Dietary Arachidonic Acid Dose-Dependently Increases the Arachidonic Acid Concentration in Human Milk\(^1,2\)

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Abstract

Lactation hampers normalization of the maternal arachidonic acid (AA) status, which is reduced after pregnancy and can further decline by the presently recommended increased consumption of (n-3) long-chain PUFA [(n-3) LCPUFA]. This may be unfavorable for breast-fed infants, because they also require an optimum supply of (n-6) LCPUFA. We therefore investigated the LCPUFA responses in nursing mothers upon increased consumption of AA and (n-3) LCPUFA. In a parallel, double-blind, controlled trial, lactating women received for 8 wk no extra LCPUFA (control group, \(n = 8\)), 200 (low AA group, \(n = 9\)), or 400 (high AA group, \(n = 8\)) mg/d AA in combination with (n-3) LCPUFA [320 mg/d docosahexaenoic acid (DHA), 80 mg/d eicosapentaenoic acid, and 80 mg/d other (n-3) fatty acids], or this dose of (n-3) LCPUFA alone [DHA + eicosapentaenoic acid group, \(n = 8\)]. Relative concentrations of AA, DHA, and sums of (n-6) and (n-3) LCPUFA were measured in milk total lipids (TL) and erythrocyte phospholipids (PL) after 2 and 8 wk and changes were compared by ANCOVA. The combined consumption of AA and (n-3) LCPUFA caused dose-dependent elevations of AA and total (n-6) LCPUFA concentrations in milk TL and did not significantly affect the DHA and total (n-3) LCPUFA increases caused by (n-3) LCPUFA supplementation only. This latter treatment did not significantly affect breast milk AA and total (n-6) LCPUFA concentrations. AA and DHA concentrations in milk TL and their changes were strongly and positively correlated with their corresponding values in erythrocyte PL (\(r^2 = 0.27–0.50; P \leq 0.002\)). We thus concluded that the consumption by lactating women of AA in addition to extra (n-3) LCPUFA dose dependently increased the AA concentration of their milk TL. J. Nutr. 138: 2190–2197, 2008.

Introduction

Arachidonic acid [AA;\(^7\) 20:4(n-6)] and docosahexaenoic acid [DHA; 22:6(n-3)] are the most abundant long-chain PUFA (LCPUFA) in the membrane phospholipids (PL) of neural tissues, including brain (1–3). Especially during rapid neurodevelopment of the fetus in the last trimester of pregnancy and in the early postnatal period, AA and DHA accumulate in large amounts in neural tissues (4), where they serve as important structural and functional components in the development of neural and synaptic networks (5).

Although newborn infants are capable of synthesizing AA and DHA from precursor fatty acids, this capacity seems insufficient to meet the high demands of the developing tissues (6,7). Consequently, for these LCPUFA, infants largely depend on an adequate dietary supply, preferably from breast milk.

Pregnancy is associated with a reduction in the relative concentrations of AA and DHA (after an initial increase) in maternal plasma PL, because the maternal-fetal LCPUFA transfer is insufficiently compensated for by increased maternal LCPUFA consumption (8,9). During lactation, women continue the transfer of their own LCPUFA to their infants, but this does not compromise the restoration of their relative plasma PL AA concentrations (10). The relative DHA concentrations in plasma PL of lactating mothers, however, become lower than those of nonlactating mothers and also lower than those before conception (11). To support maternal DHA concentrations and to stimulate the (n-3) LCPUFA intake of their infants, an interna-

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\(^7\) Abbreviations used: AA, arachidonic acid; DHA, docosahexaenoic acid; DHA + EPA group, 320 mg/d DHA provided with 80 mg/d EPA; EPA, eicosapentaenoic acid; high AA group, 400 mg/d AA provided with 320 mg/d DHA and 80 mg/d EPA; LCPUFA, long-chain PUFA; (n-3) LCPUFA, (n-3) long-chain PUFA; (n-6) LCPUFA, (n-6) long-chain PUFA; low AA group, 200 mg/d AA provided with 320 mg/d DHA and 80 mg/d EPA; LA, linoleic acid; PL, phospholipid; \(\Sigma\) (n-3) LCPUFA, sum of 20:4(n-6), 20:5(n-3), 22:6(n-3), and 22:5(n-3); \(\Sigma\) (n-6) LCPUFA, sum of 20:3(n-6), 20:4(n-6), 22:4(n-6), and 22:5(n-6); TL, total lipid.

