Acute effects of dietary fatty acids on the fatty acids of human milk[1-3]

Cindy A Francois, Sonja L Connor, Rosemary C Wander, and William E Connor

ABSTRACT Although it is known that the fatty acid profile of human milk is altered by diet, the rapidity with which this occurs has not been addressed. We hypothesized that after absorption the fatty acids of a given meal would be transferred rapidly from the chylomicrons of the blood into human milk. Fourteen lactating women drank six test formulas, each containing a different fat: menhaden oil, herring oil, safflower oil, canola oil, coconut oil, or cocoa butter. The subjects collected a midfeeding milk sample before consuming the breakfast test formula and additional samples at 6, 10, 14, and 24 h and then once daily for 4–7 d. Fatty acids of special interest included eicosapentaenoic and docosahexaenoic acids from menhaden oil, cetoleic acid from herring oil, linoleic acid from safflower oil, linolenic acid from canola oil, lauric acid from coconut oil, and palmitic and stearic acids from cocoa butter. Each of these fatty acids increased significantly in human milk within 6 h of consumption of the test formulas ($P < 0.001$). Maximum increases occurred 10 h after safflower oil; 14 h after cocoa utter, coconut oil, canola oil, and menhaden oil (eicosapentaenoic acid); and 24 h after herring oil and menhaden oil (docosahexaenoic acid). All of these fatty acids remained significantly elevated in milk ($P < 0.05$) for 10–24 h, except for docosahexaenoic acid, which remained significantly elevated for 2 d, and eicosapentaenoic acid, which remained elevated for 3 d. These data support the hypothesis that there is a rapid transfer of dietary fatty acids from chylomicrons into human milk. *Am J Clin Nutr* 1998;67:301–8.

KEY WORDS Fatty acids, breast milk, chylomicrons, cocoa butter, coconut oil, safflower oil, canola oil, menhaden oil, herring oil, palmitic acid, stearic acid, lauric acid, linoleic acid, linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, cetoleic acid, breast-feeding, women

INTRODUCTION

Human milk is especially rich in lipids. The origin of the fatty acids in milk is threefold: from mobilization of endogenous stores of fatty acids, from synthesis of fatty acids by the liver or breast tissue, and from the diet (1, 2). Mobilization occurs with deficient energy intake. When Insull et al (2) fed a low-energy, fat-free formula to breast-feeding mothers, the composition of breast milk fatty acids resembled that of adipose tissue fatty acids, indicating mobilization and transfer of these fatty acids to the breast.

Endogenous synthesis of fatty acids occurs when excess energy is fed. When Insull et al (2) fed lactating women excess energy from a fat-free diet, there was a dramatic increase in medium-chain fatty acids in breast milk, indicating endogenous synthesis. The stage of lactation may also influence synthesis. Finley et al (3) reported that the percentage of fatty acids synthesized by the breast increased with length of lactation, indicating that older infants who breast-feed may receive milk containing a higher proportion of fatty acids synthesized by the mother and fewer fatty acids derived from the diet and adipose stores.

Diet composition also influences the fatty acid composition of breast milk. Past studies addressed the effects of the long-term maternal intake of fatty acids (> 5 d) on the composition of human milk (2–10). However, few studies examined the acute effects of a single dose of dietary fat on human milk fatty acids (11, 12). Because breast tissue is especially rich in the lipolytic enzyme lipoprotein lipase, we hypothesized that during lactation, dietary fatty acids would be transferred quickly from the plasma into human milk after their ingestion, absorption, and subsequent incorporation into chylomicrons. Peak lipemia or chylomicronemia usually occurs with 3–5 h of fat ingestion. However, the action of lipoprotein lipase to hydrolyze chylomicron triacylglycerol is extremely rapid and the released fatty acids are then available for uptake by the tissues (eg, breast).

The purpose of this study was to investigate the acute effects of single doses of six different dietary fats on human milk fatty acids. The fats and their fatty acids of interest were as follows: eicosapentaenoic acid (EPA, 20:5n−3) and docosahexaenoic acid (DHA, 22:6n−3) from menhaden oil, cetoleic acid (22:1n−11) from herring oil, linoleic acid (18:2n−6) from safflower oil, linolenic acid (18:3n−3) from canola oil, lauric acid (12:0) from coconut oil, and palmitic (16:0) and stearic acids (18:0) from cocoa butter. The different fats were given to lactating women to determine the acute effects of fatty acids with a

[1] From the Division of Endocrinology, Metabolism and Clinical Nutrition, Department of Medicine, Oregon Health Sciences University, Portland, and the Department of Nutrition and Food Management, Oregon State University, Corvallis.

[2] Supported in part by the Oregon Health Sciences University Foundation, General Clinical Research PHS grant 5 M01 RR00334, Oregon State University Department of Nutrition and Food Management, and Oregon Dairy Council Nutrition Education Services.

[3] Address reprint requests to CA Francois, Department of Medicine, L465, Oregon Health Sciences University, 3181 Southwest Sam Jackson Park Road, Portland, OR 97201-3098. E-mail: freerc@ohsu.edu.

Received March 10, 1997.

Accepted for publication September 26, 1997.

wide range of chain lengths and degrees of unsaturation on the fatty acid composition of human milk.

