Current Research

Maternal Diet and Exercise: Effects on Long-Chain Polyunsaturated Fatty Acid Concentrations in Breast Milk

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ABSTRACT

Background Long-chain polyunsaturated fatty acids (LC-PUFA) are essential for infant growth and development. The amount of long-chain PUFA in breast milk depends on maternal diet and body stores. Because exercise increases mobilization and utilization of fatty acids, maternal activity may also influence the amount of LC-PUFA in breast milk.

Objective To investigate the effects of exercise on α-linolenic acid (LNA), linoleic acid (LA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (AA) concentrations in maternal plasma and breast milk and to determine if lactating women consume adequate amounts of LC-PUFA to compensate for those used for energy during exercise.

Design LC-PUFA in plasma and breast milk were measured at 12 weeks postpartum in exercising and sedentary women. Dietary intake was recorded for 3 days. A subsample of women participated in exercise and rest sessions to examine the acute effects of exercise on breast milk LC-PUFA.

Results There were no differences in dietary intake between the two groups. Mean intake (±standard error of the mean) of LA was 11.05±1.39 and 9.34±0.97 and LNA was 0.96±0.12 and 0.82±0.09 g/day by the sedentary and exercise groups, respectively. These amounts are close to the Adequate Intakes of LA and LNA for lactation (13 and 1.3 g/day, respectively). No differences were found in LC-PUFA in plasma and breast milk between groups. After 30 minutes of exercise, there was a trend for an increase in LA and LNA concentrations in breast milk, with no change in DHA, EPA, and AA concentrations.

Conclusions These results suggest that women consuming adequate amounts of LC-PUFA can exercise moderately without decreasing the LC-PUFA in their breast milk.

J Am Diet Assoc. 2005;105:1098-1103.

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0002-8223/05/10507-0004$30.00/0

doi: 10.1016/j.jada.2005.04.004

It is well-established that breast milk is the optimal food for infants, providing all the nourishment they need to grow physically and mentally for the first 6 months of life (1,2). Long-chain polyunsaturated fatty acids (LC-PUFA), specifically α-linolenic acid (LNA, 18:3 n-3), linoleic acid (LA, 18:2 n-6), docosahexaenoic acid (DHA, 22:6 n-3), eicosapentaenoic acid (EPA, 20:5 n-3), and arachidonic acid (AA, 20:4 n-6) are some of the many components in human milk that promote infant growth and development.

LNA and LA are essential fatty acids that can be desaturated and elongated into the longer chain fatty acids EPA, DHA, and AA. DHA is an important nutrient in breast milk that is crucial for neural and visual development of the infant (3). During lactation, 70 to 80 mg of DHA is utilized per day for breast milk production (4). AA is necessary for infant growth and development (5).

Intake of LC-PUFA by the breast-fed infant depends on maternal diet, body fat stores, and possibly also the mother's activity level. Dietary fatty acids of lactating women can be utilized in three ways: (a) stored in the adipose tissue, (b) transferred to the mammary gland for incorporation into milk, and/or (c) used for energy, especially during exercise. Exercise mobilizes fatty acids from body stores for energy. Increased metabolism during exercise may lead to a decrease in concentrations of LC-PUFA available for incorporation into breast milk. However, the increased loss due to oxidation of the fatty acids may be countered by an increase in fatty acid mobilization from adipose stores. This phenomenon has not been investigated in lactating women.

Current recommendations for the general population from the Centers for Disease Control and Prevention and the American College of Sports Medicine (6) are to exercise moderately 30 minutes per day, every day of the week, whereas the Institute of Medicine (7) recommends incorporating 60 minutes of moderate exercise per day to achieve optimal health benefits. In addition, the Ameri-

can Academy of Pediatrics and the American Dietetic Association recommend that all women breastfeed their infants during the first year of life (1,2). However, there is a paucity of research about the effects of exercise by lactating women on the composition of their breast milk. Therefore, the purpose of this study was to investigate the effects of chronic and acute exercise on plasma and breast milk LC-PUFA. Another objective was to determine if breastfeeding women consume adequate amounts of LC-PUFA to compensate for those used for energy during exercise.

