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Human Lactation II: Endogenous Fatty Acid Synthesis by the Mammary Gland[1]

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ABSTRACT. We studied the effects of a diet that was low in fat, high in carbohydrate (CHO) on milk lipid composition and *de novo* endogenous fatty acid synthesis by the mammary gland in five lactating women. The women consumed either a low fat (LF) (5% fat, 80% CHO) diet or a high fat (HF) (40% fat, 45% CHO) diet. Fat synthesis was determined after an oral dose of 500 mg/kg D_2O by measuring the incorporation of deuterium into C10:0 to C18:0 saturated fatty acids of milk fat and plasma triglycerides by gas chromatography-mass spectrometry. Synthesis of plasma C16:0 and C18:0 triglycerides was barely detectable while women consumed the HF diet, but was increased 6-fold during the LF diet. Medium chain fatty acids secreted by the mammary gland increased from 12.8% (HF diet) to 16.3% (LF diet) in milk fat from four of five subjects ($p = 0.027$). Medium chain fatty acid secretion, however, increased from 13.9% (HF diet) to 29.9% (LF diet) in one subject. The primary fatty acids synthesized during lactation were C10:0, C12:0, and C14:0 in the majority of women studied. The LF diet significantly increased the apparent synthesis of C14:0 ($p = 0.05$), whereas no changes were observed in C12:0, C16:0, or C18:0. One subject had highly enriched C16:0 and C18:0 fatty acids in her milk on the LF diet, which could have been the result of mammary synthesis or of transport and secretion of hepatically synthesized lipids. (*Pediatr Res* 25:63–68, 1989)

Abbreviations

CHO, carbohydrate
LF, low fat
HF, high fat
FA, fatty acids
FAME, fatty acid methyl esters
GC-MS, gas chromatography-mass spectrometry
ApoB, apolipoprotein B
TG, triglycerides
MCT, medium chain triglycerides (C10, C12, C14)
CM, chylomicrons
VLDL, very low density lipoproteins

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Milk lipid is comprised of 98% TG, 1% phospholipid, and 0.5% cholesterol and cholesterol ester, and provides 50 to 60% of an infant's energy intake (1). Milk TG contain FA derived from three sources: *de novo* mammary synthesis, dietary lipids, and mobilized adipose or hepatic lipids (2). Variations in the FA intake and composition of the mother's diet are known to alter the FA composition of her milk (3); for example, a LF, high CHO diet increased proportions of C10:0, C12:0, and C14:0 FA whereas the proportion of C16 and C18 FA was reduced (4–6). This diet, however, did not change total milk fat content significantly, and these studies were unable to distinguish between altered mammary synthesis, changes in whole body lipogenesis, and lipid transport induced by the high CHO diet.

The incorporation of deuterium into cholesterol and FA during lipogenesis was first demonstrated in the classic work of Rittenberg and Schoenheimer (7), and 22 of 31 protons in palmitic acid are known to be derived from body water (8). Despite the widespread use of deuterium to measure total body water (9), it has not been applied to the systematic study of lipogenesis in humans.

Isotopic tracers are required to distinguish between FA synthesized *de novo* by the body and those of dietary origin. We have described the use of specifically labeled FA to measure the transport of dietary fat across the lactating mammary gland (10). The low level of lipogenesis in most tissues, however, and the dilution of newly synthesized lipid into a large endogenous pool require sensitive, high precision analytical techniques for measurements of small changes in isotopic enrichment above the natural abundance.

We have developed methods to measure the incorporation of deuterium from body water into specific milk FA. These methods were used to study the effect of HF, high CHO diets on *de novo* mammary lipid synthesis and the attendant changes in milk lipid composition.

MATERIALS AND METHODS

Deuterium oxide (99.8 atom % D) was purchased from MSD Isotopes (Montreal, Canada). The ^2H_2O was administered orally without further purification. HPLC quality solvents and derivatization reagents were purchased from Fisher Chemical Co. (Houston, TX) and from Aldrich Chemical Co. (Milwaukee, WI). Lipid internal standards were obtained from Sigma Chemical Co. (St. Louis, MO).