SUBJECTS AND METHODS

Subjects

Fourteen healthy, lactating women aged 19–43 y participated in this study. Subjects were recruited during the first 6 mo of lactation by Oregon Health Sciences University campus newsletter announcements, from a prenatal clinic, and by direct contact with study investigators. The women remained in the study for ≈10 wk to allow for a 2-wk washout period between consumption of the test fats. Subjects were encouraged to keep their energy intake and physical activity constant so that they would not gain or lose weight during the study. Paired *t* tests indicated that the weights of the women did not change significantly between their initial and final visits (67.0 ± 11.7 and 66.9 ± 12.2 kg, respectively), nor did their body mass indexes (in kg/m^2; 24.4 ± 3.9 and 24.3 ± 4.2, respectively).

The study was approved by the Oregon Health Sciences University Institutional Review Board, Committee on Human Research. Informed consent was obtained from each volunteer.

Fat-rich meals

Six fats were selected for this study: menhaden oil, herring oil, safflower oil, canola oil, coconut oil, and cocoa butter. The fatty acid content of the test fats is given in **Table 1**. For each test fat, a 2933-kJ (700-kcal) liquid formula was prepared by the Clinical Research Center kitchen staff (**Table 2**). The safflower oil, canola oil, coconut oil, and cocoa butter formulas each contained 40 g fat. The menhaden and herring oil formulas contained no fat because these oils were administered in capsules (either 7- or 20-g doses) along with the formula. Smaller amounts of the fish oils, which had a distinct fishy odor, were used because higher doses were not tolerated by study participants.

The order of administration of the safflower oil, canola oil, coconut oil, and cocoa butter formulas was randomly assigned. Because of the unpleasant taste, the menhaden and herring oil formulas were given last to increase subject retention. The night before the test meals were given, subjects were asked to fast from 2000. Subjects then consumed the meals in the morning between 0800 and 1000 in place of breakfast. All subjects drank the formulas at the Clinical Research Center under the supervision of a study investigator. Subjects were asked to consume each of the six formulas once at 2-wk intervals. They were advised to avoid fats containing the unique fatty acid for 1 wk before and 1 wk after its consumption. Dietary compliance was validated by patient report. Six of the 14 women did not consume all six of the test formulas because of cessation of lactation or conflict of schedules. Thirteen women consumed the menhaden oil formula, 7 consumed the 7-g herring oil formula, 2 consumed the 20-g herring oil formula, 13 consumed the safflower oil for-

TABLE 1
Fatty acid composition of the test fats[1]

Fatty acid	Menhaden oil	Herring oil	Safflower oil	Canola oil	Coconut oil	Cocoa butter
			% by wt			
8:0[2]	—	—	—	—	5.4	0.0
10:0[2]	—	—	—	—	5.8	0.0
12:0	0.1	0.0	0.0	0.0	48.5	0.0
14:0	7.5	4.8	0.1	0.0	18.4	0.1
16:0	16.0	9.0	6.9	3.7	8.8	25.7
18:0	3.0	0.6	2.4	1.5	2.6	33.4
20:0	0.0	0.1	0.4	0.0	0.2	1.2
ΣSFAs[3]	27.8	15.4	9.8	5.5	89.8	60.6
18:1n−9	10.9	7.2	14.8	58.4	6.7	32.6
20:1n−9	1.1	17.4	0.0	0.1	0.0	0.1
22:1n−11	0.6	28.0	0.0	0.0	0.0	0.0
ΣMUFAs[4]	22.1	63.0	14.8	59.0	6.7	33.0
18:2n−6	1.1	0.7	73.6	19.8	1.7	3.0
20:3n−6	0.1	0.0	0.2	0.3	0.0	0.3
20:4n−6	1.1	0.1	0.0	0.1	0.0	0.0
Σn−6[5]	4.1	1.5	74.0	20.8	1.7	3.3
18:3n−3	0.7	0.5	0.2	8.2	0.1	0.1
20:5n−3	16.6	5.9	0.0	0.0	0.0	0.0
22:5n−3	2.6	0.8	0.0	0.1	0.0	0.0
22:6n−3	11.5	4.0	0.0	0.0	0.0	0.0
Σn−3[6]	33.7	12.6	0.3	9.2	0.1	1.0

[1] $\bar{x}$. SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids.
[2] Analyzed in coconut oil and cocoa butter only because of time-consuming laboratory procedures.
[3] Total SFAs calculated by adding 8:0, 10:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, and 24:0.
[4] Total MUFAs calculated by adding 14:1n−5, 16:1n−7, *t*-18:1n−9, 18:1n−9, 18:1n–7, 20:1n−9, 22:1n−11, 22:1n−9, and 24:1n−9.
[5] Total n−6 fatty acids calculated by adding 18:2n−6, 18:3n−6, 20:2n−6, 20:3n−6, 20:4n−6, 22:2n−6, 22:3n−6, 22:4n−6, and 22:5n−6.
[6] Total n−3 fatty acids calculated by adding 18:3n−3, 18:4n−3, 20:4n−3, 20:5n−3, 22:5n−3, and 22:6n−3.