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tional working party recently recommended increased maternal consumption of (n-3) LCPUFA during pregnancy and lactation of ~200 mg/d (12). Because recommendations for the general population are noticeably higher (13,14), the (n-3) LCPUFA consumption of pregnant and lactating women can be expected to increase considerably in the near future. However, an increase in the consumption of (n-3) LCPUFA often causes a concomitant decrease of circulating (n-6) LCPUFA concentrations and of AA in particular (15,16), although this is not a consistent finding (17). AA is considered important for fetal and infant growth and development (18,19), possibly because of its multiple physiological functions such as involvement in eicosanoid synthesis (20), diacylglycerol cell-signaling pathways (21), and transcription regulation of fat metabolizing enzymes (22).

Observational studies indicate that AA concentrations in human milk are less variable than DHA levels (23), which may be due to its putative resistance to changes in the consumption of AA and its precursors (24–26). However, data from supplementation studies are limited. We thus decided to further investigate the responsiveness of human milk to the additional supply of (n-3) and (n-6) LCPUFA. For this purpose, we first investigated the impact of the added consumption of 400 mg/d DHA + eicosapentaenoic acid (EPA) on the concentrations of AA and other LCPUFA in breast milk total lipids (TL) of lactating mothers. Subsequently, we studied the influence of coconsumption of 200 or 400 mg/d AA. To check the effects of these supplemental LCPUFA on the general LCPUFA status of the women, we also measured LCPUFA concentrations in their erythrocyte PL.

Subjects and Methods

Subjects. A total of 52 healthy women in wk 34 or 35 of pregnancy and intending to breast-feed their babies for at least 3 mo were recruited at the University Hospital Maastricht and the Atrium Hospital Heerlen (both The Netherlands) through their obstetricians or midwives during their regular checks and by public relation activities. Further inclusion criteria for study entry were: pregnant for the first, 2nd, or 3rd time; apparently healthy; prepregnancy BMI between 18 and 27 kg/m²; no vegetarian lifestyle, fish consumption <2 times/wk, no use of supplements or products rich in LCPUFA, and an alcohol consumption of ≤63 g/wk; no use of medication, drugs, or supplements (iron and folic acid allowed); smoking ≤5 cigarettes/d; no participation in another study ≤2 mo ago. Reasons for exclusion after enrolment were delivery before wk 37 or after wk 43 of gestation, failure to comply with the demands of the study, and suffering from an adverse event that might impair the reliability of the results. Subjects donated a blood sample in wk 36 of pregnancy to check their essential fatty acid status and were randomly assigned to 1 of the 4 test groups at wk 3 after delivery. The study was approved by the Medical Ethics Committee of the University Hospital Maastricht and conducted in accordance with the Helsinki Declaration. A written informed consent was obtained from each subject before enrolment.

Dietary supplementation. The subjects were asked to consume twice per day 200 mL of 1 of the 4 milk powder-based test drinks (in the morning and in the evening). For a 200-mL test product, a portion of 38 g powder was dissolved in a glass (Table 1). Per 38 g, all powders (Friesland Coberco Dairy Foods) contained ~5.7 g protein, 21.5 g carbohydrate, 5.5 g fat, and vitamins and minerals. The control group received a control product without added LCPUFA. Subjects allocated to the 3 treatment groups (DHA + EPA, low AA, and high AA groups) received the control product enriched with (n-3) LCPUFA from Dry n-3) (BASF Health and Nutrition). The test products of the low and high AA groups also AA contained from Optimar single-cell oil (DSM Food Specialties). The consumption of 2 portions of the test drinks per day resulted in an additional daily intake of 320 mg DHA, 200 mg EPA, and 80 mg of other (n-3) fatty acids (all 3 treatment groups), and 200 mg/d AA (low AA group) or 400 mg/d AA (high AA group). Treatment compliance was assessed on the difference between distributed full and returned (partly) empty cans and expressed in percentage.

Study visits and sample collections. At wk 3 postdelivery (baseline) and 2 and 8 wk later (i.e. at wk 3 and 11 postdelivery), the women were visited at their homes to assess their well-being and that of their children, to check the potential occurrence of adverse events and to determine their treatment compliance. During these visits, blood from a forearm vein was sampled into EDTA-containing tubes. Moreover, the mothers had been asked beforehand to collect, midway during the morning feeding, ~10 mL milk from each breast by manual expression into 2 collection tubes and store these in a refrigerator. The blood and breast milk samples were kept in a cool-box during transportation to the laboratory.

