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Associations between neonatal birth dimensions and maternal essential and *trans* fatty acid contents during pregnancy and at delivery

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Since birth dimensions have prognostic potential for later development and health, possible associations between neonatal birth dimensions and selected maternal plasma fatty acid contents were investigated, using data from 782 mother–infant pairs of the Maastricht Essential Fatty Acid Birth cohort. Unadjusted and multivariable-adjusted regression analyses were applied to study the associations between birth weight, birth length or head circumference and the relative contents of DHA, arachidonic acid (AA), dihomo-γ-linolenic acid (DGLA) and 18 : 1*trans* (18 : 1*t*) in maternal plasma phospholipids sampled during early, middle and late pregnancies, and at delivery. Where appropriate, corrections were made for relevant covariables. Significant 'positive' associations were observed between maternal DHA contents (especially early in pregnancy) and birth weight ($B = 52{\cdot}10$ g, 95 % CI 20·40, 83·80) and head circumference ($B = 0{\cdot}223$ cm, 95 % CI 0·074, 0·372). AA contents at late pregnancy were 'negatively' associated with birth weight ($B = -44{\cdot}25$ g, 95 % CI −68·33, −20·16) and birth length ($B = -0{\cdot}200$ cm, 95 % CI −0·335, −0·065). Significant 'negative' associations were also observed for AA contents at delivery and birth weight ($B = -27{\cdot}08$ g, 95 % CI −47·11, −7·056) and birth length ($B = -0{\cdot}207$ cm, 95 % CI −0·330, −0·084). Maternal DGLA contents at delivery were also significantly 'negatively' associated with neonatal birth weight ($B = -85{\cdot}76$ g, 95 % CI −130·9, −40·61) and birth length ($B = -0{\cdot}413$ cm, 95 % CI −0·680, −0·146). No significant associations were observed for maternal 18 : 1*t* contents. We conclude that during early pregnancy, maternal DHA content may programme fetal growth in a positive way. Maternal AA and DGLA in late pregnancy might be involved in fetal growth limitation.

Birth outcome: Maternal long-chain PUFA: Maternal *trans* fatty acids

Increasing evidence suggests that birth dimensions have prognostic values for later development and health. Indeed, birth weight and birth length have been shown to be associated with later cardiovascular risk[1], whereas head circumference at birth seems a significant predictor of later intelligence[2]. Although the causality of these associations has not yet been demonstrated, fetal growth should be optimised.

The essential long-chain PUFA (LCPUFA), DHA (22 : 6*n*-3) and arachidonic acid (AA, 20 : 4*n*-6), are thought to be of critical importance for brain development and fetal growth, respectively[3,4]. Therefore, the availability of DHA and AA to the fetus needs to be adequate. To obtain these fatty acids, fetuses depend on their mothers, as is indicated by the positive correlation between the maternal and neonatal LCPUFA status at birth[5–7]. Mothers receive the LCPUFA mainly from their diet or synthesise them from their respective precursors[8].

Dietary *trans* fatty acids are unsaturated fatty acids with at least one double bond in the *trans* configuration. They mainly result from industrial hydrogenation of edible oils and cannot be synthesised by humans[9]. As is the case for LCPUFA, maternal and neonatal *trans* fatty acids are positively correlated[10,11], indicating that the neonatal *trans* status is also determined by the maternal *trans* intake. Dietary *trans* unsaturated fatty acids have been shown to inhibit the conversion of parent essential fatty acids into their LCPUFA, especially when the essential fatty acids contents are low[12,13]. They may also impair placental LCPUFA transfer[14]. Thus, *trans* fatty acids may lower the fetal LCPUFA status, and thereby they could compromise fetal development.

Recently, the term birth weight appeared to be positively associated with most maternal *n*-3 LCPUFA proportions early in pregnancy[15]. The relationship with maternal AA contents was found to be negative. Interestingly, maternal contents of the AA precursor dihomo-γ-linolenic acid (DGLA, 20 : 3*n*-6) is positively related to the term birth weight. The birth-weight relationship with elaidic acid (the main dietary

Abbreviations: AA, arachidonic acid; DGLA, dihomo-γ-linolenic acid; LCPUFA, long-chain PUFA; MEFAB, Maastricht Essential Fatty Acid Birth.

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trans fatty acid, 18 : 1*n*-9*trans*) was negative in unadjusted analyses, but this association lost significance after adjustment for covariables.

The aim of the present study was to extend and confirm these findings with three birth outcome measures (birth weight, birth length and head circumference at birth) and maternal plasma fatty acid proportions from four different sampling times (about 16, 22 and 32 weeks of pregnancy, and immediately upon delivery), available from a different birth cohort.

Subjects and methods

General design of the study

On the basis of distinct inclusion criteria, relevant data (dependent variables, independent variables and covariables) of eligible mothers and their infants were extracted from the database of the Maastricht Essential Fatty Acid Birth (MEFAB) cohort. This database contains the data of pregnant women and their newborns who participated in several observational studies, conducted in our institute between 1990 and 1997[6,16]. In short, three antenatal clinics located in The Netherlands participated and recruited pregnant women at their first antenatal medical visit. Selection criteria for inclusion were gestational age of <16 weeks at study entry, singleton pregnancy, Caucasian race, diastolic blood pressure <90 mmHg and the absence of any metabolic, cardiovascular, neurological or renal disorder at the time of recruitment. 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. Unadjusted and multivariable-adjusted linear regression analyses were conducted to study the associations between relative DHA, AA, DGLA and 18 : 1*trans* (18 : 1*t*) contents in phospholipids of maternal plasma, collected at approximately 16, 22 and 32 weeks of pregnancy, and directly after delivery (independent variables), and the birth outcome measures birth weight, birth length or head circumference (dependent variables). In multivariable-adjusted analyses, these relationships were corrected for relevant covariables.