TABLE 2
Composition of each test formula[1]

Test fat	Menhaden and herring oils	Cocoa butter	Coconut, canola, and safflower oils
		g	
Egg white	38	38	38
Calcium caseinate	23	19[2]	23
Moducal	67	—[3]	20
Sucrose	13	21	13
Baker's sweet chocolate	—	38	—
Fat	—[4]	20[5]	40
Flavoring extract	5–8	5–8	5–8

[1] Baker's sweet chocolate (Kraft General Foods, Inc, White Plains, NY) was used as a cocoa butter source and to add chocolate flavor to the formula. Moducal (Mead Johnson Nutritionals; Evansville, IN) is 100% maltodextrin, a readily digestible carbohydrate produced by hydrolysis of cornstarch. Flavoring extracts included vanilla, coconut, strawberry, and mint.

[2] Baker's sweet chocolate (used in cocoa butter formula) contains protein, thus altering the amount of calcium caseinate (a protein source) added to the test formula.

[3] Baker's sweet chocolate contains carbohydrate, thus altering the amount of Moducal (carbohydrate source).

[4] Fats were not included in the formula because they were administered separately in gelatin capsules: 20 g menhaden oil and either 20 or 7 g herring oil.

[5] Baker's sweet chocolate contains cocoa butter, thus altering the amount of cocoa butter (fat source).

mula, 11 consumed the canola oil formula, 12 consumed the coconut oil formula, and 13 consumed the cocoa butter formula.

Dietary assessment

To monitor dietary consistency, maternal diets were assessed six times, once at each visit to the Clinical Research Center, with use of an eating habit questionnaire, the Diet Habit Survey (13), and the n−3 Fatty Acid Food Frequency Questionnaire. Both questionnaires were developed by the Lipid-Atherosclerosis Nutrition Staff in the Section of Clinical Nutrition and Lipid Metabolism, Department of Medicine, at Oregon Health Sciences University. The Diet Habit Survey assessed dietary intakes of cholesterol, saturated fat, total fat, carbohydrate, and fish; it was scored by a trained dietitian. The n−3 Fatty Acid Food Frequency Questionnaire assessed average monthly intake of EPA and DHA in g/mo. In addition, subjects were weighed at each visit to the Clinical Research Center.

Breast milk collection and analysis

Subjects collected milk samples once in the morning before consuming the test formula; once at each of the following time points after consuming the formula: 6, 10, 14, and 24 h; and once daily for 4–7 d. Subjects were instructed to collect midfeeding milk samples by breast-feeding until the first breast was partially emptied, switching to the second breast, and then expressing the milk from the partially emptied first breast into a 5-mL plastic vial, either manually or with a pump. Immediately after collection, milk samples were placed upright in the subject's freezer until the next study appointment, when they were brought on ice to the Clinical Research Center. The samples were stored at −70 ºC until fatty acids were analyzed (from 1 d to 3 mo later).

The fatty acids of breast milk and the test fats were saponified in alcoholic KOH and extracted into hexane. Fatty acid methyl esters were prepared with 12% BF3 in methanol. They were analyzed by gas-liquid chromatography as described by Anderson et al (14, 15) on a Perkin-Elmer (Norwalk, CT) instrument equipped with a hydrogen flame-ionization detector and a 30-m SP-2330 fused silica capillary column with a 0.25-mm internal diameter and a 0.2-mm film thickness. To measure caprylic acid (8:0) and capric acid (10:0), the procedure was modified to include isothermal analysis (100 min at 150 °C). The data are reported as percentage of total fatty acids by weight.

Statistical methods

Data are reported as means ± SDs; where nonparametric statistics were used, data are reported as medians and interquartile ranges. Statistical significance was defined as $P < 0.05$. To test for an overall significant difference among time points in human milk fatty acid response (individual fatty acids as well as saturated, monounsaturated, and polyunsaturated categories) to a dietary fat supplement, Friedman's repeated-measures analysis of variance on ranks was used (16). This nonparametric statistic was selected because normality and equal variance tests failed for many fatty acids when one-way repeated-measures analysis of variance was used, even after log and reciprocal transformations (16). Dunnett's test was then used to determine which time points were significantly different from baseline (16). Paired *t* tests were performed to compare initial and final scores on the Diet Habit Survey and initial and final body mass index and weight (16). The initial and final scores on the n−3 fatty acid survey were analyzed by signed-rank tests (16). SIGMA STAT FOR WINDOWS version 1.02 (Jandel Scientific, San Rafael, CA) was used for all statistical computations.

RESULTS

The results of this study showed an acute response, especially within the first 24 h, in a variety of human milk fatty acids after single test meals of six different dietary fats, each providing a specific fatty acid. Most of the fatty acids we studied had increased by 6 h after their ingestion (the first time point at which milk was collected after consumption of the test fat), peaked between 10 and 24 h, and remained significantly elevated for 1–3 d after the given fat-rich meal. These results are described below, as is the fatty acid composition of milk at baseline.