Sample processing and fatty acid analysis. Blood was processed and erythrocytes and plasma were stored until analysis as detailed before (27). The breast milk samples from both tubes were pooled and redistributed over 2 new tubes, which were tightly closed under nitrogen and stored at ~80°C until analysis.

All erythrocyte samples of a given subject were analyzed within the same analysis to ensure uniformity of the analytical conditions. The same strategy was applied to the analysis of the breast milk samples.

Lipids were extracted, PL isolated, and fatty acid methyl esters prepared as described earlier (27). Fatty acid compositions were analyzed by capillary GC with flame-ionization detection using a polar and a nonpolar column (50-m BPX70 polar column, 0.22 × 0.25 μm, and 50-m BP1 nonpolar column, 0.22 × 0.10 μm, SGE, Bester BV) and optimized injection, oven, and detection temperatures (28). Helium was used as carrier gas.

Fatty acids were quantified by the amount of internal standard recovered and expressed in absolute concentrations (g/L erythrocyte suspension or breast milk) and relative concentrations as g/100 g of total identified fatty acids in erythrocyte PL and milk TL.

In total, 45 and 42 different fatty acids were identified and quantified in milk TL and erythrocyte PL, respectively. For this study, we concentrated on the following fatty acids and fatty acid combinations: AA [20:4(n-6)], DHA [22:6(n-3)], sum of (n-6) LCPUFA (Σ(n-6) LCPUFA) calculated as the sum of 20:4(n-6), 20:5(n-6), 22:5(n-6), and 22:6(n-3)], and sum of (n-3) LCPUFA (Σ(n-3) LCPUFA) calculated as the sum of 20:4(n-3), 20:5(n-3), 22:5(n-3), and 22:6(n-3)]. Based on 8

### Table 1. Composition of the 4 test products per portion

<table>
<thead>
<tr>
<th>Group</th>
<th>Control group&lt;sup&gt;2&lt;/sup&gt;</th>
<th>DHA + EPA group&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Low AA group&lt;sup&gt;4&lt;/sup&gt;</th>
<th>High AA group&lt;sup&gt;5&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk protein, g</td>
<td>5.5</td>
<td>5.9</td>
<td>5.7</td>
<td>5.6</td>
</tr>
<tr>
<td>Carbohydrate&lt;sup&gt;6&lt;/sup&gt;, g</td>
<td>22.4</td>
<td>20.7</td>
<td>21.2</td>
<td>21.7</td>
</tr>
<tr>
<td>Total fat&lt;sup&gt;7&lt;/sup&gt;, g</td>
<td>5.6</td>
<td>5.5</td>
<td>5.4</td>
<td>5.4</td>
</tr>
<tr>
<td>Saturated fat, g</td>
<td>2.6</td>
<td>2.5</td>
<td>2.5</td>
<td>2.4</td>
</tr>
<tr>
<td>Unsaturated fat, g</td>
<td>2.9</td>
<td>2.9</td>
<td>2.9</td>
<td>3.0</td>
</tr>
<tr>
<td>18:2(n-6) (LA), mg</td>
<td>822</td>
<td>633</td>
<td>587</td>
<td>543</td>
</tr>
<tr>
<td>20:4(n-6) (AA), mg</td>
<td>—</td>
<td>—</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>18:3(n-3) (ALA), mg</td>
<td>89</td>
<td>93</td>
<td>93</td>
<td>93</td>
</tr>
<tr>
<td>20:5(n-3) (EPA), mg</td>
<td>—</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>22:6(n-3) (DHA), mg</td>
<td>—</td>
<td>161</td>
<td>161</td>
<td>161</td>
</tr>
</tbody>
</table>

<sup>1</sup> One portion = 38 g powder dissolved in ~180 mL water yielding ~200 mL test product; 2 portions were consumed each day.
<sup>2</sup> Intake per day: no LCPUFA.
<sup>3</sup> Intake per day: 320 mg DHA + 80 mg EPA + 80 mg other (n-3) fatty acids.
<sup>4</sup> Intake per day: as DHA + EPA group + 200 mg AA.
<sup>5</sup> Intake per day: as DHA + EPA group + 400 mg AA.
<sup>6</sup> 33% Lactose, 50% sucrose syrup, and 17% sucrose.
<sup>7</sup> 87% Fat blend FFOO (Friesland Coberco Dairy Foods), 12% medium-chain triglyceride oil, and 1% milk fat.
samples of a single milk pool that were analyzed together with the study samples, we calculated CV values of 1.16% for AA and 0.66% for DHA. By means of 6 erythrocyte pool samples, we determined CV values of 0.78% for AA and 1.07% for DHA, respectively.

