

Maternal plasma fatty acid composition and pregnancy outcome in adolescents

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Abstract

Adolescents are at a greater risk of adverse pregnancy outcome, including spontaneous preterm delivery and fetal growth restriction, and typically have a poorer-quality diet than adults have. In the present study, we addressed the hypothesis that low maternal dietary intake of *n*-3 long-chain PUFA (LCP) status adversely influences pregnancy outcome. A total of 500 adolescents (14–18 years) were recruited at ≤20 weeks' gestation. The frequency of consumption of oily fish was determined by questionnaire (at recruitment and during the third trimester). The fatty acid composition of plasma lipids during the third trimester was determined in 283 subjects. Principal components analysis (PCA) was used to derive components, which were divided into tertiles. The pregnancy outcomes were then compared by tertile, adjusting for potentially confounding variables. Of the participants, 69% reported never eating oily fish during pregnancy, although consumption was not associated with a shorter duration of gestation ($P=0.33$), lower customised birth weight ($P=0.82$) or higher incidence of small-for-gestational age (SGA) birth ($P=0.55$). PCA of the fatty acid composition of maternal plasma lipids identified a 'low PUFA:SFA (P:S) ratio' component and a 'high *n*-3 LCP' component. There were no differences between tertiles of the 'high *n*-3 LCP' component and gestational age at delivery ($P=0.62$), customised birth weight ($P=0.38$) or incidence of SGA birth ($P=0.25$), nor were there any associations between the 'low P:S' ratio component and pregnancy outcome. Lower proportions of *n*-3 LCP in plasma lipids are not associated with greater risk of adverse pregnancy outcomes in UK adolescents.

Key words: Adolescent pregnancy: *n*-3 PUFA: Fatty acids: Diet

Pregnancy during adolescence carries a greater risk of preterm delivery and small-for-gestational age (SGA) birth compared with pregnancy during adulthood^(1,2) and this has been attributed in part to poorer maternal nutritional status⁽³⁾. Recent meta-analyses of randomised controlled trials in adults have suggested that supplementation with *n*-3 long-chain PUFA (LCP) extends the duration of gestation^(4–6) and observational data have also suggested a protective effect on fetal growth^(7–10).

During the third trimester of pregnancy, fetal accretion of LCP increases greatly due to the accelerated development of the central nervous system and deposition of adipose tissue⁽¹¹⁾. Fetal demand for LCP, mostly as arachidonic acid (20:4*n*-6) and DHA (22:6*n*-3), can be met by maternal–fetal transfer of these fatty acids and additionally by fetal conversion of the parent fatty acids, linoleic acid

(LA; 18:2*n*-6) and α -linolenic acid (ALA; 18:3*n*-3)^(12,13). The essentiality of LA and ALA has been established and the physiological requirement for *n*-6 PUFA is met by the LA content of most mixed diets⁽¹⁴⁾. However, intakes of ALA are more variable and depend largely upon the types of culinary fats and cooking oils used in food preparation. In some countries such as the USA, partial hydrogenation of cooking oils substantially decreases the availability of ALA, whereas in the UK, most vegetable oils used in food processing are not partially hydrogenated and two of the most widely used oils, rapeseed and soybean oil, contain 10 and 7 wt% ALA, respectively⁽¹⁵⁾. A small amount of pre-formed *n*-3 LCP is typically provided in the diet, primarily by seafood and particularly oily fish, which supplies EPA (20:5*n*-3), docosapentaenoic acid

Abbreviations: ALA, α -linolenic acid; ATE, about teenage eating; GA, gestational age; IQR, interquartile ranges; LA, linoleic acid; LCP, long-chain PUFA; P:S, PUFA:SFA; Q, quantile; SGA, small-for-gestational age.

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(22:5 n -3) and DHA. Eggs, poultry and other meats also contribute to DHA intake⁽¹⁴⁾.

