ABSTRACT

Background: Pregnancy is associated with increased absolute amounts of docosahexaenoic acid (DHA; 22:6n−3) in plasma phospholipids. Expressed as a proportion of total fatty acids, DHA declines slightly in late pregnancy but little information is available on the normalization of DHA postpartum, which may be different in lactating and nonlactating women.

Objective: The aim was to investigate maternal plasma and erythrocyte long-chain polyunsaturated fatty acids (long-chain polyenes; LCPs) postpartum, particularly DHA, in relation to lactation and dietary LCP intake.

Design: Healthy pregnant women who intended to breast-feed or exclusively bottle-feed their infants were studied at 36–37 wk of pregnancy. Blood samples were collected at entry, after parturition on days 2 and 5, and 1, 2, 4, 8, 16, 32, and 64 wk postpartum. Fatty acid profiles were analyzed in plasma and erythrocyte phospholipids. Dietary intakes were assessed 4 and 32 wk postpartum with a validated food-frequency questionnaire.

Results: After delivery, the percentages of plasma linoleic, arachidonic, eicosapentaenoic, and docosapentaenoic acids increased over time, whereas the percentage of docosapentaenoic acid decreased; the patterns of change did not differ significantly between the lactating and nonlactating groups. The percentage of DHA in plasma and erythrocyte phospholipid fatty acids declined significantly in the 2 groups, more so in the lactating women, and was enhanced when the lactation period was extended. Despite the apparent higher dietary intake of essential fatty acids in the lactating group at week 4, it was not significantly different from that of the nonlactating group.

Conclusion: Normalization of maternal plasma and erythrocyte phospholipid n−3 LCPs differs significantly between lactating and nonlactating women postpartum but that of n−6 LCPs does not. Am J Clin Nutr 2001;73:1074–9.

KEY WORDS Lactation, docosahexaenoic acid, DHA, phospholipids, women, long-chain polyunsaturated fatty acids, PUFA, maternal diet, postpartum

INTRODUCTION

Pregnancy is generally associated with hyperlipidemia (1), which is thought to facilitate the maternal supply of lipids and essential fatty acids for energy and structural use by the growing fetus (2, 3). Concentrations of plasma phospholipid-associated fatty acids also rise during pregnancy, particularly docosahexaenoic acid (DHA; 22:6n−3; 4, 5)—the most prominent n−3 fatty acid and an important component of the developing central nervous system. Between 10 and 40 wk of pregnancy, the DHA content of maternal plasma phospholipids was shown to increase by ≈52%, from 47.1 to 71.7 mg/L, whereas the increase in the other n−3 fatty acids combined was only 19.2%, from 21.3 to 25.4 mg/L (6).

The postpartum course of DHA has not been described in detail. About 6 mo after delivery, the maternal plasma phospholipid DHA content was shown to decrease to values well below those seen in early pregnancy (4), comparable with values of nonpregnant women (7, 8). However, no information is available on the course of this normalization. This course may be different between lactating and nonlactating women because, after delivery, breast-feeding women continue to supply their own DHA and other long-chain polyunsaturated fatty acids (long-chain polyenes; LCPs) to their infants. Therefore, it was decided to compare the postpartum course of LCPs between lactating and nonlactating women. In addition, the dietary fatty acid intake was examined.

SUBJECTS AND METHODS

Subjects

Healthy pregnant women between 36 and 37 wk of pregnancy were recruited through midwives in the area of Southern Limburg, Netherlands. Only women meeting the following criteria were included: no metabolic, cardiovascular (including hypertension), neurologic, or renal disorders; no use of medications, except multivitamins and iron supplements; singleton pregnancy; term delivery; and no blood transfusion in the perinatal period. Sixty-eight women were enrolled; however, 11 women were not followed-up: 6 subjects did not deliver at term, 2 subjects...
received a blood transfusion immediately after delivery, and 3 subjects withdrew after 4 wk postpartum because of hospitalization of the baby, fainting during blood collection, or personal reasons. Of the remaining 57 women, 22 exclusively bottle-fed their children and 35 breast-fed their infants. The study was approved by the Medical Ethics Committee of the University Hospital Maastricht and written, informed consent was obtained from each participant.

Blood sample and dietary data collection

Venous blood samples were collected into EDTA-containing tubes at entry, 2 and 5 d after parturition, and 1, 2, 4, 8, 16, 32, and 64 wk postpartum. After blood collection, plasma was separated from the erythrocytes by centrifugation (1000 × g, 10 min, 4°C). Aliquots of the plasma samples were divided into 2 storage cups, closed tightly under nitrogen, and stored at −80°C until fatty acid analysis. Erythrocytes underwent the same procedure, but were first washed twice with EDTA-containing saline. Butylated hydroxytoluene was added to all erythrocyte samples before storage (9).