Study design. Five women were studied using a two-part crossover design in which each subject served as her own control. Written informed consent was obtained from each volunteer. The experimental protocol was approved by the Baylor College of Medicine Institutional Review Board for Human Research and by the Texas Children's Hospital Committee on Investigations and Publications. All subjects were normal, healthy, nonsmoking women, 28 to 36 y old, whose pregnancies and deliveries

had been normal. They were studied at 3 mo postpartum, except for subject 3 who was studied at 1.7 mo. Subjects were asked to keep a 24-h diet record before each of the two phases of the study. This record was used to determine their average daily caloric intake.

Subjects consumed a controlled, LF diet (5% fat, 15% protein, and 80% CHO) for 5 days; 1 mo later, they consumed a controlled, HF diet (40% fat, 15% protein, and 45% CHO) for 5 days. The energy content of these diets was adjusted to match the prestudy energy intake of each woman. The subjects consumed their usual diets between the two phases of the study and were provided with all foods consumed during the controlled diet periods. Subjects were admitted to the Clinical Research Center at Texas Children's Hospital on the evening of the 4th day of the controlled diet. On the morning of the 5th day, each subject was given an oral dose of ^2H_2O (250 or 500 mg/kg). Milk was collected from alternate breasts at 0, 3, 6, 9, 12, 15, and 24 h using an Egnell Cam I electric breast pump (Egnell, Inc., Cary, IL) and was flash-frozen for subsequent analysis. Blood (5 ml) was collected by venipuncture at 0, 8, and 24 h, and the plasma was collected by centrifugation and was frozen. Subjects were released on the morning of the 6th day after the 24-h postdose milk and blood collections.

Plasma lipid analysis. Plasma cholesterol, TG, and apolipoprotein B were measured by the Atherosclerosis Laboratory at The Methodist Hospital, Houston, TX. Total plasma cholesterol and TG were measured using a COBAS-BIO analyzer (Roche Diagnostics, Montclair, NJ) using the respective enzymatic kits (Boehringer Mannheim Diagnostics, Indianapolis, IN). Total ApoB was quantitated by RIA (10).

Isolation and sample derivatization. Total milk lipids were extracted using chloroform:methanol (2:1) and were converted to FAME using methods described previously (11). An internal standard mixture that contained heptadecanoic acid (10 μg), triheptadecanoin (100 μg), cholesteryl heptadecanoate (100 μg), and di-heptadecanoyl-L-α-phosphatidylcholine (100 μg) was added to plasma before extraction with chloroform:methanol (2:1). The plasma lipids were separated into free FA, TG, cholesterol esters, and total phospholipids using an aminopropyl solid phase extraction cartridge before transesterification (11).

Gas chromatography. FAME were analyzed using a 30-m × 0.25-mm, 0.20-μm SP-2330 capillary column (Supelco Inc., Bellefonte, PA). Chromatographic temperatures and conditions were: injector 230°C, detector 230°C, and column oven program 140 to 225°C at 5°C/min. Quantitation was based on the amount of C17:0 internal standard added to plasma.

Isotope-ratio mass spectrometry. Deuterium enrichment in milk water was quantitated by gas-isotope-ratio mass spectrometry. From each defatted milk sample, 10 μl were reduced to hydrogen gas over zinc shot at 450°C, according to the method of Wong *et al.* (12), and analyzed using a Finnigan MAT Delta E mass spectrometer (Finnigan MAT, San Jose, CA). Body water enrichment data were used to calculate total body water, and, subsequently, to derive the percentage of body fat in these subjects using the equation:

$$\text{Body fat (\%)} = 100\left[1 - \frac{(\text{Total body water})/0.72}{\text{Body weight}}\right] \quad (1)$$