It has been argued that the fetal demand for DHA during late gestation cannot be met by biosynthesis alone and that optimal fetal development requires either a sufficient dietary intake of pre-formed DHA or adequate maternal reserves⁽¹⁶⁾. Endogenous conversion of ALA to DHA and the incorporation of DHA in membranes may be inhibited by a high LA intake, since both parent fatty acids compete for access to Δ -6 desaturase, and this may markedly increase reliance upon dietary pre-formed DHA to meet fetal requirements⁽¹⁷⁾. A typical UK diet supplies only a small amount of DHA (50–100 mg/d) since oily fish is not widely consumed, particularly by adolescents. The UK Diet and Nutrition Survey found that only 33% of 15–18-year-old girls consumed oily fish during the course of a 7 d weighed food record⁽¹⁸⁾. A more recent survey in low-income households found only 3% of adolescent girls (aged 11–18 years) to be the consumers of oily fish⁽¹⁹⁾. While limited assessment periods may have underestimated consumption to some extent, these reports suggest that the majority of adolescents, especially those in low-income groups most likely to become pregnant as adolescents, derive little or no dietary n -3 LCP from oily fish. Adolescents are therefore potentially at greater risk of sub-optimal n -3 LCP status, which may in turn affect pregnancy outcome.

The About Teenage Eating (ATE) study prospectively assessed the diet and nutritional status of pregnant adolescents from London and Manchester, UK, and determined their relationships with pregnancy outcome. A previous report from this study described the associations between maternal micronutrient status and SGA birth⁽²⁰⁾. Here, we report the relationships between maternal plasma fatty acid composition measured during the third trimester and pregnancy outcomes.

Subjects and methods

Subjects

From 2004 to 2007, study-specific midwives recruited 500 pregnant adolescents from antenatal clinics at four hospitals in London and Manchester, UK. The criteria for inclusion were singleton pregnancy, age 14–18 years and gestational age (GA) \leq 20 weeks. The exclusion criteria were inability to provide informed consent, previous pre-eclampsia, clotting disorders, HIV/AIDS, haemoglobinopathies, pre-existing diabetes, renal disease, hypertension, multiple gestations or a history of three or more previous miscarriages. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the Central Manchester Local Research Ethics Committee (local reference no: 03/CM/032). A written consent was obtained from all the participants, with those aged

< 16 years being assessed for capacity to provide informed consent according to accepted UK criteria⁽²¹⁾.

Blood samples and analyses

During the early third trimester (median GA 29.9 weeks; interquartile ranges (IQR) 29.1, 30.6 weeks), a 30 ml venous blood sample was collected into EDTA. After centrifugation (1500 g, 10 min, 5°C), plasma was stored in aliquots at -40°C until analysis. For ethical reasons, subjects were not fasted, but instead were asked not to eat fatty foods before blood collection, which was confirmed at interview by self-report. Time of blood sample collection ranged from 09.30 to 17.30 hours and was determined largely by subject availability. The samples were checked for evidence of lipaemia by measurement of plasma TAG concentration using an enzymatic assay on an ILab 650 analyser (Instrumentation Laboratory UK, Warrington, Cheshire, UK).

The fatty acid composition of plasma total lipids was determined by GLC as described previously⁽²²⁾ with minor modification. Fatty acid methyl esters were analysed on a 25 m \times 22 mm internal diameter silica column (BP70X; SGE, Melbourne, VIC, Australia) using H_2 as a carrier gas on an Agilent chromatograph 6890 (Agilent, Stockport, Cheshire, UK) in split mode (50:1) and integrated using ChemStation (revision B4.01) software. Fatty acids were identified by comparison with reference standards (Sigma, Poole, Dorset, UK). Minor components, for which standards were not available, were identified by individual mass spectra on a similar column by electron impact mass spectrometry on an Agilent gas-chromatograph/mass spectrometer 6872. Tobacco exposure was determined by the measurement of plasma cotinine concentration using a solid-phase competitive chemiluminescence immunoassay (DPC, Caernarfon, Gwynedd, UK).