METHODS

Participants

Healthy (absence of chronic disease), nonsmoking, exclusively breastfeeding women were recruited from prenatal classes and obstetricians' offices. Participants were eligible if: (a) their body mass index was between 20 and 30, (b) their infants' birth weight was more than 2,500 g, and (c) there were no birth complications. Mothers were assigned to one of two groups based on their self-reported exercise history. The exercise group consisted of mothers who exercised at least 30 minutes 3 days per week for the past 6 weeks (n=30). Women were assigned to the sedentary group if they exercised once per week or less (n=23). A subsample (n=14) of women from the exercise group completed a second study examining effects of acute exercise, in which subjects participated in an exercise session and a rest session. The Institutional Review Board of the University of North Carolina at Greensboro approved this research, and all participants provided written, informed consent. This research was conducted prior to establishment of the Health Insurance Portability and Accountability Act guidelines.

Experimental Design

This was a cross-sectional study that measured the LC-PUFA concentrations in plasma and breast milk of sedentary and exercising lactating women at 12 (±2) weeks postpartum. Breast milk was collected by each woman on the morning of her laboratory measurements. Milk (30 mL) from the first feed after 5 AM was expressed and stored in the subject's home freezer until transported to the lab, where it was frozen at −80°C until analysis. Blood samples were drawn and then body composition and cardiorespiratory fitness were measured.

Fourteen of the exercising women returned for two more tests to determine the effects of acute exercise on concentrations of fatty acids in breast milk. The women participated in both a rest and an exercise session, with the order of sessions randomized. Sessions were separated by approximately 2 days. Exercise consisted of brisk walking or jogging at an intensity of approximately 75% of predicted maximum heart rate for 30 minutes. During the exercise session, breast milk was expressed before the onset of exercise and 10 and 60 minutes after the exercise bout. During the rest session, milk was collected at the same time points, with the women resting in a seated position for the 30-minute test period. All milk was completely expressed with a Medela electric breast pump (McHenry, IL) from both breasts at each time point. Samples were frozen at −80°C until analysis.

Dietary Analysis

Prior to laboratory measurements, women weighed their food and beverage intake on a portable digital gram scale (OHAUS, Florham Park, NJ) and recorded their consumption for 3 days. The Food Processor Analysis Program (version 2.2, ESHA Research, Salem, OR), along with manufacturer information, food label nutrient content information, and other food composition tables (8) were used for dietary analysis.

Anthropometrics

Weight to the nearest 0.1 kg was measured on a stationary beam balance scale (Healthometer, Continental Scale Corp, Bridgeview, IL) with women wearing only a bathing suit. Height was measured, using a stadiometer (Genentech, Inc, San Francisco, CA), to the nearest 0.1 cm. Body composition was measured by hydrostatic weighing. Body density and percentage body fat were calculated using the equations of Brozek and colleagues (9).

Cardiorespiratory Fitness Level

To assess the cardiorespiratory fitness level of the participants, a submaximal graded treadmill test was used (10). Women wore a heart rate monitor (Polar Inc, Woodbury, NY). Heart rate and perceived exertion were measured and recorded every minute for the duration of the test. Participants exercised until their heart rate reached 85% of their predicted maximal heart rate, and predicted oxygen consumption (VO_2) was determined using the formulas of the American College of Sports Medicine (10). Linear regression was used to determine the subject's predicted VO_2 max using heart rate as the independent variable and predicted VO_2 as the dependent variable.

Plasma and Breast Milk Fatty Acid Analysis

Blood was drawn by venipuncture from an antecubital vein by a trained phlebotomist, after a 12-hour fast. The plasma layer was separated and stored at −80°C until analyzed for fatty acid content. Plasma fatty acids were extracted by an adapted method of Bligh and Dyer (11). Breast milk was extracted using a modified Folch procedure as detailed by Bitman and colleagues (12). The fatty acid profiles of plasma and breast milk were determined by a gas chromatograph (5890 Gas Chromatograph, Hewlett Packard, Avondale, PA). Plasma was collected from a subsample of 38 women (sedentary group, n=14; exercise group, n=24), because measurement of plasma fatty acids was started after 15 women had already completed the study of breast milk fatty acids. Milk was not collected from one participant in the sedentary group due to laboratory error.

