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Evaluation of the relation between n−3 and n−6 fatty acid status and parity in nonpregnant women from the Netherlands[1,2]

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ABSTRACT

Background: We previously observed an inverse relation between parity and docosahexaenoic acid (DHA) status in pregnant women in the Netherlands. This implies that maternal DHA status may not fully normalize after a mature pregnancy.

Objective: The objective was to investigate the relation between the essential fatty acid status (in particular the DHA status) of nonpregnant women and the number of completed pregnancies and whether the number of previous pregnancies is associated with a lower DHA status in women from the Netherlands.

Design: This was a cross-sectional study of 129 healthy nonpregnant women who completed 0, 1, 2, 3, or 4 mature, uncomplicated, singleton pregnancies.

Results: The relative amount of DHA in the plasma phospholipids of nulliparous women and of mothers who completed 1–4 pregnancies (duration since last pregnancy: 3.9 ± 2.4 y) was not significantly different; a significant correlation between parity and the percentage of DHA in the phospholipids was not observed either. The percentage of DHA in the phospholipids of erythrocytes of mothers was significantly lower than the percentage in the erythrocytes of the nulliparas ($P = 0.013$), but no significant correlation between the percentage of DHA in the phospholipids of erythrocytes and parity was found. The time interval between the different pregnancies did not influence maternal DHA status.

Conclusions: No relation was found between DHA status and parity in the nonpregnant Dutch women whose last pregnancy was completed ≥1 y previously. Maternal DHA status, as reflected in plasma and erythrocyte phospholipids, probably normalized within 1 y after the last partus. Whether this is true for other tissues remains to be determined. *Am J Clin Nutr* 2001;73:622–7.

KEY WORDS Essential fatty acids, docosahexaenoic acid, DHA, phospholipids, nonpregnant women, pregnancy, parity, maternal docosahexaenoic acid–depletion phenomenon, Netherlands

INTRODUCTION

Essential fatty acids (EFAs) and their longer-chain polyenes (LCPs) are important constituents of all cell membranes, especially those in the brain and in the nervous and vascular systems; furthermore, some of these fatty acids provide the precursors for prostaglandins and leukotrienes (1, 2). During the last trimester of pregnancy, rapid synthesis of brain tissue occurs and the LCPs arachidonic acid (20:4n−6) and docosahexaenoic acid (DHA; 22:6n−3) are incorporated into the structural lipids of the developing brain (3, 4). The developing fetus depends on its mother for an adequate supply of EFAs (2). Therefore, it is important that the maternal EFA status during pregnancy is adequate to meet fetal requirements.

Maternal DHA status was shown to decrease during normal pregnancy (5) and seems to decrease further with each following pregnancy (6), which implies that maternal DHA status may not fully normalize after the first pregnancy. Carlson and Salem (7) found that birth order is a significant negative predictor of the red blood cell DHA content in preterm children, which also suggests that the supply of DHA to the fetus might be decreased by repeated pregnancies.

If repeated pregnancies cause DHA depletion because of incomplete recovery of DHA stores after delivery, then there probably is a negative relation between the DHA status of nonpregnant women and the number of completed pregnancies. We investigated this relation in a cross-sectional study as well as the influence of the duration between different pregnancies on maternal DHA status.

SUBJECTS AND METHODS

Subjects

Healthy nonpregnant women (age: 20–45 y) living in the Maastricht area of the Netherlands were recruited to participate in the study via the local media and from primary schools, day nurseries, and the pediatric outpatient clinic of the Maastricht University Hospital. In the women who agreed to participate, the fatty acid composition of plasma and erythrocyte phospholipids was measured in a venous blood sample to assess EFA status. The also women completed a questionnaire about their reproductive histories and their state of health. The study population consisted of 129 healthy white women with a parity of 0 ($n = 41$),

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1 (n = 21), 2 (n = 34), 3 (n = 26), or 4 (n = 7). All women had uncomplicated, singleton pregnancies. The Medical Ethics Committee of the Maastricht University approved the study and written, informed consent was obtained from all participants.

