Incorporation of Deuterium-Labeled *cis*- and *trans*-9-Octadecenoic Acids in Humans: Plasma, Erythrocyte, and Platelet Phospholipids

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ABSTRACT

The objective of this study was to follow the uptake and distribution of oleic and elaidic acids into human erythrocytes, platelets, and plasma phospholipids. The use of dual and triple labeling methodology permitted a precise comparison of elaidic and oleic acid utilization. Elaidic acid (EI) was selectively concentrated in all the plasma phospholipids except for lysophosphatidylcholine. Three times more elaidic than oleic acid (OI) accumulated in the 1-acyl position of phosphatidylcholine, as determined by hydrolysis with phospholipase A_2. Rapid incorporation and removal of elaidate were observed for all samples. These results support the concept that enzymes responsible for acylation of phospholipids are sensitive to double bond configuration and the physical properties of the fatty acid moieties. Labeled fatty acid levels in red cell and platelet phospholipids were much lower than for plasma phospholipids, indicating a relatively slow rate for the in vivo incorporation of fatty acids into blood cell membrane phospholipids. No isotope effect was found when oleic acid labeled with deuterium on the double bond was used.

Over 5.5 billion lb. of vegetable oil is hydrogenated annually for use in margarines, salad oils, cooking fats, and shortening (1). This hydrogenation process results in the formation of ca. 15% *trans* and positional fatty acid isomers (2-8). These oils are a major source of dietary fat, and it is estimated the average American diet contains ca. 8% *trans* fatty acid. Metabolism and biological effects of isomeric fatty acids have been studied in rats, monkeys, chickens, swine, rabbits, and man. The uptake and deposition of *trans* isomers into rat (9), swine (10), and human tissue (11) has been documented. Specific geometric and positional fatty acid isomers have been shown to be incorporated into egg yolk lipids (12-14), human blood plasma (15), rat liver (16-20), rat fetuses (21), rabbit serum lipids (22), and *Escherichia coli* membranes (22-27).

Hydrogenated fats have been correlated with an increase in serum cholesterol and triglyceride levels in humans (28-32), increase in fragility of erythrocytes (17), alteration of the lipid transport function of rat mitochondria (17), increase in essential fatty acid requirements (33-34), increase in long chain unsaturated fatty acids content of rat phospholipid (35-36), increases in serum cholesterol levels in swine (37) and rabbits (38,39), alterations in membrane function in *E. coli* (26,27), and increase in rate of de novo fatty acid synthesis from acetate in rat liver (40).

However, contradictory results have been reported by several researchers. For example, serum cholesterol levels in man have been reported both to be elevated and to remain unchanged by diets containing hydrogenated fats. Long term feeding studies have indicated no change in the growth, reproduction, longevity, or size of mice and rats fed hydrogenated fats (41-46).

The results reported in previous investigations with human subjects have all involved hydrogenated fat that is a mixture of isomeric fats. This study specifically compares the utilization of oleic to elaidic acid in human phospholipids and is an extension of previous data reported for human plasma neutral lipids (47).

EXPERIMENTAL

Experimental Design

The metabolism of elaidic (E1) acid and oleic (O1) acid in man was compared by feeding pairs of deuterated analogues of these acids. In these dual-labeled experiments, the deuterium label on at least one of the pairs of fatty acids was placed in a different position, and the number of deuterium labels per mole-

[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.

TABLE I

Amount and Identity of Dueterated Triolein and Trielaidin Fed to Humans

Subject	Fatty acid fed[a]	Total wt fed (g)	El(%)	01(%)	El/01 ratio
1	El-9,10-d_2 + 01-12,12,13,13-d_4	36.2	48.7	51.3	0.95
2	El-8,8,11,11,-d_4 + 01-9,10-d_2	34.0	46.5	53.5	0.87
3[b]	El-13,13,14,14-d_4 + 01-9,10-d_2 + 01-8,8,13,13,14,14-d_6	35.5	31.4	68.6[c]	0.91

[a]El = elaidic acid and 01 = oleic acid; numbers indicate position of deuterium on the acyl chain of the fatty acid.