**Statistics.** We checked fatty acid data for the presence of outliers by using the Studentized deleted residual test (29). Subsequently, variables not following normal distribution were log-transformed.

Differences between the 4 groups, with respect to the clinical variables and baseline fatty acid concentrations, were tested for statistical significance by ANOVA with Tukey’s continuation to locate differences, if any. For variables not normally distributed, the Kruskal-Wallis test was applied and continued by a Bonferroni-corrected Mann-Whitney test. ANCOVA was used to compare the differences between groups regarding selected fatty acid concentrations after 2 and 8 wk of intervention. Baseline values of the respective variables were used as covariates in these ANCOVA. Because the linoleic acid (LA) status is known to affect uptake and/or incorporation of (n-3) LCPUFA upon supplementation (30,31), LA concentrations of erythrocyte PL measured at baseline (ranging between 8.47 and 11.08 g/100 g) were applied as covariates for these ANCOVA as well. Significant differences between the groups were located by Tukey’s post hoc testing. Within each group, changes in fatty acid concentrations between baseline and after 2 and 8 wk of supplementation were tested for significance by Student’s paired samples t test (normal distribution) or Wilcoxon’s signed-rank test (distribution not normal). Differences were considered significant at $P < 0.05$, unless indicated otherwise. These statistics were performed with and without outliers.

Relationships between AA and DHA concentrations in breast milk TL and erythrocyte PL at baseline and their changes after 2 and 8 wk of intervention were studied in the combined data of all 4 test groups by linear regression analyses. Normal distributions of the residuals were required for acceptance of the regression outcomes.

All statistical analyses were performed using SPSS 12.0.1 for Windows. Results are expressed as group means ± SD, unless otherwise specified.

**Results**

**Characteristics of the study population at baseline**

Fifty-two subjects were randomly allocated to the 4 test groups. Thirty-four subjects completed the study [n = 9 (control and low AA groups) or 8 (DHA + EPA and high AA groups)], whereas 18 withdrew. Reasons for withdrawal were mainly related to problems with breast-feeding or illness of the child. In general, compliance was adequate (88–98%) and did not differ between the groups. However, 1 subject from the control group was excluded from all data analyses because of an irregular intake of the supplement.

Baseline characteristics did not differ among the groups with the exception of age at delivery, which was significantly lower in the DHA + EPA group than in other groups (Table 2).

**Fatty acid composition of breast milk TL**

At baseline (wk 3 postdelivery), breast milk total fatty acid concentrations did not differ among the groups ($P = 0.085$; Table 2). AA, $\Sigma$(n-6) LCPUFA, DHA, and $\Sigma$(n-3) LCPUFA concentrations also did not differ significantly among the 4 groups ($P > 0.05$; Table 3).

**Effect of DHA + EPA consumption on AA and $\Sigma$(n-6) LCPUFA concentrations.** As demonstrated by the control group, lactation was associated with significant reductions of the AA ($P = 0.011$ after 2 wk, $P = 0.0004$ after 8 wk) and $\Sigma$(n-6) LCPUFA ($P = 0.028$ after 8 wk) concentrations (Fig. 1A,B). Comparison between the control and the DHA + EPA groups revealed that the extra consumption of 400 mg/d DHA + EPA did not affect breast milk AA or $\Sigma$(n-6) LCPUFA concentrations after 2 or 8 wk of lactation ($P > 0.05$; Table 3).

**Effect of low AA and high AA consumption on AA and $\Sigma$(n-6) LCPUFA concentrations.** Compared with the consumption of the extra (n-3) LCPUFA only (DHA + EPA group), the additional AA intake increased the AA concentration in breast milk TL (low AA and high AA groups; $P < 0.05$; Table 3). For both AA doses, the effect was already significant after 2 wk and persisted during the entire study period of 8 wk. The effect of the high AA dose was greater than that of the low AA dose at 8 wk ($P < 0.05$).