Blood sampling

Venous blood samples were collected into EDTA-treated tubes and were stored at 4°C. Within 48 h after collection, plasma was separated from the erythrocytes by centrifugation (500 × g, 10 min, 4°C) and was stored (under nitrogen) at −80°C until analyzed for fatty acid concentrations. Erythrocytes were washed with EDTA-containing saline and stored (under nitrogen) at −50°C until lipid extraction within 1 wk.

Fatty acid analysis

The fatty acid composition of the phospholipid fraction was determined in 100-μL plasma samples and 200-μL erythrocyte samples. Total lipid extraction was performed as described by Bligh and Dyer (8). L-α-Dinonadecanoyl lecithin was used as an internal standard to calculate absolute fatty acid concentrations. Phospholipids were separated from the total lipid fractions by column chromatography according to the method of Kaluzny et al (9). Carryover of fatty acids during the phospholipid separation procedure was checked by measuring the amount of 17:1n−7 (heptadecaenoic acid) in phospholipids, the exact quantity of which was known in the internal standard. The phospholipid fraction was hydrolyzed and the fatty acids were methylated with boron trifluoride in methanol according to the method of Morrison and Smith (10). The fatty acid methyl esters (FAMEs) were separated and quantified by capillary gas-liquid chromatography with a CPSIL88 column (Chrompack; Middelburg, Netherlands). A standard FAME mixture was used to identify FAMEs on the basis of retention times. The amount of each FAME (mg/L) was quantified by relating the peak area of each FAME to the peak area of 19:0 (L-α-dinonadecanoyl lecithin; internal standard). Both absolute and relative amounts (% by wt) were calculated.

Fatty acids and EFA status

The EFAs linoleic acid (18:2n−6), 20:4n−6, adrenic acid (22:4n−6), osbond acid (22:5n−6), eicosapentaenoic acid (20:5n−3), and DHA; the sum of n−6 and n−3 fatty acids (Σn−6 and Σn−3, respectively); the sum of the LCPs of the n−6 family (Σn−6 LCPs = 20:3n−6, 20:4n−6, 22:4n−6, and 22:5n−6); the sum of the LCPs of the n−3 family (Σn−3 LCPs = 20:5–3, 22:5n−3, and DHA); and the sum of all saturated fatty acids (ΣSFA) are reported.

Besides the individual fatty acids mentioned above, 3 indexes were calculated to describe the EFA status of the women. The DHA deficiency index (DHADI) is reflected by the ratio between 22:5n−6 and 22:4n−6. The DHADI is considered a reliable marker of DHA deficiency (11) because a deficit in DHA is accompanied by an increased conversion of 22:4n−6 to 22:5n−6, resulting in higher DHADI values (12). The DHA sufficiency index (DHASI), defined as the ratio between DHA and 22:5n−6, is used as a biochemical indicator of DHA status (13). Factors that reduce DHA status lower DHASI values. The EFA index is defined as the ratio of Σn−6 + n−3 to Σn−9 + n−7 fatty acids (14, 15). The higher the EFA index, the higher the amounts of the n−3 and n−6 fatty acids or the lower the amounts of the n−9 and n−7 fatty acids.

Statistics

The clinical characteristics and amounts of fatty acid and fatty acid combinations in the plasma and erythrocyte phospholipids of the parity groups (ie, 0, 1, 2, 3, and 4) were compared; significant differences were determined by using Bonferroni-corrected analysis of variance. P values < 0.005 were considered significant.

Differences in plasma and erythrocyte phospholipid fatty acid composition between the nulliparas and the total group of mothers were tested by multiple regression analysis. The dichotomous variable "mother" (code = 1) or "nonmother" (code = 0) was the independent variable and the different fatty acids were the dependent variables. Possible confounding factors such as age, smoking habits (dichotomous variable: yes or no), alcohol consumption, and uncommon dietary habits were added to the regression model. If a confounding factor was shown to have a significant influence, the model was corrected for this factor (backward analysis). Smoking appeared to be the only confounding factor that had a significant influence.

