Human lactation: maternal transfer of dietary triglycerides labeled with stable isotopes[1]

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Abstract A stable isotope tracer method was utilized to measure quantitatively the secretion of diet-derived fatty acids (FA) into human milk. A mixture of [$^2H_6$]tripalmitin, [$^2H_{18}$]-triolein, and [$^2H_{12}$]trilinolein was administered to three healthy, lactating women 22 to 30 years of age. Milk and blood samples were collected sequentially for 72 hr. The FA composition and concentration of total plasma, lipoprotein, and milk triglycerides were determined by gas-liquid chromatography (GLC) and the isotopic enrichment was determined by gas-liquid chromatography-mass spectrometry (GLC-MS). There were no statistically significant differences in mammary secretion of the individual fats, either by a single individual or between subjects. The mean secretion of fat by one breast was 5.11 ± 1.26% of the dose (CV = 25%). There was a significant 6.0-hr delay between peak occurrence of the tracer in plasma and its occurrence in milk. The lipids are transported to the mammary gland primarily by the chylomicron and very low density lipoprotein triglycerides.—**Hachey, D. L., M. R. Thomas, E. A. Emken, C. Garza, L. Brown-Booth, R. O. Adlof, and P. D. Klein.** Human lactation: maternal transfer of dietary triglycerides labeled with stable isotopes. *J. Lipid Res.* 1987. **28:** 1185–1192.

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Human milk is a complex mixture of lipids, carbohydrates, proteins, and vitamins that should provide the infant with a nutritionally complete food (1, 2). The milk lipid composition, which consists of 98% triglycerides, 1% phospholipids, and 0.5% cholesterol and cholesteryl esters, is remarkably constant (3). These lipids contain long-chain fatty acids (> 16) derived from the diet and from mobilized adipose stores. Milk triglycerides also contain shorter chain (< 16) saturated fatty acids that are synthesized by the mammary gland. The relative contributions from these different lipid sources are unknown, but there are strong diet- and species-dependent factors that determine the fatty acid composition of milk lipids (4–6).

Chylomicrons, very low density, and low density lipoproteins transport dietary triglycerides to the mammary gland where they are hydrolyzed by lipoprotein lipase to free fatty acids and 2-monoglycerides. The free fatty acids are resynthesized into milk triglycerides in a positionally selective sequence that is characteristic of the mammary gland. These lipid transport pathways were established in animal models using either radiolabeled lipids or by measuring arteriovenous (A-V) differences in lipoprotein lipid concentrations across the mammary gland (7, 8). The conduct of similar studies in humans is impossible because of the hazards of radioisotope usage in normal healthy women and because of the invasive nature of A-V difference studies.

The purpose of our study was to determine the mechanisms that govern the transport of diet-derived fatty acids into human milk, to determine whether differences exist in the transport of specific fatty acids, to describe the dynamics of milk triglyceride synthesis, and to measure quantitatively the amount of dietary fatty acids incorporated into milk triglycerides. To achieve these goals, we have developed stable isotope tracer techniques using deuterium-labeled triglycerides of palmitic acids, and oleic and linoleic acids selectively labeled at different positions in the molecule.

Abbreviations: FA, fatty acid; GLC, gas-liquid chromatography; MS, mass spectrometry; A-V, arteriovenous; TLC, thin-layer chromatography; FAME, fatty acid methyl esters; HDL, high density lipoprotein; VLDL, very low density lipoprotein; LDL, low density lipoprotein; TG, triglyceride; PL, phospholipids; CE, cholesteryl ester; FFA, free fatty acid.

[1]Portions of this work were presented at the Twenty-fourth Annual Meeting of the American Society for Clinical Nutrition, where it was published in abstract form (Hachey, D. L., et al. 1984. *Am. J. Clin. Nutr.* **39:** 670).

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MATERIALS AND METHODS

Materials

The deuterium-labeled triglycerides were synthesized as described previously (9-11). A mixture of three triglycerides which contained 3.0 g each of the following was used in the study; $[^2H_6]$tripalmitin was prepared from $[9,10\text{-}^2H_2]$palmitic acid (86.7 atom %), $[^2H_{18}]$triolein was prepared from $[14,14,15,15,17,18\text{-}^2H_6]$oleic acid (91.0 atom %), and $[^2H_{12}]$trilinolein was prepared from $[15,15,16,16\text{-}^2H_4]$ linoleic acid (95.6 atom %). The isotopic enrichments of the pure triglycerides were determined by electron ionization gas-liquid chromatography-mass spectrometry (GLC-MS) analysis of the molecular ion. The wax-like mixture of triglycerides was melted at 85°C, then poured rapidly into 236 ml of Sustacal liquid (Mead Johnson, Evansville, IN) in a blender heated to 70°C. The Sustacal liquid contained 14.5 g of fat. The solution was blended for 20 min, then stored at 4°C in a covered container until used the following morning. This procedure produced a microdispersed lipid preparation that did not separate on standing overnight. Heptadecanoic acid, cholesteryl heptadecanoate, triheptadecanoin, and diheptadecanoyl-L-α-phosphatidylcholine (Sigma Chemical Co., St. Louis, MO) were used as internal standards for quantitation by GLC.

