Early Human Development 85 (2009) 525–530

Contents lists available at ScienceDirect

Early Human Development

journal homepage: www.elsevier.com/locate/earlhumdev

Associations between term birth dimensions and prenatal exposure to essential and *trans* fatty acids

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ARTICLE INFO

Article history:
Received 19 December 2008
Received in revised form 1 May 2009
Accepted 1 May 2009

Keywords:
Birth outcome
Neonatal long-chain polyunsaturated fatty acids
Neonatal *trans* fatty acids
Neonatal plasma
Umbilical arteries
Umbilical vein

ABSTRACT

Background: Certain essential long-chain polyunsaturated fatty acids (LCPUFAs) are considered important for fetal growth and brain development, whereas industrial *trans* fatty acids (mainly 18:1*trans*) have been associated with negative effects. The aim of this study was to investigate associations between term birth dimensions and prenatal exposure to some of these fatty acids, reflected by neonatal fatty acid concentrations at birth.
Methods: Data of up to 700 infant–mother pairs from the Maastricht Essential Fatty Acid Birth Cohort were used for the present study. Unadjusted and multivariable-adjusted linear regression analyses were performed to investigate associations between birth weight, birth length or head circumference and relative concentrations of docosahexaenoic acid (DHA), arachidonic acid (AA), dihomo-γ-linolenic acid (DGLA) and *trans*-octadecenoic acids (18:1*t*) measured in phospholipids of the walls of umbilical arteries and veins, and in umbilical cord plasma and erythrocytes.
Results: After optimal adjustment, a significant negative association was observed between birth weight and umbilical plasma DHA concentrations. Negative associations were also found for AA concentrations measured in umbilical plasma and in arterial and venous vessel walls. Birth length was negatively related to arterial vessel wall AA concentrations only. A significant negative association was observed for the relationship between 18:1*t* in cord erythrocytes and birth weight. For DGLA no significant associations were observed.
Conclusions: Results seem to preclude a role of DHA and AA as growth factors *per se*. Their negative relationships with birth dimensions may result from a limited maternal–fetal LCPUFA transfer capacity. Potential effects of 18:1*t* and DGLA on birth dimensions are probably small or non-existing.

1. Introduction

The essential long-chain polyunsaturated fatty acid (LCPUFA) docosahexaenoic acid (DHA) is an important fetal nutrient, given its active accumulation in the developing brain and retina during the last trimester of gestation [1,2]. Recent research also indicates maternal n-3 LCPUFAs to be important for the programming of fetal growth [3,4]. The role of another LCPUFA, arachidonic acid (AA), in early growth is not clear, since both promoting [5,6] as well as limiting [3,4] potentials have been suggested. Although causality of these associations has not yet been ascertained, it seems important to optimize the neonatal LCPUFA status, since low birth weight is thought to be associated with later occurrence of coronary heart disease and other chronic illnesses [7].

Dietary *trans* fatty acids, mainly formed during industrial hydrogenation of unsaturated edible oils, may interfere with the conversion of the parent essential fatty acids (EFAs) into their LCPUFAs, especially when the parent EFA concentrations are low [8,9]. In this way, *trans* fatty acids may lower the fetal LCPUFA status and, thereby, affect fetal development.

Rump et al. demonstrated that birth weight of term neonates was negatively related to DHA and AA concentrations in umbilical cord plasma phospholipids (PLs), whereas it was positively associated with concentrations of dihomo-γ-linolenic acid (DGLA), the precursor of AA [10]. In contrast, Elias and Innis, observed positive associations between neonatal triacylglycerol AA concentrations and birth weight and birth length, and between cholesteryl ester AA concentrations and birth weight [11]. In addition, no significant associations were found between neonatal plasma *trans* fatty acid concentrations and birth weight and birth length. However, the applicability of this latter study is limited since no corrections were made for relevant covariables. Moreover, Rump et al. restricted their study to birth weight and to cord plasma polyunsaturated fatty acids and did not consider *trans* fatty acids [10]. In addition, they

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doi:10.1016/j.earlhumdev.2009.05.001

only partially adjusted for potential confounders. Therefore, the present study was conducted to refine and extend these findings with additional birth outcome variables, fatty acid domains and relevant covariables.