Although the differences in $\Sigma$(n-6)LCPUFA concentrations between the 3 experimental groups followed a pattern comparable to the differences in AA, most were not significant ($P > 0.05$). One exception was the concentration after 8 wk in the group receiving the high AA dose, which was higher than that in the women receiving additional (n-3) LCPUFA only ($P < 0.05$).

**Effect of low AA and high AA consumption on DHA and $\Sigma$(n-3) LCPUFA concentrations.** In contrast to the (n-6) LCPUFA concentrations, the DHA and $\Sigma$(n-3) LCPUFA concentrations did not differ during the course of lactation (Fig. 1C,D, control group: $P = 0.254$ and 0.930 [at wk 2], $P = 0.148$ and 0.224 [at wk 8] for DHA and $\Sigma$(n-3) LCPUFA, respectively). Compared with this group, the additional consumption of 400 mg/d DHA + EPA significantly increased the DHA concentration (Table 3). This increase was already maximal

### Table 2  Baseline characteristics of the 4 test groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control group</th>
<th>DHA + EPA group</th>
<th>Low AA group</th>
<th>High AA group</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Age at delivery, y</td>
<td>33.9 ± 1.1</td>
<td>28.8 ± 3.2</td>
<td>32.3 ± 3.8</td>
<td>31.9 ± 2.6</td>
<td>0.008</td>
</tr>
<tr>
<td>Prepregnancy weight, kg</td>
<td>65.5 ± 7.4</td>
<td>70.2 ± 7.2</td>
<td>63.3 ± 6.4</td>
<td>66.6 ± 6.9</td>
<td>0.251</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.70 ± 0.07</td>
<td>1.71 ± 0.06</td>
<td>1.69 ± 0.05</td>
<td>1.71 ± 0.07</td>
<td>0.829</td>
</tr>
<tr>
<td>Prepregnancy BMI, kg/m²</td>
<td>22.6 ± 2.5</td>
<td>23.9 ± 1.8</td>
<td>22.1 ± 1.7</td>
<td>22.9 ± 3.0</td>
<td>0.492</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>114.3 ± 7.4</td>
<td>120.4 ± 10.5</td>
<td>109.4 ± 8.3</td>
<td>113.6 ± 5.9</td>
<td>0.077</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>68.2 ± 6.6</td>
<td>69.3 ± 8.1</td>
<td>64.5 ± 5.7</td>
<td>70.0 ± 3.4</td>
<td>0.280</td>
</tr>
<tr>
<td>Pregnancies, n</td>
<td>1.8 ± 0.9</td>
<td>1.9 ± 0.6</td>
<td>1.9 ± 0.6</td>
<td>1.6 ± 0.5</td>
<td>0.842</td>
</tr>
<tr>
<td>Total fatty acids in milk TL, g/L</td>
<td>33.7 ± 16.7</td>
<td>38.2 ± 8.6</td>
<td>23.2 ± 7.3</td>
<td>36.5 ± 15.2</td>
<td>0.085</td>
</tr>
<tr>
<td>Total fatty acids in erythrocyte PL, g/L</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>0.625</td>
</tr>
</tbody>
</table>

1 Values are means ± SD. *Different from the other groups (ANOVA followed by Tukey’s post hoc test, $P < 0.05$).

2 Week 3 after parturition.

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after 2 wk of intervention and coadministration of 200 or 400 mg/d AA did not significantly affect it after either 2 or 8 wk. This outcome was completely independent of 2 DHA values identified as outliers.

After 8 wk of intervention, results for \( \Sigma(n-6) \) LCPUFA (which also included 2 outliers due to the outlying DHA concentrations) were virtually identical to the DHA outcomes, showing a significant increase after the consumption of additional (n-3) LCPUFA, which was not significantly altered by AA coadministration. Again, this outcome was independent of inclusion or exclusion of both outliers.

Also after 2 wk, the results for the \( \Sigma(n-3) \) LCPUFA were qualitatively comparable to the findings for DHA. The only exception was the significant increase in \( \Sigma(n-3) \) LCPUFA concentration in the low AA group compared with the control group. However, this significant increase could be attributed to an outlier, because after its removal significance was lost.

**Fatty acid composition of erythrocyte PL**

At baseline (wk 3 postdelivery), total amounts of fatty acids associated with maternal erythrocyte PL did not differ among the groups \((P > 0.05; \) Table 2). The same was true for the relative amounts of selected LCPUFA and their sums (Table 4).