[b]El-d_4/01-d_6 ratio fed = 0.91; 01-d_2/01-d_6 ratio fed = 0.98; El-d_4/(01-d_2 + 01-d_6) ratio fed - 0.46.

[c]Contains 34.0% 01-9,10-d_2 and 34.6% 01-8,8,13,13,14,14-d_6.

cule of fatty acid was varied as shown in Table I. This technique was used to detect analytical or biological isotope effects due to the position or number of deuterium labels. In the case of the third subject, 01-d_2, 01-d_6, and El-d_4 were fed in a triple-labeled experiment in order to detect specific deuterium isotope effects that may occur because of the presence of deuterium on the 9,10-double bond position. In dual- and triple-labeled feeding experiments, the same analytical and biological variation must occur equally to both fatty acids since they are fed to the same subject at the same time. Each subject acts as his own control and thus, a comparison of El and 01 incorporation and disappearance in a dual-labeled experiment is more accurate than if only one labeled fatty acid was fed to each subject. Consequently, the effect of dietary, genetic, or biological variation on comparison of fatty acid utilization is reduced in the dual-labeled experiments.

Calculation of selectivity factors. Selectivity factors for comparing the relative utilization of El vs. 01 can be calculated and used to indicate the preference for elaidic acid uptake compared to oleic acid. Such calculations are not simple for experiments in which a single fatty acid is fed to a limited number of subjects, because variations in the fatty acid metabolism between subjects can be so large that the results are not statistically significant. Selectivity factors used in this paper are calculated by dividing the El/01 ratio found in the lipid fraction by the El/01 ratio of the fed mixture. A selectivity factor greater than 1 indicates preferential incorporation of elaidic acid, and a value less than 1 indicates preferential incorporation of oleic acid. Selectivity factors are used also to compare data when subjects are fed mixtures containing different El/01 ratios.

Deuterated Elaidic and Oleic Acids Fed

Oleic acid-9,10-d_2 (15), oleic acid-12,12,13,13-d_4 (15), oleic acid-8,8,13,13,14,14-d_6 (47), elaidic acid-9,10-d_2 (15), elaidic acid-8,8,11,11-d_4 (47), and elaidic acid-13,13,14,14-d_4 (15) were synthesized and converted to their respective triglycerides as previously described.

Feeding and blood sampling. Three male Caucasians, ages 23-27, were given mixtures of labeled triglycerides (Table I) emulsified with casein (30 g), dextrose (30 g), sucrose (15 g), and water (200-250 ml). The subjects were medical students who were in good health as judged by their general appearance, no apparent illness, or congenital ailments. Their weight, blood pressure, serum cholesterol, and triglyceride levels were normal. The diets were not controlled before or after administering the deuterated triglycerides except that food was withheld 10 hr before feeding and the subjects ate nothing for 4 hr after consuming the labeled fats except for juice or coffee. The subjects were requested not to eat high-fat meals for 12 hr after they drank the mixture of deuterated triglycerides.

Blood samples (40 ml) were drawn at 0, 2, 4, 6, 8, 12, 16, 24, and 48 hr intervals after the mixture was consumed. the 0-hr sample was used to determine fatty acid composition before the deuterated fat was ingested. Blood samples (50 to 100 ml) were also drawn at 6, 12, and 24 hr for platelet isolation. Procedures for drawing blood samples, isolating red cells, plasma, and platelets, and extracting lipids have been described previously (15,48).