Inclusion of participants

For the present study, clinical data of 1238 mothers and their infants were available in the MEFAB database. The mother–infant pairs were excluded if infants were born preterm (gestational age <37 weeks, *n* 90), mothers had diabetes (*n* 35) or developed pregnancy-induced hypertension (*n* 96), mothers had reported specific health problems in the past (e.g. diabetes mellitus, hypertension and heart, kidney, liver, gall bladder or thyroid gland disorders, *n* 128), one or both parents were non-Caucasians (*n* 40) or values for any of the afore-mentioned exclusion criteria were missing (*n* 73). The mother–infant pairs were also excluded if fatty acid analyses were not reported (*n* 148) or values were missing for birth weight, birth length and head circumference (*n* 3). After exclusion, the data of 782 mother–infant pairs were left for analysis.

Birth dimensions and fatty acid profiles

Local hospital staff members recorded birth weight, birth length and head circumference on standardised datasheets. Maternal venous blood samples were collected in EDTA tubes at about 16, 22 and 32 weeks of pregnancy, and immediately after delivery. The plasma was separated from the blood cells by centrifugation (2000 ***g***, 4°C, 15 min) and stored under nitrogen at -80°C until analysis. The fatty acid composition of plasma phospholipids was determined by GLC, as described previously[17]. The separation of the various 18 : 1*t* isomers was incomplete; therefore, they are reported together as 18 : 1*t*. The fatty acids are expressed as relative contents (percentage by weight of the total amount of identified fatty acids).

Covariables

Maternal age[18], height and BMI [weight (kg)/height (m^2)] at study entry[19], parity[20], smoking and drinking during pregnancy[21], weight increase during pregnancy[22], socio-economic status (income)[23], gestational age[19] and infant sex[24] were included in the multivariable-adjusted analyses as potential confounding factors. These data were retrieved from the original study questionnaires and medical records. In the multivariable-adjusted regression analyses, two dummy variables for parity were used, one for parity $=1$ and the other for parity ≥ 2, with parity $=0$ as a reference category. Smoking and drinking during pregnancy were both categorised as $0=$ no and $1=$ yes and infant sex as boy $=0$ and girl $=1$. Exact information on socio-economic status was not available. Therefore, parental socio-economic status was measured by proxy, using 'income' as an socio-economic status indicator, based on the parental postal code at the time of delivery (Geomarktprofiel; Wegener DM, Nieuwegein, The Netherlands). This information was classified into five groups ranging from 1 (twice or more modal income) to 5 (minimum income); socio-economic status values in the categories unknown (0) and diverse (6) were omitted and thus reported as missing values. Gestational age at birth was calculated from the self-reported first day of the last menstrual period. If the last menstrual period was uncertain, gestational age was based on early ultrasound measurements.

Statistical analyses

Associations between various birth outcome measures and the maternal fatty acid contents of interest were analysed with unadjusted and multivariable-adjusted linear regression analyses. In these analyses, birth weight, birth length and head circumference were the dependent variables and the relative proportions of the maternal fatty acids DHA, AA, DGLA and 18 : 1*t* measured in plasma phospholipids were the independent variables. In the multivariable-adjusted models, the four fatty acids were included simultaneously to allow for their usual metabolic interactions[3,8]. Furthermore, since fatty acids are reported in relative contents, any change in the proportion of one fatty acid will result in a change in the proportions of the other fatty acids included in the analysis. Maternal age, height, BMI at study entry, parity, weight increase during pregnancy, socio-economic status (income), smoking and drinking habits during pregnancy,

British Journal of Nutrition

gestational age and infant sex were included as potential confounders. 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.

Unadjusted regression analyses were performed with the same subjects as included in the corresponding multivariable-adjusted regression analyses. These twelve regression analyses (three dependent variables and fatty acid contents at four time points) were performed in subjects with complete datasets for all (co)variables included in each of the respective regression models. Because of occasionally missing observations, the number of cases for analysis was limited. Ultimately, to remove irrelevant covariables (and thereby increase the number of available cases, see later), stepwise-backward multivariable-adjusted regression analyses were performed for each of the twelve birth outcome-fatty acid combinations. Starting with the full multivariable-adjusted model, the covariable with the highest P value was removed. If this removal resulted in a change in the B value of 10 % or more for at least one of the four fatty acids included (DHA, AA, DGLA and 18 : 1*t*), and if this change amounted to 20 % or more of the standard error of this B value, then this variable was considered a confounder and retained in the regression model, even if it was not significant ($P \geq 0{\cdot}050$)[25]. If the removal of the covariable resulted in a smaller change in the B values for all the four fatty acids included and/or in their standard errors, then its removal was permanent. Subsequently, this procedure was repeated with the covariable with the next largest P value and so on, until the remaining covariables were either significant (in which case they are called 'predictors') or characterised as confounders. For each particular birth outcome-fatty acid combination, these various steps were performed with the same dataset. However, since the 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 that birth outcome-fatty acid combination.

