Essential fatty acid composition of plasma phospholipids and birth weight: a study in term neonates[1–3]

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ABSTRACT

Background: Essential fatty acids (EFAs) in umbilical cord blood samples are associated with attained birth weight in premature infants and low-birth-weight neonates.

Objective: The objective was to investigate relations between the EFA composition of cord and maternal plasma phospholipids and birth weight in term neonates.

Design: This was a cross-sectional study in 627 singletons born at term. The plasma phospholipid EFA composition of the mothers was determined by gas-liquid chromatography at study entry (≤16 wk gestation), at delivery, and in cord plasma at birth. Birth weights were normalized to SD scores.

Results: In cord plasma, the dihomo-γ-linolenic acid concentration was positively related to weight SD scores. Both arachidonic acid (AA) and docosahexaenoic acid (DHA) were negatively related to weight SD scores. EFA-status indicators showed similar negative associations, whereas eicosatrienoic acid concentrations were positively related to neonatal size. In maternal plasma, proportions of n–3 long-chain polyenes (LCPs) and n–6 LCPs decreased during pregnancy. Larger decreases in AA, DHA, n–3 LCP, and n–6 LCP fractions were observed in mothers of heavier babies. Higher concentrations of LCPs in maternal plasma were, however, not related to a larger infant size at birth.

Conclusions: A lower biochemical EFA status in umbilical cord plasma and a larger decrease in maternal plasma LCP concentrations are associated with a higher weight-for-gestational-age at birth in term neonates. Our findings do not support a growth-stimulating effect of AA or DHA; however, they do suggest that maternal-to-fetal transfer of EFAs might be a limiting factor in determining neonatal EFA status. *Am J Clin Nutr* 2001;73:797–806.

KEY WORDS Essential fatty acids, umbilical cord, plasma phospholipids, infants, pregnancy, gestational age, birth weight, nutrition, arachidonic acid, docosahexaenoic acid

INTRODUCTION

Growth retardation is one of the prominent features of essential fatty acid (EFA) deficiency in both animals (1, 2) and humans (3, 4). Linoleic acid (18:2n−6) and the longer-chain polyunsaturated fatty acids (PUFAs) of the n−6 family are believed to be important for optimal growth. Lower concentrations of EFAs were found in red blood cell membranes, plasma phospholipids, and the walls of the umbilical artery of low-birth-weight neonates (5). In premature infants, birth weight was positively associated with the proportions of arachidonic acid (AA; 20:4n−6) and dihomo-γ-linolenic acid (20:3n−6) in plasma triacylglycerols and choline phosphoglycerides (6, 7). In some studies, positive associations between birth weight and docosahexaenoic acid (DHA; 22:6n–3) were described as well (7–9). On the basis of these findings, a smaller size at birth seems to be related to a lower EFA status.

Besides individual EFA concentrations in plasma, erythrocytes, and tissue phospholipids, there are other indicators of biochemical EFA status. When the availability of EFAs does not meet functional requirements, the human body produces more fatty acids of comparable chain length and degree of unsaturation, such as eicosatrienoic acid (20:3n−9). Therefore, higher concentrations of eicosatrienoic acid indicate a lower EFA status. Similarly, docosapentaenoic acid (22:5n−6) is produced when DHA availability is marginal, and the ratio between DHA and docosapentaenoic acid can be used as an indicator of DHA status (10). A more general marker of EFA status is the ratio between the sum of all n–3 and n–6 fatty acids and the sum of all non-EFAs from the n−7 and n−9 families (11). Crawford et al (5) reported "grossly abnormal" concentrations of 2 of these indexes in the umbilical artery walls of low-birth-weight neonates (birth weight <2500 g), which suggests again that a small size at birth is associated with a lower EFA status.

Conclusions drawn from observations in premature infants or in low-birth-weight neonates might, however, not be applicable to the more common situation of term birth. Few comparable studies in healthy term neonates have been conducted. In the present study we determined both EFA concentrations and EFA-status indexes in umbilical cord plasma phospholipid samples obtained from term neonates. These indexes of biochemical EFA status

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were used to investigate relations with infant birth weight. In addition, we studied relations between neonatal weight and observed changes in maternal plasma EFA composition during pregnancy.

SUBJECTS AND METHODS

Study population

As part of other investigations, pregnant women were asked to participate in longitudinal observational studies of changes in EFA status during pregnancy and the relation of these changes to pregnancy outcome (12–15). Three antenatal clinics located in the province of Limburg in the southern part of the Netherlands participated: the University Hospital in Maastricht, Hospital "De Wever" in Heerlen, and the School for Midwifery in Kerkrade. Selection criteria for inclusion in these studies were a gestational age of <16 wk at entry, a diastolic blood pressure <90 mm Hg, and no signs of cardiovascular, neurologic, renal, or metabolic disorders at the time of recruitment. In the present analysis, the available data for 752 singletons born between January 1990 and January 1994 during these observational studies were used. After exclusion of infants with unknown gestational age or birth weight (n = 6), who were born prematurely (gestational age <37 wk; n = 43), or who died (n = 2) and of mothers with diabetes (n = 14) or pregnancy-induced hypertension (n = 71), a total study population of 627 infants was left for analysis. Approval for these studies was obtained from the Ethics Committee of the University Hospital Maastricht, and all participating women gave their written, informed consent.

Gestational age and birth weight

Local hospital staff members recorded individual maternal and infant characteristics on a standardized data sheet. Additional information was obtained from medical records or by using questionnaires. Gestational age at birth (in wk) was calculated from the recorded date of delivery and the self-reported first day of the last menstrual period; fractions were expressed in decimals. If the last menstrual period was unknown, gestational age was based on early ultrasound measurements. Infants were categorized into 5 weight-for-gestational-age categories. Infants with a birth weight ≤10th percentile were classified as small for gestational age (SGA) and those with a birth weight ≥90th percentile as large for gestational age (LGA). Because most infants were classified as appropriate for gestational age (AGA), this category was divided into 3 subcategories: *1*) a birth weight >10th percentile but ≤25th percentile, *2*) a birth weight >25th but <75th percentile, and *3*) a birth weight ≥75th but <90th percentile. This classification was based on the percentiles given by the Dutch reference standard (appropriate for length of gestation, infant sex, and birth order) (16). In addition, recorded birth weights were converted into SD scores (17):

$$\text{SD score} = (\text{weight}_{\text{observed}} - \text{mean weight}_{\text{reference}})/\text{SD}_{\text{reference}} \quad (1)$$

In this way, a continuous measure for weight-for-gestational-age was created. An SD score of −2 corresponds to the 2.3rd percentile and an SD score of 2 corresponds to the 97.7th percentile of weight-for-gestational-age, respectively.