TABLE 3
Selected fatty acids from human milk at baseline[1]

Fatty acid	Baseline
	% of total fatty acids
8:0[2]	0.1 ± 0.1
10:0[2]	0.9 ± 0.3
12:0	4.7 ± 1.7
14:0	5.5 ± 1.8
16:0	18.6 ± 2.1
18:0	7.2 ± 1.3
20:0	0.2 ± 0.1
ΣSFAs[3]	37.2 ± 4.1
18:1n–9	33.6 ± 3.5
20:1n–9	0.6 ± 0.1
22:1n–11	0.0 ± 0.0
ΣMUFAs[3]	40.9 ± 3.6
18:2n–6	14.6 ± 2.6
20:3n–6	0.4 ± 0.1
20:4n–6	0.5 ± 0.1
Σn–6[3]	16.2 ± 2.6
18:3n–3	1.2 ± 0.4
20:5n–3	0.1 ± 0.0
22:5n–3	0.2 ± 0.1
22:6n–3	0.2 ± 0.1
Σn–3[3]	1.7 ± 0.5
n–6:n–3	9.8 ± 2.2
ΣPUFAs[4]	18.6 ± 2.9

[1] $\bar{x}$ ± SD. n = 14 (69 samples). SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.
[2] Data reported for the 10 samples taken before ingestion of coconut oil.
[3] Total determined by using the same calculations as in Table 1.
[4] Total PUFAs determined by adding total n–6, total n–3, and other PUFAs (16:2n–4, 16:4n–1, 20:3n–9, and 22:3n–9).

Milk fatty acid composition at baseline

The fatty acid composition of the milk samples before each test fat was ≈37.2 ± 4.1% saturated, 40.9 ± 3.6% monounsaturated, and 18.6 ± 2.9% polyunsaturated (**Table 3**). The amount of *trans* 18:1n−9, included in the total monounsaturated fatty acid content, was 4.0 ± 2.1%. The baseline amounts of the fatty acids specifically tracked in the milk were 0.1 ± 0.0% EPA, 0.2 ± 0.1% DHA, 0.0 ± 0.0% cetoleic acid, 14.6 ± 2.6% linoleic acid, 1.2 ± 0.4% linolenic acid, 4.7 ± 1.7% lauric acid, 18.6 ± 2.1% palmitic acid, and 7.2 ± 1.3% stearic acid.

Changes in milk fatty acid composition after consumption of fatty meals containing specific fatty acids

After ingestion of the test formulas, significant changes in the fatty acid composition of human milk were observed. Fatty acid amounts are reported as medians.

Menhaden oil

The ingestion of 20 g menhaden oil significantly increased the long-chain polyunsaturated fatty acids EPA and DHA over time ($P < 0.001$). These fatty acids were also significantly increased at several time points (**Figure 1**). EPA increased from 0.1% at baseline to a peak of 0.8% at 24 h and remained significantly elevated at 0.2% until 3 d ($P < 0.05$). DHA increased significantly ($P < 0.05$) from 0.2% at baseline to 1.0% at 14 h, 1.1% at 24 h, and 0.5% at 2 d. Menhaden oil did not significantly change the total percentages of saturated, monounsaturated, or polyunsaturated fatty acids (data not shown).

Herring oil

The two doses of herring oil differed in their degree of effect on milk fatty acids. Ingestion of 7 g herring oil significantly increased the monounsaturated fatty acid cetoleic acid over time ($P < 0.001$), although it did not significantly affect the monounsaturated fatty acids generally (data not shown). Cetoleic acid increased from 0.0% at baseline to 0.4% at 14 and 24 h ($P < 0.05$; Figure 1). In comparison, the higher dose of herring oil (20 g) produced a peak cetoleic acid value of 2.4% of milk fatty acids at 14 h (the significance of this value could not be determined because only one subject ingested the higher dose and collected all the breast milk samples). In subjects who consumed 7 g herring oil, both EPA and DHA increased significantly over time as well ($P < 0.001$). Although DHA was not significantly higher at any time point, EPA increased from 0.06% at baseline to 0.12% at 14 h ($P < 0.05$). The total polyunsaturated fatty acid