GC-MS. The isotopic abundance of C10:0, C12:0, C14:0, C16:0, and C18:0 FAME was measured using a Hewlett-Packard 5988A GC-MS system (Hewlett-Packard Co., Palo Alto, CA). Samples were chromatographed using a 30-m × 0.32-mm, 0.25-μm DB-225 capillary column (J & W Scientific Inc., Folsom, CA). Chromatographic temperature conditions were: injector, 250°C, GC-MS interface, 200°C, column oven program 100–250°C at 10°C/min. GC-MS conditions were: 70 eV electron ionization, ion source temperature 200°C, electron multiplier 2000 to 2400 V. Deuterium isotopic abundances were measured by selected ion recording using the molecular ion of each FAME. Specific ratios were quantitated for C10:0 (m/z 187/186 and 188/186), C12:0 (m/z 215/214 and 216/214), C14:0 (m/z 243/242 and 244/242), C16:0 (m/z 271/270 and 272/270), and C18:0 (m/z 299/298 and 300/298). Five replicate sets of isotope ratio measurements were performed on each sample for statistical purposes. Isotopic enrichment in each FA was calculated by subtracting the isotope ratio measured at time zero from that measured for each subsequent sample.

Computation of deuterium enrichment of FA. The intensities of satellite ions in the mass spectra of FA reflect their natural isotopic enrichments in ^2H, ^{13}C, ^{17}O, and ^{18}O and can be predicted from their molecular composition (13). Calculation of the isotopic enrichment of deuterium in organic FA from changes in these intensities requires knowledge of the deuterium enrichment of body water, the numbers of hydrogen atoms in the FA that are derived from body water, and the contribution (if any) of deuterium isotope effects. A program to compute the maximum anticipated enrichment for each FA from the observed enrichment of total body water was written in Pascal for the IBM personal computer. This program assumes *1*) that any isotope effects have equal consequences on all FA, *2*) that body water-derived hydrogens on all FA are equivalent, and *3*) that there are two populations of hydrogen atoms within the FA molecule. One population is derived from carbon-bound precursors and has a constant enrichment at natural abundance levels; the second is derived from water; its enrichment reflects total body water enrichment. The program calculates the cumulative probability that the M + 1 ion will contain at least 1 deuterium atom and that the M + 2 ion will have at least 2 deuterium atoms. Using the deuterium enrichment of water measured in milk at 3 h, it was thus possible to calculate the maximum theoretical increase in the ratio of ion intensities of M + 1/M and M + 2/M for each saturated FA from C10:0 to C18:0. The relationship between body water deuterium enrichment and the enrichment calculated for palmitate is shown in Figure 1. These values showed that the GC-MS analytical precision would be sufficient to measure 0.1 mol % excess (MPE) deuterated FAME for measurements using the M + 1 ion and 0.03 MPE for measurements using the M + 2 ion. All FA enrichment data were normalized to the maximum value for that FA given the total body water enrichment of that subject, and the data were expressed as a percentage. This calculation permitted a direct comparison between the shorter chain FA and the longer chain FA that circumvented the absolute differences in numbers of body water-derived hydrogens reflected in the raw ion intensity ratios.

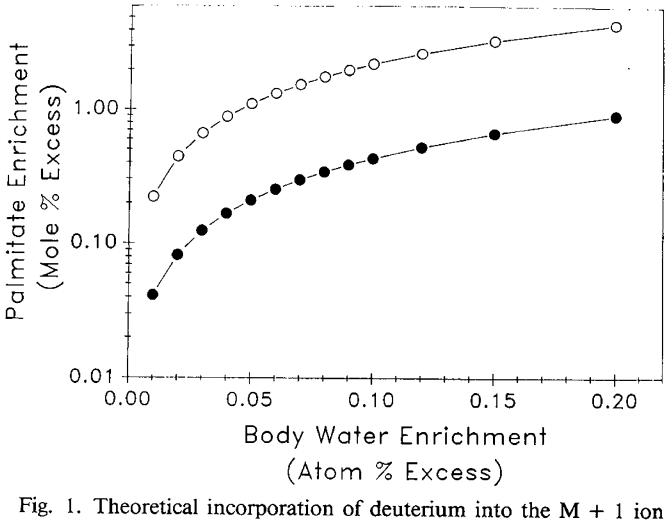

Fig. 1. Theoretical incorporation of deuterium into the M + 1 ion (O) and the M + 2 ion (●) of palmitic acid *versus* the deuterium enrichment in body water.