Subjects

Three healthy women were recruited at 2 to 5 months postpartum. The women were 22 to 30 years of age, and were lactating successfully; they were nonsmokers, on no medications, had normal dietary habits, and were within ± 20% of ideal body weight. The infants were full-term, appropriate for gestational age, and the products of normal pregnancies and deliveries. Informed consent was obtained from each subject after we explained the purpose of the study, the diet regimen, and the procedures for collecting blood and milk samples. No procedures were performed on the infants; they were test-weighed before and after feeding to determine milk production. The protocol was approved by the Institutional Review Boards for Human Research of Baylor College of Medicine and Texas Children's Hospital.

Study protocol

Subjects were asked to keep a 3-day dietary record before the study, which was used to determine their usual daily caloric intakes. Three days before admission to the Clinical Research Center (CRC) at Texas Children's Hospital, each subject was asked to replace her usual diet with a controlled, chemically defined diet of Sustacal that was based on an analysis of her dietary records. The Sustacal diet was continued during the 4-day study period in the CRC. Study participants were permitted to select a maximum of 15% of their calories from an approved list of fruits, vegetables, and cereals which did not contain any significant quantities of fat. The caloric intakes and nutrient analyses of this diet are summarized in **Table 1.** Sustacal liquid contains 14.6 g of protein (26 cal %), 33.6 g of carbohydrate (55 cal %), and 5.5 g of fat (19 cal %) per can. Sustacal pudding contains 6.0 g of protein (11 cal %), 32.0 g of carbohydrate (53 cal %), and 10.0 g of fat (36 cal %) per can.

Subjects were admitted to the CRC on the evening before the study. The following morning a heparin lock was placed into the antecubital vein of the forearm. In place of their usual breakfast, the subjects consumed the test meal of Sustacal liquid that contained a microdispersion of 9.0 g of deuterated triglycerides. The test meal contained 22.6 g of total fat, which consisted of 4.4 g of 16:0, 0.6 g of 18:0, 9.3 g of 18:1, 7.94 g of 18:2, and 0.33 g of 18:3. The calculated isotopic enrichment of the test meal lipids was 68.2 atom % $[^2H_2]$16:0, 32.2 atom %, $[^2H_6]$18:1, and 37.8 atom % $[^2H_4]$18:2. Total milk from one breast and blood samples (3 ml) were collected at 0, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 36, 48, and 72 hr. A larger blood sample (10 ml) was collected at 4,6,8,12, and 16 hr for lipoprotein lipid analyses. A small aliquot of the total milk was flash frozen (-70°C) and saved for analytical purposes. Plasma was obtained from blood by centrifugation. The smaller samples were frozen for a subsequent total lipid analysis. The larger plasma samples were stored at 4°C until the 16-hr sample was collected. The plasma samples were delivered by courier later that day to Dr. E. A. Emken at the USDA Northern Regional Research Laboratory, Peoria, IL, for lipoprotein separations.

Milk samples were collected from one breast using an Egnell electric breast pump and the infant was nursed from the other. Milk analyzed during the study was col-

TABLE 1. Subject data, diet analysis, and milk production

	Subject number			
	1	2	3	Mean ± SD
Subject data				
Age (yr)	22.0	30.5	26.2	26.2 ± 4.3
Height (cm)	160	163	163	16 ± 1.7
Weight (cm)	65.9	67.8	61.7	65.1 ± 3.1
Infant's age (wk)	25.3	28.0	7.4	20.2 ± 11.2
Diet analysis				
Caloric intake (kcal)[a]	1687	1643	2565	1965 ± 520
% Protein[b]	17.3	17.7	16.0	17.0 ± 0.9
% Carbohydrate[b]	57.3	55.7	55.3	56.1 ± 1.1
% Fat[b]	25.3	26.7	28.7	26.9 ± 1.7
Milk production				
Mean sample volume (ml)	38.5	45.0	51.2	44.9 ± 6.4
Output (ml/day)	610	805	747	721 ± 100
% Fat (24-hr mean)	3.3	2.8	3.0	3.0 ± 0.2

[a]Three-day mean.
[b]Cal %.