**Effect of DHA + EPA consumption on AA and \( \Sigma(n-6) \) LCPUFA concentrations.** As demonstrated by the control group, lactation did not significantly reduce the concentrations of AA and \( \Sigma(n-6) \) LCPUFA compared with baseline values (AA: \( P = 0.829 \) after 2 wk and 0.064 after 8 wk; \( \Sigma(n-6) \) LCPUFA: \( P = 0.782 \) and 0.315 after 2 and 8 wk, respectively; Fig. 2A, B). The consumption of 400 mg/d DHA + EPA did not significantly affect the AA concentrations after either 2 or 8 wk (comparison between control and DHA + EPA groups; Table 4; \( P > 0.05 \)). However, consumption of the extra (n-3) LCPUFA significantly reduced the \( \Sigma(n-6) \) LCPUFA concentrations, both after 2 wk as well as after 8 wk.

**Effect of low AA and high AA consumption on AA and \( \Sigma(n-6) \) LCPUFA concentrations.** Compared with the consumption of the extra (n-3) LCPUFA only (DHA + EPA group), coadministration of AA increased the AA concentration \((P < 0.05; \) Table 4). For both AA doses, the effect was already

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**TABLE 3** Selected fatty acid concentrations in milk TL of lactating women at baseline and after 2 and 8 wk of dietary intervention with different doses of AA and (n-3) LCPUFA

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time</th>
<th>Control group</th>
<th>DHA + EPA group</th>
<th>Low AA group</th>
<th>High AA group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>g/100 g total fatty acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td></td>
<td>0.52 ± 0.03</td>
<td>0.45 ± 0.08</td>
<td>0.50 ± 0.11</td>
<td>0.47 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.45 ± 0.06</td>
<td>0.43 ± 0.09</td>
<td>0.53 ± 0.08</td>
<td>0.55 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.41 ± 0.06</td>
<td>0.40 ± 0.04</td>
<td>0.49 ± 0.10</td>
<td>0.56 ± 0.07</td>
</tr>
<tr>
<td>( \Sigma(n-6) ) LCPUFA</td>
<td></td>
<td>1.12 ± 0.05</td>
<td>1.08 ± 0.08</td>
<td>1.04 ± 0.18</td>
<td>1.01 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.06 ± 0.10</td>
<td>1.05 ± 0.12</td>
<td>1.06 ± 0.19</td>
<td>1.08 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.89 ± 0.10</td>
<td>0.90 ± 0.11</td>
<td>0.99 ± 0.19</td>
<td>1.04 ± 0.14</td>
</tr>
<tr>
<td>DHA</td>
<td></td>
<td>0.30 ± 0.06</td>
<td>0.34 ± 0.10</td>
<td>0.30 ± 0.11</td>
<td>0.34 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.25 ± 0.08</td>
<td>0.55 ± 0.23</td>
<td>0.60 ± 0.26</td>
<td>0.46 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.24 ± 0.08</td>
<td>0.53 ± 0.13</td>
<td>0.54 ± 0.17</td>
<td>0.50 ± 0.14</td>
</tr>
<tr>
<td>( \Sigma(n-3) ) LCPUFA</td>
<td></td>
<td>0.64 ± 0.08</td>
<td>0.74 ± 0.15</td>
<td>0.62 ± 0.16</td>
<td>0.68 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.65 ± 0.20</td>
<td>0.99 ± 0.35</td>
<td>1.02± ± 0.42</td>
<td>0.80 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.57 ± 0.17</td>
<td>0.88 ± 0.18</td>
<td>0.92 ± 0.26</td>
<td>0.84 ± 0.22</td>
</tr>
</tbody>
</table>

1 Values are means ± SD. Means in a row with superscripts without a common letter a, b, or c, ANCOVA, control and DHA + EPA groups or x, y, or z, ANCOVA, DHA + EPA, low AA and high AA groups or all 4 groups) differ, \( P < 0.05 \). *Includes 1 outlier; results after removal: 0.89 ± 0.21 \((n = 8)\). **Includes 1 outlier; results after removal: 0.89 ± 0.10 \((n = 7)\). *Includes 1 outlier; results after removal: 0.89 ± 0.21 \((n = 8)\). \( P < 0.05 \).
significant after 2 wk and persisted during the entire study period of 8 wk. At both points in time, the effect of the high AA dose did not differ from that of the low AA dose (P > 0.05; Table 4).