The dietary fatty acid intake was assessed with a validated food-frequency questionnaire, specifically designed to collect data on fat consumption (10). The subjects completed the questionnaire at 4 and 32 wk postpartum. The food consumption data were encoded according to the system of the Dutch nutrient data bank (NEVO) and converted into energy and nutrients by using the computerized version of the Dutch Year Food Table 1996 II (11). This table enabled calculation of the intake of total fat; total saturated, monounsaturated, and polyunsaturated fatty acids; linoleic acid (18:2n−6); α-linolenic acid (18:3n−3); total n−6 fatty acids; eicosapentaenoic acid (20:5n−3); DHA; and total n−3 fatty acids.

Fatty acid analyses

The fatty acids of phospholipids isolated from maternal plasma and erythrocytes were analyzed as previously described (12). The composition of these fatty acids was determined by capillary gas chromatography with a 50-m BP1 nonpolar column (0.22 × 0.10 μm) and a 50-m BPx70 polar column (0.22 × 0.25 μm), both from SGE, Bester BV, Amstelveen, Netherlands (13). The amount of each fatty acid was calculated on the basis of the amount of fatty acids recovered with use of the internal standard 1,α-dinonadecanoyl phosphatidylcholine. The results were expressed as mg/L in plasma or the erythrocyte suspension and as a percentage of total fatty acids (% by wt) for 18:2n−6, arachidonic acid (20:4n−6), n−6 docosapentaenoic acid (22:5n−6), 18:3n−3, 20:5n−3, docosapentaenoic acid (22:5n−3), and DHA.

Statistical analysis

The data are presented as means ± SEMs. Comparison of the clinical characteristics (continuous variables) between the 2 groups was performed with the unpaired t test, whereas the chi-square test was used for the variable parity. Repeated-measures analysis of variance (ANOVA) was used to examine the effects of lactation on the fatty acid composition of plasma and erythrocyte phospholipids. The ANOVA model included a grouping factor (lactating and nonlactating women), a time factor (fatty acid data from day 2 through 64 wk postpartum), an interaction term between time and group (time × group), and the covariate parity. Parity was previously shown to correlate inversely with the DHA content of plasma phospholipids (14). If the interaction term was significant, changes in the fatty acid composition over time within each group were further studied by repeated-measures ANOVA, and between-groups comparisons were made with one-way ANOVA. To correct for baseline (36 wk of pregnancy), the fatty acid data included in the ANOVA models were the postpartum data at each sampling point minus the baseline value during pregnancy (∆fatty acid). The lactating group consisted of all women who had breast-fed their infants after delivery; when a woman stopped breast-feeding, she remained in this group. A further comparison was made between nonlactating and lactating women, who were divided into 4 subgroups based on duration of lactation: subgroup 1, ≤9 wk, n = 5; subgroup 2: 10–15 wk, n = 10; subgroup 3: 16–21 wk, n = 9; and subgroup 4: 32–50 wk, n = 11 (ANOVA with post hoc Bonferroni procedure for multiple comparisons).

Dietary intakes at 4 and 32 wk of pregnancy were examined within groups by using Wilcoxon’s signed-rank test and between groups by using the Mann-Whitney U test. The relation between dietary fatty acid intake (% of total fat) and plasma and erythrocyte fatty acid composition (% of total fatty acids) was evaluated by using Spearman’s rank-correlation test. Nonparametric tests were used because the data were not normally distributed.

Because of multiple testing, P values <0.01 were considered significant for plasma and erythrocyte fatty acid data, whereas for dietary intakes the significance level was set at 0.05. All statistical analyses were performed by using STATVIEW (version 5.0 for Macintosh PPC; SAS Institute Inc, Cary, NC).

RESULTS

Clinical characteristics

The characteristics of the 2 groups are presented in Table 1. Except for age, the groups were not significantly different. The nonlactating women were significantly younger than the lactating women. All women had uncomplicated pregnancies and delivered full-term singletons: gestational ages of 40.2 ± 0.2 wk in the lactating group and 40.4 ± 0.3 wk in the nonlactating group. There were no significant differences in mean birth weights, birth lengths, or head circumferences between the 2 groups.