To check whether the relationships between dependent and independent variables are comparable for the added cases and the initial study population (a prerequisite for acceptance of this procedure), interaction analyses were performed. To this end, two new variables were introduced into the final regression model. The first variable, named A, was coded '0' for cases from the initial (full) model and '1' for the cases that were added after the deletion of the irrelevant covariables. The second variable was the interaction term, calculated as 'the respective independent fatty acid variable × variable A'. The significance of this interaction term ($P < 0{\cdot}010$, to correct for multiple testing) implies that the regression models are fundamentally different for both sets of cases. In that case, 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 the final backward models could be approved. To check possible influential cases in the regressions, all data points were checked by calculating their Cook's distance and removed if this value was ≥1. Such influential data points were not observed, however.

For the regression analyses, $P < 0{\cdot}010$ were considered statistically significant, to correct for multiple testing. $P < 0{\cdot}050$ was considered to indicate a (non-significant) trend. The values are reported as median (25th–75th percentile), unless specified otherwise. All statistical analyses were performed using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA).

Results

A total of 782 mother–infant pairs were enrolled in the present study. Their relevant characteristics are listed in Table 1. The relative contents (%, w/w) of the four maternal plasma phospholipid fatty acids of interest are reported in Table 2.

In Tables 3–5, the results of the unadjusted, multivariable-adjusted and final backward regression analyses are shown.

Relationship between maternal 18 : 1trans contents and birth dimensions

None of the associations between relative maternal 18 : 1*t* contents and birth weight, birth length or head circumference reached statistical significance or indicated a trend (results not shown). In addition, the backward regression analyses demonstrated that for none of the twelve birth outcome-fatty acid combinations, 18 : 1*t* was either a predictor or a confounder. Since a considerable number of 18 : 1*t* values were missing from the database, which lowered the number of cases and, consequently, the power of the regression, it was decided to remove 18 : 1*t* from the dataset and to restrict further analyses to the combination of the three LCPUFA, DHA, AA and DGLA. This decision appeared justified after the last check, demonstrating that also in the final backward analyses with the three LCPUFA included, 18 : 1*t* was neither a confounder nor a predictor (data not shown).

Table 1. Subject characteristics

	n	
Maternal characteristics		
Age (years)	782	29·0 (26·2–31·7)
Height (cm)	740	167 (162–170)
BMI at study entry (kg/m^2)	709	23·0 (21·2–25·3)
Parity (*n*) 0/1/≥2	782	574/173/35
Weight increase during pregnancy (kg)	736	11·7 (9·2–14·3)
Socio-economic status (income class)*	559	3 (2–3)
Smoking during pregnancy (*n*) no/yes	778	570/208
Alcohol during pregnancy (*n*) no/yes	779	761/18
Infant characteristics		
Gestational age (weeks)	782	40·1 (39·3–41·0)
Sex (*n*) male/female	782	421/361
Birth weight (g)	780	3331 (448)
Birth length (cm)	661	50·1 (2·2)
Head circumference (cm)	580	34·2 (1·6)

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

* Ranges from minimum (5) to ≥2 × modal (1).

British Journal of Nutrition

Table 2. Relative contents (%, w/w) of selected fatty acids in maternal plasma phospholipids collected at several pregnancy durations (weeks) and at delivery

Fatty acids	*n*	16 weeks	*n*	22 weeks	*n*	32 weeks	*n*	Delivery
DHA	749	3·88 (3·34–4·49)	697	3·98 (3·46–4·51)	722	3·84 (3·39–4·44)	676	3·75 (3·31–4·24)
AA	749	9·59 (8·68–10·53)	697	8·60 (7·75–9·51)	722	8·14 (7·35–8·94)	676	8·47 (7·60–9·43)
DGLA	749	3·06 (2·67–3·54)	697	3·36 (2·98–3·74)	722	3·34 (2·96–3·76)	676	3·40 (3·05–3·82)
18 : 1*t*	574	0·45 (0·33–0·59)	534	0·44 (0·32–0·58)	553	0·42 (0·31–0·54)	516	0·37 (0·27–0·49)

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

Relationship between maternal DHA contents and birth dimensions

Unadjusted regression analyses revealed significant positive relationships between maternal DHA contents at week 16 of pregnancy and birth weight and head circumference (Table 3). After entering all covariables, these associations remained significant. After the removal of irrelevant covariables by the stepwise-backward procedure, the final models explained 37·5 and 20·0 % of the variability in birth weight and head circumference, respectively. The contributions of DHA stayed significant, explaining 1·0 and 1·4 % of these variabilities, respectively.

For birth length, unadjusted regression analyses revealed a significant positive association with maternal DHA, measured at 32 weeks of pregnancy. This association just lost significance after adjustment for relevant covariables. The relationships between birth length and DHA proportions measured at 16 and 22 weeks were of the same order of magnitude, but they only showed non-significant positive trends in unadjusted analyses as well as after adjustment for relevant covariables.

None of the birth outcome variables were significantly associated or showed trends with DHA values measured at delivery.

Relationship between maternal arachidonic acid contents and birth dimensions

No significant associations were observed in unadjusted analyses between AA contents in maternal plasma phospholipids and birth outcome variables (Table 4). After adjustment for covariables, associations with neonatal head circumference hardly changed and remained insignificant. The adjustment 'did' enhance most associations of birth weight and birth length with maternal AA proportions at 16 and 22 weeks of pregnancy, but only non-significant negative trends became apparent between maternal AA contents at 16 and 22 weeks of pregnancy and birth weight and between AA contents at 22 weeks of pregnancy and birth length. When related to maternal AA proportions in late pregnancy (week 32) and at delivery, however, 'significant' negative relationships were observed. The significant final multivariable-adjusted backward models (with only relevant covariables included) explained, respectively, up to 36·8 and 28·7 % of the variability in birth weight and birth length, up to 1·5 % of which was due to the contribution of AA.