2. Subjects and methods

2.1. General design of the study

According to pre-specified inclusion criteria (see below), information with respect to birth outcome variables, fatty acid concentrations and clinical characteristics of eligible infants and their mothers was taken from the database of the Maastricht Essential Fatty Acid Birth Cohort (MEFAB database, [3]). Associations between selected fatty acid concentrations in PLs of the walls of umbilical arteries and veins, and of umbilical cord plasma and erythrocytes (independent variables) and birth weight, birth length or head circumference (dependent variables) were studied by unadjusted and multivariable-adjusted linear regression analyses.

2.2. Inclusion of participants

The study was executed with data of pregnant women and their newborns who participated in several observational studies, conducted in our institute between 1990 and 1997 in The Netherlands [12,13]. Approval for these studies was obtained from the Medical Ethics Committee of the University Hospital Maastricht and the University of Maastricht, and all participating women gave their written informed consent. Data of infant–mother pairs were included in the present study if the infants were born at term to healthy Caucasian parents after an uncomplicated pregnancy, as detailed before [3]. In addition, PL fatty acid profiles of the four neonatal lipid domains needed to be available. This resulted in 703, 158, 484 and 479 cases available for regression analyses related to cord plasma, erythrocytes, arterial and venous walls, respectively.

2.3. Birth dimensions and fatty acid analyses

Birth weight, birth length and head circumference were recorded directly after birth on standardized sheets. Directly after delivery, umbilical cord blood samples were collected, plasma was separated from erythrocytes by centrifugation and after plasma collection the erythrocytes were washed with physiological saline. Furthermore, a piece of umbilical cord was collected and rinsed with saline (NaCl, 0.9% w/v). Plasma, erythrocytes and umbilical tissue samples were stored under nitrogen at −80 °C until analysis. The fatty acid composition of PLs isolated from plasma, erythrocytes and cord vein and artery walls were determined as described earlier [14,15]. Separation of the various *trans* isomers of octadecenoic acid (C18:1) was incomplete; therefore, they are reported together as 18:1*t*. Fatty acids are expressed as relative contents (% by wt of total amount of identified fatty acids).

2.4. Covariables

For reasons explained in the respective references provided below, the following covariables were included in the multivariable-adjusted analyses as potential confounding factors: maternal age [16], height and body mass index (BMI, weight (kg)/height (m)^2) at study entry [17], parity [18], smoking and drinking during pregnancy [19], weight increase during pregnancy [20], socio-economic status (income) [21], gestational age [17] and infant sex [22]. Methodological details have been described in detail before [3].

2.5. Statistical analyses

Before starting the analyses, outliers (±4 SD outside the mean) of the dependent variables were removed and the normality of their distributions was checked and confirmed by histograms. Thereafter, associations between various birth outcome measures (the dependent variables; birth weight, birth length and head circumference) and the neonatal fatty acid concentrations of interest, *viz.* DHA, AA, DGLA and 18:1*t*, were analyzed with unadjusted and multivariable-adjusted linear regression analyses. The unadjusted analyses were performed with the same subjects as included in the corresponding multivariable-adjusted analyses. Because of occasionally missing observations, this limited the number of cases for analysis. Therefore, to increase the number of available cases, irrelevant covariables were removed by stepwise backward multivariable-adjusted regression analyses, performed for each fatty acids–birth outcome combination. This procedure has been described in detail before [3] and the successive steps were continued until all remaining covariables were either significant or were characterized as confounders. For each particular combination of fatty acids and birth outcome, these various steps were performed with the same dataset. However, since removal of the irrelevant covariables implied less missing values and, consequently, a larger number of cases available for analysis, the ultimate regression analyses were finally repeated with the maximum number of complete cases available for each combination. To check whether the relationships between the dependent and independent variables were comparable for the added cases and the initial study population (a prerequisite for acceptance of this procedure), interaction analyses were performed as detailed before [3]. If the added cases were significantly different from the initially included ones, the final model with the larger number of cases could not be accepted. Since these interaction analyses revealed no significant differences between initial and additional cases, all final backward models could be approved.

To determine possible influential cases in the regressions, all data points were checked by calculating their Cook's distance and removed if values were ≥1. Such influential data points were not observed, however.