Fatty acid changes in the nonlactating and lactating women

No significant differences were observed in plasma and erythrocyte fatty acid compositions at entry (data not shown) between the lactating and nonlactating women. After delivery, total fatty acids in plasma phospholipids decreased significantly over time in the lactating and nonlactating groups (Table 2). Comparable results were observed for the n−6 fatty acids (Figure 1). Results were less consistent for plasma n−3 fatty acids. The amounts of 18:3n−3, DHA (Figure 1), and total n−3 fatty acids (data not shown) showed significant downward trends postpartum in both groups, whereas the amounts of 20:5n−3 (Figure 1) and 22:5n−3 (data not shown) increased significantly after delivery. However, the observed patterns of change in n−3 and n−6 LCPs were not significantly different between lactating and nonlactating women (no significant interaction terms).

When expressed as a percentage of total phospholipid–associated plasma fatty acids (Figure 2), the changes over time in 18:2n−6 and 20:4n−6 were different from those expressed as absolute
TABLE 1
Clinical characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Lactating group (n = 35)</th>
<th>Nonlactating group (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>31.9 ± 0.43</td>
<td>29.1 ± 0.68</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.69 ± 0.01</td>
<td>1.68 ± 0.01</td>
</tr>
<tr>
<td>Mother’s weight (kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before pregnancy</td>
<td>64.8 ± 1.5</td>
<td>69.0 ± 2.7</td>
</tr>
<tr>
<td>Gain during pregnancy</td>
<td>12.0 ± 0.8</td>
<td>13.3 ± 1.2</td>
</tr>
<tr>
<td>64 wk postpartum</td>
<td>66.2 ± 1.7</td>
<td>69.0 ± 2.9</td>
</tr>
<tr>
<td>Duration of breast-feeding (wk)</td>
<td>20.4 ± 2.3</td>
<td>—</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At entry</td>
<td>36.5 ± 0.1</td>
<td>36.2 ± 0.1</td>
</tr>
<tr>
<td>At delivery</td>
<td>40.2 ± 0.2</td>
<td>40.4 ± 0.3</td>
</tr>
<tr>
<td>Parity (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Birth outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>3543.1 ± 83.4</td>
<td>3593.6 ± 118.5</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>50.9 ± 0.4</td>
<td>51.2 ± 0.4</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>34.8 ± 0.3</td>
<td>34.9 ± 0.4</td>
</tr>
</tbody>
</table>

1 ± SEM.
2 Significantly different from the lactating group, P = 0.0004 (unpaired t-test).
3 n = 29 in the lactating group and 17 in the nonlactating group.

Further analysis showed no significant differences between the 2 groups at any time point.

There were no significant changes over time in the percentages of 18:3n−3 after delivery (data not shown), whereas the percentages of 20:5n−3 and 22:5n−3 increased significantly (Figure 2). For the percentage of plasma DHA (Figure 2), there was a significant effect of time and group and a significant time×group interaction. The percentage of DHA decreased significantly over time in both groups and was significantly lower in the lactating than in the nonlactating group (P < 0.001) between 1 and 16 wk postpartum.

In general, the postpartum changes in the percentages of erythrocyte phospholipid fatty acids (Figure 3) were not significantly different from the changes in plasma. However, the increasing patterns of 18:2n−6 and 22:5n−3 were now significantly different between lactating and nonlactating women. The postpartum downward course of DHA resembled that seen in plasma and was significantly different between the 2 groups.

Fatty acid changes in relation to duration of breast-feeding

As mentioned previously, during the observational period of 64 wk there was a wide variation in the duration of lactation. Therefore, the effect of duration of lactation was studied by evaluating the pattern of change in the fatty acids between the nonlactating group and the 4 subgroups of lactating women. No significant outcomes were found for the interaction terms of the n−6 fatty acids in either plasma or erythrocyte phospholipids (data not shown). In contrast, the patterns by which plasma and erythrocyte phospholipid 22:5n−3 and DHA changed over time were significantly different between the lactating and nonlactating groups (Figure 4). These significant interaction terms were further studied by comparing the nonlactating group with each of the 4 subgroups of lactating women at the different sampling times. Erythrocyte phospholipid 22:5n−3 values were significantly higher in subgroup 3 than in the nonlactating women at both 16 and 32 wk postpartum. At 32 wk postpartum, subgroup 4

FIGURE 1. Mean (±SEM) postpartum changes in plasma phospholipid n−6 and n−3 fatty acid concentrations in the lactating (□; n = 35) and nonlactating (▲; n = 22) women. The straight dotted lines represent the mean values of 80 healthy nonpregnant women. There was a significant effect of time for all 6 fatty acids, P < 0.0001 (repeated-measures ANOVA). Pr, 36 wk of pregnancy.
The present study showed that the elevated concentrations (mg/L) of plasma phospholipid total fatty acids during pregnancy declined in both lactating and nonlactating women after parturition. There were no significant differences in the changes in plasma phospholipid total fatty acids (Table 2) or in the individual n-3 and n-6 fatty acids (Figure 1) between the 2 groups. These results indicate that the postpartum normalization of plasma phospholipid fatty acid concentrations occurs in the same manner in all women, regardless of the practice of breastfeeding or bottle-feeding.

