPC	0	0	6.8	40.5	6.1	12.4	1.6	10.2	17.9	3.5	1.0	0.36
1	LPC	12	2.2	3.5	38.5	2.3	12.0	3.2	14.6	20.3	1.4	4.1	0.40
2	LPC	0	0	9.9	37.4	13.1	11.2	1.0	10.0	9.7	5.8	1.8	0.26
2	LPC	15	1.5	6.6	36.0	9.0	11.5	1.4	12.8	17.8	2.8	2.0	0.38
1	PE	0	0	7.8	21.2	7.4	14.3	1.7	8.5	8.7	22.1	8.3	0.71
1	PE	12	3.6	4.4	17.0	6.6	11.4	4.5	18.2	11.7	15.5	10.8	0.83
2	PE	0	0	8.6	21.4	10.4	13.5	1.0	10.4	7.4	15.1	12.3	0.52
2	PE	8	5.3	3.8	16.7	5.4	12.6	3.5	15.5	11.6	21.7	9.1	1.01

[a] Percent of deuterated *cis* plus *trans* 13-18:1 in total sample at maximum incorporation.

acids plus a control (9*c*-18:1) were fed rather than one fatty acid plus control. A feature of this multiple-labeled approach is that each subject serves as his own control and analytical errors are normally cancelled or are at least similar for each fatty acid. The major advantage of multiple-labeled studies over single isotope experiments is that factors that are responsible for subject variation should have an identical effect on the metabolism of each fat, and thus the comparison of data for one fat to the other results in data with greater precision. A further advantage of this method is that almost the same amount of effort provides data for three fats rather than for only one fat as is the case in single-labeled studies.

Assessment of the subjects lipid metabolism status

Based on the following criteria, the metabolism of dietary fatty acids by the subjects used in these experiments was considered to be typical of young adult males. Clinical data for plasma triglyceride and cholesteryl ester and lipoprotein levels were normal. The rates of uptake and disappearance of the control fatty acid (9*c*-18:1) in the various plasma and lipoprotein lipid classes were similar to those observed previously for five different subjects (21–23). The maximal incorporation of labeled 9*c*-18:1 into the various lipid classes was also fairly consistent for these and previous subjects.

Absorption of 13*t*- and 13*c*-18:1 isomers from the intestinal tract

Based on the curves for chylomicron data in Fig. 1 for 13*t*-, 13*c*-, and 9*c*-18:1, absorption of both 13-18:1 positional isomers was similar to that of 9*c*-18:1. The curves in Fig. 1 were typical of Chylo-TG data previously obtained for 9*t*-, 12*t*-, and 12*c*-18:1. The wide variation in the melting point of the fed TG's (5°C to 46°C) did not affect the absorption of these fatty acids. This implies that pancreatic lipase hydrolyzes TG's containing these isomers at the same rates.

The presence of a "double peak" in the chylomicron curve (Fig. 1) for subject 1 was also observed when 9*t*-18:1 was fed in a previous study (21, 22). This non-Gaussian absorption of the fat mixtures also occurred in Blomstrand's early work (31) and may be a fairly

common occurrence, but it does not appear to be a function of the fatty acid composition of the fed mixture. No physiological differences between these subjects or previous ones were identified that would explain the difference in the rate of dietary fat absorption. Variation in the width and height of the Chylo-TG curves was inversely related as expected, since the total area under the curves represents the total amount of deuterated fat absorbed.

Absorbed short-chain but not long-chain fatty acids are reported to be transported directly to the liver by the portal vein (32–33). Recent studies in rats have shown portal venous transport of small amounts of long-chain fatty acids can occur, although the main route is by the lymphatic system (34). The appearance of substantial amounts of deuterated fatty acids in the 2-hr samples of human plasma lipids (Figs. 2–4) was sooner than commonly reported (30) and suggests that a portion of the dietary fat may be transported by the portal venous pathway in humans. This study supports earlier studies in humans that suggested that dietary oleic acid may enter the circulatory system via the portal system or by way of lymphatic-venous anastomoses (31).