Assessment of frequency of oily fish consumption

Frequency of oily fish consumption was assessed twice by eating behaviour questionnaire; first, during early pregnancy (\leq 20 weeks' GA) and again during the third trimester alongside blood sample collection. The subjects were asked how often during the previous 3 months they had eaten oily fish, to which there were seven possible responses: 'more than once a day', 'once a day', 'most days', 'at least once a week', 'at least once a month', 'less than once a month' or 'never'. Examples of oily and non-oily fish were provided for clarification. Frequency of non-oily fish consumption was also assessed, with the subjects being asked how often they ate white fish, such as cod or haddock, and canned tuna, which were not classified as oily fish.

Maternal BMI and sociodemographic data

Maternal height and weight were measured at the first interview (median 13.7 weeks' GA; IQR 12.3, 16.2 weeks)

and BMI z -score was calculated using age-adjusted methods^(23,24). Socio-economic status was assessed using the Index of Multiple Deprivation 2004, which ranks areas of residence by affluence⁽²⁵⁾, and head of household occupation⁽²⁶⁾. Tobacco use was assessed twice: by self-report at first interview; by measurement of plasma cotinine concentration during the third trimester (see above). Maternal ethnicity was assessed by self-report using categories from the most recent UK national census, which were then condensed into 'white', 'black' and 'mixed black-white' and 'other ethnicity', the latter incorporating the subjects of South Asian, Far-East Asian and any other ethnicities.

Pregnancy outcome

Pregnancy outcome data were obtained from patient records. Gestational age was confirmed by ultrasound assessment during early pregnancy (median GA 12.4 weeks; IQR 11.3, 13.7 weeks). Customised birth weight percentiles were calculated using GROW-CENTILE software version 6.2 (Gestation Network, Birmingham, UK), which predicts birth weight based upon maternal height, weight, ethnicity, parity, infant sex and GA, using coefficients derived from a large UK reference sample⁽²⁷⁾. The program generates a birth weight z -score that reflects actual relative to predicted birth weight and converts this into a percentile value. SGA birth was defined as <10th birth weight centile and preterm birth as delivery at <37 weeks' GA.

Statistical analyses

Statistical analyses were performed using STATA 9.2 (Stata-Corp LP, College Station, TX, USA). A total of 280 subjects had complete data relating to plasma fatty acid composition, oily fish consumption, confounding variables and pregnancy outcomes. Comparisons of relevant parameters between the entire ATE cohort and the subgroup providing samples for plasma fatty acid analysis were made by unpaired t test for continuous variables and χ^2 test for proportions. Differences in plasma fatty acid proportions between ethnic groups were assessed by one-way ANOVA. Linear trends in plasma proportions of LCP were determined by χ^2 test for trend. Differences in the consumption of oily fish were determined by χ^2 test.

The distributions of plasma fatty acids were log-transformed where appropriate (14:0, 16:0, 16:1 n -7, 18:0, 18:1 $trans$, 18:3 n -3, 20:5 n -3 and 22:6 n -3) to improve normality. Principal components analysis on the correlations of plasma fatty acids reduced the data into clusters, as reported previously⁽²⁸⁾. Principal components analysis forms linear combinations of original variables into groups of correlated variables, which in turn identify underlying dimensions in the data. Fatty acids were selected for inclusion if they were essential fatty acids or

their metabolites (18:2 n -6, 18:3 n -3, 18:3 n -6, 20:3 n -6, 20:4 n -6, 20:5 n -3, 22:5 n -3 and 22:6 n -3), constituted >2% of total plasma fatty acids (16:0, 16:1 n -7, 18:0 and 18:1 n -9) or were relevant biomarkers of dietary intake (14:0, $trans$ 18:1). Components were subjected to varimax rotation to obtain an orthogonal solution and scores were calculated for each participant. These scores were then converted to three categories by quantile (Q) for comparison of pregnancy outcomes.