However, on the basis of relative fatty acid composition (% by wt), postpartum changes in n-3 fatty acids but not in n-6 fatty acids were significantly different between the nonlactating and lactating groups (Figures 2 and 3).

Compared with the values measured at 36 wk of pregnancy, DHA values in maternal plasma and erythrocyte phospholipids decreased significantly postpartum in both lactating and nonlactating women, probably representing the postpartum normalization process of the plasma DHA content. This finding is supported by the work of Sanjurjo et al (15) and our recent longitudinal study (8) in which we showed that plasma DHA increases continuously from prepregnancy through week 10 of pregnancy. However, our present observation is at odds with the previous work of Holman et al (16), in which the DHA values of...
pregnant women at term were found to be lower than those of nonpregnant women.

The decline in plasma phospholipid DHA values was enhanced in the lactating group. As a result, the women who were still breast-feeding their infants 8 wk postpartum had values (2.44 ± 0.12%) significantly lower than the mean reference value (2.95 ± 0.08%) of nonpregnant women (n = 80) of similar parity, whose last pregnancy was ≥1 y before blood collection (7). These findings most likely reflect the utilization of DHA for breast milk. Breast-milk DHA concentrations were shown to correlate positively with the content in maternal plasma phospholipids (17, 18), which is largely dependent on dietary intake.

The mean estimated dietary intake of DHA in the lactating group was not significantly different between the 2 assessment periods (91 mg/d at 4 wk and 81 mg/d at 32 wk postpartum). On the basis of average Dutch figures for breast-milk production (19) and composition (71.7 mg DHA/L, n = 65; unpublished observations, 2000), the daily transfer of DHA from a breast-feeding mother to her infant is ≈50–53 mg. Comparable amounts of DHA were estimated in the diet of the nonlactating women (52 and 55 mg/d at 4 and 32 wk postpartum, respectively). These data suggest that the DHA intake of the lactating women was probably sufficient to meet the demand for breast-feeding, but not to meet the women’s own requirements or to maintain plasma phospholipid DHA at values observed in the nonlactating women.

When our paper was in its final phase of preparation, Makrides and Gibson (20) reported postpartum changes in maternal plasma DHA. They also observed a decrease in maternal plasma DHA in both lactating and nonlactating women after parturition, which they suggested was probably related to a hormonal effect or to increased utilization of maternal reserves, independent of lactation. It is conceivable that either or both processes occur in women after parturition, but our longitudinal comparison between lactating and nonlactating women strongly suggests that the reductions in DHA are related, at least in part, to lactation. First, our data showed that the reductions in maternal DHA became stronger the longer the duration of breast-feeding (Figure 4). Furthermore, our data showed that at 16 wk postpartum, plasma and erythrocyte phospholipid DHA values were significantly lower in the women still breast-feeding than in the nonlactating group.

Unlike DHA, the other plasma phospholipid n–3 LCPs rose after parturition. This effect was particularly evident in the women whose DHA values decreased the most (Figure 4), suggesting that DHA is selectively transferred to breast milk compared with the other n–3 LCPs. The postpartum changes observed for DHA and the other n–3 LCPs showed patterns opposite those observed for these fatty acids during pregnancy (6). Maternal plasma phospholipid 22:5n–3 decreased during pregnancy, whereas DHA declined after an initial increase until the second trimester (6). These observations suggest an