Associations with *p*-values <0.010 were considered statistically significant, to correct for multiple testing. A *p*-value <0.050 was regarded to indicate a (non-significant) trend. Variables are reported as median (25th–75th%), unless specified otherwise. All statistical analyses were performed using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

Table 1 shows the maternal and infant characteristics of the included participants. The relative concentrations (% wt/wt) of DHA,

Table 1
Subject characteristics.

	n	
Maternal characteristics		
Age (yrs)	730	29.0(26.1–31.7)
Height (cm)	698	167(162–170)
BMI at study entry (kg/m^2)	686	22.8(20.9–25.1)
Parity (*n*) 0/1/≥2	730	551/151/28
Weight increase during pregnancy (kg)	687	11.8(9.3–14.5)
Socio-economic status (income class)[a]	576	3(2–3)
Smoking during pregnancy (*n*) no/yes	726	527/199
Alcohol during pregnancy (*n*) no/yes	727	706/21
Infant characteristics		
Gestational age (weeks)	730	40.1(39.3–41.1)
Sex (*n*) male/female	730	392/338
Birth weight (g)	728	3323(437)
Birth length (cm)	629	50.0(2.2)
Head circumference (cm)	554	34.2(1.6)

Birth weight, birth length and head circumference were normally distributed and are therefore expressed as mean ± SD. The distributions of the other characteristics were not checked for normality and are, therefore, given as median (25th–75th%).

[a] Ranges from minimum (5) to ≥2×modal (1).

Table 2
Relative concentrations (% wt/wt) of fatty acids of interest in neonatal PLs collected in several domains directly after birth.

Fatty acid	*n*	Umbilical plasma	*n*	Arterial wall	*n*	Venous wall	*n*	Erythrocytes
DHA	703	6.07(5.11–7.14)	484	5.11(4.50–5.63)	479	4.89(4.37–5.50)	158	4.65(4.18–5.25)
AA	703	16.99(15.87–18.03)	484	13.68(12.46–15.35)	479	18.19(17.16–19.28)	158	14.23(13.45–14.85)
DGLA	703	5.10(4.60–5.67)	483	1.26(1.09–1.44)	479	1.86(1.62–2.12)	158	2.40(2.11–2.69)
18:1*t*	401	0.11(0.08–0.14)	329	0.14(0.12–0.17)	328	0.10(0.08–0.12)	116	0.10(0.07–0.13)

The relative fatty acid results are expressed as median (25th–75th%). PLs, phospholipids; AA, arachidonic acid; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; 18:1*t*, 18:1*trans* isomers.

AA, DGLA and 18:1*t* in plasma, erythrocyte and umbilical tissue PLs are given in Table 2. Outcomes of the regression analyses are shown in Tables 3–5. To reduce the complexity of these tables, only results are shown for those relationships in which significant associations and/or trends were found in either the unadjusted, multivariable-adjusted or final backward analyses. Full results are available on request.

3.1. Relationship between neonatal 18:1t concentrations and birth dimensions

Unadjusted regression analyses revealed no significant associations between neonatal 18:1*t* concentrations in the four umbilical cord domains and the birth dimensions. However, after full adjustment a significant negative association was observed for the relationship between neonatal 18:1*t* in erythrocyte PLs and birth weight ($p=0.009$). The complete model explained 39.2% of the variability in birth weight ($R^2=.392$), 6% of which was contributed by 18:1*t* ($r^2=.060$). In the final backward model the association remained significant and was of the same order of magnitude ($p=0.004$, $R^2=.334$, $r^2=.058$). No other associations or trends were found between 18:1*t* concentrations and birth dimensions.

3.2. Relationship between neonatal DHA concentrations and birth dimensions (Table 3)

After adjustment for all covariables, a significant negative association was found for the relationship between DHA concentrations in umbilical plasma PLs and birth weight. After removal of irrelevant covariables by the stepwise backward procedure, this association remained significant and the model explained 40.8% of the variability in birth weight, 3.1% of which was contributed by DHA.

For DHA in umbilical vein wall PLs, unadjusted regression analyses revealed a positive trend with birth length. However, this trend disappeared after full adjustment and after correction for the relevant covariables only.

With respect to DHA in erythrocyte PLs, a negative trend was found in the final backward model for birth weight.

Other associations between birth dimensions and neonatal DHA concentrations were not significant and no additional trends were observed either.

3.3. Relationship between neonatal AA concentrations and birth dimensions (Table 4)

For umbilical plasma PL AA concentrations, a significant negative relationship with birth weight was observed in unadjusted regression analyses. This negative association remained significant after correction for all covariables. The complete model explained 42.4% of the variability in birth weight and the contribution of AA was 2.4%. After removal of the irrelevant covariables, the final model explained 40.8% of the variability in birth weight, and the contribution of AA (1.7%) remained significant.