The relation between the fatty acid composition of plasma and erythrocyte phospholipids and the number of completed pregnancies was investigated by multiple regression analysis. The various fatty acids and fatty acid combinations were used as dependent variables, whereas the number of completed pregnancies (a continuous variable: 0, 1, 2, 3, or 4) was used as the independent variable. Age, smoking habits, alcohol consumption, and uncommon dietary habits were added as possible confounding factors. If a confounding factor had a significant influence, the model was corrected for this factor (backward analysis). Smoking appeared to be the only confounding factor that had a significant influence.

It was not possible to adjust for possible confounding factors associated with pregnancy [eg, lactation, duration of lactation, duration between pregnancies (ie, sum of the time between individual pregnancies, with the duration between the time of last partus and of blood sampling included, divided by the number of completed pregnancies), and the duration between the time of the last partus and of blood sampling] because these factors are not applicable to nulliparas. Therefore, another multiple regression analysis was performed to investigate the relation between parity and fatty acid composition of the phospholipids and the influence of the duration between the different pregnancies on maternal EFA status. Again, various fatty acids and fatty acid combinations were used as dependent variables. Parity (a continuous variable: 1, 2, 3, or 4) was used as the independent variable. Besides age, smoking habits, alcohol consumption, and uncommon dietary habits, lactation, duration of lactation, duration between pregnancies, and duration between the time of the last partus and of blood sampling were added as possible confounding factors (backward analysis). Smoking appeared to be the only confounding factor that had a significant influence.

In the multiple regression analyses, P values ≤0.05 were considered significant. Statistical analyses were performed by using STATVIEW (version 5.0 for Macintosh PPC; SAS Institute Inc, Cary, NC).

RESULTS

Clinical characteristics of the study population are given in **Table 1**. The nulliparas were significantly younger than the primi- and multiparas. The duration between the time of the

TABLE 1
Clinical characteristics of the study population

Characteristic	Nulliparas (n = 41)	Parity: Para 1 (n = 21)	Para 2 (n = 34)	Para 3 (n = 26)	Para 4 (n = 7)
Age (y)	27.51 ± 3.82 (21.6–35.8)[1,2]	34.40 ± 4.63 (27.0–41.9)	34.70 ± 3.12 (26.1–39.2)	37.22 ± 2.25 (32.8–41.8)	36.75 ± 2.98 (33.2–42.6)
Duration between last delivery and blood sampling (y)	—	4.31 ± 3.14 (1.0–12.3)	3.93 ± 2.18 (0.9–8.2)	3.69 ± 2.22 (1.0–10.4)	3.58 ± 2.23 (0.8–6.2)
Duration between pregnancies (y)[3]	—	4.31 ± 3.14 (1.0–12.3)[4]	3.32 ± 1.12 (1.7–4.8)	2.87 ± 1.00 (1.5–5.0)	2.60 ± 0.79 (1.6–3.8)
Duration of lactation (mo)	—	7.47 ± 9.38 (0.5–33.0)	4.06 ± 3.04 (0.1–11.0)	5.40 ± 3.61 (0.2–13.0)	7.57 ± 5.96 (1.8–18.0)
Number of women who					
Breast-fed	—	13	27	22	6
Smoked	4	6	9	2	0
Consumed alcohol[5]	21	14	19	19	6
Had common dietary habits	38	21	31	25	7
Were vegetarians	3	0	3	1	0

[1] $\bar{x}$ ± SD; range in parentheses.
[2] Significantly different from para 1, 2, 3, and 4, $P < 0.001$.
[3] Duration between the time of last partus and of blood sampling included.
[4] Significantly different from para 3, $P < 0.005$.
[5] At least one alcoholic beverage per week.

last partus and of blood sampling was not significantly different between the 4 groups of mothers. The mean duration between pregnancies in the para 3 group was significantly lower than that in the para 1 group. Although the duration of lactation increased significantly with an increase in parity, the mean duration of lactation was not significantly different between the 4 groups of mothers.