RESULTS

Dietary effects on milk fat composition. Table 1 summarizes the subjects' clinical data and their diet analyses. Figure 2 illustrates the relative composition of milk lipid FA on the LF and HF diets. The data in Figure 2*A* are the mean FA compositions of eight individual milk samples collected from subjects 1, 2, 4, and 5 during the 5th day of the study. MCT increased from 12.8 ± 2.3% (HF diet) to 16.3 ± 1.8% (LF diet) in these four subjects ($p = 0.027$). We observed a rise in the percentage of 16:1 ($p = 0.05$) and in 20:4 ($p = 0.1$) on the LF diet and a decrease in C18:0 ($p = 0.01$) and C18:3 ($p = 0.1$). Figure 2*B* shows the mean FA composition of milk samples collected from subject 3 who had the most exaggerated response to the LF diet. Her milk FA composition was similar to that of the other subjects during the HF diet period.

Plasma lipid measurements. Table 2 summarizes the mean fasting plasma levels of cholesterol, TG, and ApoB that were obtained from the 0- and 24-h samples under both dietary conditions. Statistically significant differences, based on a paired *t* test, were observed between dietary periods for all plasma lipids. Figure 3*A* and *B* show the magnitude of the postprandial rise in plasma TG during the LF and HF diet periods, respectively. The maximum rise occurred 4 h after the noon meal, which should be near the maximum postprandial plasma TG levels, based on our earlier work (11) and 8 h after the subjects consumed the ^2H_2O.

Total body water measurements. Table 3 summarizes the body water ^2H enrichment, the percentage of body fat, the milk fat concentration, milk volume, and MCT production. The wide range in MCT production is explained by differences in the volume of milk secreted by individual subjects. Body water enrichment data were used to calculate the maximum enrichment in specific FA to be expected if two thirds of the organic hydrogen were derived from body water. A change in the percentage of body fat and body weight (> 1 kg) was observed in subject 1 between the two diet periods. The body fat and body weight of the other subjects remained constant during both diet periods. We observed a significant positive correlation ($r = 0.79$) between the percentage of body fat and the percentage of milk fat during the LF diet period. This relationship was not as strong ($r = 0.51$) during the HF diet.

Milk fatty acid synthesis. The incorporation of deuterium into C12:0, C14:0, C16:0, and C18:0 FA is shown in Figure 4 for women who displayed the "typical" diet response. The data for C10:0 FA were obtained in these subjects, but the low amounts of C10:0 FA present in human milk prevented precise measurement of isotopic enrichment in most cases. The isotopic enrichment pattern in C10:0 FA was similar to the C12:0 FA enrichment in two subjects where the analytical precision permitted such comparisons. A statistically significant difference in the relative enrichment of C14:0 ($p = 0.05$) was observed at 24 h; no significant differences were observed in C12:0, C16:0, or C18:0. Figure 5 shows the isotopic enrichment pattern in subject 3, who displayed the greatest response to the HF, high CHO diet.

Plasma lipid synthesis. The isotopic enrichment observed in plasma TG is shown in Figure 6. The data are expressed as the relative isotopic enrichment above the baseline measurement at time 0, normalized to the theoretical ^2H enrichment calculated for C16:0 and C18:0 based on body water ^2H enrichment. Only the data for C16:0 and C18:0 FA are shown. Virtually no C10:0 and C12:0 FA were detected in plasma lipids, and the amount of C14:0 was usually too low to permit accurate isotopic enrichment measurements. Figure 6*A* shows the incorporation of ^2H from body water into plasma TG in subjects 4 and 5, both of whom responded similarly to the LF, high CHO diet. Similarly, Figure 6*B* shows the incorporation of ^2H into plasma TG of subject 3, whose response to the LF diet was greater than that of the other subjects.

DISCUSSION

Our investigation of the effects of two levels of lipid intake on milk FA synthesis in five lactating women showed that their milk fat contained more MCT when they consumed the LF diet compared with the HF diet. Slightly more energy (1.6 kcal/kg) was consumed during the HF diet, but the difference was not statistically significant. Total milk volumes secreted during the LF diet period were slightly greater than those during the HF diet, although milk fat concentration was decreased. The greater volumes of milk secreted, however, were insufficient to maintain total fat secretion at levels observed during the HF diet period. Whereas we had expected the LF diet to induce greater changes in milk lipid composition, additional changes were not observed beyond the 3rd day of the LF diet. Klein *et al.* (14) have described rapid adjustments in the ^{13}C isotopic composition of the major components in bovine milk that were due to changes in the isotopic composition of the bovine diet.