Relationship between maternal dihomo-γ-linolenic acid contents and birth dimensions

Unadjusted regression analyses did not demonstrate any significant relationship between maternal DGLA contents and birth outcome variables (Table 5). However, after adjustment for only the relevant covariables, a negative trend was found for the relationship between maternal DGLA proportions at 32 weeks of pregnancy and birth weight. After full adjustment, the maternal DGLA content at delivery was significantly and negatively associated with neonatal birth weight. The complete model explained about one-third (34·2 %) of the variability in birth weight, 1·4 % of which was contributed by DGLA. After the removal of the irrelevant covariables by the stepwise-backward procedure, the final model explained 36·7 % of the variability in birth weight and the contribution of DGLA (1·5 %) remained significant. For the association between maternal DGLA proportions at delivery and neonatal birth length, comparable results were obtained. The full multiple regression model explained 26·9 % of the variability in birth length with an almost significant contribution of 0·8 % from DGLA. This contribution increased to 1·3 % and became significant after the removal of irrelevant covariables by the backward regression analysis.

Other associations between birth outcome variables and maternal DGLA contents were not significant and no trends were found either.

Discussion

In the present study, with the data of mother–infant pairs present in the MEFAB database, we observed that the plasma phospholipid DHA contents of mothers, especially when measured early in pregnancy, were significantly and 'positively' related to birth weight and head circumference of their neonates, whereas maternal AA and DGLA proportions in late pregnancy were 'negatively' related to birth weight and birth length. No significant associations were observed for maternal contents of 18 : 1*t*, a group of industrial *trans* unsaturated fatty acids present in the diet.

In the literature, most observational studies relating neonatal birth outcome to maternal *n*-3 fatty acid intake inferred from the intake of fish and marine mammals assessed using FFQ. A few of these studies reported positive associations between *n*-3 fatty acids and birth dimensions[26–28], whereas other studies found no[29,30] or even negative associations[31]. No significant associations were reported between birth weight or birth length and maternal DHA contents measured in plasma lipid fatty acids at 35 weeks of pregnancy[7] or the fatty acid ratio (EPA + DPA + DHA)/AA in erythrocytes sampled at 30 weeks of pregnancy and used as a biochemical marker of the marine *n*-3 fatty acid intake[29].

The reasons for these inconsistent results probably include the use of different methods to estimate the *n*-3 LCPUFA status of the women, as well as incomplete adjustment for

Table 3. Unadjusted and multivariable-adjusted (backward) regression analyses of the relationships between birth outcome variables and DHA contents in phospholipids of maternal plasma, collected at different times during pregnancy and at delivery

		Unadjusted model (no covariables included)				Multivariable-adjusted model (all covariables included)*†				Final multivariable-adjusted backward model (only relevant covariables included)*‡						
															95 % CI (*B*)	
Pregnancy duration (weeks)	Birth outcome	*n*	R^2	*B*	*P*	R^2	*B*	r^2	*P*	*n*	R^2	*B*	r^2	*P*	Low	High
16	BW§	473	0·018	64·12	0·003	0·357	61·97	0·015	0·001	665	0·375	52·10	0·010	0·001	20·40	83·80
	BL§	410	0·016	0·313	0·011	0·279	0·282	0·011	0·014	569	0·287	0·210	0·006	0·025	0·026	0·393
	HC‖	369	0·022	0·255	0·005	0·181	0·275	0·022	0·002	510	0·200	0·223	0·014	0·003	0·074	0·372
22	BW§	441	0·001	17·09	0·462	0·337	23·75	0·002	0·263	623	0·362	31·18	0·003	0·085	−4·301	66·67
	BL¶	384	0·011	0·277	0·040	0·275	0·295	0·010	0·021	536	0·283	0·270	0·009	0·010	0·065	0·476
	HC**	343	0·005	0·133	0·188	0·155	0·102	0·003	0·322	472	0·165	0·142	0·005	0·107	−0·031	0·314
32	BW§	457	0·003	28·41	0·272	0·355	32·01	0·003	0·164	644	0·368	33·08	0·003	0·094	−5·699	71·86
	BL††	396	0·017	0·379	0·008	0·286	0·329	0·011	0·015	551	0·285	0·276	0·008	0·012	0·060	0·491
	HC‖	353	0·006	0·162	0·134	0·162	0·181	0·007	0·100	489	0·177	0·192	0·007	0·039	0·010	0·373
Delivery	BW§	467	0·002	−22·45	0·362	0·342	−12·63	0·000	0·578	608	0·367	3·423	0·000	0·861	−34·95	41·80
	BL††	407	0·001	0·069	0·629	0·269	0·131	0·002	0·346	526	0·287	0·157	0·003	0·171	−0·068	0·382
	HC‖	363	0·000	0·042	0·690	0·148	0·044	0·000	0·688	467	0·187	0·191	0·007	0·050	0·000	0·382

BW, birth weight; BL, birth length; HC, head circumference. R^2, coefficient of determination of the 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. $P<0{\cdot}010$ refer to a significant relationship and $0{\cdot}010 \leq P<0{\cdot}050$ refer to a non-significant trend.