Although unadjusted regression analyses did not demonstrate any significant relationship between AA concentrations measured in arterial wall PLs and birth outcome variables, adjustment for covariables revealed significant negative associations between neonatal AA levels and birth weight and birth length. The final multivariable models (with relevant covariables included only) explained 43.3 and 32.9% of the variability in birth weight and birth length, respectively. The contributions of AA were 4.8% (for birth weight) and 2.3% (for birth length). For the association between arterial wall AA concentrations and head circumference, a negative trend was found after full adjustment. The complete model explained 29.2% of the variability in head circumference with an almost significant contribution of 2.9% of AA. After adjustment for only the relevant covariables results remained the same.

Table 3
Unadjusted and multivariable-adjusted (backward) regression analyses of the relationships between birth outcome variables and DHA concentrations in PLs, collected in different neonatal fatty acid umbilical domains directly after birth.

Umbilical cord domain	Birth outcome	Unadjusted (no covariables included)				Multivariable-adjusted model (all covariables included)[a,b]				Final multivariable-adjusted backward model with (relevant covariables included only)[a]						
		n	R^2	*B*	*p*	R^2	*B*	r^2	*p*	*n*	R^2	*B*	r^2	p	95% CI (B)	
															Low	High
Plasma	BW[c]	291	.001	−8.767	.644	.424	−65.21	.033	*.000*	366	.408	−63.23	.031	*.000*	−92.13	−34.32
Venous wall	BL[d]	189	.028	0.392	.021	.331	0.215	.004	.294	252	.334	0.225	.005	.180	−0.105	0.555
Erythrocytes	BW[e]	90	.001	−15.66	.762	.392	−65.13	.015	.188	110	.334	−93.82	.030	.036	−181.6	−6.071

BW, birth weight; BL, birth length; HC, head circumference. R^2 = coefficient of determination of total model; r^2 = square of the semi-partial correlation coefficient of fatty acid concerned; *B* = regression coefficient of fatty acid of interest; *p* = *p*-value of fatty acid concerned; CI = Confidence interval.
[f]maternal height, BMI at study entry, parity, smoking during pregnancy, alcohol during pregnancy, weight increase during pregnancy, socio-economic status, gestational age.
[g]maternal height, alcohol during pregnancy, weight increase during pregnancy, gestational age, infant sex.
[h]maternal height, parity, gestational age, infant sex.
[i]maternal age, maternal height, BMI at study entry, parity, smoking during pregnancy, weight increase during pregnancy, socio-economic status, gestational age, infant sex.
[j]maternal age, maternal height, parity, gestational age, infant sex.
[k]maternal height, BMI at study entry, parity, alcohol during pregnancy, weight increase during pregnancy, infant sex.
Italic numbers refer to a significant relationship ($p<0.010$), $0.010\leq p<0.050$ refer to a non-significant trend.
[a] The total model *p*-values of the (final) multivariable-adjusted analyses were all <0.000, except for Erythrocytes-BL (.011 and .003, for respectively the multivariable and the backward model).
[b] Same cases as included in unadjusted model. Relevant covariables in final backward model.
[c] maternal height, BMI at study entry, parity, alcohol during pregnancy, weight gain during pregnancy, gestational age, infant sex.
[d] maternal height, alcohol during pregnancy, weight gain during pregnancy, gestational age, infant sex.
[e] gestational age, body mass index (BMI) at study entry, parity, and weight increase during pregnancy.

Table 4
Unadjusted and multivariable-adjusted (backward) regression analyses of the relationships between birth outcome variables and AA concentrations in PLs, collected in different neonatal fatty acid umbilical domains directly after birth.