Differences in pregnancy outcome between tertiles were determined by multiple regression using robust standard errors and adjusting for potentially confounding variables, including maternal underweight and obesity (adjusted for age), ethnicity, smoking, maternal age, socio-economic deprivation (by Index of Multiple Deprivation ranking and head of household occupation) and GA at blood sampling. Adjustment for maternal smoking used self-reported smoking and plasma cotinine concentration, the latter divided into three ordinal categories: <10 ng/ml, 10–99 ng/ml and \geq 100 ng/ml. The inclusion of these factors was based on well-documented *a priori* evidence of an independent association with the duration of gestation or fetal growth⁽²⁹⁾. Kruskal–Wallis tests were used to determine the associations between frequency of oily fish consumption and pregnancy outcome. Pregnancies not resulting in liveborn infants were excluded from the analyses. Significant P -values were two sided at $\alpha < 0.05$, with the exception of comparisons between multiple ethnic groups, for which significance was deemed to be two sided at $\alpha < 0.001$.

Results

Sample description

Detailed characteristics of the ATE study cohort have been described previously⁽²⁰⁾. Of the 500 participants initially recruited, 497 (99%) participants completed the first questionnaire during early pregnancy. During the third trimester, 377 (75%) participants completed the questionnaire and 283 (58%) provided blood samples for the analysis of plasma fatty acids, of which 280 (56%) had complete pregnancy outcome data. Five pregnancies resulted in fetal death, either *in utero* or at delivery. The baseline characteristics of the subjects providing samples for fatty acids analysis did not differ significantly from those of the main cohort, as shown in Table 1. More than a quarter (28%) of the subjects reported smoking during early pregnancy and 35% had a plasma cotinine concentration indicative of exposure to tobacco smoke (>15 μ g/l) during the third trimester. Between the white and black subjects, there was a marked difference in BMI z -score (0.43, SD 0.10 *v.* 1.00, SD 0.12, respectively; $P < 0.001$) and in the proportions who smoked (44 *v.* 9%, respectively; $P < 0.001$). All the subjects reported consuming either meat or fish at some point during pregnancy. Most of

Table 1. Baseline characteristics of the About Teenage Eating Study cohort and sub-cohort with plasma fatty acid data* (Median values and interquartile ranges)

	Main cohort			Sub-cohort		
	Available data (n)	Median	Interquartile range	Available data (n)	Median	Interquartile range
Maternal age (years)	500	17.8	17.1–18.4	283	17.8	17.1–18.4
BMI z-score mean (sd)†	500	0.60	1.20	283	0.71	1.23
Ethnic group (n, %)				283		
White	500	282	56	283	142	50
Black	500	129	26	283	91	32
Mixed black–white	500	59	12	283	32	11
Other ethnicity	500	30	6	283	19	6
Smoking (n, %‡)						
Never smoked	498	211	42	283	112	40
Ex-smoker pre-conception	498	41	8	283	32	11
Ex-smoker post-conception	498	87	17	283	60	21
Smoking at recruitment	498	159	32	283	79	28
Head of household status (n, %§)						
Managerial/professional	499	49	10	283	33	12
Intermediate	499	76	15	283	46	16
Routine/manual	499	270	54	283	135	48
Unemployed/student/other	499	104	21	283	69	24
Index of multiple deprivation (%)	475	14	6–23	283	15	10–24
Marine oil supplement use (n, %¶)	384	6	2	280	5	2
Gestational age at recruitment (weeks)	500	13.7	12.3–16.2	283	14.1	12.4–16.3
Parity (n, %)						
Nulliparous	499	475	95	283	271	96
Primiparous	499	24	5	283	12	4
Gestational age at delivery (weeks)	483	40.0	38.9–41.0	280	40.0	38.9–41.0
Birth weight z-score mean (sd)	478	–0.33	1.12	280	–0.30	1.13
Spontaneous delivery (n, %)	483	383	79	280	221	79
Small-for-gestational age (n, %)	483	84	18	280	45	16

* All the comparisons between main cohort and sub-cohort were not significantly different ($P > 0.10$).

† Age-adjusted BMI classification^(21,22).

‡ Self-reported at recruitment.

§ Categorized according to the UK National Statistics Socio-Economic Classification criteria⁽²⁴⁾.

|| Higher values indicate greater affluence⁽²³⁾.