Statistical Analysis

SPSS-PC (Version 10.0, SPSS, Chicago, IL) statistical software was used for all data analysis. Student's *t* test was used to assess differences between experimental groups. Repeated measures analysis of variance was used to determine if breast milk LC-PUFA concentrations changed during exercise or rest sessions. Pearson correlations were identified for relationships between breast

Table 1. Characteristics of sedentary and exercising breastfeeding women at 12 weeks postpartum

Characteristic	Sedentary group (n=23)	Exercising group (n=30)
	← mean± SEM[a] →	
Age (y)	31.5±1.0	31.5±0.6
Parity	2.26±0.2	1.90±0.2
Height (cm)	163.1±1.1	164.6±1.2
Weight (kg)	66.0±1.6	64.0±1.5
Body mass index (calculated as kg/m^2)	24.9±0.7	23.6±0.5
% Body fat	28.0±1.4	25.5±1.1
Predicted maximal O_2 consumption (mLO_2/kg/min)	32.2±1.0	39.6±1.0*

[a]SEM=standard error of the mean.
*Significantly different from sedentary group, $P<.01$.

milk fatty acids and diet, plasma, parity, and percentage body fat. Multiple linear regression analysis was used to predict concentrations of breast milk fatty acids from the variables mentioned. Statistical significance was set at $P<.05$.

RESULTS

There were no significant differences between groups in characteristics of participants, except that exercising women exhibited a higher level of cardiorespiratory fitness (Table 1). They reported exercising a mean of 47 minutes per day, 4 days per week. The majority of the women walked briskly and/or participated in aerobic exercise classes. Their average fitness level was in the 80th percentile, according to values given by the American College of Sports Medicine (10) for cardiorespiratory fitness. The sedentary women had an average fitness level in the 40th percentile.

There were no differences in percentage body fat (Table 1) or dietary intake (Table 2) between the two groups; except that AA intake was significantly higher in the sedentary group. Both groups consumed a diet of approximately 55% carbohydrates, 15% protein, and 30% fat; and the ratio of LA to LNA was approximately 11.5:1.

There were no significant differences in the concentrations of the LC-PUFA in the breast milk of exercising and sedentary lactating women (Table 3). Breast milk and plasma of the subgroup (n=38) were also not significantly different between groups (data not shown). Due to the lack of difference between groups, the data from all subjects were pooled together for further analysis. Breast milk AA and dietary AA were correlated ($r=0.29$, $P=.04$); however, there were no correlations between breast milk and dietary intake of the other LC-PUFA. Plasma LA and AA were significantly correlated with milk LA ($r=0.33$, $P=.04$) and AA ($r=0.45$, $P=.005$), respectively. There was no correlation between parity or percentage body fat and breast milk fatty acid concentrations in this group. When these were controlled for, there were still no significant differences in dietary fatty acid concentrations between groups. In multiple regression analysis, dietary intake, parity, and percentage body fat did not predict fatty acid concentrations in breast milk.

Characteristics of the subsample who participated in the second study were similar to the characteristics of all the women in the exercise group, shown in Table 1. Between-session changes over time were noted (Figure) for LA and LNA; however, these changes were not significant (LA, $P=.18$; LNA, $P=.23$). During the exercise session, LA increased from time 0 to 60, but in the rest session, LA decreased from time 0 to 60. Similarly, LNA increased in

Table 2. Macronutrient and fatty acid intake of breastfeeding women at 12 weeks postpartum