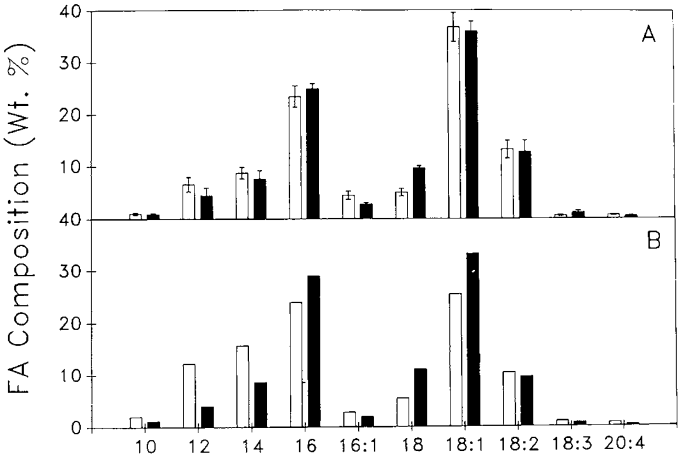

Fig. 2. The FA composition of human milk lipids in women receiving either a LF, high CHO diet (□) or a HF diet (■). *A*, typical mean lipid composition from four women who responded similarly. *B*, lipid composition from an individual who had an anomalous response.

Table 1. *Individual subject characteristics*

Subject	Ht (cm)	Wt (kg)	Age (y)	Postpartum age (mo)	LF nutrient intakes (kcal/kg)	LF (% fat)	LF (% CHO)	HF nutrient intakes (kcal/kg)	HF (% fat)	HF (% CHO)
1	167.6	70.3	34.8	2.9	34.9	5.0	79.4	36.8	46.1	40.3
2	158.8	58.5	32.6	3.0	40.5	5.2	78.6	45.9	46.4	40.1
3	154.9	56.7	30.3	1.7	45.9	5.7	79.0	45.2	47.2	39.4
4	154.9	55.3	35.9	3.8	45.4	4.6	79.8	43.8	39.6	46.7
5	165.1	89.4	27.9	3.3	26.9	5.3	78.5	29.6	45.5	41.3
Mean	160.3	66.0	32.3	2.9	38.7	5.2	79.1	40.3	45.0	41.6
SD	5.9	14.3	3.3	0.8	8.0	0.4	0.5	7.0	3.1	3.0
CV (%)	3.7	21.7	10.1	25.8	20.6	7.5	0.7	17.3	6.8	7.1

Subject 3 demonstrated the greatest response to the LF diet; her medium chain fat increased from 13.9 to 29.9% of the milk lipids, and her milk fat decreased from 2.1 to 1.1%. Two conditions have been associated with an increased MCT content of human milk, but only one with both an increased MCT content and a significant decline in total fat content. Type I hyperlipoproteinemia has been reported to increase MCT and to decrease total fat secretion by restricting the lipolysis and transport of plasma TG across the mammary gland, because of the inherited absence of lipoprotein lipase (15). Nothing in the clinical history of this subject, *e.g.* diabetes or type I hyperlipoproteinemia, suggested an explanation for her response to the LF diet. Consumption of a high CHO diet at levels 50% above energy requirements has been associated with increased MCT concentration, but no change in total fat (4, 6). Although this subject had the lowest percentage of body fat of the group, her energy intakes, when normalized to body weight, were among the highest in the study, and she was one of two subjects whose percentage of body fat increased during the month that separated both diet periods. The increased MCT content of this woman's milk may have resulted from the effects of insulin, which would have been elevated by the high CHO, hypercaloric diet. Insulin stimulates glucose transport across cell membranes, thus providing greater availability of glucose for lipid synthesis, which is stimulated by insulin as well. At the same time, elevated insulin levels will suppress lipolysis of adipose tissue lipids, thus restricting the supply of a major source of milk lipids. Her response to the HF diet was identical to that of the other women.