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lected entirely from a single breast to minimize any lateral differences in milk production or composition which may exist (12). Total milk production was determined on the basis of both the volume collected by pump and the amount consumed by the infant, which was measured by test-weighing before and after each feed.

Milk and plasma lipid extractions and separations

Triheptadecanoin (5 mg in chloroform) internal standard was added to 1 ml of human milk. The milk was extracted with 2 ml of water, 1 ml of 0.6 N ammonium hydroxide, and 5 ml of chloroform-methanol 3:1. The organic layer was washed once with 5 ml of 2% potassium diphosphate and 5 ml of ethyl ether was added to bring the total volume to approximately 10 ml. The organic layer was dried over anhydrous magnesium sulfate. This procedure gives nearly quantitative recovery of nonpolar lipids, but it may not recover all of the polar phospholipids and free fatty acids. The methyl esters were prepared by standard techniques (13). This sample was analyzed both by GLC and by GLC-MS.

Blood samples for total plasma lipid analysis were drawn into standard EDTA tubes (Vacutainer; Becton, Dickinson, and Co., Rutherford, NJ) and kept on ice until plasma could be separated by centrifugation. A cocktail of internal standards containing 100 µg each of 17:0 triglyceride (TG), phospholipid (PL), cholesteryl ester (CE), and 10 µg of the free fatty acid (FFA) standards was added to 0.5 ml of plasma. The plasma was extracted with 10 ml of chloroform-methanol 2:1 using a vortex mixer or rotator. The organic layer was filtered, and the residue was washed once with 1 ml of chloroform-methanol and then evaporated to approximately 0.5 ml. The lipid extract was dissolved in 4 ml of chloroform-methanol 19:1, dried over 4 g of anhydrous sodium sulfate, filtered, and evaporated to dryness. The lipids were redissolved on 0.4 ml of chloroform for lipid class separations.

The individual plasma lipid classes were separated on a disposable aminopropyl solid phase extraction column (Bond Elut #611313, Analytichem International, Harbor City, CA) using a modified procedure (14). Fresh columns were washed twice with 2 ml of hexane and the chloroform extract containing the lipids was applied to the column. Both TG and CE were eluted with 4 ml of chloroform-isopropanol 2:1. FFA was eluted with 4 ml of 2% acetic acid in diethyl ether. The total phospholipid fraction was eluted with 4 ml of methanol. The fraction containing TG and CE was evaporated to dryness; all residual chloroform was carefully removed to prevent incomplete separation of the TG from the CE in the subsequent steps. This fraction was dissolved in 0.2 ml of hexane and applied to a freshly washed extraction column. The CE was eluted with two 8-ml hexane washes. Two rinses with hexane were required to insure complete recovery of CE and to prevent any cross-contamination of the TG that eluted afterward. The TG was eluted with 8 ml of 1% diethyl ether and 10% methylene chloride in hexane. The completeness of the separations was monitored by TLC on a silica gel G plate (Supelco Inc., Bellefonte, PA) and developed with 1% acetic acid and 10% diethyl ether in hexane. TLC plates were visualized with iodine vapor. The separated lipid fractions were converted to the methyl esters for analysis by GLC and by GLC-MS.

Lipoprotein separations and lipid isolation

Blood samples for lipoprotein lipid analysis were drawn into standard EDTA tubes (Vacutainer; Becton, Dickinson, and Co., Rutherford, NJ). Plasma was separated by centrifugation within a few minutes of collection. Lipoproteins were separated into chylomicron, very low density (VLDL), low density (LDL), and high density (HDL) fractions by preparative ultracentrifugation. Total lipids were extracted from the individual lipoprotein fractions with chloroform-methanol 2:1 after the internal standards were added. The lipids were separated into TG, CE, FFA, and PL classes. The details of these methods have been described elsewhere (15). No attempts were made to separate milk lipids into individual classes for these studies. Any differences between fatty acid composition or isotopic enrichment of the minor PL and CE lipids and the major TG would not significantly alter the total FA composition.