During the fatty acid analysis, the erythrocyte samples of 6 women (1 in the para 0 group, 4 in the para 1 group, and 1 in the para 2 group) were not used because of a technical mistake; nevertheless, the plasma samples of these women were analyzed. Furthermore, the plasma phospholipid DHADI values of 2 women in the para 1 group were excluded from the statistical analysis because they were extremely high (because of a relatively low percentage of 22:5n−6 and a relatively high percentage of DHA).

The total amount of fatty acids in the plasma and erythrocyte phospholipids of the nulli-, primi-, and multiparas was not significantly different; therefore, only relative amounts of fatty acids were considered and were analyzed statistically. The total amount of fatty acids, the relative amounts of the analyzed fatty acids, and the EFA status indexes in the plasma and erythrocyte phospholipids of the nulli-, primi-, and multiparas are given in **Tables 2** and **3**.

DHA status in plasma and erythrocyte phospholipids

The percentage of DHA in the plasma phospholipids was not significantly different between the nulliparas and the mothers. The DHADI in the plasma phospholipids, after correction for smoking habits, was significantly lower in the mothers than in the nulliparas ($r = -0.324$, $P = 0.0004$). No significant differences were observed between the plasma DHASI of the nulliparas and mothers. The EFA index in plasma phospholipids was significantly higher in the mothers than in the nulliparas ($r = 0.247$, $P = 0.0047$).

The percentage of DHA in the erythrocyte phospholipids was significantly lower in the mothers than in the nulliparas ($r = -0.223$, $P = 0.013$). The DHADI in the erythrocyte phospholipids, after correction for smoking habits, was also significantly lower in the mothers than in the nulliparas [final model: DHADI (% by wt) = 0.117 − (0.027 × parity) + (0.003 × smoking habits); $r = -0.388$, $r^2 = 0.151$, $P < 0.0001$]. Parity was coded as 0 for the nulliparas and as 1 for the mothers; smoking habit was coded as 0 for nonsmokers and as 1 for smokers. Neither the DHASI nor the EFA index in the erythrocyte phospholipids of the nulliparas and mothers was significantly different.

The relative amount of DHA in plasma phospholipids did not change significantly as parity increased. Neither the DHADI nor the DHASI was related to parity. The EFA index in plasma phospholipids, however, increased significantly as parity increased ($r = 0.213$, $P = 0.0155$).

In erythrocyte phospholipids, there was no significant relation between parity and the relative amount of DHA, the DHASI, or the EFA index. However, the DHADI in erythrocyte phospholipids decreased significantly as parity increased ($r = -0.245$, $P = 0.0063$).

Other EFAs in plasma and erythrocyte phospholipids

The percentage of 18:2n−6 in plasma phospholipids, after correction for smoking habits, was significantly higher in the mothers than in the nulliparas ($r = 0.296$, $P = 0.0013$). The percentages of Σn−6 LCPs, after correction for smoking habits, and of 22:5n−6 were significantly lower in the mothers than in the nulliparas ($r = -0.244$, $P = 0.0082$ and $r = -0.217$, $P = 0.0136$, respectively). The percentages of 20:4n−6, 22:4n−6, Σn−6, 20:5n−3, Σn−3 LCPs, Σn−3, and ΣSFA were not significantly different between the nulliparas and the mothers.