Separation and Derivatization of Phospholipids

The phospholipids were extracted (15) and separated by preparative thin layer chromatography (TLC) on 2-mm silica gel plates. A chloroform/methanol/petroleum ether/water (8:8:6:1) solvent mixture was used as the developing system (49). Phospholipid bands were visualized by exposing the edges of the TLC plates to iodine vapor. The bands were then identified by comparing them to authentic standards that were chromatographed on the

same plate. The phospholipid bands were scraped from the TLC plate and extracted two times from the silica gel using 2:1 $CHCl_3$/MeOH followed by a single extraction with 3:5:2 $CHCl_3$/MeOH/H_2O (50). After extraction of the phospholipids either from the blood samples or from silica gel, the temperature of the extract was kept below 30 C and never evaporated to dryness. All phospholipid extracts were stored in 19:1 chloroform/methanol at -25 C under nitrogen. These precautions are required to prevent phospholipid oxidation, poor resolution by TLC, and loss of sample. Antioxidants were not needed to prevent autoxidation if the samples were isolated according to the above procedure.

Phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylcholine (PC), and lysophosphatidylcholine (lyso PC) were methylated by mixing with 1 ml dry benzene and 2 ml 5% HCl-methanol, flushing with nitrogen, and heating at 65 C for 2 hr in screw-cap culture tube. Sphingomyelin (SM) was methylated by mixing with 1 ml dry benzene and 2 ml 10% HCl-methanol and heating at 65 C for 5 hr (51).

Phospholipase A_2 hydrolysis of PC with *Ophiophagus hannah* venom (Ross Allen Reptile Institute, Silver Springs, FL) was used to remove the 2-acyl fatty acid (52). Products from the reaction mixture were purified by streaking the entire reaction mixture on a 2 mm preparative silica gel TLC plate and developing with the chloroform/methanol/petroleum ether/water (8:8:6:1) solvent system. The lysophosphatidylcholine (PC-1) and free fatty acid (PC-2) reaction products from phospholipase A_2 hydrolysis of PC were extracted from the silica gel, recovered, and methylated by the benzene-5% HCl-methanol procedure.

Analysis of Phospholipids

The methyl esters of the separated phospholipids were analyzed for deuterium-labeled fatty acid content by combined gas chromatography-mass spectrometry (GC-MS) using selected ion monitoring of the molecular ion or the molecular ion-31 peaks. A Nuclide Model 12 90 DF mass spectrometer equipped with a silicon rubber membrane separator was employed to separate the carrier gas from the fatty methyl esters. The small sample size and large number of analyses did not permit extensive replication and classic statistical analysis of the data in Table II. The data was determined to be accurate and significant because of three factors: (1) data plotted in Figures 1 through 6 yield curves which are generally smooth; (2) for those samples where there was sufficient sample

TABLE II

Analysis of Plasma Phospholipids

Subject	Phospholipid[a]	Time, hr	Selectivity for E1[b]
1	PE	6	1.50
2	PE	8	1.55
3	PE	6	2.82[c]
1	PS	8	0.80
2	PS	12	1.08
3	PS	8	2.29[c]
1	PC	12	1.40
2	PC	12	1.08
3	PC	16	1.54[c]
1	PC-1	12	3.39
2	PC-1	16	3.25
3	PC-1	12	4.40[c]
1	PC-2	12	0.83
2	PC-2	16	0.31
3	PC-2	12	0.90[c]
1	SM	12	1.24
2	SM	12	3.50
3	SM	12	1.24[c]
1	Lyso-PC	8	0.73
2	Lyso-PC	8	0.92
3	Lyso-PC	8	4.35[c]

[a]Phospholipid abbreviations: PE = phosphatidylethanolamine; PS = phosphatidylserine; PC - phosphatidylcholine; PC-1 = 1-acyl phosphatidylcholine; PC-2 = 2-acyl phosphatidylcholine; SM = sphingomyelin; Lyso-PC = lysophosphatidylcholine.

[b]E1 = elaidic acid.