### TABLE 3

<table>
<thead>
<tr>
<th>Dietary intakes of fatty acids in the lactating and nonlactating groups&lt;sup&gt;1&lt;/sup&gt;</th>
<th>4 wk (n = 33)</th>
<th>32 wk (n = 28)</th>
<th>4 wk (n = 17)</th>
<th>32 wk (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lactating group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total fat (g/d)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>101.92 ± 5.57</td>
<td>87.43 ± 5.93&lt;sup&gt;3&lt;/sup&gt;</td>
<td>79.19 ± 4.55&lt;sup&gt;4&lt;/sup&gt;</td>
<td>73.39 ± 6.50</td>
</tr>
<tr>
<td>Total SFA (g/d)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>41.37 ± 2.37</td>
<td>35.50 ± 2.33&lt;sup&gt;3&lt;/sup&gt;</td>
<td>30.35 ± 2.28&lt;sup&gt;5&lt;/sup&gt;</td>
<td>26.48 ± 2.69&lt;sup&gt;1,5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total MUFA (g/d)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>36.02 ± 2.13</td>
<td>30.88 ± 2.15</td>
<td>28.71 ± 1.46&lt;sup&gt;4&lt;/sup&gt;</td>
<td>27.29 ± 2.36</td>
</tr>
<tr>
<td>Total PUFA (g/d)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>17.51 ± 1.07</td>
<td>14.95 ± 1.28&lt;sup&gt;3&lt;/sup&gt;</td>
<td>14.59 ± 1.04</td>
<td>14.44 ± 1.31</td>
</tr>
<tr>
<td>18:2n–6 (g/d)</td>
<td>14.88 ± .97</td>
<td>12.62 ± 1.13&lt;sup&gt;3&lt;/sup&gt;</td>
<td>12.36 ± 1.01</td>
<td>12.44 ± 1.23</td>
</tr>
<tr>
<td>Total n–6 (g/d)</td>
<td>14.92 ± 0.97</td>
<td>12.66 ± 1.13</td>
<td>12.41 ± 1.01</td>
<td>12.48 ± 1.24</td>
</tr>
<tr>
<td>18:3n–3 (g/d)</td>
<td>1.23 ± 0.09</td>
<td>0.96 ± 0.09&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.93 ± 0.08</td>
<td>0.85 ± 0.09&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>20:5n–3 (mg/d)</td>
<td>47.27 ± 11.05</td>
<td>45.00 ± 9.25</td>
<td>28.82 ± 8.31</td>
<td>33.68 ± 13.36</td>
</tr>
<tr>
<td>22:6n–3 (mg/d)</td>
<td>90.61 ± 18.08</td>
<td>80.71 ± 13.27</td>
<td>51.77 ± 11.48</td>
<td>55.26 ± 14.54</td>
</tr>
<tr>
<td>Total n–3 (g/d)</td>
<td>1.40 ± 0.10</td>
<td>1.11 ± 0.10</td>
<td>1.05 ± 0.08</td>
<td>0.96 ± 0.10</td>
</tr>
</tbody>
</table>

<sup>1</sup>SEM. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

<sup>2</sup>Dutch National Food Consumption Survey 1997–1998: total fat = 85 g/d, SFA = 33 g/d, MUFA = 30 g/d, and PUFA = 15 g/d.

<sup>3</sup>Significantly different from 4 wk, 0.01 < P < 0.05 (Wilcoxon’s signed-rank test).

<sup>4</sup>Significantly different from the respective lactating group (Mann Whitney U test): *<sup>0.01 < P < 0.05, 0.001 < P < 0.01.</sup>
enhanced synthesis of DHA from the conversion of 22:5n–3 to DHA, which is no longer supported when pregnancy ends at the time of delivery. Therefore, besides a selective transfer of DHA to breast milk, the formation of DHA is possibly altered, resulting in both a reduction in DHA and an increase in 22:5n–3, which is enhanced further by lactation.

The mean daily intake of saturated fatty acids in the lactating women was significantly higher than that in the nonlactating women. Although the estimated dietary intake of the essential fatty acids in the lactating group at 4 wk postpartum seemed higher than the intake recorded at 32 wk postpartum and in the nonlactating group, these differences were not significant (Table 3). Notwithstanding, compared with values from the Dutch National Food Consumption Survey (21) for women in the same age category (n = 1472), the intake of total fat and saturated, monounsaturated, and polyunsaturated fatty acids in the lactating women at week 4 postpartum appeared to be higher. This observation suggests that the practice of exclusive breast-feeding might have an influence on the habitual maternal diet.

In summary, normalization of the postpartum course of maternal plasma and erythrocyte phospholipid n–6 LCPs did not differ between lactating and nonlactating women, whereas that of n–3 LCPs did. Plasma and erythrocyte phospholipid DHA values decreased by ≈20% in nonlactating women, whereas a further reduction was observed in the lactating women that was augmented by duration of lactation. These markedly reduced DHA values had returned to nonlactating values at 32 wk postpartum. Further studies are required to elucidate the underlying mechanism involved in these postpartum changes in DHA.

We thank Hasibe Aydeniz for her technical assistance, Arnold Kester for his advice on the statistical analyses, and the participants and the midwives A Heuts, C Smeets, and A Merkx for their cooperation.

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