Blood collection and determination of fatty acid composition

Maternal venous blood samples were collected in EDTA-treated evacuated tubes at study entry [≤16 wk; mean (±SD) gestational age at entry was 11 ± 3 wk] and after delivery. Directly after parturition, a blood sample was obtained from the umbilical vein. Plasma was separated from blood cells by centrifugation (2000 × g, 4°C, 15 min) and stored under nitrogen at −80°C until analyzed (18). The fatty acid composition of maternal and umbilical cord plasma phospholipids was determined as described previously (12). In short, after the addition of 1,2-dinonadecanoyl phosphatidylcholine (internal standard), total lipid extracts from 100 μL plasma were prepared by using a modified (19) version of Folch et al's (20) extraction method. Phospholipids were isolated by solid-phase extraction of total lipid extracts on aminopropyl-silica columns (21). To check for carryover of other lipid fractions during this procedure, heptadecaenoic acid (17:1) was added to the samples. After saponification of the isolated phospholipids, fatty acids were converted to the corresponding fatty acid methyl esters (22). The fatty acid methyl esters were analyzed by capillary gas-liquid chromatography with use of a 50-m CP-Sil 5 CB nonpolar capillary column (Chrompack, Middelburg, Netherlands). Plasma total phospholipid fatty acids were expressed in absolute concentrations (mg/L) and the individual fractions of fatty acids and fatty acid groups as relative values (% by wt of total fatty acids). In total, 39 fatty acids were identified but, for clarity, only the concentrations of 9 individual fatty acids and 8 fatty acid groups are reported (**Table 1**). The concentrations of γ-linolenic acid (18:3n−6) were <0.1% of total fatty acids and were therefore not reported. In addition to the reported concentrations, 2 indexes of EFA status were calculated: the DHA-status index (ratio of DHA to docosapentaenoic acid; a higher ratio indicates a higher DHA status) (10) and the EFA-status index (ratio of Σn−3 + n−6 to Σn−7 + n−9; a higher ratio indicates a higher EFA status) (11).

Statistical analysis

Values are reported as means ± SEMs, unless specified otherwise. Differences between means were evaluated by either paired or unpaired two-tailed Student's t tests. Relations between variables were analyzed with simple and multiple regression models. To test for linear trends, the continuous variable weight SD score was used instead of the weight-for-gestational-age categories. Maternal age, maternal weight at study entry, weight increase during pregnancy, smoking habits, parity (ordinal), mode of delivery (dummies for extraction and cesarean section with vaginal delivery as a reference), and the 5-min Apgar score were included as potential confounding factors. When the total study sample was analyzed, infant sex was introduced as an additional factor. Because of a skewed distribution of some variables (EPA, eicosatrienoic acid, and the DHA-status index), $\log_{10}$ or square-root transformed data were used in the analyses. Because of incomplete data records, not all analyses were based on the same number of subjects. A two-tailed P value <0.05 was considered statistically significant. All statistical analyses were performed by using STATVIEW (version 4.5; Abacus Concepts Inc, Berkeley, CA).

RESULTS

Characteristics of the women and their neonates are listed in **Table 2**. Birth weight ranged from 1875 to 4350 g in girls and from 2050 to 4620 g in boys. In total, 81 (13%) infants were born SGA according to Dutch references (16). Forty-one (7%) neonates were born LGA. On average, birth weight deviated from the reference mean by −0.16 SDs (range in weight SD score: −3.23 to 2.57). Weight SD scores did not differ signifi-

TABLE 1
Fatty acid composition of umbilical cord plasma phospholipids of term neonates[1]

	Boys (n = 347)	Girls (n = 280)	All (n = 627)
Total fatty acids (mg/L)	571.5 ± 6.07[2]	613.9 ± 7.4	590.4 ± 4.79 (251.5 –1218.5)
Fatty acids (% by wt of total)			
18:2n−6	7.54 ± 0.07	7.41 ± 0.07	7.48 ± 0.05 (4.30–11.98)
20:3n−6	5.06 ± 0.05	5.18 ± 0.05	5.11 ± 0.03 (2.55–8.04)
20:4n−6	16.74 ± 0.08	16.89 ± 0.09	16.81 ± 0.06 (11.04–21.30)
22:4n−6	0.81 ± 0.01	0.79 ± 0.01	0.80 ± 0.01 (0.47–1.38)
22:5n−6	0.86 ± 0.02	0.83 ± 0.02	0.85 ± 0.01 (0.25–1.93)
Σn−6	32.08 ± 0.09	32.17 ± 0.10	32.12 ± 0.07 (24.11–36.51)
Σn−6 LCPs	23.46 ± 0.08	23.68 ± 0.08	23.56 ± 0.06 (18.38–27.79)
18:3n−3	ND	ND	ND
20:5n−3	0.23 ± 0.01	0.23 ± 0.01	0.23 ± 0.00 (0.00–1.07)
22:5n−3	0.47 ± 0.01	0.47 ± 0.01	0.47 ± 0.01 (0.14–1.21)
22:6n−3	6.20 ± 0.07	6.21 ± 0.08	6.21 ± 0.05 (3.12–10.48)
Σn−3	7.04 ± 0.08	7.04 ± 0.09	7.04 ± 0.06 (3.57–11.69)
Σn−3 LCPs	6.98 ± 0.08	6.70 ± 0.09	6.99 ± 0.06 (3.54–11.69)
20:3n−9	0.49 ± 0.02	0.44 ± 0.02	0.47 ± 0.01 (0.00–2.32)
Σn−7 + n−9	12.44 ± 0.10	12.28 ± 0.12	12.37 ± 0.08 (6.09–24.45)
ΣSFAs	47.60 ± 0.08	47.69 ± 0.08	47.64 ± 0.06 (39.33–57.27)
ΣMUFAs	11.89 ± 0.09	11.77 ± 0.10	11.83 ± 0.07 (5.75–23.53)
ΣPUFAs	39.52 ± 0.09	39.59 ± 0.10	39.55 ± 0.07 (31.47–43.73)

[1] $\bar{x}$ ± SEM; range in parentheses. Σn−6 and Σn−3, the sum of all n−6 and n−3 fatty acids, respectively; Σn−6 LCPs, the sum of all n−6 long-chain polyenes (20:3n−6, 20:4n−6, 22:4n−6, and 22:5n−6); Σn−3 LCPs, the sum of all n−3 LCPs (20:4n−3, 20:5n−3, 22:5n−3, and 22:6n−3); Σn−7 + n−9, the sum of all n−7 and n−9 fatty acids; ΣSFAs, ΣMUFAs, and ΣPUFAs, the sum of all saturated, monounsaturated, and polyunsaturated fatty acids, respectively; ND, not detected.