* The total modal *P* values of the (final) multivariable-adjusted analyses were all <0.000.

† The same cases as included in the unadjusted model.

‡ For all the models, the following covariables appeared relevant: infant sex; gestational age; maternal height.

§ Additional relevant covariables: parity, weight increase during pregnancy, BMI at study entry, smoking and drinking during pregnancy.

‖ Additional relevant covariables: parity and BMI at study entry.

¶ Additional relevant covariables: parity, smoking during pregnancy, BMI at study entry and weight increase during pregnancy.

** Additional relevant covariables: parity and weight increase during pregnancy.

†† Additional relevant covariables: weight increase during pregnancy, BMI at study entry, smoking and drinking during pregnancy.

Table 4. Unadjusted and multivariable-adjusted (backward) regression analyses of the relationships between birth outcome variables and arachidonic acid contents in phospholipids of maternal plasma, collected at different times during pregnancy and at delivery

		Unadjusted model (no covariables included)				Multivariable-adjusted model (all covariables included)*†				Final multivariable-adjusted backward model (only relevant covariables included)*‡						
															95 % CI (*B*)	
Pregnancy duration (weeks)	Birth outcome	*n*	R^2	*B*	*P*	R^2	*B*	r^2	*P*	*n*	R^2	*B*	r^2	*P*	Low	High
16	BW§	473	0·000	1·468	0·912	0·357	−22·14	0·005	0·060	665	0·375	−20·75	0·004	0.037	−40·22	−1·274
	BL§	410	0·000	0·008	0·914	0·279	−0·103	0·004	0·141	569	0·287	−0·090	0·003	0·117	−0·203	0·023
	HC‖	369	0·000	0·014	0·791	0·181	−0·067	0·004	0·214	510	0·200	−0·078	0·004	0·094	−0·169	0·013
22	BW§	441	0·001	−7·785	0·604	0·337	−31·84	0·008	0·024	623	0·362	−25·90	0·005	0·030	−49·32	−2·478
	BL¶	384	0·001	−0·051	0·554	0·275	−0·207	0·012	0·014	536	0·283	−0·174	0·009	0·012	−0·310	−0·038
	HC**	343	0·006	0·090	0·167	0·155	0·031	0·001	0·650	472	0·165	0·020	0·000	0·723	−0·091	0·131
32	BW§	457	0·009	−32·85	0·042	0·355	−49·22	0·017	0·001	644	0·368	−44·25	0·013	0·000	−68·33	−20·16
	BL††	396	0·004	−0·105	0·232	0·286	−0·220	0·013	0·009	551	0·285	−0·200	0·011	0·004	−0·335	−0·065
	HC‖	353	0·000	0·020	0·772	0·162	−0·022	0·000	0·762	489	0·177	−0·072	0·003	0·231	−0·190	0·046
Delivery	BW§	467	0·008	−25·05	0·052	0·342	−38·91	0·016	0·001	608	0·367	−27·08	0·008	0·008	−47·11	−7·056
	BL††	407	0·004	−0·098	0·186	0·269	−0·192	0·013	0·008	526	0·287	−0·207	0·015	0·001	−0·330	−0·084
	HC‖	363	0·001	0·029	0·611	0·148	0·008	0·000	0·893	467	0·187	−0·033	0·001	0·520	−0·132	0·067

For explanations of symbols, see Table 3.

Table 5. Unadjusted and multivariable-adjusted (backward) regression analyses of the relationships between birth outcome variables and dihomo-γ-linolenic acid contents in phospholipids of maternal plasma, collected at different times during pregnancy and at delivery

		Unadjusted model (no covariables included)				Multivariable-adjusted model (all covariables included)*†				Final multivariable-adjusted backward model (only relevant covariables included)*‡						
															95 % CI (*B*)	
Pregnancy duration (weeks)	Birth outcome	*n*	R^2	*B*	*P*	R^2	*B*	r^2	*P*	*n*	R^2	*B*	r^2	*P*	Low	High
16	BW§	473	0·002	24·75	0·400	0·357	8·829	0·000	0·732	665	0·375	−5·410	0·000	0·811	−49·78	38·96
	BL§	410	0·000	0·026	0·875	0·279	−0·035	0·000	0·819	569	0·287	−0·041	0·000	0·750	−0·294	0·212
	HC‖	369	0·000	0·028	0·825	0·181	0·106	0·002	0·395	510	0·200	0·126	0·002	0·243	−0·086	0·337
22	BW§	441	0·003	37·64	0·237	0·337	−3·640	0·000	0·899	623	0·362	−16·20	0·000	0·520	−65·64	33·25
	BL¶	384	0·001	0·121	0·496	0·275	0·029	0·000	0·862	536	0·283	−0·055	0·000	0·703	−0·340	0·229
	HC**	343	0·006	0·202	0·143	0·155	0·257	0·008	0·071	472	0·165	0·101	0·001	0·393	−0·132	0·335
32	BW§	457	0·000	10·31	0·748	0·335	−38·30	0·003	0·190	644	0·368	−54·55	0·005	0·029	−103·6	−5·471
	BL††	396	0·000	−0·053	0·764	0·286	−0·161	0·002	0·345	551	0·285	−0·269	0·005	0·053	−0·542	0·004
	HC‖	353	0·002	0·100	0·462	0·162	0·177	0·004	0·215	489	0·177	0·005	0·000	0·969	−0·228	0·237
Delivery	BW§	467	0·002	−28·96	0·337	0·342	−84·00	0·014	0·002	608	0·367	−85·76	0·015	0·000	−130·9	−40·61
	BL‖	407	0·003	−0·187	0·271	0·269	−0·340	0·008	0·033	526	0·287	−0·413	0·013	0·003	−0·680	−0·146
	HC‖	363	0·001	−0·083	0·518	0·148	−0·050	0·000	0·698	467	0·187	−0·136	0·003	0·236	−0·361	0·089