Gas chromatographic analysis

Fatty acid methyl esters (FAME) were analyzed using one of two cyanopropyl silicone capillary GLC columns in a model 5840 gas chromatograph equipped with a flame ionization detector (Hewlett-Packard, Avondale, PA). Individual FAME were identified by comparison of their retention times with those of authentic commercial standards (Supelco, Inc., Bellefonte, PA). The first column was a 25 m × 0.5 mm, 1.0 µ phase Silar-10C glass column (Alltech Associates, Inc., Deerfield, IL). The injector and detector temperatures were 230°C; the column temperature was programmed from 120°C to 230°C at 5°C/min at a helium flow rate of 2.5 ml/min. The second column was a 30 m × 0.32 mm DB-225, 0.25 µ phase fused silica column (J and W Scientific, Inc., Rancho Cordova, CA). The injector and detector were 200°C; the column temperature was programmed from 150° to 220°C/min at helium flow rate of 1.5 ml/min. Methyl *trans*-9-octadecenoate was not completely separated from the *cis* isomer (methyl oleate) on either of these columns. All of the lipid samples contained a known amount of a 17:0 internal standard which was used to calculate the amount of a specific fatty acid present in a given sample. No corrections were applied to these data to account for the small amount (0.2 to 0.4%) of 17:0 normally present in human milk.

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Isotopic analysis by GLC-MS

Isotopic analysis was performed by capillary GLC-MS (MAT 212 GC-MS with the SS-200 data system, Finnigan MAT, San Jose, CA) using 70 ev electron ionization at a source temperature of 200°C. Two time windows for ion quantitation were established with the data system: one to measure the isotopic abundances of methyl palmitate at m/z 270 and 272, and the second to measure the isotopic abundances of methyl oleate at m/z 296 and 302 and methyl linoleate at m/z 294 and 298. These ions, which represent the molecular ion region, were used for quantitation of FAME obtained from milk samples. Ammonia chemical ionization MS, which enhances the ionization of unsaturated fatty acids, was used to analyze samples containing small quantities of plasma lipoprotein FAME samples. The ammonia adduct ions that were used for quantitation were: methyl palmitate, m/z 288 and 290; methyl oleate, m/z 314 and 320; methyl linoleate, m/z 312 and 316.

Isotopic enrichment of palmitate was determined by subtracting the isotopic ratio of each subject's zero time sample for m/z 272 or 288 ([M + 2], 2.1%) from all subsequent timed samples. Isotopic enrichments of oleate and linoleate were derived directly from the measured isotope ratios since there is no significant natural isotopic background present at the [M + 4] and [M + 6] masses (16). The data are expressed as atom percent composition.

Computations

The actual mass of specific fatty acids present in a sample was determined by GLC, based on a known amount of a 17:0 internal standard added to a measured sample volume. The mass of a deuterated fatty acid present in the sample was obtained by multiplying the total mass of a specific FA by the isotopic enrichment of that FA. The results are expressed in μg/ml for total plasma and lipoprotein lipids, in mg/ml for milk TG, and in percent dose/ml. A two-way analysis of variance was done using either of two methods to account for a missing value (17).

RESULTS

Milk fatty acid composition

The physical characteristics, dietary intakes, milk output, and total milk fat content for each subject are summarized in Table 1. **Table 2** compares the FA composition of milk obtained from each subject, together with the weighted average of the Sustacal liquid and pudding lipids. The subject data are the mean values of the individual samples that were collected according to the schedule described earlier. The coefficients of variation (CV) for these averaged data were typically 2 to 5% for the major FA (16:0, 18:1, and 18:2) and were due primarily to diurnal variations after meals. The CV was 5 to 10% for the minor FA. We observed normal diurnal variations in milk fat concentration (18). The total fat concentration (2.0 to 4.3%) in individual milk samples obtained from these subjects was within normal limits.

Isotope enrichment in milk fatty acids

Representative isotopic enrichments of 16:0, 18:1, and 18:2 in milk from subject 3 are illustrated in **Fig. 1.** The peak enrichment was observed 8 to 10 hr after the labeled TG meal was consumed. Peak enrichments were 6.5 to 15.5 atom percent excess (range for all three subjects). The variations in enrichment were due to the dilution of the tracer by the test meal lipids, which was constant for these studies, and subsequently by intestinal and plasma lipids before secretion into milk. The second factor was the major source of variability between subjects in the isotopic enrichment data. The milk TG isotope ratios decreased monoexponentially from 10 to 48 hr, but there was visual evidence of a second, slower exponential component from 48 to 72 hr.

Fig. 2 summarizes the mean isotopic enrichment data for each of the fatty acids, normalized to the percent dose of the tracer per ml of milk. These data were derived from the milk fatty acid concentrations determined by GLC and the isotopic enrichments determined by GLC-MS.