	Sedentary group (n=23)	Exercising group (n=30)
	← mean± SEM[a] →	
Total energy intake (kcal)	2,276±93	2,142±106
Kcal/kg body weight	35±2	34±2
Total protein intake (g)	85±5	83±3
% kcal from protein	15±1	16±1
Total carbohydrate intake (g)	313±13	306±18
% kcal from carbohydrate	55±1	56±2
Total fat intake (g)	80±5	69±5
% kcal from fat	32±1	29±1
Fatty acid intake (g)		
18:2 n-6 (LA)[b]	11.05±1.39	9.34±0.97
18:3 n-3 (LNA)[c]	0.96±0.12	0.82±0.09
20:4 n-6 (AA)[d]	0.10±0.01	0.06±0.01*
20:5 n-3 (EPA)[e]	0.03±0.01	0.03±0.01
22:6 n-3 (DHA)[f]	0.06±0.02	0.05±0.02

[a]SEM=standard error of the mean.
[b]LA=linoleic acid.
[c]LNA=α-linolenic acid.
[d]AA=arachidonic acid.
[e]EPA=eicosapentaenoic acid.
[f]DHA=docosahexaenoic acid.
*Significantly different from sedentary group, $P<.05$.

Table 3. Long-chain polyunsaturated fatty acid composition of breast milk at 12 weeks postpartum

% of total fat	Sedentary group (n=22)	Exercising group (n=30)
	← mean± SEM[a] →	
18:2 n-6 (LA)[b]	16.94±0.97	14.71±0.72
18:3 n-3 (LNA)[c]	1.14±0.11	1.03±0.09
20:4 n-6 (AA)[d]	0.41±0.03	0.38±0.02
20:5 n-3 (EPA)[e]	0.30±0.08	0.39±0.09
22:6 n-3 (DHA)[f]	0.21±0.08	0.43±0.09

[a]SEM=standard error of the mean.
[b]LA=linoleic acid.
[c]LNA=α-linolenic acid.
[d]AA=arachidonic acid.
[e]EPA=eicosapentaenoic acid.
[f]DHA=docosahexaenoic acid.

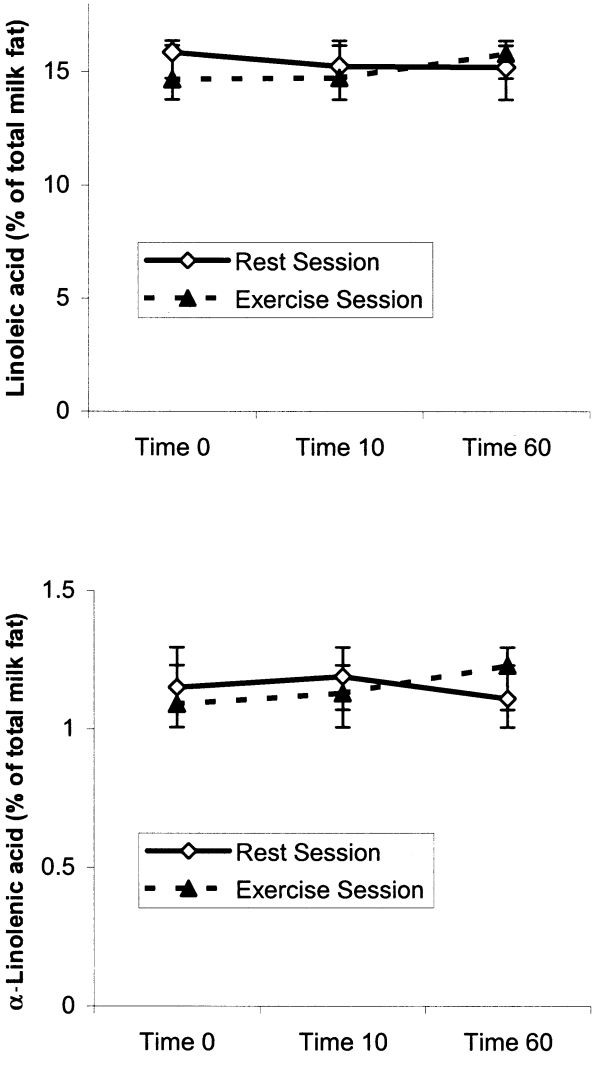

Figure. Percent linoleic acid (LA) and α-linolenic acid (LNA) in breast milk of women before (Time 0) and after (Time 10 and Time 60) the 30-minute rest session or exercise session. Values are means±standard error of the means. LA concentration decreased during rest and increased during exercise. Changes were similar with LNA concentrations. However, these changes were not significant.