¶ Self-reported during the third trimester.

them lived in areas of high social deprivation and had low levels of educational attainment.

Maternal plasma TAG concentrations

The median plasma TAG concentration was 1.58 (IQR 1.28, 2.15 mmol/l). The black subjects had a significantly lower mean TAG concentration (1.42 (SD 0.50) mmol/l; $P < 0.001$) than the white subjects (1.93 (SD 0.70) mmol/l; $P < 0.001$), the mixed black–white subjects (1.67 (SD 0.59) mmol/l; $P = 0.024$) and those of other ethnicity (2.10 (SD 0.88) mmol/l; $P < 0.001$). There were no other significant differences between the ethnic groups.

Frequency of oily fish consumption

More than two-thirds of participants (69%; $n = 498$) reported never consuming oily fish during early pregnancy, while 14% consumed it at least once a week. There was a marked difference in frequency of consumption between the ethnic groups. In the white subjects, 81% reported never eating oily fish compared with 44% of the black subjects ($P < 0.001$), 68% of the mixed black–white subjects ($P = 0.042$) and 77% of subjects of other ethnicity

($P = 0.51$). Of the black subjects, 31% consumed oily fish frequently (at least once a week) compared with 7% of the white subjects ($P < 0.001$), 7% of the mixed black–white subjects ($P < 0.001$) and 4% of the subjects from other ethnic groups ($P = 0.006$). The proportion of frequent consumers did not differ between early pregnancy and the third trimester (14 *v.* 16%; $P = 0.68$). Of those subjects with plasma fatty acid composition data, only one reported taking fish oil supplements at both interviews, while 2% reported taking them only during the third trimester.

Maternal plasma fatty acids

Plasma fatty acid data were available for 283 participants. Compared with those of white ethnicity, the black subjects had significantly higher plasma proportions of 18:0, 18:2*n*-6, 20:4*n*-6, 20:5*n*-3, 22:5*n*-3 and 22:6*n*-3 and lower proportions of 14:0, 16:0, 16:1*n*-7, 18:1*n*-9, 18:3*n*-6 and 20:3*n*-6 ($P < 0.001$) (Table 2). *Trans*-fatty acids were only present in trace amounts, consistent with the virtual absence of partially hydrogenated vegetable fats from the UK food supply. There was no association between plasma fatty acids and socio-economic deprivation, as assessed either by Indices of Multiple

Table 2. Plasma fatty acid composition (% total fatty acids) in pregnant adolescents during the third trimester⁽¹⁾
(Mean values with their standard errors)

Fatty acid	White British, Irish and other (n 142)		Black African and Caribbean (n 91)		Mixed black–white (n 32)		Other ethnicity (n 18)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
14:0	1.21	0.03	0.99*	0.04	1.06**	0.07	1.44*†	0.09
16:0	25.9	0.17	24.8*	0.18	25.4††	0.36	26.7†	0.41
18:0	5.84	0.05	6.05***	0.07	6.04†††	0.10	5.86†	0.13
ΣSFA	33.0	0.20	31.8*	0.21	32.5†††	0.44	34.0***	0.50
16:1n-7	2.41	0.07	1.61*	0.06	1.85*†	0.09	2.32†	0.19
18:1 trans	0.22	0.01	0.20	0.02	0.26†	0.02	0.21	0.04
18:1n-9	22.2	0.40	22.1*	0.27	23.8†	0.37	22.2††	0.40
18:1n-7	6.99	0.17	5.68*	0.14	6.20**	0.31	6.98†††	5.75
20:1n-9	0.33	0.01	0.33	0.01	0.32†††	0.01	0.32†††	0.01
ΣMUFA	28.8	0.25	25.9*	0.31	27.8**††	0.38	26.8†	0.49
18:3n-3	0.84	0.02	0.86	0.03	0.88††	0.04	0.94†	0.06
20:5n-3	0.32	0.02	0.59*	0.05	0.42	0.06	0.37	0.04
22:5n-3	0.33	0.01	0.38*	0.01	0.33	0.01	0.36	0.02
22:6n-3	2.07	0.04	2.96*	0.08	2.38	0.10	2.30	0.23
Σn-3 LCP	2.72	0.06	3.94*	0.13	3.12	0.16	3.02	0.29
Σn-3 PUFA	3.56	0.07	4.80*	0.15	4.00***	0.17	3.96	0.32
18:2n-6	27.1	0.29	29.0*	0.37	27.5	0.56	27.9††	0.63
18:3n-6	1.91	0.03	1.52*	0.04	1.78†††	0.06	1.95††	0.10
20:3n-6	7.88	0.20	5.14*	0.16	6.81†	0.38	7.98†	0.58
20:4n-6	5.40	0.08	6.72	0.12	6.13	0.16	5.16	0.28
Σn-6 PUFA	34.7	0.31	37.5*	0.40	35.7	0.59	35.3††	0.67
ΣPUFA	38.3	0.34	42.3*	0.39	39.7***	0.60	39.3†††	0.68
LA:ALA	35.2	0.97	39.9	2.19	32.8††	1.49	32.1†††	2.23
AA:ΣDHA + EPA	2.32	0.04	2.06*	0.06	2.32†††	0.09	2.11	0.13