TABLE 2. Weight percent composition of human milk fatty acids and Sustacal lipids[a]

Fatty Acid	Subject 1	Subject 2	Subject 3	Sustacal[b]
10:0	1.07 ± 0.06	1.10 ± 0.11	1.37 ± 0.16	0.02
12:0	3.61 ± 0.22	4.30 ± 0.38	4.28 ± 0.48	0.19
14:0	2.95 ± 0.12	3.89 ± 0.21	3.37 ± 0.35	0.24
16:0	20.07 ± 1.02	18.93 ± 0.76	16.61 ± 0.91	10.37
16:1	2.97 ± 0.31	1.34 ± 0.10	1.94 ± 0.34	0.02
18:0	5.79 ± 0.19	6.75 ± 0.32	5.51 ± 0.41	4.85
18:1	38.48 ± 1.33	35.76 ± 1.76	35.02 ± 1.76	46.48
18:2	23.43 ± 1.35	26.18 ± 1.21	29.89 ± 2.25	35.49
18.3	1.00 ± 0.11	1.08 ± 0.11	1.41 ± 0.14	2.32
20:4	0.63 ± 0.03	0.68 ± 0.02	0.60 ± 0.07	0

[a]Mean ± SD of 11 samples collected over the first 24-hr period of the isotope tracer study.
[b]Weighed mean of the fatty acids found in one can each of liquid and pudding.

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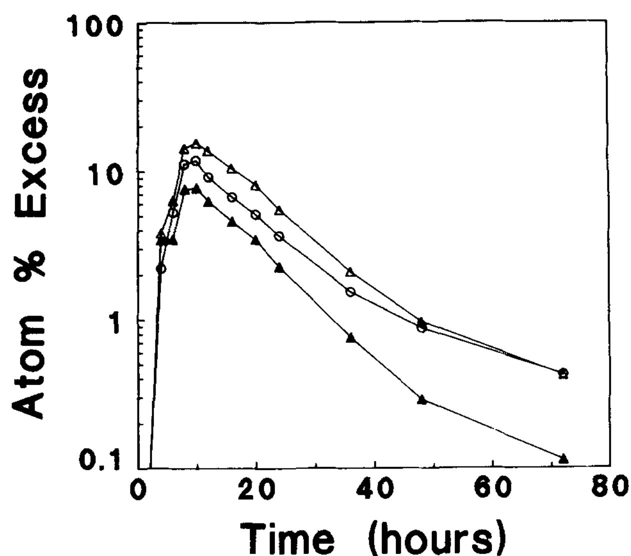

Fig. 1. Typical enrichment pattern of milk fatty acids observed in one subject (#3); (△), [$^2H_2$]16:0; (○), [$^2H_6$]18:1; (▲), [$^2H_4$]18:2.

Because a known amount of an internal standard (triheptadecanoin) was added to each milk sample, we could determine quantitatively the milk fat composition and concentration. These data illustrate the delayed secretion of the tracer into milk fat and a multiexponential decrease in isotopic enrichment. No significant differences were observed in the secretion patterns of individual fatty acids.

Fig. 3 illustrates the cumulative secretion pattern of the tracer lipids into milk. These data are the mean cumulative percent dose secretion data of all three subjects, normalized to the amount of tracer ultimately secreted into milk over the 3-day period of the study, approximately 5.1%. Approximately 97% of the test meal lipids which ultimately occur in milk are secreted within the first 24-hr period.

Table 3 summarizes the cumulative percent dose (CPD) of the labeled fatty acids secreted into milk over 72 hr. These data are the product of the percent dose per ml data shown in Fig. 2 and the volume of milk collected in a given sample. No statistically significant differences were found either for the secretion of a specific FA by all subjects or for the secretion of all FA by a single subject.

Plasma triglyceride isotope enrichment

The clearance of deuterium-labeled TG [$^2H_6$]18:1 from various plasma components is illustrated in **Fig. 4** for a typical study. The highest value we found in chylomicron TG was at 4 hr, the earliest sampling time for lipoprotein lipids. Maximum enrichment in total plasma TG was 14 to 21 atom percent excess, depending on dilution of the tracer by the test meal, and subsequently by intestinal and endogenous fat. These levels were achieved by 2 to 4 hr. The maximum chylomicron TG enrichment was 17 to 23 atom percent excess. We observed the usual course of lipid transfer and dilution of labeled FA from highly enriched chylomicron TG to VLDL TG and then to LDL and HDL TG (19).