Table 2. *Plasma lipid concentrations determined in fasting subjects*

	Cholesterol		Triglycerides		ApoB		TG/ApoB	
Subject	LF	HF	LF	HF	LF	HF	LF	HF
1	254	279	182	114	101	113	1.80	1.01
2	160	177	68	54	49	58	1.39	0.94
3	146	220	72	58	67	71	1.07	0.82
4	198	289	161	70	72	113	2.24	0.62
5	160	174	149	95	59	63	2.54	1.51
Mean	184	228	126	78	70	84	1.81	0.98
SD	44	55	53	26	20	27	0.60	0.33
p*	0.025		0.017		0.057		0.013	

* Probability, paired t, of finding a significant difference in each category of plasma lipid among individuals consuming the LF and HF diets.

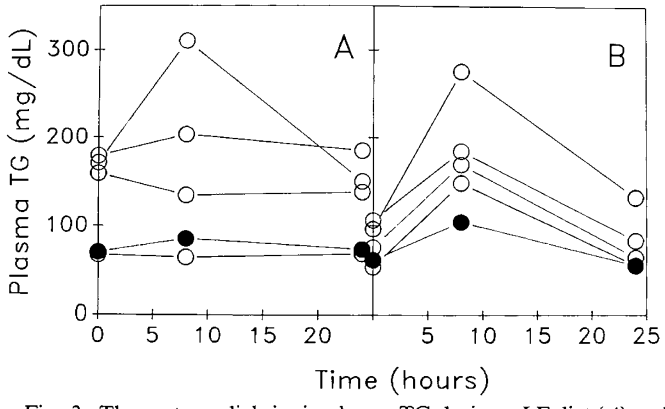

Fig. 3. The postprandial rise in plasma TG during a LF diet (*A*) and during a HF diet (*B*). Subject 3 (●). Subjects 1, 2, 4, and 5 (○).

Subject 3 differed from the other subjects in an additionally important aspect; highly enriched C16:0 and C18:0 were found in her milk when she consumed the LF diet. The highly enriched C16:0 and C18:0 found in this subject's milk probably originated as TG-rich VLDL lipids that were synthesized by the liver in response to the high CHO meal and were subsequently converted into milk TG. The isotopic enrichment of these lipids was not diluted significantly by dietary or adipose TG, as shown by the high enrichments measured in the plasma TG.

Plasma lipid levels were determined in these women on both the HF and LF diets; the results from our subjects on the HF diet were nearly identical to those reported by Knopp *et al.* (16) for lactating women on uncontrolled diets. Individual subjects invariably had lower cholesterol and ApoB levels and had higher TG levels on the LF diet. One significant change attributable to diet was the increased TG to ApoB ratio ($p = 0.013$), which was expected when subjects consumed a fat-restricted diet (17). This observation may be explained by increased hepatic synthesis and secretion of large, TG-rich VLDL particles by subjects on the LF diet.

In our studies, C12:0 had the highest incorporation of deuterium into FA that were newly synthesized by the mammary gland. On the basis of approximately identical curves (Fig. 4) for the LF diet and the HF diet, all of the C12:0 found in milk was provided by *de novo* synthesis and was not diluted by unlabeled dietary lipids. These results are probably valid as well for C10:0; we have reliable data from two subjects to support this conclusion. In contrast, more C14:0 was synthesized by the mammary gland of women when they consumed the LF diet rather than the HF diet, as shown by the difference in isotopic enrichment in C14:0 between the two diets. These data indicate that approximately 45% of the C14:0 obtained from mothers on the HF diet

Table 3. *Isotopic enrichment in body water, percentage of body fat, percentage of milk fat, and MCT production during the LF and HF diets*