Additionally, the percentage of 18:2n−6 in erythrocyte phospholipids, after correction for smoking habits, was significantly higher in the mothers than in the nulliparas ($r = 0.288$, $P = 0.0021$); the relative amounts of 20:4n−6, 22:4n−6, 22:5n−6, and Σn−6 LCPs in erythrocyte phospholipids were significantly lower in the mothers than in the nulliparas ($r = -0.226$, -0.265, -0.434, and -0.338, respectively). The

TABLE 2
Relative amounts of fatty acids and essential fatty acid (EFA) status indexes in plasma phospholipids[1]

Fatty acid	Nulliparas (n = 41)	All mothers (n = 88)	Parity: Para 1 (n = 21)	Para 2 (n = 34)	Para 3 (n = 26)	Para 4 (n = 7)
Total (mg/L)	1296.30 ± 230.03	1260.05 ± 199.58	1243.90 ± 225.86	1268.00 ± 188.79	1272.80 ± 208.24	1222.10 ± 163.66
18:2n−6 (% by wt)	22.52 ± 2.61	23.48 ± 2.58[2]	22.78 ± 3.32	23.34 ± 2.27	24.02 ± 2.14	26.24 ± 2.97
20:4n−6 (% by wt)	9.12 ± 1.38	8.97 ± 1.31	9.43 ± 1.39	9.17 ± 1.21	8.37 ± 1.19	8.84 ± 1.42
22:4n−6 (% by wt)	0.33 ± 0.06	0.32 ± 0.06	0.31 ± 0.07	0.33 ± 0.06	0.30 ± 0.06	0.34 ± 0.05
22:5n−6 (% by wt)	0.22 ± 0.12	0.18 ± 0.07[3]	0.16 ± 0.08	0.19 ± 0.08	0.17 ± 0.06	0.19 ± 0.08
Σn−6 LCP (% by wt)	12.74 ± 1.75	12.31 ± 1.58[4]	12.66 ± 1.81	12.71 ± 1.47	11.61 ± 1.32	11.94 ± 1.53
Σn−6 (% by wt)	35.88 ±1.36	36.35 ± 2.04	35.97 ± 2.47	36.63 ± 1.98	36.20 ± 1.72	36.70 ± 2.19
20:5n−3 (% by wt)	0.52 ± 0.29	0.55 ± 0.38	0.64 ± 0.47	0.52 ± 0.30	0.56 ± 0.44	0.41 ± 0.12
22:6n−3 (% by wt)	3.09 ± 0.55	2.94 ± 0.69	2.95 ± 0.78	2.95 ± 0.54	2.99 ± 0.86	2.70 ± 0.49
Σn−3 LCP (% by wt)	4.42 ± 0.77	4.34 ± 1.05	4.43 ± 1.23	4.32 ± 0.81	4.39 ± 1.26	3.96 ± 0.65
Σn−3 (% by wt)	4.62 ± 0.78	4.50 ± 1.06	4.61 ± 1.23	4.49 ± 0.82	4.55 ± 1.27	4.05 ± 0.66
ΣSFA (% by wt)	45.77 ± 0.97	45.82 ± 1.31	46.07 ± 1.13	45.65 ± 1.27	45.80 ± 1.35	46.65 ± 1.95
EFA index	3.44 ± 0.36	3.67 ± 0.46[2]	3.61± 0.53	3.74 ± 0.46	3.62 ± 0.39	3.73 ± 0.58
DHADI	0.65 ± 0.29	0.55 ± 0.19[5]	0.51 ± 0.22	0.56 ± 0.19	0.56 ± 0.17	0.56 ± 0.18
DHASI	18.13 ± 9.96	19.05 ± 10.21[6]	18.40 ± 9.70[6]	18.58 ± 8.42	20.81 ± 12.94	16.58 ± 9.08