Fatty acid domain	Birth outcome	Unadjusted (no covariables included)				Multivariable-adjusted model (all covariables included)[a,b]				Final multivariable-adjusted backward model with (relevant covariables included only)[a]						
		n	R^2	*B*	*p*	R^2	*B*	r^2	*p*	*n*	R^2	*B*	r^2	*p*	95% CI (B)	
															Low	High
Plasma	BW[c]	291	.038	−48.02	*.001*	.424	−44.63	.024	*.001*	366	.408	−36.89	.017	*.002*	−59.86	−13.93
Arterial wall	BW[f]	225	.010	−20.91	.127	.440	−61.97	.042	*.000*	225	.433	−65.52	.048	*.000*	−96.09	−34.95
	BL[g]	187	.002	−0.051	.502	.357	−0.295	.036	*.002*	251	.329	−0.232	.023	*.005*	−0.392	−0.072
	HC[h]	166	.016	−0.093	.100	.292	−0.187	.029	.014	226	.246	−0.157	.021	.015	−0.284	−0.030
Venous wall	BW[i]	226	.021	−28.00	.030	.447	−49.81	.040	*.000*	226	.440	−50.54	.041	*.000*	−75.87	−25.20
	HC[j]	168	.024	−0.110	.047	.274	−0.135	.021	.040	228	.244	−0.128	.017	.026	−0.241	−0.015
Erythrocytes	BL[k]	71	.083	−0.485	.015	.374	−0.571	.064	.021	87	.286	−0.436	.043	.035	−0.841	−0.031

For explanations of symbols, see Table 3.

In unadjusted regression analyses negative trends were observed for the associations between neonatal AA concentrations measured in umbilical vein PLs and birth weight and head circumference. After entering all covariables, these trends became clearly stronger but only for the association with birth weight it became significant. The complete model explained 44.7% of the variability in birth weight and the contribution of AA was 4.0%. In the final backward model, results were comparable.

In erythrocyte PLs, only a trend was observed between neonatal AA concentrations and birth length in the unadjusted and multivariable-adjusted models, which remained after the stepwise backward procedure.

No other associations between birth dimensions and neonatal AA concentrations showed trends or were significant.

3.4. Relationship between neonatal DGLA concentrations and birth dimensions (Table 5)

In unadjusted analyses only a positive trend was found for the association between birth weight and DGLA concentrations measured in plasma PLs. After adjustment for all covariables as well as for the relevant covariables only, this trend was lost.

In arterial walls, positive trends were observed for the DGLA associations with birth weight and birth length in the multivariable-adjusted models. Only the trend for birth weight remained after removal of irrelevant covariables by backward regression analysis.

Other associations between birth outcome variables and neonatal DGLA concentrations were not observed.

4. Discussion

In the present study, we investigated in an infant–mother birth cohort the associations between several birth dimensions and selected fatty acid concentrations in four neonatal domains. These fatty acids are considered to reflect the prenatal exposure of the fetus, since it was observed that fetuses do not possess a different EFA status than infants directly after birth at a comparable gestational age [23]. For DGLA no significant associations were observed. Umbilical plasma PL DHA and AA concentrations appeared negatively related to birth weight, just like AA concentrations in the PLs of arterial and venous umbilical walls. For umbilical erythrocyte PL concentrations of 18:1*t*, the main industrially produced *trans* unsaturated fatty acid present in the diet, also a significant negative association was found with birth weight. Birth length was only significantly associated (in a negative way) with AA concentrations in cord artery wall PLs.

For AA these results are not in line with the general opinion that this LCPUFA stimulates fetal growth. However, this opinion is based on studies in *preterm* infants [5,6]. Studies in *term* babies gave inconsistent results [10,11]. Thus, Elias and Innis observed no significant associations between plasma PL AA concentrations and birth weight and birth length [11]. Interestingly, significant *positive* associations were found between infant plasma cholesteryl ester AA and birth weight and between infant triacylglycerol AA and birth weight and birth length. For the other LCPUFAs no significant results were seen. However, in that study no corrections were made for potential confounders, which could have influenced the study outcomes. Rump et al. observed significant negative relations between AA and DHA concentrations in umbilical cord plasma PLs and birthweight in their study [10]. These results are in line with our findings, which could be expected since we used the same database (although extended) as Rump et al. However, we included the fatty acids of interest simultaneously, which enabled an additional correction for their mutual interactions (see below). Furthermore, we included more potential confounders and our analyses were broadened by including fatty acids measured in different umbilical domains.