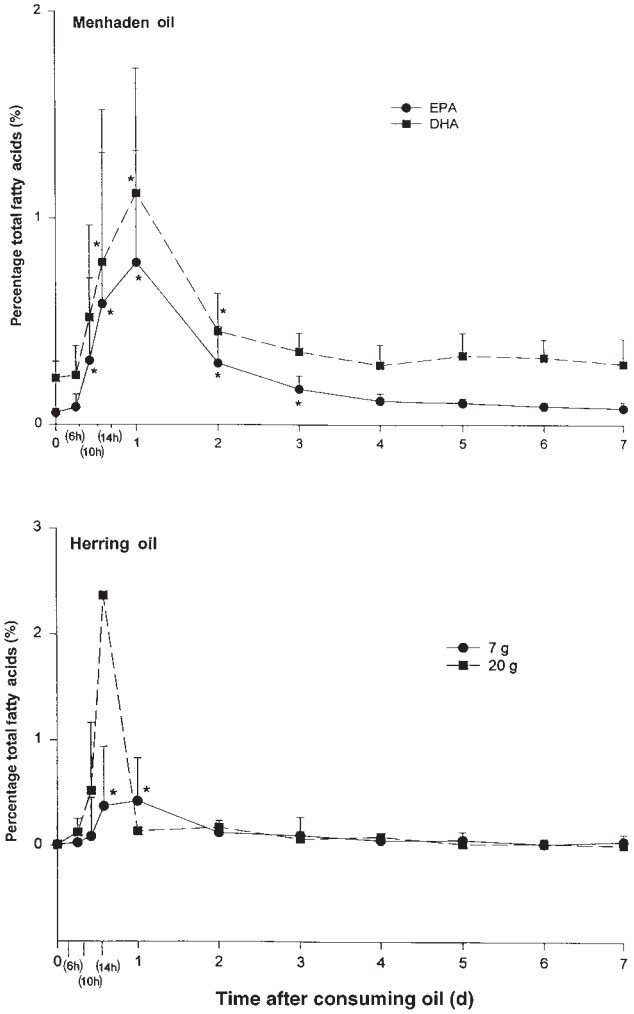

FIGURE 1. Changes in breast milk eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contents after consumption of menhaden oil (n = 13), and changes in breast milk cetoleic acid contents after consumption of either 7 (n = 7) or 20 (n = 2) g herring oil. Scales for each graph are different. *Significantly different from baseline, $P < 0.05$.

content increased significantly over time ($P < 0.05$), whereas the total saturated fatty acid content decreased significantly ($P < 0.05$) (data not shown).

Safflower oil

Ingestion of the formula containing 40 g safflower oil significantly increased the polyunsaturated fatty acid linoleic acid in milk over time ($P < 0.001$). Linoleic acid increased from 14.6% at baseline to 26.4% at 10 h, 25.4% at 14 h, and 23.0% at 24 h ($P < 0.05$; **Figure 2**). Because of this increase in linoleic acid, the total polyunsaturated fatty acid content of milk increased significantly ($P < 0.001$), whereas total saturated and monounsaturated fatty acid contents decreased over time ($P < 0.001$; data not shown).

Canola oil

The canola oil meal increased the content of linolenic acid in milk over time ($P < 0.001$). Linolenic acid increased from 1.1% at baseline to 2.3% at 10 h, 2.4% at 14 h, and 2.1% at 24 h ($P < 0.05$; Figure 2). This increase was not great enough to affect the total polyunsaturated fatty acid content because of the small amount of linolenic acid in human milk. The total monounsaturated fatty acid content did increase ($P < 0.05$), whereas the total saturated fatty acid content was not affected (data not shown).

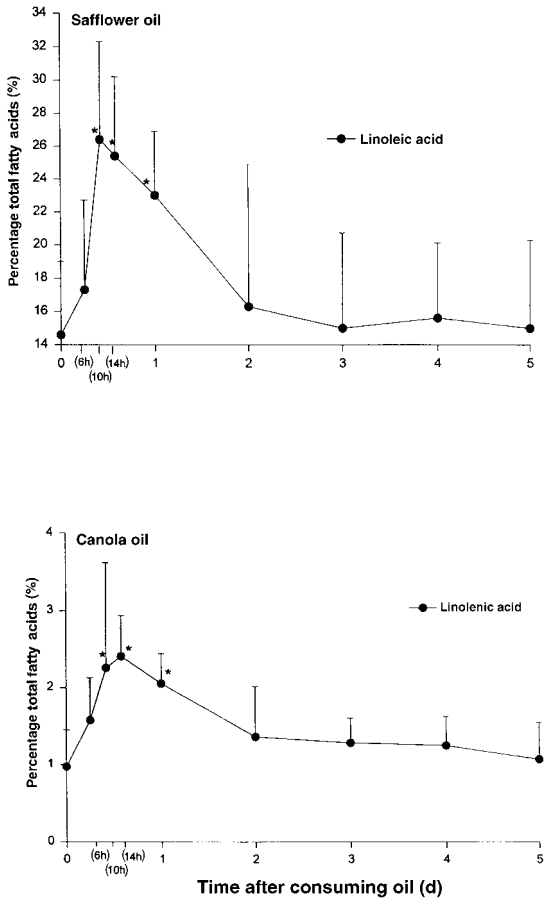

FIGURE 2. Changes in breast milk linoleic and linolenic acid contents after consumption of safflower oil ($n = 14$) and canola oil ($n = 11$), respectively. Scales for each graph are different. *Significantly different from baseline, $P < 0.05$.

Coconut oil

Ingestion of the formula containing 40 g coconut oil increased the milk content of lauric acid over time ($P < 0.001$). Lauric acid increased from 3.9% of fatty acids at baseline to 9.2% at 10 h and 9.6% at 14 h ($P < 0.05$; **Figure 3**). The breast milk content of capric acid (10:0) also increased significantly over time ($P < 0.05$), but not at any time points. This fatty acid is of interest because coconut oil contains 5.8% capric acid by weight. Ingestion of the coconut oil formula significantly increased total saturated fatty acids ($P < 0.001$), significantly decreased total monounsaturated fatty acids ($P < 0.05$), but had no effect on total polyunsaturated fatty acids over time (data not shown). The rise in total saturated fatty acid content was due primarily to the increase in lauric acid.

Cocoa butter

Ingestion of the formula containing 40 g cocoa butter increased the milk content of stearic acid over time ($P < 0.001$). It rose from 6.7% at baseline to 10.6% at 10 h and 10.1% at 14 h ($P < 0.05$; Figure 3). Surprisingly, the breast milk palmitic acid content did not change significantly over time or at any time points as might be expected because of its large content in cocoa butter (25.7% of total fatty acids). Ingestion of the cocoa butter formula decreased total polyunsaturated fatty acid content over time ($P < 0.05$), but did not significantly affect total monounsaturated or saturated fatty acid contents (data not shown).