[1] $\bar{x}$ ± SD. Σn−6 longer-chain polyenes (LCPs): 20:3n−6, 20:4n−6, 22:4n−6, and 22:5n−6; Σn−6, sum of n−6 fatty acids; Σn−3 LCPs, 20:5n−3, 22:5n−3, and 22:6n−3; Σn−3, sum of n−3 fatty acids; ΣSFA, sum of all saturated fatty acids; EFA index, the ratio of Σn−3 + Σn−6 to Σn−9 + Σn−7; DHADI, docosahexaenoic acid deficiency index (22:5n−6/22:4n−6); DHASI, docosahexaenoic acid sufficiency index (22:6n−3/22:5n−6).
[2–5] Significantly different from nulliparas: [2] $P < 0.005$, [3] $P < 0.05$, [4] $P < 0.01$, [5] $P < 0.001$.
[6] Two outliers were excluded: 5.17/0.05 = 103.4 and 3.85/0.02 = 192.5.

percentages of Σn−6, 20:5n−3, Σn−3 LCPs, and Σn−3 were not significantly different between the nulliparas and the mothers. The percentage of ΣSFA was, however, significantly higher in the mothers than in the nulliparas (r = 0.194, P = 0.0315).

The percentage of 18:2n−6 in plasma phospholipids, after correction for smoking habits, was significantly and positively associated with parity (r = 0.329, P = 0.0003); the percentage of Σn−6 LCPs, after correction for smoking habits, was significantly and negatively associated with parity (r = −0.319, P = 0.0005). There was no relation between parity and 20:4n−6, 22:4n−6, 22:5n−6, 20:5n−3, Σn−6, Σn−3 LCPs, Σn−3, and ΣSFA.

The relation between parity and all fatty acids and fatty acid indexes, except 22:4n−6 and 22:5n−6, in erythrocyte phospholipids was comparable with the findings in plasma. After correction for smoking habits, the relative amount of both 22:4n−6 and 22:5n−6 in erythrocyte phospholipids decreased significantly as parity increased (r = −0.205, P = 0.0295 and r = −0.335, P = 0.0004, respectively). Addition of possible confounding factors associated with pregnancy to the multiple regression analysis did not change the results (data not shown).

DISCUSSION

In the present study, the fatty acid composition of plasma and erythrocyte phospholipids in nonpregnant nulli-, primi, and multiparas was compared to investigate the relation between the EFA status, particularly the DHA status, and the number of completed pregnancies in nonpregnant women. The relative amount of DHA in plasma phospholipids was not significantly different between the nulli-, primi, and multiparas and there was no significant relation between parity and the percentage of DHA in plasma phospholipids. Although the percentage of DHA in erythrocyte phospholipids was significantly lower in the mothers than in the nulliparas, there was no significant relation with parity in our population. Therefore, there is hardly any evidence to suggest that pregnancy produces long-term effects on the DHA status.

This view is supported by the DHASI values in plasma and erythrocyte phospholipids, which were not significantly different between the mothers and the nulliparas. Moreover, there was no correlation between the DHASI values and parity. The findings for DHADI values were somewhat inconsistent. In plasma, this index was lower in the mothers than in the nulliparas, but there was no significant correlation with parity. In erythrocyte phospholipids, this index was lower in the mothers than in the nulliparas and was also negatively correlated with parity. These data suggest that pregnancy would cause the DHA status of erythrocyte phospholipids to improve. However, the use of these indexes in the present study is questionable because one expects that effects on DHA deficiency would have reciprocal effects on DHA sufficiency.

Our results showed that the percentage of 18:2n−6 in both plasma and erythrocyte phospholipids was significantly higher in the mothers than in the nulliparas and that the percentage of 18:2n−6 increased significantly as parity increased. Σn−6 LCPs in both plasma and erythrocyte phospholipids were also significantly, but negatively, correlated with parity. Σn−6 LCPs in plasma and erythrocyte phospholipids seemed to be lower in the mothers than in the nulliparas.