[2] Significantly different from girls, $P < 0.0001$ (unpaired Student's t test).

cantly between boys and girls ($\bar{x}$ ± SD: −0.14 ± 0.87 and −0.18 ± 0.92, respectively) by unpaired Student's t test.

Infant sex and the EFA composition of plasma phospholipids

The total amount (mg/L) of fatty acids in umbilical cord plasma phospholipids of female neonates was larger than that in males (Table 1). This difference, however, was not due to a higher concentration of one particular fatty acid or fatty acid group. The proportions of each individual fatty acid and that of specific fatty acid groups did not differ significantly between boys and girls. In addition, the total amount of fatty acids and the relative fatty acid composition of maternal plasma phospholipids did not differ significantly between boys and girls, nor was there any sex-related difference in the observed changes in relative fatty acid concentrations in maternal plasma phospholipids during pregnancy. For these reasons, further comparisons were done with boys and girls combined. Infant sex was used as an additional factor in the multiple regression models, but no major effect of sex on fatty acid fractions or relations was found.

The fatty acid α-linolenic acid (18:3n−3) was present in such low concentrations in umbilical cord plasma phospholipids that it could not be detected in most of the samples. In only 34% of the infants was α-linolenic acid detected in measurable amounts. The median α-linolenic acid concentration in these subjects was 0.12% by wt of total fatty acids [interquartile range (IQR): 0.07% by wt of total fatty acids]. In the total group of infants, the median α-linolenic acid concentration was 0.00% by wt of total fatty acids (IQR: 0.08% by wt of total fatty acids). The number of infants in whom α-linolenic acid could be measured did not differ significantly between boys and girls, gestational age groups, or weight-for-gestational-age groups (chi-square tests).

Gestational age and the fatty acid composition of umbilical cord plasma phospholipids

Infants born after a shorter duration of gestation had relatively higher linoleic acid and higher Σn−6 fatty acid concentrations in their umbilical cord plasma phospholipids (**Table 3**). In contrast, 20:4n−6 and Σn−6 long-chain polyene (LCP) concentrations were not significantly related to gestational age at birth. Most pronounced, however, were the differences in the n−3 fatty acid fractions. Neonates born at a later gestational age had higher umbilical cord plasma concentrations of EPA, docosapentaenoic acid, DHA, Σn−3, and Σn−3 LCPs, whereas the proportions of eicosatrienoic acid and Σn−7 + n−9 fatty acids were lower in these infants. The total fatty acid content was not related to gestational age at birth.

Birth weight and the fatty acid composition of umbilical cord plasma phospholipids

The relative EFA composition of umbilical cord plasma phospholipids was associated with weight-for-gestational-age at birth (**Table 4**). Fractions of AA, Σn−6, Σn−6 LCPs, and ΣPUFAs were lower in infants born LGA. Both dihomo-γ-linolenic acid and docosapentaenoic acid concentrations were, however, higher in heavier neonates. Furthermore, docosapentaenoic acid, DHA, Σn−3, and Σn−3 LCP concentrations were higher in the smaller infants, whereas the proportion of eicosapentaenoic acid (EPA; 20:5n−3) was not related to weight-for-gestational-age at birth. In addition to these observations, eicosatrienoic acid, Σn−7 + n−9 fatty acids, and the sum of monounsaturated fatty acid (ΣMUFA) concentrations were evidently higher in the umbilical cord plasma samples of heavier

TABLE 2
Characteristics of the study population according to weight-for-gestational-age percentile category at birth[1]

		AGA			
	SGA, ≤10th (*n* = 81)	>10th to ≤25th (*n* = 95)	>25th to <75th (*n* = 339)	≥75th to <90th (*n* = 71)	LGA, ≥90th (*n* = 41)
Maternal characteristics					
Age (y)	28.9 ± 4.1[2]	28.9 ± 4.6	29.5 ± 4.2	29.3 ± 4.2	29.4 ± 3.9
Height (cm)	163.7 ± 6.9	164.4 ± 5.9	166.6 ± 6.6	168.5 ± 6.0	170.8 ± 6.0
Weight at study entry (kg)	61.7 ± 11.4	61.3 ± 9.6	65.2 ± 11.0	71.4 ± 15.8	71.3 ± 12.0
Weight increase during pregnancy (kg)	9.7 ± 3.8	10.7 ± 3.7	11.8 ± 3.8	12.4 ± 4.4	12.5 ± 3.9
Parity (%)					
0	67	71	74	75	68
1	30	23	21	21	27
2	1	5	4	4	5
≥3	2	1	1	0	0
Smoking (%)	44	28	27	18	17
Mode of delivery (%)					
Vaginal	77	79	77	79	68
Extraction (vacuum/forceps)	11	12	17	13	17
Cesarean	12	9	6	8	15
Infant characteristics					
Sex (M:F)	36:45	49:46	204:135	38:33	20:21
Gestational age (wk)	40.1 ± 1.3	40.0 ± 1.0	40.1 ± 1.2	40.6 ± 1.2	40.4 ± 1.3
Birth weight (g)	2661 ± 255	3006 ± 178	3380 ± 247	3856 ± 222	4166 ± 227
Weight SD score	−1.62 ± 0.39	−0.87 ± 0.17	−0.05 ± 0.36	0.90 ± 0.19	1.63 ± 0.32
Crown-heel length (cm)	47.6 ± 2.0	48.8 ± 1.7	50.3 ± 1.7	51.8 ± 1.6	52.5 ± 1.4
Occipital-frontal circumference (cm)	33.2 ± 1.3	34.0 ± 1.2	34.4 ± 1.3	35.5 ± 1.2	36.1 ± 1.2
Apgar score after 5 min	9.3 ± 1.2	9.7 ± 0.8	9.6 ± 0.8	9.7 ± 0.6	9.6 ± 0.6
Apgar score ≤7 (%)	9	2	2	1	3

[1] SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age. Percentile categories based on Dutch reference standards (appropriate for length of gestation, infant sex, and birth order) (16).
[2] $\bar{x}$ ± SD; *n* = 627.