LCP, long-chain PUFA; LA, linoleic acid; ALA, α-linolenic acid; AA, arachidonic acid.

Mean values were significantly different compared with the white subjects (by one-way ANOVA): * $P < 0.001$, ** $P < 0.01$, *** $P < 0.05$.Mean values were significantly different compared with the black subjects (by one-way ANOVA): † $P < 0.001$, †† $P < 0.01$, ††† $P < 0.05$.**Table 3.** Plasma fatty acid concentrations (mg/l) in pregnant adolescents during the third trimester⁽¹⁾
(Mean values with their standard errors)

Fatty acid	White British, Irish and other (n 142)		Black African and Caribbean (n 91)		Mixed black–white (n 32)		Other ethnicity (n 18)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
14:0	50.9	1.9	34.5*	1.6	42.1**	4.2	58.6**†	4.2
16:0	1070	23.8	842*	18.3	978††	52.3	1090†	59.5
18:0	239	4.1	204*	3.8	231†††	10.3	237†	11.0
ΣSFA	1360	29.1	1080*	22.6	1250†††	65.5	1390***	72.1
16:1n-7	102	4.2	55.6*	2.6	71.6*†	5.6	97.0†	10.8
18:1 trans	9.1	0.6	6.9*	0.7	10.0†	0.9	9.1	2.5
18:1n-9	997	20.7	752*	18.1	912†	46.6	914††	57.8
18:1n-7	69.9	1.7	56.8*	1.4	62.0**	3.1	69.8†††	5.7
20:1n-9	13.5	3.3	11.2*	0.5	12.2†††	0.6	13.2†††	0.9
ΣMUFA	1180	25.2	875*	21.3	1060***††	54.0	1090†	71.4
18:3n-3	34.7	1.1	29.4*	1.3	33.1††	1.6	38.4†	3.6
20:5n-3	13.3	0.8	20.1*	1.9	15.5	2.3	14.8	1.9
22:5n-3	13.5	0.4	13.0	0.4	12.3	0.6	14.5	1.1
22:6n-3	84.6	2.1	100*	3.1	89.5	4.5	92.6	9.5
Σn-3 LCP	111	3.2	133*	5.1	117	6.8	122	12.0
Σn-3 PUFA	146	3.6	162**	5.8	150	7.7	160	14.2
18:2n-6	1100	17.8	979*	19.4	1040	38.9	1130††	48.3
18:3n-6	11.2	0.4	7.7*	0.3	9.5†††	0.6	9.9††	0.8
20:3n-6	78.8	2.0	51.4*	1.6	68.1†	3.8	79.8†	5.8
20:4n-6	220	4.7	226	4.7	230	8.6	206	11.2
Σn-6 PUFA	1410	21.9	1270*	28.3	1350	47.7	1430††	56.3
ΣPUFA	1560	24.4	1430*	24.9	1500	51.5	1590†††	62.1
LA:ALA	35.2	0.97	39.9*	2.19	32.8††	1.49	32.1†††	2.23
AA:ΣDHA + EPA	3.19	0.05	3.13*	0.07	3.28†††	0.11	2.93	0.10

LCP, long-chain PUFA; LA, linoleic acid; ALA, α-linolenic acid; AA, arachidonic acid.