Fig. 5 shows the percent dose of labeled TG per ml in total plasma TG lipids. These are mean values of the individual fatty acids for all subjects. These values are the product of total plasma TG concentration and the isotopic enrichment of individual fatty acids, normalized to the original tracer dose administered to each subject. The peak enrichment was reached by 2 to 4 hr in plasma TG. We observed no significant differences in the clearance of individual FA from plasma. **Table 4** summarizes the time delays between occurrence of the peak enrichment in total plasma TG and in milk. The mean delay was 6.0 ± 1.9 hr for all fatty acids and subjects. No differences were detected in the delay times of individual fatty acids within a subject.

DISCUSSION

The stable isotope tracer methods we have developed to study fatty acid transport during lactation permit us to determine quantitatively the amount of dietary fat secreted into human milk. The diet contained lipids derived from partially hydrogenated soybean oil, which differed substantially from the standard western diet. The diet contained only 27% fat, compared with 40% fat that usually is consumed by lactating women in the United States (6). The dietary lipids were also more highly unsaturated than usual. The fatty acid composition of milk

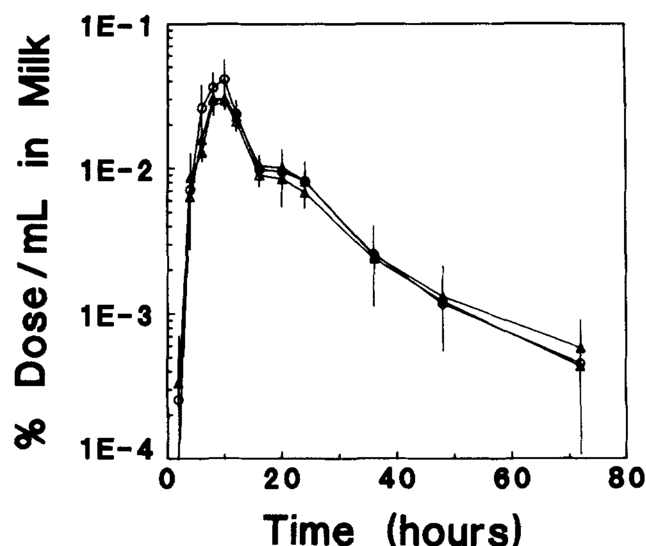

Fig. 2. Percent dose of the tracer per ml secreted into milk. These data are the mean values of all three subjects; (△), [$^2H_2$]16:0; (○), [$^2H_6$]18:1; (▲), [$^2H_4$]18:2.

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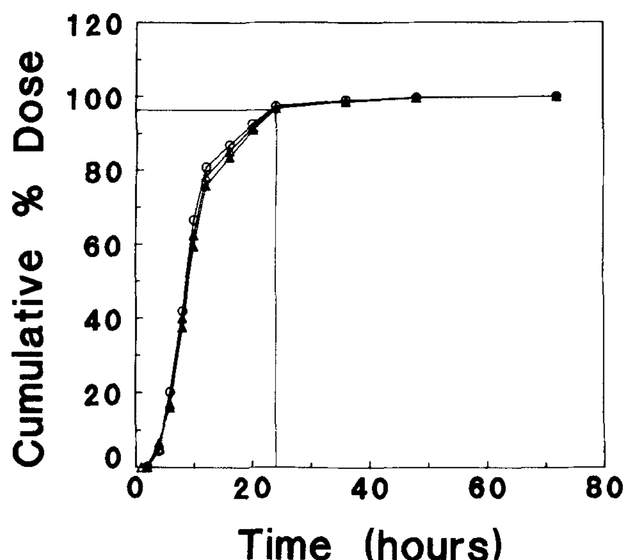

Fig. 3. Cumulative percent dose of the tracer secreted into milk over 72 hr; (△), [$^2H_2$]16:0; (○), [$^2H_6$]18:1; (▲), [$^2H_4$]18:2. The vertical line marks the 24-hr point and the horizontal line marks the 97% point.

TG was remarkably constant over the duration of the study, with only a 2 to 5% coefficient of variation in the major fatty acids. Only two notable differences were observed. Palmitic acid content was 17 to 20%, which is slightly lower than the 20 to 27% reported by others (3, 5, 6). This difference was due to the lower concentration of saturated fats in the standard meal. Linoleic acid content was twofold higher (23 to 30%) in our study than the levels of 7 to 15% reported by others (5). The oleic acid content, however, was nearly identical to published values (3, 5, 6).