TABLE 3
Fatty acid composition of umbilical cord plasma phospholipids in term infants according to gestational age at birth[1]

							P for trend[2]	
	37 wk (*n* = 26)	38 wk (*n* = 69)	39 wk (*n* = 162)	40 wk (*n* = 189)	41 wk (*n* = 143)	42 wk (*n* = 38)	Crude (*n* = 627)	Adjusted (*n* = 614)
Total fatty acids (mg/L)	594.6 ± 22.5[3]	590.8 ± 16.2	580.2 ± 10.0	582.8 ± 7.79	613.6 ± 10.1	581.9 ± 18.7	0.3335	0.3233
Fatty acids (% by wt of total)								
18:2n−6	8.35 ± 0.21	7.79 ± 0.13	7.53 ± 0.09	7.38 ± 0.08	7.39 ± 0.10	6.96 ± 0.21	<0.0001	<0.0001
20:3n−6	5.04 ± 0.18	5.33 ± 0.12	5.24 ± 0.06	5.03 ± 0.06	5.00 ± 0.07	5.01 ± 0.14	0.0105	0.0039
20:4n−6	17.04 ± 0.30	16.61 ± 0.21	16.72 ± 0.12	16.93 ± 0.11	16.69 ± 0.13	17.21 ± 0.25	0.4008	0.3204
22:4n−6	0.72 ± 0.02	0.73 ± 0.01	0.80 ± 0.01	0.81 ± 0.01	0.82 ± 0.01	0.84 ± 0.02	<0.0001	<0.0001
22:5n−6	0.78 ± 0.05	0.82 ± 0.03	0.86 ± 0.02	0.85 ± 0.02	0.85 ± 0.02	0.87 ± 0.05	0.3751	0.2357
Σn−6	33.09 ± 0.24	32.38 ± 0.18	32.26 ± 0.12	32.06 ± 0.13	31.83 ± 0.14	31.88 ± 0.27	<0.0001	0.0003
Σn−6 LCPs	23.57 ± 0.28	23.49 ± 0.18	23.62 ± 0.11	23.62 ± 0.11	23.35 ± 0.12	23.92 ± 0.21	0.9713	0.9291
18:3n−3	ND	ND	ND	ND	ND	ND		
20:5n−3	0.19 ± 0.02	0.22 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.24 ± 0.01	0.24 ± 0.02	0.0303	0.0423
22:5n−3	0.34 ± 0.03	0.38 ± 0.01	0.45 ± 0.01	0.48 ± 0.01	0.52 ± 0.01	0.57 ± 0.03	<0.0001	<0.0001
22:6n−3	5.12 ± 0.27	5.48 ± 0.13	6.07 ± 0.09	6.25 ± 0.09	6.61 ± 0.12	7.08 ± 0.19	<0.0001	<0.0001
Σn−3	5.74 ± 0.29	6.19 ± 0.14	6.90 ± 0.11	7.08 ± 0.11	7.51 ± 0.14	8.06 ± 0.22	<0.0001	<0.0001
Σn−3 LCPs	5.72 ± 0.29	6.15 ± 0.14	6.86 ± 0.11	7.04 ± 0.11	7.46 ± 0.14	7.98 ± 0.22	<0.0001	<0.0001
20:3n−9	0.44 ± 0.06	0.53 ± 0.03	0.47 ± 0.02	0.47 ± 0.02	0.43 ± 0.02	0.41 ± 0.03	0.0299	0.0060
Σn−7 + n−9	12.90 ± 0.36	13.22 ± 0.22	12.33 ± 0.11	12.27 ± 0.13	12.22 ± 0.21	11.68 ± 0.20	<0.0001	<0.0001
ΣSFAs	47.55 ± 0.18	47.31 ± 0.17	47.72 ± 0.09	47.78 ± 0.11	47.57 ± 0.14	47.44 ± 0.20	0.4809	0.6155
ΣMUFAs	12.37 ± 0.30	12.61 ± 0.20	11.78 ± 0.09	11.73 ± 0.12	11.73 ± 0.20	11.21 ± 0.17	<0.0001	<0.0001
ΣPUFAs	39.18 ± 0.34	39.02 ± 0.19	39.54 ± 0.12	39.54 ± 0.13	39.70 ± 0.16	40.28 ± 0.24	0.0003	0.0006

[1] Σn−6 and Σn−3, the sum of all n−6 and n−3 fatty acids, respectively; Σn−6 LCPs, the sum of all n−6 long-chain polyenes (20:3n−6, 20:4n−6, 22:4n−6, and 22:5n−6); Σn−3 LCPs, the sum of all n−3 LCPs (20:4n−3, 20:5n−3, 22:5n−3, and 22:6n−3); Σn−7 + n−9, the sum of all n−7 and n−9 fatty acids; ΣSFAs, ΣMUFAs, and ΣPUFAs, the sum of all saturated, monounsaturated, and polyunsaturated fatty acids, respectively; ND, not detected.
[2] *P* values for linear trends with the continuous variable gestational age are given crude and adjusted for potential confounding factors (maternal age, maternal weight at entry, weight increase during pregnancy, smoking, parity, mode of delivery, 5-min Apgar score, infant sex, and weight SD score).
[3] Unadjusted $\bar{x}$ ± SEM; *n* = 627.