	^2H_2O enrichment (APE)		Body fat (%)		Milk fat (%)		MCT (g/day/breast)		Milk vol (g/day/breast)	
Subject	LF	HF	LF	HF	LF	HF	LF	HF	LF	HF
1*	0.0447	0.0379	28.0	16.0	3.4	3.5	1.4	1.0	212	200
2*	0.0454	0.0392	28.7	20.3	0.9	2.5	0.8	1.2	230	269
3	0.0796	0.0775	20.0	22.7	1.1	2.1	1.9	1.2	466	328
4	0.0837	0.0848	25.2	27.4	2.2	4.4	2.9	2.3	418	333
5	0.0980	0.0971	38.5	37.2	4.7	4.0	1.6	nd	153	124
Mean			28.1	24.7	2.5	3.3	1.7	1.4	296	251
SD			6.8	8.1	1.6	1.0	0.8	0.6	138	89
p†			NS		0.092		NS		0.104	

* Subjects 1 and 2 received 250 mg ^2H_2O kg.

† Probability, paired t test; NS, not significant; nd, not done.

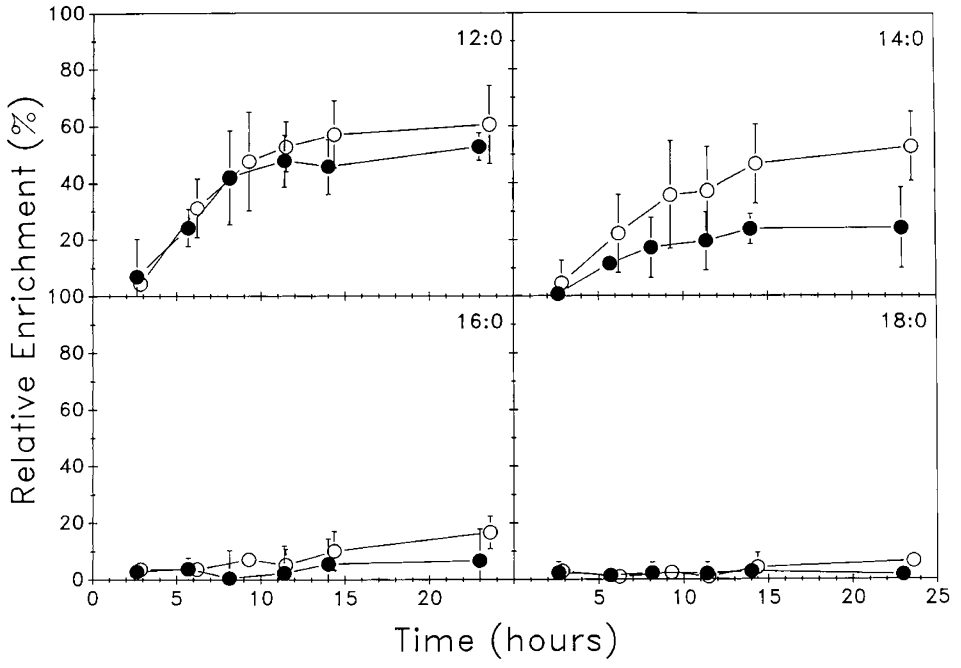

Fig. 4. The incorporation of deuterium from body water into specific milk FA in four women who received either a LF diet (O) or a HF diet (●). These data are the relative enrichments (mean ± 1 SD) normalized to the body water deuterium enrichment.