Milk production and fat concentration were within normal ranges for our population (3, 20). When we examined lateral differences in milk production, we found that the infant consumed slightly less milk (4 to 14%) from the suckled breast than we obtained from the mechanically pumped breast. These small differences were probably due to the more frequent sample collection schedule (ten per day), which may stimulate milk production, compared with the nursing schedule (five to eight feeds per day). The difference also may be due, however, to more efficient emptying of the breast by mechanical means. Because the infant nursed on his/her preferred schedule, we could not reliably estimate the isotopic enrichment of the fatty acids that were consumed. For these reasons we have described only the quantitative fat transfer data in Table 3 for milk produced by a single breast. We assume, however, that these data could be doubled to account for the fat consumed by the infants.

Milk fat contains FA derived from endogenous mammary synthesis, from direct transfer of dietary lipids, from mobilized adipose stores, and from synthesis in sites other than the mammary gland. The medium-chain FA synthesized by the breast are not important quantitatively in most western women since these fats account for less than 10% of the total FA, but they may play other functional roles (21). We chose to study the transfer of palmitate, oleate, and linoleate, which account for 75% of FA present in human milk. Palmitate is derived from dietary sources, from hepatic and adipose tissue, and to a small degree from mammary synthesis. Thompson and Smith (22) have shown that palmitate is made by isolated breast epithelial cells at less than one-half the rate of 12:0 and 14:0 synthesis. The remainder comes from the diet, mobilization of adipose TG, and tissue synthesis. The oleate found in milk may be derived either from hepatic desaturation or from mammary desaturation (23) of stearate, but the major source is most likely dietary lipid. Linoleate is derived exclusively from dietary sources.

Radioisotope-labeled compounds have been used for similar studies in animals (2, 7), but ethical concerns prohibit their use in pregnant and lactating women. Stable isotope tracer techniques were developed to determine quantitatively the amount of dietary fat secreted into milk. Results from this study suggest that 10 to 12% (twice the values listed in Table 3) of these fatty acids originate immediately from dietary sources and that the remainder come from all other sources. The uniform secretion patterns of all three fatty acids suggest that longer chain (> 14:0) dietary fats are transferred from the diet to milk in a similar manner. The small sample (n = 3) in our study, however, may not have permitted us to distinguish subtle differences in the transport and secretion of a particular fatty acid. Nevertheless, our data suggest

TABLE 3. Cumulative percent dose (CPD) of labeled fatty acids secreted into milk over a 72-hr period[a]

Subject	[$^2H_2$]16:0	[$^2H_6$]18:1	[$^2H_4$]18:2	Mean ± SD (CV)
1	3.63	4.29	4.50	4.14 ± 0.46 (11%)
2	ND[b]	6.06	6.31	6.19 ± 0.17 (3%)
3	4.77	7.20	4.09	5.35 ± 1.63 (31%)
Mean ± SD	4.20 ± 0.81	5.85 ± 1.46	4.97 ± 1.18	
CV %	19	25	24	

[a]The overall mean of all subjects and all fatty acids taken as a group was 5.11 ± 1.26 (25%).
[b]ND, no data; unlabeled tripalmitin was inadvertenly given to subject #2 in place of deuterated tripalmitin.

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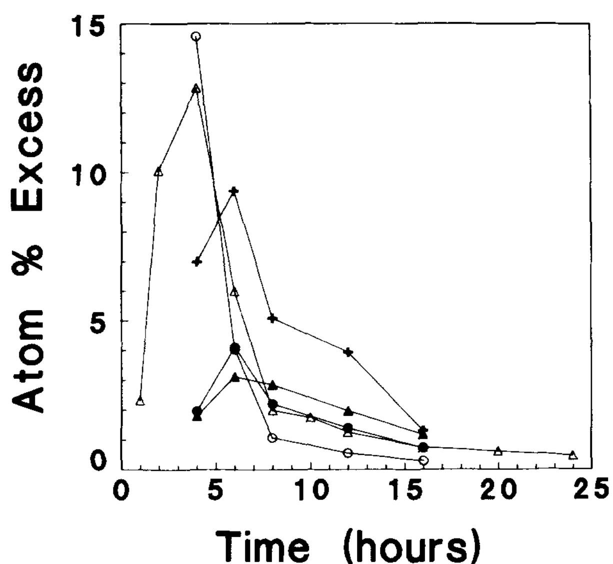

Fig. 4. Typical enrichment pattern of various plasma TG[$^2H_6$]18:1 fractions observed in one subject (#3); (△), total plasma TG; (○), chylomicron TG; (+), very low density lipoprotein TG; (▲), low density lipoprotein TG; (●), high density lipoprotein TG.

that differences do exist between individuals and indicate an approach for exploring the basis of individual variation in milk composition.