Mean values were significantly different compared with the white subjects (by one-way ANOVA): * $P < 0.001$, ** $P < 0.01$, *** $P < 0.05$.Mean values were significantly different compared with the black subjects (by one-way ANOVA): † $P < 0.001$, †† $P < 0.01$, ††† $P < 0.05$.

Table 4. Relationships between frequency of consumption of oily fish and mean proportions of long-chain PUFA in plasma lipids (% total fatty acids) during the third trimester⁽¹⁾

(Number, percentage, mean values and 95 % confidence intervals)

Frequency	Subjects (<i>n</i> 283)		AA (20:4 <i>n</i> -6)		EPA (20:5 <i>n</i> -3)		DPA (22:5 <i>n</i> -3)		DHA (22:6 <i>n</i> -3)	
	<i>n</i>	%	Mean	95 % CI	GM	95 % CI	Mean	95 % CI	GM	95 % CI
Never	180	64	5.75	5.57, 5.93	0.29	0.27, 0.31	0.33	0.32, 0.34	2.10	2.03, 2.17
< 1 per month	12	4	6.48	5.85, 7.10	0.38	0.30, 0.49	0.37	0.31, 0.43	2.46	2.19, 2.77
≥ 1 per month	36	13	6.21	5.84, 6.58	0.45	0.38, 0.54	0.38	0.35, 0.41	2.75	2.52, 3.00
≥ 1 per week	46	16	6.02*	5.68, 6.36	0.52**	0.42, 0.65	0.40**	0.37, 0.43	2.93**	2.69, 3.21
Missing	9	3	6.03	4.77, 7.29	0.27	0.17, 0.42	0.31	0.25, 0.36	2.14	1.79, 2.51

AA, arachidonic acid; DPA, *n*-3 docosapentaenoic acid.Mean values were significantly different for linear trend by frequency of consumption of oily fish: **P*<0.038, ***P*<0.001.

Deprivation (IMD) ranking or by head of household occupation. Pronounced differences in absolute plasma fatty acid concentrations were observed between the ethnic groups (Table 3). The black subjects had lower concentrations of most fatty acids compared with those of white ethnicity, with the exceptions of 20:5*n*-3 and 22:6*n*-3 which were greater. There were no differences in the LA:ALA ratio between ethnic groups. However, the black subjects had a lower 18:3*n*-3 concentration compared with the other ethnic groups. The proportion of *n*-3 LCP in plasma lipids was higher in frequent consumers of oily fish (*P*<0.001) (Table 4).

Plasma fatty acid components

Principal components analysis clearly identified two components, which together represented 47% of the total variance in plasma fatty acid proportions (Table 5). The 'low PUFA:SFA (P:S) ratio' component was determined mainly by higher proportions of 14:0, 16:0, 16:1*n*-7 and 18:1*n*-9 and lower proportions of 18:2*n*-6, 20:4*n*-6 and 22:6*n*-3. It was positively correlated with the plasma TAG concentration, although it was not associated with oily fish consumption. The 'high *n*-3 LCP' component was associated with higher plasma proportions of 20:5*n*-3, 22:5*n*-3 and 22:6*n*-3 and lower proportions of 16:0, 16:1*n*-7 and 18:1*n*-9. It was positively correlated with frequency of oily fish consumption and negatively correlated with plasma TAG concentration.