TABLE 4
Fatty acid composition of umbilical cord plasma phospholipids in term infants according to weight-for-gestational-age percentile category at birth[1]

		AGA				*P* for trend[2]	
	SGA, ≤ 10th (*n* = 81)	>10th to ≤25th (*n* = 95)	>25th to <75th (*n* = 339)	≥75th to <90th (*n* = 71)	LGA, ≥90th (*n* = 41)	Crude (*n* = 627)	Adjusted (*n* = 614)
Total fatty acids (mg/L)	573.9 ± 12.6[3]	576.7 ± 13.3	588.7 ± 6.48	621.2 ± 12.5	615.9 ± 20.2	0.0006	<0.0001
Fatty acids (% by wt of total)							
18:2n−6	7.60 ± 0.13	7.51 ± 0.12	7.48 ± 0.06	7.35 ± 0.14	7.42 ± 0.19	0.0426	0.2131
20:3n−6	4.73 ± 0.10	5.03 ± 0.09	5.18 ± 0.05	5.18 ± 0.09	5.35 ± 0.11	<0.0001	<0.0001
20:4n−6	17.61 ± 0.18	17.01 ± 0.14	16.68 ± 0.09	16.60 ± 0.18	16.23 ± 0.22	<0.0001	<0.0001
22:4n−6	0.80 ± 0.02	0.82 ± 0.02	0.79 ± 0.01	0.78 ± 0.02	0.79 ± 0.02	0.1925	0.0574
22:5n−6	0.78 ± 0.03	0.85 ± 0.03	0.85 ± 0.02	0.86 ± 0.03	0.95 ± 0.05	0.0006	0.0001
Σn−6	32.57 ± 0.17	32.32 ± 0.17	32.06 ± 0.09	31.80 ± 0.19	31.81 ± 0.23	0.0007	0.0021
Σn−6 LCPs	23.92 ± 0.16	23.71 ± 0.14	23.49 ± 0.08	23.42 ± 0.18	23.31 ± 0.21	0.0371	0.0166
18:3n−3	ND	ND	ND	ND	ND		
20:5n−3	0.23 ± 0.01	0.22 ± 0.01	0.23 ± 0.01	0.24 ± 0.02	0.22 ± 0.01	0.9161	0.5722
22:5n−3	0.51 ± 0.02	0.47 ± 0.02	0.46 ± 0.01	0.48 ± 0.02	0.45 ± 0.02	0.0373	0.0003
22:6n−3	6.56 ± 0.16	6.28 ± 0.13	6.13 ± 0.07	6.32 ± 0.17	5.74 ± 0.19	0.0072	<0.0001
Σn−3	7.42 ± 0.17	7.11 ± 0.14	6.96 ± 0.08	7.17 ± 0.19	6.52 ± 0.21	0.0108	<0.0001
Σn−3 LCPs	7.38 ± 0.17	7.06 ± 0.14	6.91 ± 0.08	7.13 ± 0.19	6.49 ± 0.21	0.0112	<0.0001
20:3n−9	0.35 ± 0.03	0.43 ± 0.03	0.48 ± 0.02	0.49 ± 0.03	0.61 ± 0.05	<0.0001	<0.0001
Σn−7 + n−9	11.67 ± 0.20	12.00 ± 0.16	12.49 ± 0.11	12.59 ± 0.21	13.16 ± 0.28	<0.0001	<0.0001
ΣSFAs	47.42 ± 0.15	47.73 ± 0.17	47.66 ± 0.08	47.57 ± 0.15	47.77 ± 0.14	0.3284	0.4106
ΣMUFAs	11.26 ± 0.19	11.50 ± 0.15	11.94 ± 0.10	12.02 ± 0.18	12.49 ± 0.23	<0.0001	<0.0001
ΣPUFAs	40.27 ± 0.16	39.79 ± 0.18	39.43 ± 0.09	39.38 ± 0.22	38.86 ± 0.23	<0.0001	<0.0001

[1] Percentile categories based on Dutch reference standards (appropriate for length of gestation, infant sex, and birth order) (16). SGA, small for gestational age; AGA, appropriate for gestational age; LGA, large for gestational age; Σn−6 and Σn−3, the sum of all n−6 and n−3 fatty acids, respectively; Σn−6 LCPs, the sum of all n−6 long-chain polyenes (20:3n−6, 20:4n−6, 22:4n−6, and 22:5n−6); Σn−3 LCPs, the sum of all n−3 LCPs (20:4n−3, 20:5n−3, 22:5n−3, and 22:6n−3); Σn−7 + n−9, the sum of all n−7 and n−9 fatty acids; ΣSFAs, ΣMUFAs, and ΣPUFAs, the sum of all saturated, monounsaturated, and polyunsaturated fatty acids, respectively; ND, not detected.

[2] *P* values for linear trends with the continuous variable weight SD score are given crude and adjusted for potential confounding factors (maternal age, maternal weight at entry, weight increase during pregnancy, smoking, parity, mode of delivery, 5-min Apgar score, infant sex, and gestation duration).

[3] Unadjusted $\bar{x}$ ± SEM; *n* = 627.

infants. The total amount of plasma phospholipid fatty acids was also higher in heavier infants.

Gestational age, birth weight, and indicators of EFA status

The EFA-status index was higher in the umbilical cord plasma of neonates born at a later time point but was lower in those born LGA (**Figure 1**, A and C). Similarly, the DHA-status index was higher in the plasma of infants born after a longer duration of gestation and lower in those with a higher weight-for-gestational-age at birth (Figure 1, B and D). In **Table 5**, the association between weight-for-gestational-age at birth and the EFA-status index is compared with the relations of key prognostic indicators of fetal growth and infant size at birth.

EFA composition of maternal plasma phospholipids

Of all 627 selected neonates, information on the EFA composition of 582 maternal plasma samples taken at study entry (≤16 wk gestation) and of 568 maternal plasma samples taken at delivery were available. In a total of 546 cases (87%), the fatty acid compositions of both samples were known. The well-known differences in relative fatty acid composition between maternal and umbilical cord plasma were observed (Table 2 and **Table 6**). The concentration of α-linolenic acid was significantly higher in the plasma phospholipids of mothers of infants in whom α-linolenic acid concentrations were measurable than in those of mothers of infants with undetectable amounts of α-linolenic acid (0.27 ± 0.10% compared with 0.19 ± 0.10% by wt of total fatty acids; $P < 0.0001$).

During pregnancy, the total fatty acid concentration increased and its composition changed significantly (Table 6). At delivery, relatively more saturated fatty acids and MUFAs were found, whereas the fraction of PUFAs was lower. The EFA-status index and the DHA-status index also decreased during pregnancy (Table 6). The observed changes in the mothers' maternal plasma EFA compositions were related to the size of their infants (**Figures 2** and **3**). The biggest decrease in plasma concentrations of AA, DHA, Σn−6, and Σn−3 LCPs were observed in mothers of heavier infants, whereas the largest reduction in the fraction of linoleic acid was found in the mothers of relatively smaller neonates. No cross-sectional association was found between maternal fatty acid concentrations and infant size at birth at study entry or at delivery. There also was no relation between maternal plasma fatty acid concentrations and the total duration of gestation. The fatty acid concentrations in maternal plasma were strong predictors of umbilical cord plasma fatty acid composition. However, the reported relations between umbilical cord plasma fatty acid composition and normalized birth weight were independent of the observed maternal concentrations.