In the present study, 18:1*t* levels in neonatal PLs (mainly elaidic acid, but including some minor positional isomers also) were negatively associated with most LCPUFA concentrations (data not shown, but available on request). This confirms earlier results obtained by us and others [8,11,24,25] and moreover demonstrates that, although *trans* fatty acid concentrations are relatively low in PLs, they are high enough for reliable correlation studies. Furthermore, other studies observed that *trans* fatty acid values in PLs exhibit, in general, the same relationships as the *trans* fatty acid values measured in triacylglycerols. Moreover, these studies also showed that *trans* fatty acid values in PLs and triacylglycerols are also strongly correlated [26,27]. Interestingly, the neonatal *trans* status was hardly associated with birth dimensions, since

Table 5
Unadjusted and multivariable-adjusted (backward) regression analyses of the relationships between birth outcome variables and DGLA concentrations in PLs, collected in different neonatal fatty acid umbilical domains directly after birth.

Fatty acid domain	Birth outcome	Unadjusted (no covariables included)				Multivariable-adjusted model (all covariables included)[a,b]				Final multivariable-adjusted backward model with (relevant covariables included only)[a]						
		n	R^2	*B*	*p*	R^2	*B*	r^2	*p*	*n*	R^2	*B*	r^2	*p*	95% CI (B)	
															Low	High
Plasma	BW[c]	291	.014	56.10	.047	.424	9.494	.000	.715	366	.408	8.830	.000	.704	−36.77	54.43
Arterial wall	BW[f]	225	.009	151.9	.157	.440	220.9	.012	.036	225	.433	220.6	.012	.036	14.24	426.9
	BL[g]	187	.012	0.874	.135	.357	1.304	.017	.034	251	.329	0.968	.009	.069	−0.076	2.012

For explanations of symbols, see Table 3.

in our multivariable-adjusted analyses only one significant (negative) association was observed (between 18:1*t* in erythrocyte PLs and birth weight). In the uncorrected study of Elias and Innis, no associations were found between *trans* fatty acids of term infants and birth weight and birth length either [11]. In their study, *trans* fatty acids were measured in triacylglycerols, cholesterol esters and PLs of umbilical cord plasma. On the other hand, Koletzko observed significant negative associations between birth weight and *trans* fatty acid concentrations measured in various neonatal plasma lipid fractions, but not in triacylglycerols [8]. However, the latter study was performed in *preterm* infants, which may explain the difference with the results of Elias and Innis. Furthermore, in both studies no corrections were made for any possible confounder. Van Houwelingen and Hornstra observed significant negative correlations between 18:1n-9*t* concentrations in umbilical artery wall PLs and birth weight and head circumference, but this was a relatively small study ($n=37$) and correction for potential confounders was incomplete [24]. Also several animal studies have been reported and, overall, no adverse effects of *trans* fatty acids on fetal growth were observed [28,29]. These rather inconsistent results indicate that under the dietary conditions tested, any potential effect of dietary *trans* unsaturated fatty acids on birth outcome is either small or non-existing and appears largely independent of the lipid fractions the *trans* fatty acids were determined in. In our neonates, the plasma PL-*trans* content was considerably lower than that of some other study populations [11,25], indicating a lower *trans*-unsaturated fatty acid consumption of their mothers [30]. Therefore, an impact of maternal *trans* consumptions during pregnancy on birth dimensions of the offspring cannot be excluded in populations with a higher habitual *trans* unsaturated fatty acid intake.

A strong aspect of our study is that fatty acid concentrations were measured in several umbilical cord domains, reflecting the fetal LCPUFA status over various gestational periods. Thus, cord plasma PLs reflect the LCPUFA availability at the very end of gestation, whereas the vessel wall and erythrocyte PLs, with presumably a lower turnover than plasma PLs [31], represent a longer-term reflection of the fetal LCPUFA status during pregnancy. Furthermore, use of the MEFAB database enabled the selection of a relatively large number of neonatal and maternal covariables. On the other hand, this was an observational study and, therefore, residual confounding cannot be excluded.

Relative high drop-out rates in cohort studies due to missing values can bias the results. The high drop-out rate in the present study, especially in the erythrocytes domain, is mainly the consequence of the lower number of fatty acid analyses performed for this domain. However, no major effects of this high drop-out rate on the results were observed for the mean birth dimensions and the median fatty acid values. Even for the maximum drop-out rate the group means of the three birth dimensions differed less than 3% (ranging from 0.9 to 2.7%) for the cases that did or did not participate in the regression analyses. It is unlikely that these small birth outcome differences are of clinical relevance. For the fatty acids DGLA, AA, DHA and 18:1*t* these differences were between 2.5 and 4.4%, averaged over the four fatty acid domains. These differences are relatively small and well within the reproducibility range of our fatty acid analysis (in plasma PL, coefficients of variations were 11.7, 6.1, 9.0 and 29.9% for DGLA, AA, DHA and 18:1*t*, respectively).