For explanations of symbols, see Table 3.

covariables[7,26,27,30,31]. Furthermore, most observational studies were restricted to relationships with maternal fatty acids in late pregnancy, whereas we observed significant associations with maternal DHA contents early in pregnancy only. This finding suggests a fetal growth-programming potential of maternal DHA, especially, early in pregnancy, which (if confirmed) could imply that DHA supplementation after this period may not significantly affect birth dimensions anymore. Indeed, as initially observed by Olsen *et al.* [32] and recently confirmed by a meta-analysis containing five additional randomised controlled trials, no significant effects on birth weight or birth length are observed when *n*-3 LCPUFA is supplemented from 15 to 30 weeks of pregnancy onwards[33].

The present results agree with those of van Eijsden *et al.* who observed a significant positive correlation between DHA proportions measured in maternal plasma phospholipids at 13 weeks of pregnancy and birth weight. However, this association lost significance after adjustment for covariables[15]. It should be mentioned that, when compared with our volunteers, their (multi-ethnic) population had relatively high DHA values, and the association they observed was mainly present at the lower range of the DHA distribution.

Previous observational studies suggested that AA has growth-promoting effects early in life in both preterm[34,35] and term neonates[7,36,37]. By contrast, we observed negative associations between AA contents in maternal plasma phospholipids and neonatal birth weight and birth length. Although present throughout pregnancy, these associations were only significant at late pregnancy and directly after delivery, which suggests the involvement of AA in fetal growth limitation. These results confirm earlier observations of van Eijsden and co-workers that higher AA proportions in plasma phospholipids of women at about 13 weeks of pregnancy are significantly related to lower birth weights of their neonates[15]. At 16 weeks of pregnancy, the negative association we observed in the present study for birth weight was not quite significant ($P=0{\cdot}037$), which may have been due to the smaller study population (*n* 665), compared with the study of van Eijsden (*n* 3706).

In general, maternal plasma contents of DGLA, the precursor of AA, were negatively associated with birth weight and birth length after correction for relevant covariables. Although not significant for fatty acid proportions early in pregnancy, the associations became significant as pregnancy progressed. From the literature, hardly anything is known about the possible association between maternal DGLA contents and birth outcome. In their (uncorrected) study, Elias and Innis did not observe a significant relationship with DGLA contents measured in maternal plasma phospholipids, TAG or cholesteryl esters at 35 weeks of pregnancy for birth weight and birth length[7]. It should be mentioned that in the present study, significant results for DGLA were observed only after correction for relevant covariables. van Eijsden and co-workers found a significant 'positive' association between maternal DGLA proportions, measured early in pregnancy, and birth weight in unadjusted and multivariable-adjusted analyses ($P<0{\cdot}010$)[15]. Although we tested several possible scenarios, we were unable to reconcile these contrasting results.

In agreement with the inhibitory effect of *trans* unsaturated fatty acids on the endogenous LCPUFA synthesis from their essential fatty acids precursors and on their placental transfer[12–14], 18 : 1*t* contents in maternal plasma phospholipids were found significantly and negatively associated with maternal DHA and AA proportions measured during pregnancy and directly after delivery (data not shown). Despite the rather strong relationships ($P\leq 0{\cdot}011$) and the significant associations we observed between maternal LCPUFA contents and birth dimensions, associations between maternal 18 : 1*t* proportions and the various birth outcome variables were not significant. This might be partly explained by the rather low 18 : 1*t* contents in the plasma phospholipids of the present study population (Table 2), resulting in a narrow exposure range, which usually impedes the detection of any association. As in the (unadjusted) analyses of Elias and Innis, the *trans* fatty acid proportions were also not significantly related to infant birth weight and birth length[7], it seems that any potential effect of industrial *trans* unsaturated fatty acids is either small or non-existing.

One of the strengths of the present study is that the plasma for fatty acid content measurement was collected at several time points during pregnancy and directly after birth. This allowed us to investigate the associations of these fatty acids with birth dimensions from early pregnancy onwards until and including delivery. Second, the database of this cohort contains a relatively large number of maternal and neonatal covariables. However, as with all observational studies, residual confounding cannot be ruled out and it is not possible to decide whether or not the associations observed are causal.

Even though significant correlations existed between DHA, AA, DGLA and 18 : 1*t*, we regarded it justified to include them in the multivariable-adjusted analyses together, because the correlation coefficients between these fatty acids were $\leq 0{\cdot}4$. In addition, the multicollinearity checks revealed a tolerance value of $>0{\cdot}1$ and a variance inflation factor of <10, which allowed us to make this decision[38]. By including the fatty acids simultaneously, it was first possible to take into consideration their metabolic interactions[3,8]. Second, since fatty acid contents are reported in proportions, any change in the proportion of one fatty acid will result in a change in the relative proportions of the other fatty acids included in the analysis.