[c]E1 selectivities for subject 3 phospholipids are based on E1-d_4/01-d_6 ratios.

for replicate analyses, the replicates agreed within several tenths of a percent; and (3) the selective-ion-monitoring analytical procedure has been extensively studied and found to be reliable (53). In a study on the accuracy of the selective-ion-monitoring GC-MS procedure, nine replications were made for each of nine dilutions, which showed that a linear regression line could be drawn through the data such that the percent relative standard deviation is equal to $2.2\sqrt{W}$, where W equals the weight of the deuterated fatty acid in the sample. If this same error is projected to Table II, the expected standard deviations would be in the range of 0.2 to 0.9%, depending on the amount of sample available for analysis and the amount of label in the sample. The larger standard deviation occurs for those samples containing the smallest amount of sample and lowest percentage of deuterated fatty acids.

The total fatty acid profile of the lipid fractions was determined on a Packard Model 7400 gas chromatograph equipped with flame ionization detectors and a 6 ft X 4 mm 10% EGSS-X column. Methyl elaidate and methyl

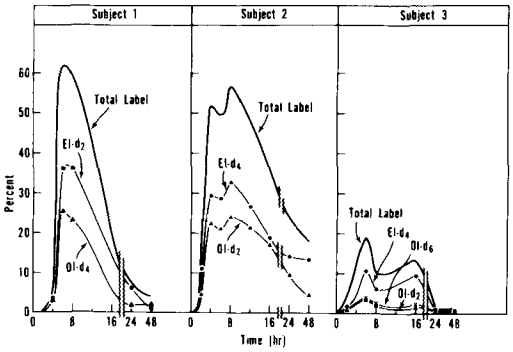

FIG. 1. Incorporation of deuterated oleic acid (O1) and elaidic acid (E1) into plasma phosphatidylethanolamine of three human subjects. The curves identified as O1, E1, and "total label" give the percent deuterated O1, E1, and O1 + E1 contained in the total octadecenoate portion of the samples. See Table I for composition of the deuterated triglyceride mixture fed.

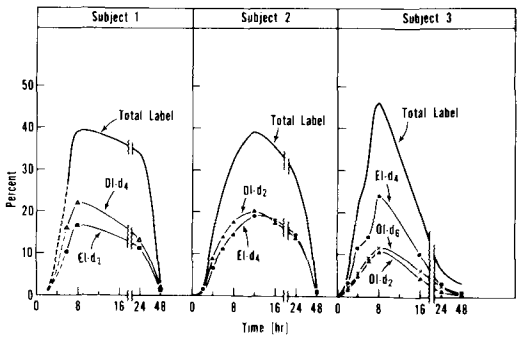

FIG. 2. Incorporation of deuterated O1 and E1 into plasma phosphatidylserine of three human subjects. The curves identified as O1, E1, and "total label" give the percent deuterated O1, E1, and O1 + E1 contained in the total octadecenoate portion of the samples. See Table I for composition of the deuterated triglyceride mixtures fed.

oleate were analyzed on a 20 ft x 4 mm 10% silar 10 C glass column or a 20 ft X 4 mm 15% OV 275 glass column, and their percentages were calculated by integration of the peak areas.

RESULTS AND DISCUSSION

Uptake of Deuterated Fatty Acids by Plasma Phospholipids

Plasma-PE. Plasma-PE data from all subjects in Figure 1 show a definite preference for incorporation of elaidic acid compared to oleic acid. Total label curves in Figure 1 indicate a considerable variation in the uptake of labeled fat by each subject. Deuterated fatty acids accounted for 61.7% of the octadecenoic acids in plasma-PE of subject 1 compared to 19.4% for subject 3. These differences in total uptake reflect variation in our subjects due to unknown causes. This variation did not change the enzyme specificity for double bond configuration since all subjects show similar selectivities for E1. O1-d_2 and O1-d_6 curves in Figure 1 for subject 3 are similar and the O1-d_2/O1-d_6 ratio is the same as in the fed mixture. These data indicate the enzymatic reactions involved in plasma-PE synthesis are not changed by the deuterium on the 9,10-double bond position of O1-d_2.