the exercise session from time 0 to 60. However, in the rest session, LNA increased from time 0 to 10, but decreased from time 10 to 60, with time 60 falling to a concentration less than the initial measurement (Figure). There were no significant differences in changes over time for AA, EPA, or DHA (data not shown). Interestingly, percentage body fat was significantly correlated with concentrations of LA in milk at time 60 after the exercise session (r=0.62, P=.02) and with concentrations of LNA in milk at time 10 (r=0.53, P=.04) and time 60 (r=0.77, P=.001) after the exercise session. There were no significant correlations between percentage body fat and milk LC-PUFA concentrations during the rest sessions.

DISCUSSION

In this study, dietary intake and exercise did not significantly affect the plasma and breast milk of lactating women. However, there was a trend toward an increase in LA and LNA after an acute exercise bout. With the exception of AA intake, there were no differences in the diets of the women in this study. On average, these women ate diets sufficient in energy, protein, and fat, and were similar to dietary intakes of lactating women reported by other researchers (13,14). It is possible that the observation of no effect of diet or exercise on the LC-PUFA concentrations (except AA) in breast milk was due to insufficient variation in intakes among the women.

The optimal ratio for general good health of n-6 to n-3 fatty acids has not been established; however, researchers suggest a ratio of less than 10:1 (15). This ratio is estimated to be 25:1 in American diets due to increased consumption of processed foods in place of natural foods (16). Women in the current study consumed diets with an n-6 to n-3 ratio at approximately 11.5:1, which is better than the reported national average. The recommended Adequate Intakes of LA and LNA for lactation are 13 g/day and 1.3 g/day, respectively (7). Diets of breastfeeding mothers in this study were close to these recommendations (Table 2). Conversion of LNA into DHA and EPA is quite inefficient (15); therefore, in addition to consuming adequate amounts of LA and LNA, breastfeeding women should make efforts to consume adequate amounts of DHA and EPA. These results suggest that active, breastfeeding women are able to meet the new dietary recommendations for LA and LNA; however, specific recommendations are not yet available for DHA, EPA, and AA.

Current recommendations for LA and LNA for infants from birth to 6 months are 4.4 and 0.5 g/day, respectively (7). Based on the recommended total fat intake of 31 g/day for this age group (7), the percentage of total fat coming from the individual fatty acids (LA and LNA) can be calculated as 14.2% and 1.6%, respectively. Mean concentrations of LA and LNA in the breast milk samples in our study were similar to these recommendations (Table 3), indicating that the women in this study were able to provide sufficient LA and LNA for their infants.

Concentrations of fatty acids were similar to those reported by others in breast milk (17) and plasma (18). Only dietary and breast milk AA concentrations were significantly related in our study. However, isotope tracer studies indicate that maternal body stores, rather than diet, are the major source of breast milk fatty acids, suggesting that dietary fatty acids are first stored in the body and later mobilized for incorporation into breast milk (19). In addition, concentrations of LC-PUFA in breast milk were less than concentrations of LC-PUFA in plasma, with the exception of LNA, which was slightly higher in breast milk than in plasma. This suggests that the availability of LC-PUFA in the plasma is not a limiting factor for concentrations in breast milk.

Fatty acid concentrations decrease as parity increases (20). The observation of no effect of parity on concentrations of fatty acids in breast milk may be due in part to the limited range of parity in this group. Most women had a parity of 1 (n=18) or 2 (n=23), with only 13 women having a parity of 3 or more. Although breast milk fatty

acid concentrations did decrease as parity increased, this relationship was not significant.

Exercise leads to the mobilization of fatty acids from the adipose tissue triacylglycerol; plasma fatty acid concentrations increase threefold compared with rest (21). We observed that 30 minutes of moderate exercise increased concentrations of LA and LNA in breast milk; however, the increase was not significant. Borsheim and colleagues (22) observed a similar increase in LA in the plasma of exercising as compared with resting men, and Mougios and colleagues (23) reported that male handball players had an increase in plasma LA after two 30-minute playing sessions. However, we observed no effect of exercise on concentrations of AA in breast milk, in contrast to the decrease in plasma AA reported by Borsheim (22).