As mentioned before, we included all four selected fatty acids in the regression models simultaneously, thereby taking into account their mutual metabolic interactions [32,33]. Furthermore, fatty acid contents are reported in relative concentrations and any change in the concentration of one fatty acid will result in a change in the relative concentrations of the other fatty acids included in the analysis. Although correlation coefficients between the selected fatty acids in the several umbilical domains varied between 0.082 and 0.796, the multicollinearity checks revealed a tolerance value of >0.1 and a variance inflation factor of <10, which allowed us to make this decision [34].

Fetuses depend on their mothers to obtain the LCPUFAs needed for optimal development, as is supported by the positive correlations between maternal and fetal LCPUFA concentrations during pregnancy [23,35] and between maternal and neonatal LCPUFA concentrations at birth [13,36,37]. Therefore, maternal plasma fatty acids measured during pregnancy can be taken to reflect the LCPUFA status of the children during the prenatal period also. In two previous studies, associations between maternal fatty acid concentrations, measured during pregnancy, and birth weight, birth length or head circumference have been investigated [3,4]. In both studies significant negative associations were observed between maternal AA concentrations and some birth outcomes, which is in line with results of the present study. For DHA concentrations, however, contrasting study results were found when birth dimensions were related to maternal (positive associations) or to neonatal fatty acid levels (negative association). As mentioned before, the negative associations for AA and DHA observed in the present neonatal study are in agreement with the findings of Rump et al. [10]. They also observed that the well-known decreases in maternal plasma n-3 and n-6 LCPUFA concentrations during pregnancy [38] were more pronounced in women who gave birth to heavier infants. Despite this larger LCPUFA supply, relative concentrations of the ePUFA shortage marker Mead Acid (MA, 20:3n-9) were higher in plasma of heavier neonates, and values for the EFA- and DHA-status indices were lower [10]. Thus, although larger fetuses are provided with more LCPUFAs than smaller ones, the placental LCPUFA transfer capacity appears insufficient to fully meet the increased requirements of higher fetal mass. As a result, a larger neonate might have less LCPUFAs available per mass unit than a smaller neonate. Further studies are needed to test this hypothesis.

In the present study the unadjusted and multivariable-adjusted analyses were performed with the same number of complete cases (all (co)variables available). In general, stronger associations were observed when unadjusted regression analyses were executed with the maximum number of cases available, thereby increasing the power of the analyses. For DGLA in cord plasma PLs this resulted in a significant positive association ($p=0.001$) with birth weight, as observed before by Rump et al. [10]. In addition, birth length appeared negatively associated with AA in cord plasma PLs ($p<0.000$). No significant associations were observed in the unadjusted analyses of the relationships between neonatal DHA concentrations and birth weight, but significant positive relationships were found with birth length ($p<0.005$ for 3 lipid domains). Although this is in line with the positive trend we observed in the initial unadjusted regression analysis for vein wall DHA (see Table 3), this trend disappeared after adjustment for relevant covariables. Therefore, it seems rather unlikely that DHA has a promoting effect on birth length. Increasing the power of the unadjusted regression analyses by including all available cases did not result in other major differences (results not shown).

In conclusion, DHA and AA concentrations measured in various umbilical domains and considered to reflect fetal LCPUFA availability during late gestation are mainly negatively related to birth weight and birth length. Although these fatty acids, as essential membrane constituents, are required to allow fetal growth to take place, our results seems to preclude their role as growth factors *per se*. The negative relationships observed may result from a limited maternal–fetal LCPUFA transfer capacity. Associations with birth dimensions are weak or non-existing for 18:1*t* and DGLA. However, for populations with a high habitual *trans* consumption it seems prudent to further investigate the potential role of *trans* fatty acids on birth outcome in these populations.

Conflict of interest statement

GH was the recipient of a project grant of the Malaysian Palm Oil Board, Kuala Lumpur, Malaysia, to perform the study. None of the authors had any personal or financial conflicts of interest.

Acknowledgements

This study was financially supported by the Malaysian Palm Oil Board, Kuala Lumpur, Malaysia. The study sponsor had no involvement

in the study design, in the collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.

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