To check whether confounding was present and in what direction it worked, the same subjects were used in the unadjusted as well as the multivariable-adjusted regression analyses. When unadjusted regression analyses were performed with the maximum number of cases available, the associations were considerably more significant than those reported in Tables 3–5 (data not shown).

The present results support earlier suggestions[15] that differences in maternal LCPUFA contents (irrespective of their causes) can have a significant impact on neonatal birth dimensions. Moreover, the present results can be taken to indicate that maternal DHA contents may programme fetal growth in a positive way, whereas maternal AA and DGLA contents later in pregnancy might be involved in fetal growth limitation. If these associations prove to be causal, they imply that birth dimensions can be optimised by the adaptation of the maternal LCPUFA intake during pregnancy, because circulating proportions of LCPUFA depend, at least partly, on the LCPUFA intake. Thus, intervention studies clearly demonstrated that plasma contents of DHA, AA and DGLA, as well as their proportions, can be modified by

changing the consumption of these LCPUFA or their precursors[8,39–41], although non-dietary factors can also play a modulating role[42–44]. Therefore, randomised clinical trials are needed to test the causality of the associations observed and to find the ideal maternal LCPUFA status for optimum fetal growth and infant development. As it is known that maternal and neonatal essential fatty acid contents are positively related with each other[5,6,17], such intervention studies would be especially appropriate if it can be confirmed that associations with birth dimensions, as observed in the present study for maternal LCPUFA contents during pregnancy, also exist for neonatal LCPUFA contents at birth. These studies are now underway.

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References

1. Godfrey KM & Barker DJ (2000) Fetal nutrition and adult disease. *Am J Clin Nutr* **71**, 1344S–1352S.
2. Gale CR, O'Callaghan FJ, Bredow M & Martyn CN (2006) The influence of head growth in fetal life, infancy, and childhood on intelligence at the ages of 4 and 8 years. *Pediatrics* **118**, 1486–1492.
3. Innis SM (1991) Essential fatty acids in growth and development. *Prog Lipid Res* **30**, 39–103.
4. Heird WC & Lapillonne A (2005) The role of essential fatty acids in development. *Annu Rev Nutr* **25**, 549–571.
5. Ghebremeskel K, Crawford MA, Lowy C, Min Y, Thomas B, Golfetto I, Bitsanis D & Costeloe K (2000) Arachidonic and docosahexaenoic acids are strongly associated in maternal and neonatal blood. *Eur J Clin Nutr* **54**, 50–56.
6. Al MD, van Houwelingen AC & Hornstra G (2000) Long-chain polyunsaturated fatty acids, pregnancy, and pregnancy outcome. *Am J Clin Nutr* **71**, 285S–291S.
7. Elias SL & Innis SM (2001) Infant plasma trans, *n*-6, and *n*-3 fatty acids and conjugated linoleic acids are related to maternal plasma fatty acids, length of gestation, and birth weight and length. *Am J Clin Nutr* **73**, 807–814.
8. Hornstra G (2001) Importance of polyunsaturated fatty acids of the *n*-6 and *n*-3 families for early human development. *Eur J Lipid Sci Technol* **103**, 379–389.
9. Stender S & Dyerberg J (2004) Influence of *trans* fatty acids on health. *Ann Nutr Metab* **48**, 61–66.
10. Koletzko B & Muller J (1990) *Cis*- and *trans*-isomeric fatty acids in plasma lipids of newborn infants and their mothers. *Biol Neonate* **57**, 172–178.
11. von Houwelingen AC & Hornstra G (1994) *Trans* fatty acids in early human development. *World Rev Nutr Diet* **75**, 175–178.
12. Sugano M & Ikeda I (1996) Metabolic interactions between essential and *trans*-fatty acids. *Curr Opin Lipidol* **7**, 38–42.
13. Koletzko B (1992) *Trans* fatty acids may impair biosynthesis of long-chain polyunsaturates and growth in man. *Acta Paediatr* **81**, 302–306.
14. Campbell FM, Gordon MJ & Dutta-Roy AK (1996) Preferential uptake of long chain polyunsaturated fatty acids by isolated human placental membranes. *Mol Cell Biochem* **155**, 77–83.
15. van Eijsden M, Hornstra G, van der Wal MF, Vrijkotte TG & Bonsel GJ (2008) Maternal essential fatty acid status early in pregnancy and term birthweight: a prospective cohort study. *Am J Clin Nutr* **87**, 887–895.
16. Hornstra G (2000) Essential fatty acids in mothers and their neonates. *Am J Clin Nutr* **71**, 1262S–1269S.
17. Al MD, van Houwelingen AC, Kester AD, Hasaart TH, de Jong AE & Hornstra G (1995) Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status. *Br J Nutr* **74**, 55–68.
18. Delbaere I, Verstraelen H, Goetgeluk S, Martens G, De Backer G & Temmerman M (2007) Pregnancy outcome in primiparae of advanced maternal age. *Eur J Obstet Gynecol Reprod Biol* **135**, 41–46.
19. Hindmarsh PC, Geary MP, Rodeck CH, Kingdom JC & Cole TJ (2002) Intrauterine growth and its relationship to size and shape at birth. *Pediatr Res* **52**, 263–268.
20. Andersson SW, Niklasson A, Lapidus L, Hallberg L, Bengtsson C & Hulthen L (2000) Sociodemographic characteristics influencing birth outcome in Sweden, 1908–1930. Birth variables in the Population Study of Women in Gothenburg. *J Epidemiol Community Health* **54**, 269–278.
21. Shu XO, Hatch MC, Mills J, Clemens J & Susser M (1995) Maternal smoking, alcohol drinking, caffeine consumption, and fetal growth: results from a prospective study. *Epidemiology* **6**, 115–120.
22. Kramer MS (2003) The epidemiology of adverse pregnancy outcomes: an overview. *J Nutr* **133**, 1592S–1596S.
23. Luo ZC, Wilkins R & Kramer MS (2006) Effect of neighbourhood income and maternal education on birth outcomes: a population-based study. *CMAJ* **174**, 1415–1420.
24. Catalano PM, Drago NM & Amini SB (1995) Factors affecting fetal growth and body composition. *Am J Obstet Gynecol* **172**, 1459–1463.
25. Maldonado G & Greenland S (1993) Simulation study of confounder-selection strategies. *Am J Epidemiol* **138**, 923–936.
26. Olsen SF, Olsen J & Frische G (1990) Does fish consumption during pregnancy increase fetal growth? A study of the size of the newborn, placental weight and gestational age in relation to fish consumption during pregnancy. *Int J Epidemiol* **19**, 971–977.
27. Olsen SF & Secher NJ (2002) Low consumption of seafood in early pregnancy as a risk factor for preterm delivery: prospective cohort study. *Br Med J* **324**, 447–450.
28. Olsen SF, Grandjean P, Weihe P & Videro T (1993) Frequency of seafood intake in pregnancy as a determinant of birth weight: evidence for a dose dependent relationship. *J Epidemiol Community Health* **47**, 436–440.
29. Olsen SF, Hansen HS, Secher NJ, Jensen B & Sandstrom B (1995) Gestation length and birth weight in relation to intake of marine *n*-3 fatty acids. *Br J Nutr* **73**, 397–404.
30. Rogers I, Emmett P, Ness A & Golding J (2004) Maternal fish intake in late pregnancy and the frequency of low birth weight and intrauterine growth retardation in a cohort of British infants. *J Epidemiol Community Health* **58**, 486–492.
31. Oken E, Kleinman KP, Olsen SF, Rich-Edwards JW & Gillman MW (2004) Associations of seafood and elongated *n*-3 fatty acid intake with fetal growth and length of gestation: results from a US pregnancy cohort. *Am J Epidemiol* **160**, 774–783.