The observation of no exercise effect on breast milk DHA may be related to DHA being one of the fatty acids stored in small quantities in the human body, and during lactation, storage concentrations are much less than normal (4). Therefore, much less DHA may be mobilized during exercise, as compared with LA and LNA. Plasma lipid fractions also contain very little EPA and AA. Turnover of these free fatty acids is quite rapid in the time between meals to provide tissues with nourishment (24).

Because exercise-stimulated lipid mobilization can continue for an extended period of time after cessation of exercise (22), fatty acids may have been transferred to breast milk from plasma after the 60-minute measurement point. In our sample, there was a trend toward an increase in the concentrations of LA and LNA in the breast milk from 10 to 60 minutes after exercise. This increase may have been more if we had continued our measurements beyond 60 minutes. These results suggest that instead of experiencing a decrease in breast milk LA and LNA concentrations after exercise, women may in fact have increased concentrations of LA and LNA in their breast milk.

The significant relationship between percentage body fat and concentrations of LA and LNA 60 minutes after the exercise session suggests a greater mobilization of LA and LNA during exercise in women who have a higher percentage body fat. This is likely due to a greater availability of fat for mobilization. The trend toward an increase in concentrations of LA and LNA after exercise indicates that LA and LNA may actually increase in breast milk after exercise, especially among women with adequate body fat stores. This may not be true for women who are underweight.

CONCLUSIONS

Moderate exercise is not only safe during lactation, but also improves the cardiorespiratory fitness levels of postpartum women and provides many other health benefits (25-27). We have previously reported no effect of exercise on breast milk concentrations of macronutrients (25,27), vitamin B-6 (28), or immunological compounds (29). This study found no effect of exercise on the LC-PUFA concentrations in breast milk. These results suggest that a lactating woman with adequate body fat stores, and consuming a diet sufficient in energy and essential fatty acids (including total fatty acid intake and LA to LNA ratio), can exercise moderately without decreasing, and may potentially increase, the concentrations of LC-PUFA in her breast milk.

The authors thank all of the women who participated in this research study. Special thanks to Ye Sun Lee, PhD, for assistance with fatty acid analysis; and to the student research assistants who assisted with data collection. This research was supported by grants from the North Carolina Institute of Nutrition and the North Carolina Agricultural Research Service.

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APPLICATIONS

Increasing Maternal Docosahexaenoic Acid Levels

The research presented in the preceding article by Bopp and colleagues (1) answers important questions about the preservation of long-chain polyunsaturated fatty acid (LC-PUFA) in human milk after maternal exercise. The role of LC-PUFA, especially docosahexaenoic acid (DHA), in the neural development of infants has been well documented (2-4). In fact, in the past decade, most infant formula manufacturers have offered at least one product fortified with DHA in an attempt to close the nutritional gap between artificial substitutes and human milk. During pregnancy, the fetus, and later the exclusively breastfed infant, relies on maternal intake of DHA for normal growth and development. Optimizing maternal dietary sources during both pregnancy and lactation to ensure desirable DHA levels is worthy of consideration. The authors noted that maternal intake of LC-PUFA was not significantly different between sedentary and exercising women and that both groups were close to the recommended intake levels for both linoleic and α-linolenic acids. An important finding for clinical practice is that DHA levels were maintained despite maternal exercise in this study.

Increasing maternal dietary sources of n-3 fatty acids is a seemingly simple solution to ensuring adequate infant intake; however, DHA levels for pregnant and lactating women in the United States often fall short of intake goals (5). The recommended intake for DHA is $\geq$300 mg/day and possibly as high as 500 mg/d of eicosapentaenoic acid and DHA combined for maximizing adult cardiovascular health benefits (6,7). In addition to eating n-3–rich seafood two times per week, other dietary sources of n-3 fatty acids such as flaxseed, canola oil,

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doi: 10.1016/j.jada.2005.05.191