In our study population, the concentrations of longer-chain, more unsaturated derivatives of 18:2n−6 decreased as parity increased. The metabolic index Σn−6 LCPs/18:2n−6 in plasma and erythrocyte phospholipids supports this suggestion (data not shown). The index was lower in the mothers than in the nulliparas, after correction for smoking habits, in both plasma and erythrocytes (P = 0.0014 and 0.0001, respectively) and was significantly negatively correlated with parity in both plasma and erythrocytes (P < 0.0001 and 0.0005, respectively). It is known that an excessive dietary intake of 18:2n−6 from vegetable oils inhibits the conversion of 18:2n−6 into longer-chain, more unsaturated fatty acids (2, 16, 17). However, there is no reason to assume differences in the dietary intake of 18:2n−6 between the mothers and the nulliparas in our study. Furthermore, there was

TABLE 3
Relative amounts of fatty acids and essential fatty acid (EFA) status indexes in erythrocyte phospholipids[1]

			Parity			
Fatty acid	Nulliparas (n = 40)	All mothers (n = 83)	Para 1 (n = 17)	Para 2 (n = 33)	Para 3 (n = 26)	Para 4 (n = 7)
Total (mg/L)	983.68 ± 157.88	1050.63 ± 161.08	1069.49 ± 175.14	1035.37 ± 126.27	1074.99 ± 148.88	986.21 ± 292.68
18:2n−6 (% by wt)	11.65 ± 1.35	12.07 ± 1.23[2]	12.10 ± 1.30	11.85 ± 1.18	12.27 ± 1.22	12.37 ± 1.44
20:4n−6 (% by wt)	11.45 ± 1.16	11.10 ± 0.94[3]	11.15 ± 0.87	11.21 ± 1.02	10.92 ± 0.70	11.20 ± 1.51
22:4n−6 (% by wt)	3.03 ± 0.55	2.83 ± 0.45[2]	2.84 ± 0.36	2.79 ± 0.48	2.82 ± 0.49	2.99 ± 0.44
22:5n−6 (% by wt)	0.33 ± 0.14	0.26 ± 0.08[4]	0.24 ± 0.08	0.26 ± 0.09	0.25 ± 0.07	0.27 ± 0.07
Σn−6 LCP (% by wt)	16.41 ± 1.72	15.61 ± 1.34[5]	15.61 ± 1.30	15.72 ± 1.40	15.40 ± 1.13	15.90 ± 1.96
Σn−6 (% by wt)	29.22 ± 1.41	28.70 ± 1.49	28.67 ± 1.38	28.57 ± 1.52	28.69 ± 1.49	29.31 ± 1.73
20:5n−3 (% by wt)	0.40 ± 0.16	0.42 ± 0.21	0.43 ± 0.26	0.43 ± 0.20	0.41 ± 0.19	0.37 ± 0.16
22:6n−3 (% by wt)	3.44 ± 0.61	3.13 ± 0.66[3]	2.91 ± 0.42	3.19 ± 0.65	3.21 ± 0.77	3.08 ± 0.74
Σn−3 LCP (% by wt)	5.67 ± 0.82	5.44 ± 0.86	5.22 ± 0.70	5.51 ± 0.80	5.48 ± 1.00	5.50 ± 1.04
Σn−3 (% by wt)	5.79 ± 0.82	5.53 ± 0.87	5.31 ± 0.70	5.60 ± 0.81	5.56 ± 1.02	5.56 ± 1.07
ΣSFA (% by wt)	45.17 ± 2.14	46.11 ± 2.28[3]	46.19 ± 2.02	46.47 ± 2.32	45.89 ± 2.43	45.01 ± 2.09
EFA index	1.91 ± 0.15	1.95 ± 0.17	1.92 ± 0.15	1.95 ± 0.15	1.94 ± 0.18	1.99 ± 0.27
DHADI	0.11 ± 0.04	0.09 ± 0.02[4]	0.08 ± 0.02	0.09 ± 0.03	0.09 ± 0.02	0.09 ± 0.02
DHASI	11.61 ± 4.05	13.27 ± 5.07	13.40 ± 6.11	13.27 ± 4.81	13.53 ± 5.22	11.96 ± 3.58