Diets

The diets of the subjects remained constant during the study. The Diet Habit Survey indicated that there were no significant differences in cholesterol–saturated fat, carbohydrate, fish, or total scores. The subjects consumed <30% of their energy as fat, 10% as saturated fat, 55% as carbohydrate, < 300 mg cholesterol/d, and 2900 mg Na/d.

Significant differences were found between initial and final EPA ($P < 0.05$), DHA ($P < 0.01$), and total EPA and DHA ($P < 0.01$) scores from the n−3 Fatty Acid Food Frequency Questionnaire. The average monthly EPA intake at the beginning of the study was 3.0 ± 2.8 g/mo and that of DHA was 3.7 ± 3.3 g/mo (total: 6.7 ± 6.0 g/mo). The average monthly EPA intake after completion of the study was 1.7 ± 2.2 g/mo and that of DHA was 2.3 ± 2.8 g/mo (total: 4.0 ± 5.0 g/mo). The lower intake of the n−3 fatty acids at the end of the study was anticipated. By design, 12 of 14 subjects consumed at least one of the fish-oil test formulas during the last month of the study. Because subjects were asked to avoid fish during the week before and after the menhaden and herring oil test meals, most subjects would have been asked to avoid fish for ≥ 2 wk during the last month of the study.

DISCUSSION

It has been well documented that the type of fatty acids in the maternal diet affects the fatty acid composition of breast milk in a predictable fashion. Consumption of polyunsaturated vegetable oils raises the milk content of linoleic and linolenic acids (3, 6, 7). In addition, both dietary EPA and DHA increase the content of these fatty acids in milk (3, 8–10). Other studies showed dif-

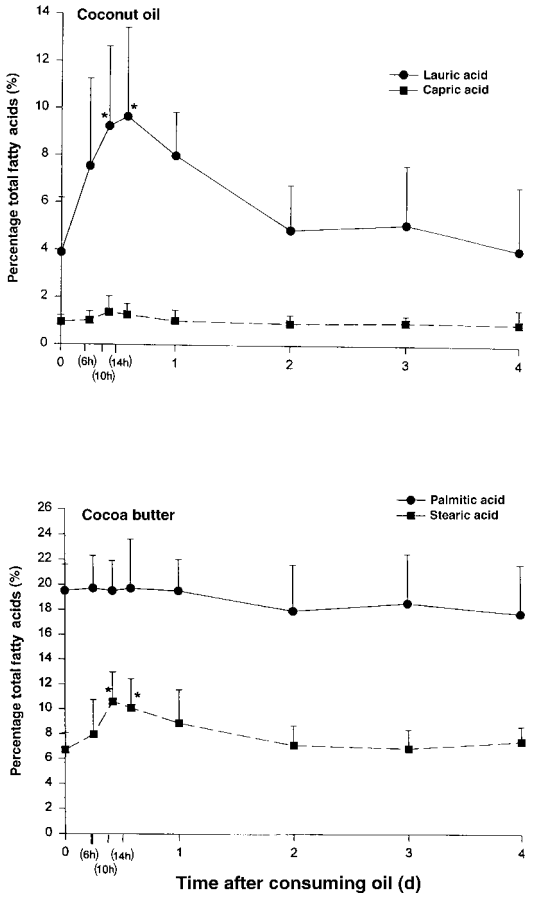

FIGURE 3. Changes in breast milk fatty acid contents after consumption of coconut oil (n = 12) and cocoa butter (n = 13). Scales for each graph are different. *Significantly different from baseline, $P < 0.05$.

ferent responses to various doses of test fats. An earlier report from our laboratory indicated dose-dependent increases in breast milk EPA and DHA in women taking 5- to 47-g fish-oil supplements for 1–4 wk (8). Henderson et al (9) reported similar dose-dependent responses in these breast milk long-chain polyunsaturated fatty acids after consumption of fish oil. Other components of the maternal diet may also influence the fatty acid content of breast milk. When lactating women consume a low-fat, high-carbohydrate diet, there are greater amounts of medium-chain saturated fatty acids in breast milk as a result of their increased synthesis in mammary tissue (4, 5, 17, 18).

Whereas these other studies reported the effects of the long-term diet on human milk fatty acid composition, the current study investigated acute changes in milk fatty acids after the consumption of six test meals containing different fats and oils. The fatty acids of specific interest usually were elevated in the breast milk within 6 h of ingestion of the test meal, peaked between 10 and 24 h, and remained significantly elevated for 1–3 d.

The time to peak triacylglycerol concentrations from dietary fatty acids differs considerably between plasma and breast milk. Hachey et al (11) reported a lag time of 6.0 ± 1.9 h between the peak increases of fatty acids in plasma and human milk. On the assumption that plasma triacylglycerol concentrations peak 3–4 h after the ingestion of fat (19), human milk fatty acids might be expected to peak between 7 and 15 h after the ingestion of fat. This lag time can be attributed to the time needed for lipolysis of chylomicron and VLDL triacylglycerol by the lipoprotein lipase in the mammary capillary bed, resynthesis into triacylglycerol by the alveolar cells of the breast, formation and extrusion of the milk fat globule, and the release of milk from the breast. During lactation, lipoprotein lipase activity decreases in adipose tissue while increasing dramatically in mammary tissue, presumably to channel more fat to the mammary gland (20, 21).