Incorporation of 13*t*- and 13*c*-18:1 isomers into plasma lipids

The rate of absorption of labeled dietary TG as reflected by the shape of the Chylo-TG curves should influence the rate of incorporation of the labeled fats into other plasma lipids and the shape of their curves. However, the double peak in the Chylo-TG curve from subject 1 is absent in the PC and PE curves. This difference could be explained by assuming that most of the fed triglycerides had entered the circulation system before a significant portion of the TG fatty acids were incorporated into the PC and PE fractions. The non-Gaussian curves for deuterated fatty acid incorporation into some plasma phospholipid lipids may reflect variation in dietary fat absorption or the fact that each plasma curve is a composite of lipoprotein curves, each having a characteristic shape.

The 1-acyl PC curve in Fig. 3 shows the expected preference for incorporation of *trans* fatty acids into the 1-acyl position of PC. Data in Table 3 showed discrimination against placement of both 13*t*- and 13*c*-18:1 into the 2-acyl position. These negative selectivities probably reflect differences in acyl transferase specificity as reported previously (35–38). In Fig. 3, the amount of 13*c*-18:1 isomer incorporated into PC-1 was intermediate between 9*c*- and 13*t*-18:1, indicating that both position and configuration of the double bond influences the relative incorporation. The data in Table 3 emphasize the marked contrast between 13*c*-18:1 data and previous data that showed preferential incorporation of 12*c*-18:1 (23) into both the 1- and 2-acyl positions of PC.

Plasma selectivity values plotted in Fig. 5 qualitatively confirm selectivity values that can be calculated from rat feeding studies (37, 38), although quantitatively there are considerable differences. These differences are not surprising considering the differences between species and tissue sources. Selectivity values for plasma 1- and 2-acyl PC data in Table 3 and in vitro data for esterification of 1- and 2-acyl PC are also qualitatively in agreement (36). In vitro selectivity values from experiments with acyl transferase using 1-acyl PC and 2-acyl PC as the substrate are +0.70 and −0.40, respectively, for esterification with 13*t*-18:1, and +0.43 and −0.18, respectively, for esterification with 13*c*-18:1. The relative selectivity values for the 13*c*-, 13*t*-, 9*c*-, 9*t*-, 12*c*-, 12*t*-18:1 did not follow the same order within the various plasma lipid classes. For example, the selectivity values for plasma PC were $12c < 13t < 9c < 9t \cong 12t < 12c$, but the order for plasma SM was $13t < 13c < 9c < 12t < 9t < 12c$. These variations in selectivity values may be a reflection of different biological or physical requirements associated with the structure of the various lipid classes. These different requirements could in turn influence the relative incorporation of the 18:1 positional isomers.

The negative selectivities for all the plasma lipid classes (Fig. 5) and for most of the lipoprotein lipid classes (Table 2) are in contrast to previous human data for the 9*t*-, 12*t*-, and 12*c*-18:1 isomers (21–23). These negative values can be explained either by postulating preferential β-oxidation of 13*t*- and 13*c*-18:1 or by preferential uptake into tissue lipids. Analysis of human tissue lipids indicates that no significant accumulation of the 13*t*- or 13*c*-18:1 isomer does occur (20). Thus a higher rate of β-oxidation is a plausible explanation for the negative selectivities observed. The problem is that a slower rate of oxidation for 13*t*- and 13*c*-18:1 relative to 9*c*-18:1 would be expected based on in vitro rat heart and liver mitochondria data (39). This dilemma may reflect a particularly high rate of β-oxidation for 13*c*- and 13*t*-18:1 by the peroxisomal pathway as suggested by Osmundsen for hydrogenated marine oils (40).

Platelet selectivity values were obtained on a 24-hr sample and reflect lipid metabolism in tissue since platelets contain the enzymes necessary for lipid metabolism. The negative platelet selectivity values for CE and PC were consistent with the negative plasma selectivity values. In contrast, the positive platelet TG selectivity values were not consistent with the negative plasma TG selectivity data. This result supports the concept that TG containing 13*t*- and 13*c*-18:1 isomers were selectively incorporated into platelets but they were then oxidized rather than converted into CE or PC.