Plasma-PS. O1 and E1 curves shown in Figure 2 for phosphatidylserine (PS) from subjects 1, 2, and 3 indicate contradictory results. O1 curves for subjects 1 and 2 show a preference for oleic acid incorporation, but the O1 curves from subject 3 indicate a selection for elaidic acid. O1-d_2 and O1-d_6 curves in Figure 2 for subject 3 show slightly less O1-d_2 is incorporated than O1-d_6 due to the deuterium on the double bond. Because O1-9,10-d_2 was fed to subject 2, the selectivity factor for O1 utilization may be slightly diminished by a small dueterium isotope effect. Maximum total labeled fatty acid incorporation is similar for all subjects in Figure 2 although the general shapes of the "total label" curves are different. As previously noted for plasma-PE, differences in the "total label" curves are caused by unknown factors. Lack of published data on labeled fat metabolism in humans prevents comparison of these curves with the results of other studies. These results indicate a specificity that varies from individual to individual for double bond configuration similar to that for plasma-PE, which is sensitive to double bond configuration.

Plasma-PC. Phosphatidylcholine data are given in Figure 3. The data are inconsistent since the preference for elaidic acid utilization observed for subject 1 disappears in the data for subject 2 and is only significant for the 16 hr sample in subject 3. Factors such as diet or difference in metabolic activity or stimulation should have an equal effect on both O1 and E1 utilization and thus are not expected to cause these inconsistencies. Variation in plasma-PC data may reflect differences in enzyme specificities, but the reason for altered enzyme specificities between subjects is unknown. The shape of the "total label" curves and the percentage of labeled fatty acid incorporated are similar except for the dip in the E1-d_4 curve for the 12-hr sample from the third subject. The "total label" curves suggest that the overall lipid metabolism of the subjects are similar, but the E1 curves indicate that there are differences

in the three subjects' abilities to specifically utilize E1.

The E1-d_4 content of the 12-hr sample from subject 3 in Figure 3 would need to be ca. 12% in order to form a smooth curve. The dip in the curve cannot be explained except to postulate that the elaidic acid was specifically mobilized to supply calories or for some other use.

Plasma PC-1. Curves in Figure 4 represent the fatty acid in the 1-acyl position of phosphatidylcholine. Analysis of the fatty acids in the one position of PC allows a more detailed understanding of the uptake of elaidate than can be obtained from the total PC data shown in Figure 3. Unfortunately the opportunity to analyze the positional fatty acids in all the phospholipid fractions was not available because of small sample size. The preferential incorporation of E1 into the 1-acyl position of PC is much larger than was found for total PC in Figure 3. These data are consistent with in vitro studies using rat liver enzymes (52,53) which showed that E1 is preferred over O1 as the substrate for 1-acyl-CoA:phospholipid acyl transferase acylation of the 1 position of PE and PC. These in vivo human studies confirm the validity of using the in vitro data to help predict the metabolism of isomeric fats in humans.

Plasma-SM. Results of the sphingomyelin analyses are given in Figure 5, and comparison of the O1 and E1 curves indicates enzyme specificities for elaidic acid. As observed for the PE "total label" curves in Figure 1, the "total label" curves in Figure 5 also show considerable variation. The 12-hr SM sample from subject 3 in Figure 5 shows a large reduction in total deuterated fat uptake. This result cannot be explained, but it correlates with the reduction in the elaidic acid level in the 12-hr PC fraction for subject 3 in Figure 3. Differences in the total deuterated fatty acid percentages shown by the "total label" curves reflect variation in the subjects' overall lipid utilization, which apparently has little effect on the preferential utilization of E1 or O1 since selectivity for E1 incorporation occurred in all plasma-SM samples. Only 2 data points are plotted for subject 1 in Figure 5 because the GC-MS data were not accurate for the other data points due to small sample size and problems with the esterification procedure. No deuterium isotope effect for O1-9,10-d_2 incorporation into SM was detected based on the O1-d_2 and O1-d_6 curves from subject 3 in Figure 5.