The acute increases in milk EPA and DHA after consumption of the menhaden oil formula are consistent with those observed in the Maternal Diet Study by our group (22). In this study, the breast milk of some of the women consuming typical diets had much higher amounts of EPA and DHA on 1 of 2 consecutive days. For example, on the second of 2 consecutive days, the EPA content of milk in one subject was about two times greater than on the previous day (0.04% compared with 0.09%) and the DHA content was about five times greater than on the previous day (0.1% compared with 0.5%). These differences were attributed to changes in dietary intake of EPA and DHA from fish. Several investigators have reported that regular intake (≥ 5 d) of fish and fish oil increases the breast milk content of these fatty acids (3, 8–10). The two doses of herring oil in the current study suggested a dose-dependent increase in milk cetoleic acid as well as in EPA and DHA.

Of note in the present study was the delay in peak values of EPA and DHA compared with the other milk fatty acids. Most of the other fatty acids from a given test meal peaked at 10 and 14 h, whereas EPA and DHA peaked at 24 h. In addition, these fatty acids remained significantly elevated in breast milk longer than the other fatty acids. EPA remained elevated for 3 and DHA for 2 d after consumption of menhaden oil, compared with the other fatty acids, which remained elevated for only a maximum of 24 h. One possible explanation for the prolonged elevation of EPA and DHA in milk is that the body's pool size of these fatty acids is small, except for the large content of DHA in the brain, which is an isolated compartment. EPA and DHA as prostaglandin precursors and membrane constituents are possibly not as readily utilized for energy sources as are other fatty acids.

In this study, cetoleic acid from herring oil was identified as an excellent marker for tracking the appearance of fatty acids from the diet in breast milk. This fatty acid is not usually found in either the diet or the milk of US women because its chief source, herring, is typically not consumed. Cetoleic acid is, however, found in the milk of Inuits, who consume a variety of foods of marine origin containing this fatty acid (23).

The ingestion of the saturated fatty acid palmitic acid produced inconsistent results. We, as well as Mellies et al (7), found no change in milk palmitic acid after its ingestion. On the other hand, Connor et al (WE Connor, S Smith, S Van Winkle, et al, unpublished observations, 1987) reported a slight but significant decrease of breast milk palmitic acid after 10 d of consumption of a cocoa butter formula containing 10 g of this fatty acid, and Hachey et al (11) reported an increase after the ingestion of a Sustacal formula (Mead Johnson, Evansville, IN) containing 4.4 g palmitic acid. Although it is difficult to explain these discrepant findings, it appears that the magnitude of the changes was slight and that the effect of a daily intake of 4–10 g palmitic acid has a minimal effect on the amount of this fatty acid in

breast milk. This is most likely because of the large pool size of palmitic acid in the body. In our study, the fatty acids with a large plasma pool size were less affected by dietary sources than were the fatty acids with a small pool size. We also observed this phenomenon after consumption of oleic acid (18:1n−9), another fatty acid with a large pool size in the body. After ingestion of the canola oil and cocoa butter formulas, which contained 58.4% and 32.6% oleic acid, respectively, the oleic acid content of milk did not increase significantly at any time point.

On the other hand, we observed that the milk content of stearic acid increased significantly 14 h after ingestion of the cocoa butter formula. Stearic acid has a much smaller plasma and adipose tissue pool size than palmitic or oleic acid. Although no other studies have looked at acute effects of diet on the stearic acid content of milk, WE Connor et al (unpublished observations, 1987) and Mellies et al (7) reported no change in the breast milk content of stearic acid after its long-term ingestion. This lack of effect of dietary stearic acid, as well as of palmitic acid, on the composition of milk concentrations of these fatty acids could be partially explained by desaturation. Both stearic (18:0) and palmitic acid (16:0) can be desaturated via acyl desaturase to 16:1n−7 or 18:1n−9. Lin et al (24) observed that in rabbits fed palm oil and cocoa butter, good sources of palmitic acid and stearic acid respectively, these fatty acids were desaturated to form 16-carbon and 18-carbon monounsaturated fatty acids. They suggested that this desaturation occurs to allow the adipose tissue to maintain a certain degree of unsaturation for cell membrane fluidity.

The findings of this study are particularly relevant for investigators conducting studies on human milk. Because single meals can significantly affect milk fatty acid composition, investigators using single samples could report amounts of fatty acids in milk that reflect not chronic intake but only a recent meal. An average of several breast milk samples days apart would better reflect habitual dietary intake of the mother. Furthermore, no studies have addressed the acute effects of the breast-feeding infant's milk intake on the infant's blood or tissue fatty acids at different time points after a feeding. Additional studies that measure the infant's blood fatty acids at various time points after a given fat-rich meal would be helpful to better understand this relation.

In summary, this study provides new information about the effect of the maternal diet on the availability of fatty acids for the breast-feeding infant. A single meal of a particular fat may significantly affect the breast milk fatty acid composition for 1–3 d, and the maximum increase will probably occur during the first 24 h. However, the habitual diet of the mother has a greater influence on the breast milk fatty acid composition generally. If a lactating woman consumes fish regularly, her milk will contain greater amounts of the long-chain polyunsaturated fatty acids EPA and DHA for a longer period of time than will the milk of a woman who consumes fish only occasionally. Therefore, the diet of the mother directly influences the fatty acid composition of the milk both acutely and chronically.