DISCUSSION

To our knowledge, this was the first study to show relations between umbilical cord plasma phospholipid EFA composition and size at birth in term neonates. Both the pattern of individual fatty acid fractions and the EFA-status indexes seemed to indicate

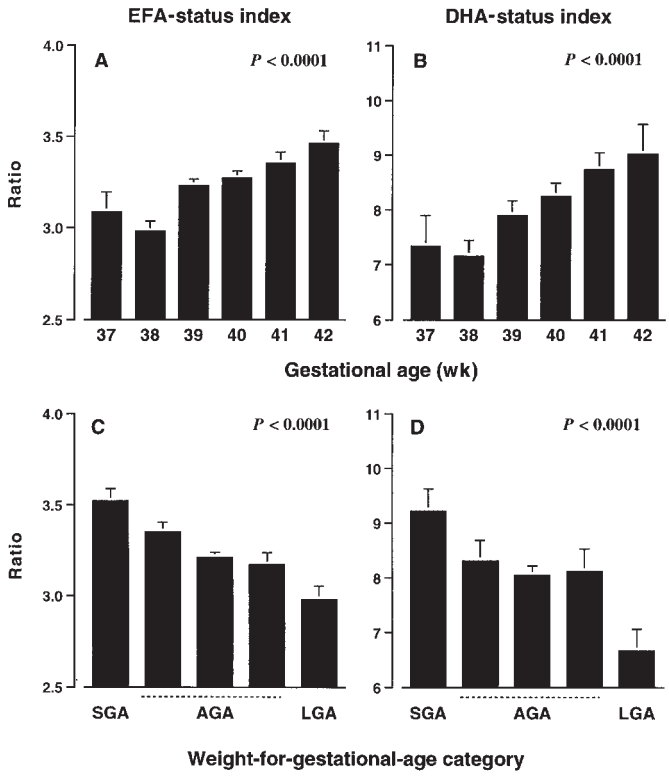

FIGURE 1. Mean (±SEM) essential fatty acid (EFA)–status index (ratio of Σn−3 + n−6 to Σn−7 + n−9) and docosahexaenoic acid (DHA)–status index (ratio of DHA to docosapentaenoic acid) in umbilical cord plasma phospholipids of infants according to gestational age at birth (A and B) and infant birth weight, ie, weight-for-gestational-age category (C and D). The categories are small for gestational age (SGA; birth weight ≤10th percentile), appropriate for gestational age (AGA; divided into 3 subcategories: *1*) birth weight >10th but ≤25th percentile, *2*) >25th but <75th percentile, and *3*) ≥75th but <90th percentile), and large for gestational age (LGA; ≥90th percentile). All percentiles were based on Dutch reference standards (appropriate for length of gestation, infant sex, and birth order) (16). *P* values shown are for linear trends with the continuous variable weight SD score.

a higher biochemical EFA status in the umbilical cord plasma of smaller infants than in the plasma of larger ones.

EFAs as determinants of fetal growth

The concept that EFAs such as AA and DHA serve as potential fetal growth factors was not supported by our results. Proportions of AA and DHA in umbilical cord plasma phospholipids were negatively related to neonatal size at birth (Table 4). In addition, no relation was found between the concentrations of these fatty acids in maternal plasma and infant birth weight. These findings are in contrast with previous observations in premature infants and low-birth-weight babies (5–7, 23). In most of these studies, lower proportions of AA, DHA, or both were found in smaller neonates. An explanation for this inconsistency between studies could be that additional (pathologic) factors associated with premature birth or severe growth

TABLE 5
Association of weight-for-gestational-age at birth with the essential fatty acid (EFA)–status index measured in umbilical cord plasma phospholipids and prognostic indicators of fetal growth[1]

	Univariate analysis			Multivariate analysis		
	β	SE	*t*	β	SE	*t*
EFA-status index[2]	−0.343[3]	0.060	−5.8	−0.369[3]	0.055	−6.7
Maternal weight at study entry (kg)	0.019[3]	0.003	6.5	0.018[3]	0.003	6.3
Weight increase during pregnancy (kg)	0.049[3]	0.009	5.6	0.051[3]	0.008	6.3
Maternal height (cm)	0.041[3]	0.005	7.7	0.024[3]	0.005	4.7
Maternal smoking (yes = 1, no = 0)	−0.353[3]	0.079	−4.5	−0.266	0.072	−3.7
Maternal age (y)	0.010	0.008	1.2	0.004[4]	0.008	0.5

[1] Results of simple and multiple regression analyses with weight SD score [appropriate for length of gestation, infant sex, and birth order (16)] as the dependent variable and the EFA–status index and prognostic indicators of fetal growth as independent variables.
[2] Ratio of Σn−3 + n−6 to Σn−7 + n−9 fatty acids.
[3] $P < 0.0001$.
[4] $P < 0.001$.

TABLE 6
Fatty acid composition of maternal plasma phospholipids[1]