Plasma Lyso-PC. GC-MS analyses of plasma lyso-PC samples are plotted in Figure 6. In this figure, selective incorporation of oleic acid into plasma lyso-PC was observed in subject 1

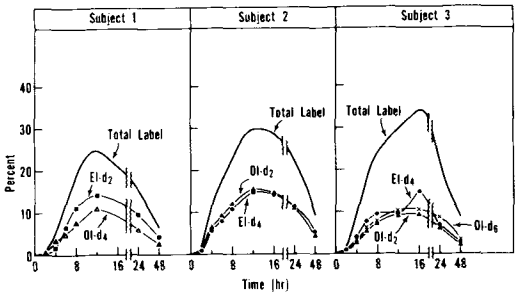

FIG. 3. Incorporation of deuterated O1 and E1 into plasma phosphatidylcholine of three human subjects. The curves identified as O1, E1, and "total label" given the percent deuterated O1, E1 and O1 + E1 contained in the total octadecenoate portion of the samples. See Table I for composition of the deutrated triglyceride mixtures fed.

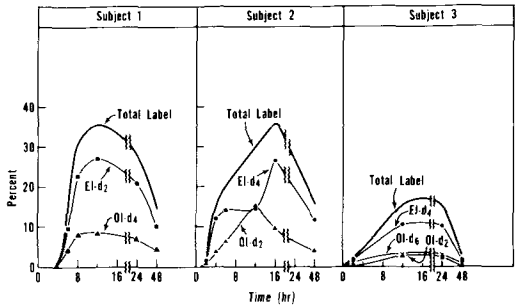

FIG. 4. Incorporation of deuterated O1 and E1 into plasma 1-acyl phosphatidylcholine of three human subjects. The curves identified as O1, E1, and "total label" give the percent deuterated O1, E1, and O1 + E1 contained in the total octadecenoate portion of the samples. See Table I for composition of the deuterated triglyceride mixtures fed.

and 2, but in contrast, a large selectivity for elaidic acid was found for lyso-PC data from subject 3. This inconsistency is similar to the one noted for the PS data in Figure 2 where the E1 selectivity was also reversed for subject 3 compared to the other two subjects. The percent total labeled fatty acid found in the lyso-PC fractions varied from 20 to 43%. The shape of the total label curves was similar in all experiments. These data support the concept that incorporation of individual fatty acids into lyso-PC is effected by double bond configuration. However, these data also indicate that other factors, such as diet or differences in the subject's metabolism, may be involved in controlling the fatty acids utilized in synthesis of lyso-PC.

Selectivity Factors

Selectivity factors are summarized in Table

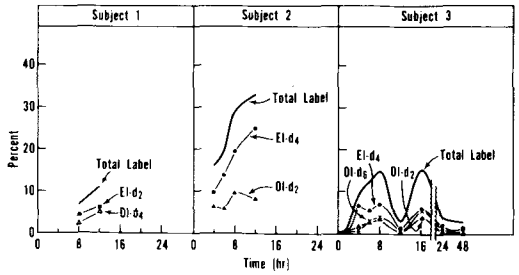

FIG. 5. Incorporation of deuterated O1 and E1 into plasma sphingomyelin in three human subjects. The curves identified as O1, E1, and "total label" give the percent deuterated O1, E1, and O1 + E1 contained in the total octadecenoate portion of the samples. See Table I for composition of the deuterated triglyceride mixtures fed.