British Journal of Nutrition

32. Olsen SF, Sorensen JD, Secher NJ, Hedegaard M, Henriksen TB, Hansen HS & Grant A (1992) Randomised controlled trial of effect of fish-oil supplementation on pregnancy duration. *Lancet* **339**, 1003–1007.
33. Szajewska H, Horvath A & Koletzko B (2006) Effect of *n*-3 long-chain polyunsaturated fatty acid supplementation of women with low-risk pregnancies on pregnancy outcomes and growth measures at birth: a meta-analysis of randomized controlled trials. *Am J Clin Nutr* **83**, 1337–1344.
34. Koletzko B & Braun M (1991) Arachidonic acid and early human growth: is there a relation? *Ann Nutr Metab* **35**, 128–131.
35. Carlson SE, Werkman SH, Peeples JM, Cooke RJ & Tolley EA (1993) Arachidonic acid status correlates with first year growth in preterm infants. *Proc Natl Acad Sci U S A* **90**, 1073–1077.
36. Xiang M & Zetterstrom R (1999) Relation between polyunsaturated fatty acids and growth. *Acta Paediatr Suppl* **88**, 78–82.
37. Xiang M, Harbige LS & Zetterstrom R (2007) Breast milk levels of zinc and omega-6 polyunsaturated fatty acids and growth of healthy Chinese infants. *Acta Paediatr* **96**, 387–390.
38. Field A (2005) *Discovering Statistics Using SPSS*, 2nd ed. London: Sage Publications Ltd.
39. Otto SJ, van Houwelingen AC & Hornstra G (2000) The effect of supplementation with docosahexaenoic and arachidonic acid derived from single cell oils on plasma and erythrocyte fatty acids of pregnant women in the second trimester. *Prostaglandins Leukot Essent Fatty Acids* **63**, 323–328.
40. De Vriese SR, Matthys C, De Henauw S, De Backer G, Dhont M & Christophe AB (2002) Maternal and umbilical fatty acid status in relation to maternal diet. *Prostaglandins Leukot Essent Fatty Acids* **67**, 389–396.
41. Geppert J, Demmelmair H, Hornstra G & Koletzko B (2008) Co-supplementation of healthy women with fish oil and evening primrose oil increases plasma docosahexaenoic acid, gamma-linolenic acid and dihomo-gamma-linolenic acid levels without reducing arachidonic acid concentrations. *Br J Nutr* **99**, 360–369.
42. Schaeffer L, Gohlke H, Müller M, Heid IM, Palmer LJ, Kompauer I, Demmelmair H, Illig T, Koletzko B & Heinrich J (2006) Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. *Hum Mol Genet* **15**, 1745–1756.
43. Baylin A, Ruiz-Narvaez E, Kraft P & Campos H (2007) Alpha-Linolenic acid. Delta6-desaturase gene polymorphism, and the risk of nonfatal myocardial infarction. *Am J Clin Nutr* **85**, 554–560.
44. Malerba G, Schaeffer L, Xumerle L, *et al.* (2008) SNPs of the FADS gene cluster are associated with polyunsaturated fatty acids in a cohort of patients with cardiovascular disease. *Lipids* **43**, 289–299.

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