A significant delay (6.0 ± 1.9 hr) was observed between the peak enrichment in milk and that in plasma TG. This delay was due to the time lag between delivery of the tracer to the breast, lipolysis of chylomicron and VLDL TG in the mammary gland capillary bed, resynthesis into triglycerides, extrusion of the milk fat globule, and expression of the milk from the breast. This value is similar to the 4-hr value reported for the formation of the milk fat globule in the goat (24, 25).

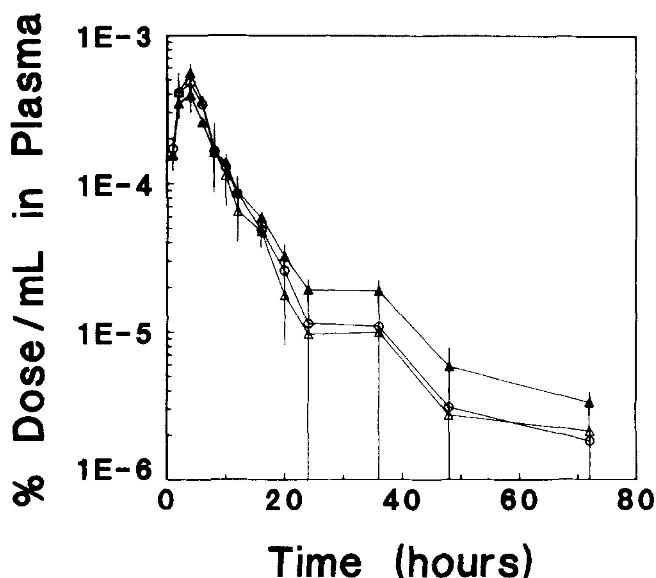

Fig. 5. Percent dose of the tracer per ml of plasma. These data are the mean values of three subjects total plasma TG enrichment data; (△), [$^2H_2$]16:0; (○), [$^2H_6$]18:1; (▲), [$^2H_4$]18:2.

Dietary fatty acids are delivered to the breast by the plasma lipoprotein TG transport system. Plasma has a lower triglyceride content (0.5 to 1 mg/ml) than milk (30 to 40 mg/ml). The relative efficiency of lipid transport across the mammary gland is a reflection of the relative efficiency of a 60:1 concentration difference between the two fluids. We measured the isotopic enrichment of various lipoprotein lipids in order to determine their relative importance for fat transport. The plasma chylomicron TG was the most highly enriched lipid fraction at 4 hr, our earliest time sample. VLDL triglycerides were the most highly enriched lipids observed thereafter. The peak isotopic enrichments in the LDL and HDL triglycerides were approximately 20 to 30% of the maximum enrichment observed in milk. The enrichment observed in milk was intermediate between the enrichment of the chylomicron triglycerides and the VLDL triglycerides. These results indicate that chylomicron and VLDL triglycerides are the major sources of milk lipids, and that LDL and HDL triglycerides are less important quantitatively. At present, we are unable to assign numerical values to the amount of TG transported by the various lipoproteins. A compartmental model that will describe quantitatively the lipid transport mechanisms involved in human lactation may be constructed by using the methods tested in this study.

Our gas chromatographic data and the more extensive work by others (1-6) suggest that the medium-chain, saturated fatty acids synthesized by the mammary gland account for 10 to 12% of the total fat in milk. The long-chain, diet-derived fatty acids account for 29% of the fat in milk. This value is based on twice the percentage of the tracer dose secreted into milk by one breast (2 × 5.1% from Table 3), the mean daily caloric intake of the subjects studied, and percentage of fat in the diet. We assumed that dietary contributions to milk fat from the noon and evening meals were identical to those of the morning meal which contained the tracer. This assumption requires experimental validation in future studies. The remaining 59% is derived from tissue synthesis and adipose stores and approximates the 60% value reported-

TABLE 4. Time delay (hr) observed between maximum enrichment in milk triglycerides (TG) and the maximum enrichment in total plasma TG

Subject	[$^2H_2$]16:0	[$^2H_6$]18:1	[$^2H_4$]18:2
1	6	2	6
2	ND[a]	8	8
3	6	6	6

[a]No data; see Table 3.

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ly contributed to milk fat by circulating blood lipids in ruminants (26). Our estimate of the dietary contributions to milk fat reflects only the current study population; thus values may differ considerably in subjects who receive low-fat, high-carbohydrate diets.

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