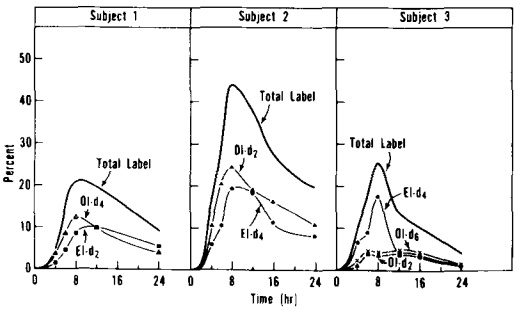

FIG. 6. Incorporation of deuterated O1 and E1 into plasma lysophosphatidylcholine in three human subjects. The curves identified as O1, E1, and "total label" give the percent deuterated O1, E1, and O1 + E1 contained in the total octadecenoate portion of the samples. See Table I for the composition of the deuterated triglyceride mixtures fed.

II for individual phospholipid samples that contained the maximum amounts of labeled fatty acid. All phospholipids selectively incorporated elaidic acid except for PS samples from subject 1, lyso PC samples from subjects 1 and 2, and the 2-acyl phosphatidylcholine samples from all three subjects. The selectivity factors indicate that our third subject had a greater tendency to incorporate elaidic acid than the first and second subjects. The fact that elaidic acid is concentrated in many of the phospholipids indicates dietary elaidic acid may have a larger impact on the structure of the phospholipids than would be predicted based on the *trans* content of hydrogenated fats.

Average E1 selectivity for the total PC samples listed in Table II is 1.34. E1 selectivity for the 1-acyl position of PC averages 3.7 compared to 0.68 for E1 at the 2-acyl position. Since phosphatidylcholine:cholesterol acyl transferase utilizes the fatty acids from the 2-acyl phosphatidylcholine position, the low E1 content of the PC-2 samples is partly responsible for the very low (0.19) E1 selectivity we previously reported for cholesteryl ester samples (48). Thus, incorporation of E1 into plasma cholesteryl ester samples is limited by the reduced activity of 2-acyl-CoA:phospholipid acyl transferase and phosphatidylcholine:cholesteryl acyl transferase for utilization of E1 as their substrate. This concept is supported by the low *trans* content of the cholesteryl ester samples from rabbits fed hydrogenated olive oil containing 37% E1 (55).

trans-Octadecenoic Acid Percentages

The total percent E1 (labeled plus unlabeled) in phospholipids containing the maximum amounts of deuterated fatty acids was determined by gas chromatography and compared to the total percent E1 in the 0-hr phospholipid fractions (Table III).

All 0-hr phospholipids from subjects 1 and 3 contained unlabeled E1. The 0-hr samples from subject 2 contained considerably less E1, and three of the phospholipid fractions (PC, PC-1, lyso PC) contained no unlabeled E1. The source of the E1 in these samples is probably hydrogenated vegetable oil. In all subjects, the total percent E1 (labeled and unlabeled) increase after deuterated E1 was fed. Maximum E1 levels in these phospholipid samples (see Table III) generally coincided with the maximum incorporation of deuterated E1 as determined by mass spectroscopy (see Figs. 1-6). In all the phospholipid samples, the total E1 determined by GC was equal to or greater than deuterated E1 as measured by mass spectrometry. This comparison of GC and MS data indicates there were no large errors in the mass spectrometry data since total E1 as measured by GC cannot be less than deuterated E1 if the mass spectrometry data is correct. In most of the phospholipid samples, total E1 percentage was approximately equal to the 0-hr E1 percentage plus percent deuterated E1. The correlation suggests deuterated E1 is added to the undeuterated E1 already present in the phospholipids. PC fractions generally contained less E1 in the 0-hr samples than other 0-hr phospholipid fractions, and PC fractions also accumulated less total deuterated E1. PC-1 samples had large E1 percentages in the 12- and 16-hr fractions, which reflects the selective incorporation of E1 into the 1-acyl position by 1-acyl-CoA:phospholipid transferase as mentioned earlier.