We gratefully acknowledge Gary Sexton of the Clinical Research Center for his expertise in statistics, Greg Anderson for his expert advice about gas chromatography, and the study participants for their cooperation with the study procedures.

REFERENCES

1. Jensen RG. The lipids in human milk. Prog Lipid Res 1996;35:53–92.
2. Insull W, Hirsch T, James T, Ahrens EH. The fatty acids of human milk. II. Alterations produced by manipulation of caloric balance and exchange of dietary fats. J Clin Invest 1959;38:443–50.
3. Finley DA, Lonnerdal B, Dewey KG, Grivetti LE. Breast milk composition: fat content and fatty acid composition in vegetarians and non-vegetarians. Am J Clin Nutr 1985;41:787–800.
4. Silber GH, Hachey DL, Schanler RJ, Garza C. Manipulation of maternal diet to alter fatty acid composition of human milk intended for premature infants. Am J Clin Nutr 1988;47:810–4.
5. van Beusekom CM, Martini IA, Rutgers HM, Boersma ER, Muskiet FA. A carbohydrate-rich diet not only leads to incorporation of medium-chain fatty acids (6:0–14:0) in milk triglycerides but also in each milk-phospholipid subclass. Am J Clin Nutr 1990;52:326–34.
6. Sanders TAB, Reddy S. The influence of a vegetarian diet on the fatty acid composition of human milk and the essential fatty acid status of the infant. J Pediatr 1992;120:S71-7.
7. Mellies MJ, Ishikawa TT, Gartside PS, et al. Effects of varying maternal dietary fatty acids in lactating women and their infants. Am J Clin Nutr 1979;32:299–303.
8. Harris WS, Connor WE, Lindsey S. Will dietary omega-3 fatty acids change the composition of human milk? Am J Clin Nutr 1984;40:780–5.
9. Henderson RA, Jensen RG, Lammi-Keefe CJ, Ferris AM, Dardick KR. Effect of fish oil on the fatty acid composition of human milk and maternal and infant erythrocytes. Lipids 1992;27:863–9.
10. Chulei R, Xiaofang L, Hongsheng M, et al. Human milk composition in women from five different regions in China: the great diversity of milk fatty acids. J Nutr 1995;125:2993–8.
11. Hachey DL, Thomas RM, Emken EA, et al. Human lactation: maternal transfer of dietary triglycerides labeled with stable isotopes. J Lipid Res 1987;28:1185–92.
12. Freer CA, Connor WE, Connor SL, Wander RC. Acute effects of dietary fatty acids on human milk fatty acids. Am J Clin Nutr 1996;61:893 (abstr).
13. Connor SL, Gustafson JR, Sexton G, Becker N, Artaud-Wild S, Connor WE. The Diet Habit Survey: a new method of dietary assessment that relates to plasma cholesterol changes. J Am Diet Assoc 1992;92:41–7.
14. Anderson GJ, Connor WE, Corliss JD, Lin DS. Rapid modulation of the n−3 docosahexaenoic acid levels in the brain and retina of the newly hatched chick. J Lipid Res 1989;30:433–41.
15. Anderson GJ. Developmental sensitivity of the brain to dietary n−3 fatty acids. J Lipid Res 1994;35:105–11.
16. Winer BJ. Statistical principles in experimental design. 2nd ed. New York: McGraw-Hill, 1971.
17. Hachey DL, Silber GH, Wong WW, Garza C. Human lactation II: endogenous fatty acid synthesis by the mammary gland. Pediatr Res 1989;25:63–8.
18. Read WWC, Lutz PG, Tashjian A. Human milk lipids. II. The influence of dietary carbohydrates and fat on the fatty acids of mature milk. A study in four ethnic groups. Am J Clin Nutr 1965;17:180–3.
19. Harris W, Connor W, Alam N, Illingworth D. Reduction of postprandial triglyceridemia in humans by dietary n−3 fatty acids. J Lipid Res 1988;29:1451–60.
20. Subcommittee on Nutrition During Lactation, Food and Nutrition Board. Nutrition during lactation. Washington, DC: National Academy Press, 1991.
21. Hamosh M, Clary TR, Chernick SS, Scow RO. Lipoprotein lipase activity of adipose and mammary tissue and plasma triglyceride in pregnant and lactating rats. Biochim Biophys Acta 1970; 210:473–82.
22. Freer CA, Connor WE, Connor SL. The impact of maternal dietary n−3 fatty acids upon blood levels of docosahexaenoic acid in the newborn and breast-fed infant. J Am Diet Assoc 1995;95:A-19

(abstr).
23. Innis S, Kuhnlein H. Long-chain n−3 fatty acids in breast milk of Inuit women consuming traditional foods. Early Hum Dev 1988;18:185–9.
24. Lin DS, Connor WE, Spenler CW. Are dietary saturated, monounsaturated, and polyunsaturated fatty acids deposited to the same extent in adipose tissue of rabbits? Am J Clin Nutr 1993;58:174–9.