[1] $\bar{x}$ ± SD. Σn−6 longer-chain polyenes (LCPs): 20:3n−6, 20:4n−6, 22:4n−6, and 22:5n−6; Σn−6, sum of n−6 fatty acids; Σn−3 LCPs, 20:5n−3, 22:5n−3, and 22:6n−3; Σn−3, sum of n−3 fatty acids; ΣSFA, sum of all saturated fatty acids; EFA index, the ratio of Σn−3 + Σn−6 to Σn−9 + Σn−7; DHADI, docosahexaenoic acid deficiency index (22:5n−6/22:4n−6); DHASI, docosahexaenoic acid sufficiency index (22:6n−3/22:5n−6).
[2–5] Significantly different from nulliparas: [2] $P < 0.005$, [3] $P < 0.05$, [4] $P < 0.0001$, [5] $P < 0.001$.

no evidence that the women in the present study consumed excessive amounts of 18:2n−6, although we did not measure it. Competitive inhibition of the conversion of 18:2n−6 into longer-chain, more unsaturated fatty acids by n−3 fatty acids is not likely because both the percentage of Σn−3 LCPs and Σn−3 did not change significantly as parity increased.

To our knowledge, the relation between the EFA status of nonpregnant women and the number of completed pregnancies had not been investigated previously. Al et al (18) investigated the relation between maternal DHA status (during pregnancy) and parity in a cross-sectional design. They found that the percentage of DHA in the plasma phospholipids of multigravidas is on average ≈10% lower than in primigravidas. In addition, a significant negative relation was observed between the gravida number and the percentage of DHA in the maternal plasma phospholipids. These results indicate that maternal DHA status diminishes with each subsequent pregnancy or does not normalize completely after a mature pregnancy. The influence of the duration between different pregnancies on maternal DHA status was not investigated by Al et al; however, the shorter this duration, the shorter the time to replenish fatty acid stores.

The results of the present study indicate that maternal DHA status normalizes after a mature pregnancy. A maternal DHA-depletion phenomenon was not observed. However, the relatively long duration between the time of blood sampling and the last partus in the para 1, 2, 3, and 4 groups (Table 1) has to be taken into account. It can be argued that after such a long time, no influence of the last pregnancy would be expected; the mother could have replenished her fatty acid supply. Therefore, the relatively long duration between the time of blood sampling and the last partus may have influenced the results of our study.

In a subgroup of 27 mothers (para 1: n = 7; para 2: n = 11; para 3: n = 6; and para 4: n = 3), the duration between the time of blood sampling and the last partus was between 1 and 2 y (mean duration: 1.5 ± 0.4 y). There was no significant relation between parity and the percentage of DHA in the plasma and erythrocyte phospholipids of this subgroup. There were also no significant differences between the percentage of DHA in the plasma and erythrocyte phospholipids of these 27 mothers and that in the plasma (n = 41) and erythrocytes (n = 40) of the nulliparas. These observations indicate that the DHA status after a mature pregnancy normalizes within 1 y. More research is needed to explain the results found by Al et al (18).

Human milk contains significant amounts of DHA. Therefore, there is an extra drain on DHA stores in women who breast-feed their children and it will probably take them longer to replenish their maternal DHA stores. We investigated the influence of breast-feeding on the EFA status of both the whole group of mothers and of the above-mentioned subgroup of 27 mothers. Interestingly, the EFA status in both the plasma and erythrocyte phospholipids was not significantly different between breast-feeding and non-breast-feeding mothers (data not shown). The results of our study also showed that the duration between different pregnancies was not related to maternal DHA status. This relation was the same for the variables lactation, duration of lactation, age, alcohol consumption, and dietary habits of the mothers.

In conclusion, there was no relation between DHA status and parity in the nonpregnant women whose last pregnancy was completed ≥1 y previously. Maternal DHA status, as reflected by plasma and erythrocyte phospholipids, probably normalizes with 1 y after the last partus. Therefore, no maternal DHA-depletionin phenomenon was observed. Whether these conclusions pertain to women in countries other than the Netherlands remains to be determined.

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