Fatty acid compositions (16:0, 18:0, 18:1, 18:2, and 20:4) in 0-hr samples were similar to the fatty acid compositions found at maximum deuterated fat incorportion after the data

TABLE III

Percent *trans*-Octadecenoic Acid in Plasma Phospholipid Fractions at 0 hr and at Maximum Deuterium Incorporation

Phospholipid[a]	Subject	E1[a] (%) (0 hr)	E1[a] %	E1[a] Time, hr
PE	1	10.7	37.2	6
PE	2	4.8	32.9	6
PE	3	9.6	17.4	6
PS	1	11.0	21.6	8
PS	2	8.5	24.2	12
PS	3	12.5	38.0	8
PC	1	2.6	13.6	12
PC	2	0.0	16.0	12
PC	3	4.7	15.0	16
PC-1	1	13.1	37.9	12
PC-1	2	0.0	26.6	16
PC-1	3	12.0	25.0	12
SM	1	19.3	35.0	12
SM	2	ND[b]	ND	---
SM	3	13.1	16.7	6
Lyso PC	1	15.1	37.0	8
Lyso PC	2	0.0	20.0	8
Lyso PC	3	7.1	19.5	8

[a]Abbreviations the same as in Table II.
[b]ND = not determined.

are normalized for the increase in deuterated 18:1. Thus, increases in E1 percentages are the result of the deuterated E1 diluting existing fatty acids and are not the result of selective replacement of any one fatty acid in the phospholipid samples.

Platelet and Red Blood Cell Data

Total platelet and red blood cell (RBC) phospholipids were analyzed by GC-MS for deuterated fatty acids and for total E1 by GC. Total deuterated fatty acid incorporation into platelet phospholipids was low (4 to 6% of the total octadecenoates). Platelet phospholipid samples (0-hr) contained 10-15% E1. The deuterated O1 and E1 percentages as determined by GC-MS contained an error of ±2% and, consequently, were not accurate enough to permit meaningful selectivity factors to be calculated. The poor accuracy was caused by a combination of small total sample size and low levels of deuterated fatty acid incorporation into the platelet phospholipids.

Red blood cell (RBC) phospholipids contained smaller levels of deuterated fatty acids than the platelet phospholipid samples. Total deuterated fatty acid percentages in the RBC phospholipids ranged from 0 to 4% of the total octadecenoates with an error of ±1.5%. These data were not useful for calculation of meaningful selectivity factors. Data summarized in this section provide direct evidence for in vivo incorporation of dietary fatty acids into platelet and RBC phospholipids. Since low levels (less than 6%) of deuterated fat were found in platelet and RBC phospholipids, this indicates that either incorporation of deuterated fatty acids or exchange of platelet or RBC phospholipids for plasma phospholipids occurs during the 10- to 12-hr period that plasma phospholipids contain over 10% deuterated fatty acids. The platelet and RBC phospholipid data from these studies are consistent with previous in vitro investigations where human RBC were incubated for 3-5 hr with ^{14}C-labeled fatty acids (56,57) or rat RBC with ^{32}P-labeled phospholipids (58). These in vitro studies were not designed to determine the total amounts of RBC phospholipid fatty acid that were replaced. Neehout (59) reported fatty acid profiles of human RBC phospholipids were not measurably changed during incubation with linoleic acid for 48 hr. Our data provide direct evidence that fatty acid incorporation into human RBC and platelet phospholipids also occurs in vivo at an estimated rate of 2-8% per 24 hr although the rate may be governed by the lipid level of the plasma.

The total E1 percentages for RBC total phospholipids ranged from 0 to 15%, which is approximately the same level as the plasma-0 hr E1 percentages given in Table III. The RBC

and platelet data, summarized in this section, indicate that phospholipid composition of the RBC and platelet membrane phospholipids reflects the plasma lipid fatty acid composition and that the same E1 selectivities observed for individual plasma phospholipids probably occur in platelet and RBC membrane phospholipids.

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