	Study entry (n = 582)	Delivery (n = 568)	Change[2] (n = 546)
Total fatty acids (mg/L)	1329.5 ± 10.5	1759.4 ± 13.0	433.0 ± 14.6 (404.2, 461.7)
Fatty acids (% by wt of total)			
18:2n−6	21.48 ± 0.11	20.72 ± 0.10	–0.76 ± 0.10 (−0.96, −0.57)
20:3n−6	3.08 ± 0.03	3.46 ± 0.03	0.37 ± 0.03 (0.32, 0.42)
20:4n−6	9.61 ± 0.06	8.55 ± 0.06	−1.07 ± 0.05 (−1.17, −0.97)
22:4n−6	0.39 ± 0.00	0.38 ± 0.00	−0.02 ± 0.00 (−0.02, −0.01)
22:5n−6	0.35 ± 0.01	0.53 ± 0.01	0.18 ± 0.01 (0.17, 0.19)
Σn−6	35.55 ± 0.08	34.33 ± 0.08	−1.24 ± 0.08 (−1.40, −1.08)
Σn−6 LCPs	13.44 ± 0.07	12.92 ± 0.07	−0.53 ± 0.06 (−0.64, −0.42)
18:3n−3	0.21 ± 0.01	0.22 ± 0.00	0.01 ± 0.01 (−0.01, 0.02)
20:5n−3	0.55 ± 0.02	0.35 ± 0.01	−0.20 ± 0.02 (−0.23, −0.17)
22:5n−3	0.75 ± 0.01	0.55 ± 0.01	−0.20 ± 0.01 (−0.22, −0.19)
22:6n−3	4.04 ± 0.04	3.87 ± 0.03	−0.18 ± 0.03 (−0.24, −0.11)
Σn−3	5.69 ± 0.05	4.93 ± 0.04	−0.77 ± 0.04 (−0.85, −0.68)
Σn−3 LCPs	5.48 ± 0.05	4.91 ± 0.04	−0.58 ± 0.04 (−0.66, −0.49)
20:3n−9	0.19 ± 0.01	0.25 ± 0.01	0.06 ± 0.00 (0.05, 0.06)
Σn−7 + n−9	11.95 ± 0.05	13.08 ± 0.06	1.13 ± 0.06 (1.02, 1.25)
ΣSFAs	44.36 ± 0.05	45.60 ± 0.04	1.24 ± 0.05 (1.14, 1.33)
ΣMUFAs	11.72 ± 0.05	12.80 ± 0.06	1.08 ± 0.06 (0.97, 1.19)
ΣPUFAs	41.44 ± 0.06	39.63 ± 0.06	−1.83 ± 0.07 (−1.96, −1.67)
DHA status index[3]	12.85 ± 0.25	8.21 ± 0.24	−4.71 ± 0.21 (−5.11, −4.30)
EFA status index[4]	3.50 ± 0.02	3.05 ± 0.02	−0.45 ± 0.02 (−0.50, −0.41)

[1] $\bar{x}$ ± SEM; 95% CI in parentheses. Numbers of subjects differ because of incomplete data records. Σn−6 and Σn−3, the sum of all n−6 and n−3 fatty acids, respectively; Σn−6 LCPs, the sum of all n−6 long-chain polyenes (20:3n−6, 20:4n−6, 22:4n−6, and 22:5n−6); Σn−3 LCPs, the sum of all n−3 LCPs (20:4n−3, 20:5n−3, 22:5n−3, and 22:6n−3); Σn−7 + n−9, the sum of all n−7 and n−9 fatty acids; ΣSFAs, ΣMUFAs, and ΣPUFAs, the sum of all saturated, monounsaturated, and polyunsaturated fatty acids, respectively.

[2] Difference between fatty acid concentrations at delivery and at study entry (≤16 wk). All differences were significant at $P < 0.0001$ (paired Student's t test), except for α-linolenic acid.

[3] Ratio of docosahexaenoic acid to docosapentaenoic acid.

[4] Ratio of Σn−3 + n−6 to Σn−7 + n−9 fatty acids.

retardation affected both EFA concentrations and intrauterine growth in these populations.

Another factor that might play a role is gestational age at birth. Both birth weight and the biochemical EFA status of newborns are related to the duration of gestation (24, 25). No adjustment for differences in gestational age at birth might therefore have confounded some of the previously reported associations. When birth weights were interpreted in relation to gestation duration in comparisons of SGA with AGA or LGA infants, no differences in plasma or vessel wall AA concentrations were found by several investigators (7, 8, 26, 27). In one study, the reported relative DHA concentration was even significantly higher in SGA babies (7). However, most of these findings were based on observations in relatively small numbers of infants.

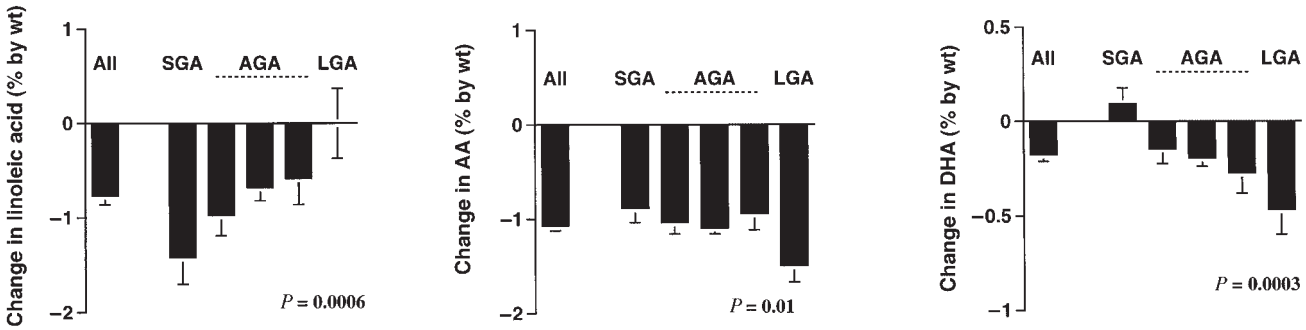

FIGURE 2. Mean (±SEM) changes in linoleic acid (18:2n−6), arachidonic acid (AA; 20:4n−6), and docosahexaenoic acid (DHA; 22:6n−3) concentrations in maternal plasma phospholipids during pregnancy for the total study group and according to infant birth weight, ie, weight-for-gestational-age category. The categories are small for gestational age (SGA; birth weight ≤10th percentile), appropriate for gestational age (AGA; divided into 3 subcategories: *1*) birth weight >10th but ≤25th percentile, *2*) >25th but <75th percentile, and *3*) ≥75th but <90th percentile), and large for gestational age (LGA; ≥90th percentile). All percentiles were based on Dutch reference standards (appropriate for length of gestation, infant sex, and birth order) (16). *P* values shown are for linear trends with the continuous variable weight SD score.