Results for $\Sigma$ (n-6) LCPUFA concentrations were identical to those observed for AA, showing significant increases after 2 as well as 8 wk of intervention that were not significantly different for both AA doses (Table 4).

Effect of low AA and high AA consumption on DHA and $\Sigma$ (n-3) LCPUFA concentrations. In the control group, receiving no additional LCPUFA, the DHA concentration strongly decreased during lactation (P < 0.001 after 2 wk, P < 0.001 after 8 wk; Fig. 2C). Comparable changes were observed for total (n-3) LCPUFA, although less pronounced (P < 0.001 and 0.0126 after 2 and 8 wk, respectively; Fig. 2D).

Compared with the control group, the additional consumption of 400 mg/d DHA + EPA significantly increased the DHA concentration after 2 and 8 wk of intervention (Table 4). This effect was not significantly altered by coadministration of 200 mg/d AA. Coconsumption of 400 mg/d AA, however, attenuated this DHA increase, although the difference with the control group was still significant. Coconsumption of the high AA dose resulted in a significant difference compared with the low AA dose after 2 wk and compared with the DHA + EPA group after 8 wk.

Results for the $\Sigma$ (n-3) LCPUFA were largely comparable (Table 4). After 2 and 8 wk, the daily administration of 400 mg DHA + EPA significantly increased the $\Sigma$ (n-3) LCPUFA concentration compared with the control group. Coadministration of 200 mg/d AA did not significantly influence this effect. In contrast, the daily coadministration of 400 mg AA considerably reduced the $\Sigma$ (n-3) LCPUFA increase, as demonstrated by the absence of a significant difference with the control group but significant differences with the DHA + EPA group (at 8 wk only) and the low AA group (at 2 wk).

Relationships between breast milk TL and erythrocyte PL with respect to AA and DHA concentrations and their changes

When data of all 4 test groups were combined, linear regression analyses revealed significant relationships between erythrocyte PL and breast milk TL for AA and DHA concentrations at baseline (P < 0.001; Fig. 3A, B) as well as after 2 and 8 wk of LCPUFA consumption (data not shown). Significant relationships between both domains were also observed for changes in

\[\text{Effect of low AA and high AA consumption on DHA and } \Sigma\text{(n-3) LCPUFA concentrations.} \]

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AA and DHA concentrations both over the first 2 wk of intervention ($P < 0.001$; Fig. 3C,D) as well as over the entire intervention period of 8 wk [$P = 0.002$ (AA change), $P = 0.001$ (DHA change); Fig. 3E,F).

**Discussion**

Because of their multiple health benefits, it can be expected that the intake of (n-3) LCPUFA will increase in the general population and in pregnant and lactating women, in particular. Elevated (n-3) LCPUFA consumption often coincides with decreased AA status of an individual. In pregnant and lactating women, however, this may not be desirable, because AA is considered essential for fetal and neonatal development (18,19). Hence, in a group of lactating women we investigated the effects of the extra consumption of 320 mg/d DHA and 80 mg/d EPA on the (n-6) and (n-3) LCPUFA concentrations in their milk over a period of 8 wk. In addition, we studied the effects of 200 and 400 mg/d AA added to these (n-3) LCPUFA. To monitor changes in the general LCPUFA status of the participating women, the response of the LCPUFA concentrations were measured in erythrocyte PL.

Compared with the control product without added LCPUFA, the daily consumption of 400 mg extra (n-3) LCPUFA did not significantly alter the AA and (n-6) LCPUFA concentrations in breast milk TL (Table 3). Consistent with earlier reports (32–34), we observed that the milk DHA concentrations significantly increased within 2 wk upon the intake of 320 mg/d DHA and 80 mg/d EPA. The extra consumption of 200 or 400 mg/d AA significantly increased the AA concentration in breast milk TL both after 2 and 8 wk of intervention in a dose-dependent manner. However, it required $>2$ wk before the dose effect became significant, which might have been due to the rather small sizes of our test groups. The $\Sigma$(n-6) LCPUFA concentrations of breast milk TL also increased upon AA consumption. This effect was only significant for the high AA dose and an intake period of $>2$ wk (Table 3).