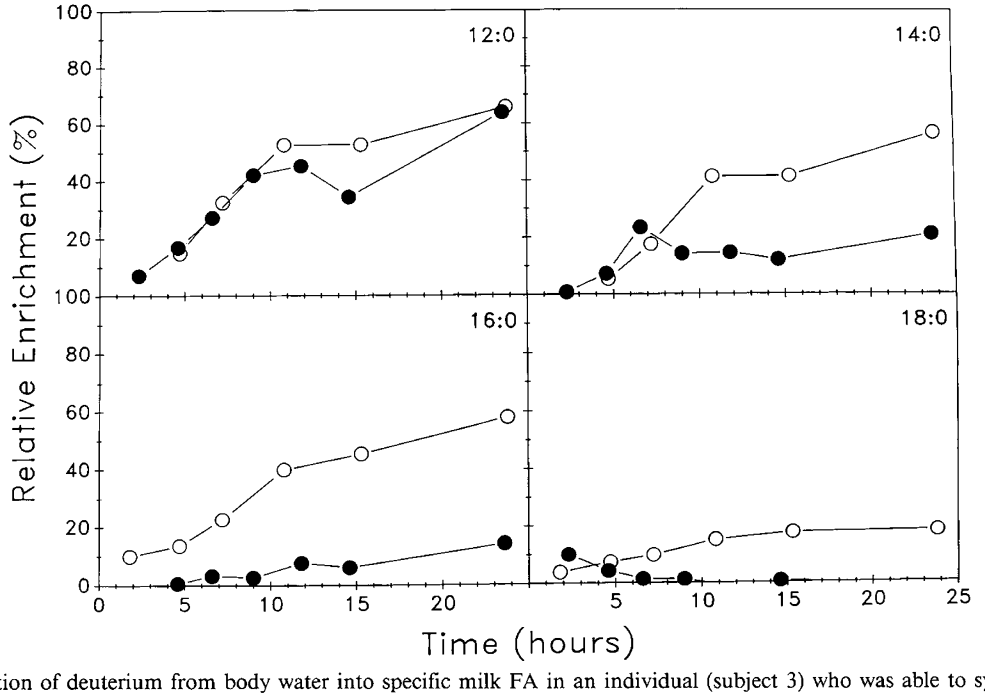

Fig. 5. Incorporation of deuterium from body water into specific milk FA in an individual (subject 3) who was able to synthesize C16:0 and C18:0. LF diet (O) and HF diet (●).

was synthesized by the breast. This value is the difference in isotopic enrichment between the two diets. If all of the C14:0 enrichment observed during the LF diet period were assumed to result from *de novo* synthesis by the breast, then any decrease in isotopic enrichment should reflect the entry of unlabeled dietary fat into milk. A similar response to dietary manipulation was observed in all five subjects. The human mammary gland is known to synthesize C10:0, C12:0, and C14:0 FA, as demonstrated by Thompson and Smith (18) in mammary gland epithelial cell cultures.

The mammary gland apparently has a limited capacity to synthesize C16:0 and C18:0, even on the stringent LF diet. We observed a slight rise in the isotopic enrichment of milk C16:0 in four of the women during the LF diet which we suspect was hepatic TG; however, no corresponding rise in C18:0 enrichment was observed. FA are synthesized by the FA synthetase complex. Chain elongation is stopped by a species-dependent enzyme, thioesterase II, which in the human mammary gland terminates the chain at C14:0 (19).

The studies we have described are unique in human lactation research. They illustrate the complex relationships between diet, energy metabolism, and milk composition. In most instances, alterations in maternal diet composition will have little effect on the lipid composition of the milk produced, provided that adipose stores are maintained. Only one subject was identified in whom the effects of altered nutrient intake had significant results.

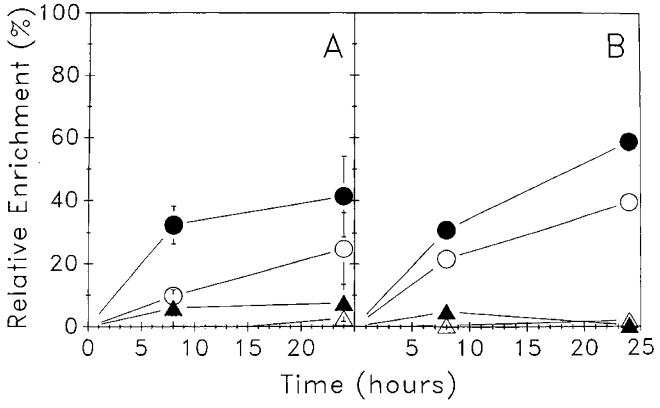

Fig. 6. Incorporation of deuterium from body water into plasma TG from normal women (*A*) and from a woman (subject 3) (*B*) who had the apparent ability to synthesize C16:0 and C18:0 in the mammary gland. Diet LF C16:0 (○) and C18:0 (●). Diet HF C16:0 (△) and C18:0 (▲). Deuterium enrichment.

The methods we have developed are safe and may have application to other areas of lipid metabolism.

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