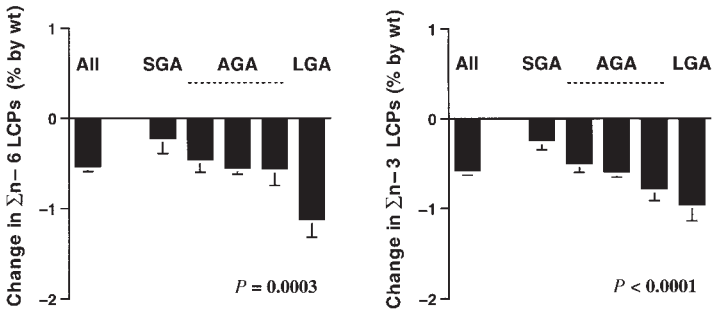

FIGURE 3. Mean (±SEM) changes in n−6 long-chain polyenes (Σn−6 LCPs) and n−3 long-chain polyenes (Σn−3 LCPs) in maternal plasma phospholipids during pregnancy for the total study group and according to infant birth weight, ie, weight-for-gestational-age category. The categories are small for gestational age (SGA; birth weight ≤10th percentile), appropriate for gestational age (AGA; divided into 3 subcategories: *1*) birth weight >10th but ≤25th percentile, *2*) >25th but <75th percentile, and *3*) ≥75th but <90th percentile), and large for gestational age (LGA; ≥90th percentile). All percentiles were based on Dutch reference standards (appropriate for length of gestation, infant sex, and birth order) (16). *P* values shown are for linear trends with the continuous variable weight SD score.

AA and DHA were also shown to be related to postnatal growth. During the first year after birth, negative effects of fish-oil-supplemented formula (rich in DHA and EPA) on infant growth were described in premature infants (28). A reduction in the AA status was regarded as a causative factor in the observed growth restriction (29). However, some intervention studies in term neonates found no such effect of DHA supplementation (with or without AA) on postnatal growth (30–33). To our knowledge, no studies of the effects of formulas supplemented with AA alone on infant growth have been conducted. A potential effect of AA and DHA on neonatal growth, therefore, is still controversial.

In contrast with AA and DHA concentrations, concentrations of dihomo-γ-linolenic acid were positively related to normalized birth weight (Table 4). Lower concentrations of dihomo-γ-linolenic acid in the blood or vessel walls of smaller than of larger neonates were reported previously and were also found in premature infants (6–8, 26, 27, 34). The positive association between dihomo-γ-linolenic acid concentration and size at birth seems to be more consistent than that reported for AA or DHA. Therefore, dihomo-γ-linolenic acid may be more important for intrauterine growth than is AA. No study has yet evaluated the effect of dihomo-γ-linolenic acid supplementation on intrauterine or postnatal growth.

Maternal-to-fetal EFA supply

The n−3 and n−6 long-chain PUFAs (especially AA and DHA) are important structural and functional components of cell membranes. Therefore, a larger infant probably accretes more of these substances than does a smaller one. Because the fetal capacity to convert linoleic acid and α-linolenic acid into LCPs is limited (35–38), most of these LCPs are obtained from the maternal circulation via the placenta. The observation that umbilical cord plasma EFA concentrations are positively associated with both maternal plasma EFA concentrations and maternal dietary EFA intake (14) supports this notion.

In a subgroup of the participating women, previously published information on the dietary intake of fatty acids (14, 15) was available (based on food-frequency questionnaires and dietary history). The most important finding was a relatively high intake of linoleic acid (±6% of total energy intake and ±85% of total PUFA intake). Such a high linoleic acid intake might explain the low α-linolenic acid concentrations found in umbilical cord plasma. Indeed, the dietary ratio of linoleic acid to other PUFAs (mainly α-linolenic acid) was significantly lower in the mothers of infants in whom α-linolenic acid could be detected than in the mothers of infants with undetectable amounts of α-linolenic acid ($P < 0.05$). Moreover, higher maternal plasma α-linolenic acid concentrations were found in the mothers of infants with detectable α-linolenic acid concentrations. The maternal intake of fatty acids, however, did not differ significantly between the weight-for-gestational-age groups (data not shown).

The current finding that decreases in maternal plasma n−3 and n−6 LCP fractions were more pronounced in women who gave birth to larger infants (Figures 2 and 3) implies that the EFA transfer from the mother to the fetus is related to fetal growth. It is possible that larger infants are born when the maternal-to-fetal transfer of LCPs is more efficient. However, an increased LCP transfer could also be an adaptation to an increased fetal LCP accretion. Because umbilical cord plasma EFA concentrations are positively related to maternal plasma EFA concentrations, whereas birth weights of infants are not, the latter explanation seems more likely.

The observed lower relative concentrations of AA and DHA, higher concentrations of plasma eicosatrienoic acid (Table 4), and lower EFA-status and DHA-status indexes (Figure 1) in the plasma of heavier neonates suggest that the maternal-to-fetal supply of EFAs is limited. It seems that even an increased maternal-to-fetal LCP flux cannot prevent a lower biochemical EFA status in the plasma of heavier neonates. We showed previously that the biochemical EFA status determined on the basis of EFA concentrations in the cord plasma and vessel walls of twins and triplets is lower than that observed in singletons (39, 40). These observations seem to support the concept that a larger total fetal tissue mass is related to an increased EFA accretion and the idea that the supply of EFA is limited. However, these hypotheses are based on observed associations and further evidence is needed to validate them. Studies using more advanced techniques, such as stable isotopes, are needed to evaluate complex dynamic processes like LCP accretion and placental transport efficiency.

The pregnancy-associated decrease in linoleic acid concentrations was more pronounced in the mothers of smaller infants than in the mothers of larger infants (Figure 2). The reason for this is not clear. Relative linoleic acid concentrations tend to remain stable until the end of pregnancy and the decrease shown

in Figure 2 occurs mainly around the time of delivery (12). In contrast, the observed decreases in the fractions of AA and DHA start before 16 and 22 wk of gestation, respectively (12). Thus, it seems unlikely that these opposite patterns of observed decreases are directly related, eg, because of competitive or selective placental transfer of fatty acids.

Nutritional sufficiency of EFA status in the plasma of term neonates

Although low EFA concentrations did not seem to be associated with limited growth in the present study, the specific neonatal demand for EFAs may not have been met (41, 42). We are presently conducting a long-term follow-up study of the children born during our studies to investigate potential functional consequences of early EFA status in later life. Without such information, statements about the nutritional sufficiency of the EFA status of term infants, on the basis of EFA concentrations found in umbilical cord plasma, remain speculative.

In summary, under the present dietary conditions, EFAs such as AA and DHA do not seem to be important determinants of fetal growth in term neonates. The biochemical EFA status measured in umbilical cord plasma of term neonates is even negatively associated with size at birth. This lower EFA status in the plasma of heavier infants occurred despite a larger decrease in LCP fractions in maternal plasma. These findings suggest that the maternal-to-fetal EFA transfer capacity is a limiting factor in determining neonatal EFA status. However, the implications of these findings for mothers and children are not known and remain to be investigated.

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