Lipoprotein lipids

Differences between lipoprotein lipids were found for both selectivity values and percentage of 13*t*- and 13*c*-18:1 isomers incorporated into specific lipoprotein lipids (Table 2). These values reflect differences in the turnover of those lipid structures containing the deuterated fatty acids. The selectivity values indicate that during incorporation of chylomicron fatty acids into VLDL, LDL, and HDL, the relative turnover or affinity of the lipid classes containing 13*t*, 13*c*, and 9*c*-18:1 vary depending on the lipid class, fatty acid structure, and lipoprotein fraction. These differences are surprising since saturated and polyunsaturated fat diets were reported to have little effect on lipoprotein phospholipid fatty acid composition in humans (41). The presence of significant amounts of deuterated fatty acids in chylomicron phospholipids is probably the result of incorporation during formation of the chylomicron, rather than due to exchange of deuterated lipids between the various lipoprotein fractions. Both the higher percentage of deuterated fatty acid in the chylomicron phospholipid fractions compared to the other lipoprotein phospholipids and the selectivity values support this argument.

The TG in the 4-hr VLDL, LDL, and HDL fractions contained a significant level (29.1%, 16.7% and 20.1%) of total deuterated 18:1, which indicates these lipoproteins may also be formed by the intestinal cells. Equilibration of lipid classes associated with chylomicron and the various lipoproteins would not explain the presence of deuterated fatty acids in the various lipoprotein fractions, because selectivity values and percentages of deuterated fatty acids vary. Alternatively, exchange of FFA would produce differences in selectivity values if the rates at which FFA is formed by hydrolysis and reesterified into phospholipids were dependent on the specific lipoprotein fraction associated with the various lipid classes.

Fatty acid composition

Fatty acid composition of plasma-PE and LPC (Table 4) suggests that the 13*c*/13*t*-18:1 isomers apparently replace saturated fatty acids and increase polyunsaturated fatty acids but to a lesser degree than previously reported for the 12*c*/12*t*-18:1 isomers (42). The polyunsaturate to saturate ratio is not decreased in the plasma phospholipids and cannot be used to support the concept that 13*c*/13*t*-18:1 isomers enhance the atherogenic properties associated with saturated fats.

The plasma cholesteryl ester fraction exhibited no change in fatty acid composition, which was not surprising since only low levels of deuterated fatty acid were incorporated. The low percentage of 13*t*- and 13*c*-18:1 fatty acids, plus the strong negative selectivity values, indicated cholesterol esterase and/or lecithin:cholesterol acyl transferase (LCAT) activity is relatively low for the 13*t*- and 13*c*-18:1 isomers. Glomset (43) has suggested that most of the CE synthesized in human plasma is catalyzed by LCAT. Therefore, these results would indicate LCAT rather than cholesterol esterase activity for 13*t*- and 13*c*-18:1 is low compared to 9*c*-18:1. The strong specificity against incorporation of the 13*t*- and 13*c*-18:1 isomers could also be the consequence of the low percentage of 13*t*- and 13*c*-18:1 in the 2-acyl position of PC. In fact, the selectivity values for 2-acyl PC correlated with the CE selectivities. A similar correlation was previously reported for CE and 2-acyl PC data with the 12*t*-18:1 and 9*t*-18:1 isomers but not with the 12*c*-18:1 isomer (21, 23).

Nutritional impact

The respective average daily consumption of the 13*c*- and 13*t*-18:1 isomers is estimated to be 0.14 g and 0.48 g (26). These amounts are small compared to the approximately 168 g of fat consumed daily in U.S. diets (44). The low level of consumption coupled with evidence for no selective accumulation (other than in the 1-acyl position of PC), turnover rates comparable to 9*c*-18:1, negative selectivity values that imply β-oxidation rates for 13-18:1 isomers are higher than for 9*c*-18:1, and no unfavorable effect on fatty acid composition (decrease in P/S ratio) do not support the premise that 13*t* and 13*c*-18:1 isomers would have undesirable nutritional effects in humans.

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