Based on indirect evidence, it has been suggested that maternal AA intake influences the AA content of breast milk only to a minor extent (24,26). However, the effect of AA consumption on breast milk AA concentration has hardly been investigated. The only study we are aware of was performed by Smit et al. (25), who supplied lactating women with 300 mg AA either alone or in combination with 400 mg DHA and 110 mg EPA per day for 1 wk. No significant effects were found on the AA concentration of breast milk TL, which was recently attributed to the short study period (35).

In general, the LCPUFA responses in breast milk TL were in good agreement with those observed in erythrocyte PL (Table 4) but not in all aspects. Although consumption of extra (n-3) LCPUFA did not significantly affect AA concentrations in erythrocyte PL, the $\Sigma$(n-6) LCPUFA concentrations progressively decreased. This decline appeared mainly attributed to the significantly decreasing concentrations of adrenic acid [22:4(n-6); results not shown, but available on request], the elongation product of AA.

As expected, the DHA and $\Sigma$(n-3) LCPUFA concentrations increased significantly in the RBC PL upon the consumption of the extra (n-3) LCPUFA (Table 4). Interestingly, the concomitant intake of AA attenuated this effect considerably, which indicates that AA may compete with DHA for incorporation into erythrocyte membrane PL. Such a competition likely explains results, reported by Nelson et al. (36), that the daily consumption of 1.5 g AA for 50 d significantly lowers the DHA content of erythrocyte PL. In our study, the extra AA also raised the AA status of the women, as demonstrated by the AA increase in their erythrocyte PL.

During the entire study period, breast milk TL concentrations of AA and DHA as well as their diet-induced changes were positively and significantly correlated with AA and DHA concentrations and changes in erythrocyte PL (see Fig. 3). Dietary AA and DHA are generally acknowledged to directly affect the AA and DHA concentrations in erythrocyte PL and the correlation with concentrations and changes in milk TL could suggest that they may also directly influence the breast milk concentrations of these LCPUFA.

Remarkably, the regression slopes for the diet-induced changes, both after 2 and 8 wk of intervention, were about twice as steep for DHA as for AA. Prima facie this could be taken as evidence for a less efficient incorporation of AA into breast milk TL compared to DHA.
with DHA, which would be in agreement with most observational studies (23). However, this interpretation would ignore the considerable differences between the intercepts of the AA and DHA regressions. Moreover, different incorporation efficiencies are not supported by our observation that a comparable daily LCPUFA intake [400 mg AA (high AA group) or 320 mg DHA + 80 mg EPA (DHA + EPA group)] raised the breast milk AA and DHA concentrations after 8 wk by about the same extent (−0.2 g/100 g compared with the control group; see Fig. 1A, high AA vs. control group for AA, and Fig. 1C, DHA + EPA vs. control group for DHA). This observation rather suggests that the incorporation efficacies of dietary AA and DHA in breast milk TL are of the same magnitude. To fully elucidate, however, the efficiency by which dietary AA is incorporated into breast milk TL, studies with stable isotope-labeled AA are required.

In line with earlier studies (37−39), breast milk total fatty acid concentrations decreased considerably during the course of lactation (Table 3). In the control group, AA and the $\Sigma$(n-6) LCPUFA also declined (Fig. 1A,B). Although these changes were not significant for DHA and the $\Sigma$(n-3) LCPUFA (Fig. 1C,D), the breast milk AA:DHA and $\Sigma$(n-6):$\Sigma$(n-3) LCPUFA ratios (1.81 ± 0.37 and 1.77 ± 0.21 at baseline, respectively) remained essentially unchanged. From the 3 experimental groups, it became obvious that additional LCPUFA consumption can substantially alter these ratios. Thus, the extra intake of 400 mg/d DHA + EPA resulted in a reduction of the AA:DHA ratio of 45.8% within the 8-wk intervention, which brings it within the lower range of the AA:DHA ratio distribution worldwide (23). In the same period, the $\Sigma$(n-6):$\Sigma$(n-3) LCPUFA ratio declined by 31.3%. The extra intake of 400 mg/d AA attenuated the decreases of both ratios to 28.6 and 22.1%, respectively, but did not prevent them. Whether these ratio changes in breast milk composition are of any functional importance needs to be considered in future studies.

In conclusion, we demonstrated that the consumption by lactating women of additional AA and (n-3) LCPUFA increased the AA and DHA concentrations of their milk TL. For AA, this effect appeared dose dependent. Nonetheless, higher amounts of AA than DHA would be required to keep the breast milk AA:DHA ratio constant at habitual values upon DHA supplementation.

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Literature Cited