Pregnancy outcome in the About Teenage Eating cohort and sub-sample

In the cohort as a whole, 18% delivered SGA infants and 10% were preterm. Of the 280 subjects with both plasma fatty acids and pregnancy outcome data, 16% delivered SGA infants and 8% were preterm. The median GA at delivery for both the main cohort and the sub-cohort was 40.0 (IQR 38.9, 41.0) weeks. Compared with the white subjects, the duration of gestation was shorter in the black subjects (difference: -0.71 (95% CI -1.29, -0.14) d; *P*=0.015) and mixed black-white subjects (difference:

-1.42 (95% CI -2.63, -0.21) d; *P*=0.022) and was non-significantly shorter in the subjects of other ethnicity (difference: -0.81 (95% CI -1.74, 0.10) d; *P*=0.08).

Consumption of oily fish and pregnancy outcome

There was no association between pregnancy outcome and the frequency of oily fish consumption as measured by questionnaire, either during early pregnancy (duration of gestation: *P*=0.33, *n* 480; customised birth weight: *P*=0.82, *n* 475; SGA birth: *P*=0.55, *n* 475) or during the third trimester (duration of gestation: *P*=0.33, *n* 373; customised birth weight: *P*=0.80, *n* 373; SGA birth: *P*=0.58, *n* 373).

Table 5. Correlations between plasma fatty acids (w/w %), related variables and principal components in About Teenage Eating Study participants⁽¹⁾

	Component 1 Low P:S	Component 2 LCP <i>n</i> -3
Variance explained (%)	25.9	20.9
Eigen value	4.04	2.64
14:0	0.78*	-0.01
16:0	0.77*	-0.28*
16:1 <i>n</i> -7	0.89*	-0.26*
18:0	-0.15	0.21*
18:1 <i>trans</i>	0.34*	-0.19
18:1 <i>n</i> -9	0.32*	-0.57*
18:2 <i>n</i> -6	-0.78*	0.14
18:3 <i>n</i> -3	0.06	0.26*
18:3 <i>n</i> -6	0.56*	0.17
20:3 <i>n</i> -6	0.51*	-0.07
20:4 <i>n</i> -6	-0.46*	0.55*
20:5 <i>n</i> -3	-0.13	0.84*
22:5 <i>n</i> -3	0.05	0.83*
22:6 <i>n</i> -3	-0.36*	0.85*
Plasma TAG (<i>n</i> 269)	0.56*	-0.39*
Oily fish consumption (<i>n</i> 274)	-0.19*	0.42*
BMI z-score (<i>n</i> 283)	0.00	0.09
Smoking status (<i>n</i> 281)	0.03	-0.24*
Ethnicity		
White	0.40*	-0.41*
Black	-0.45*	0.44*
Mixed black-white	-0.20*	0.26*
Other ethnicity	0.09	-0.05

P:S, PUFA:SFA ratio; LCP, long-chain PUFA.

**P*<0.001 (Pearson correlation).

Principal components and pregnancy outcome

There was no difference in the mean duration of gestation between the top and bottom tertiles of the 'high *n*-3 LCP' component (Q3: 39.8 (95% CI 39.4, 40.1) weeks *v.* Q1: 39.9 (95% CI 39.5, 40.2) weeks; $P=0.62$, n 280, by multiple linear regression). Restriction of the analyses to spontaneous deliveries did not alter this finding ($P=0.59$; n 221). There was no difference in duration of gestation between tertiles of the 'low P:S' component ($P=0.39$).

There was no difference in customised birth weight between tertiles of the 'high *n*-3 LCP' component (n 280). The mean customised birth weight percentile in the highest Q was 42.0 (95% CI 32.9, 51.5)% compared with 38.7 (95% CI 29.7, 48.3)% in the lowest tertile ($P=0.086$, by simple linear regression). Adjustment for confounding variables did not alter this finding ($P=0.38$, by multiple linear regression). There was no difference in customised birth weight between tertiles of the 'low P:S' component ($P=0.44$).

The incidence of SGA birth in the highest tertile of the 'high *n*-3 LCP' component appeared lower than those of Q1 and Q2, although these differences were NS (Q1: 18.1 (95% CI 11.5, 27.2)%; Q2: 19.1 (95% CI 12.4, 28.3)%; Q3: 10.8 (95% CI 5.9, 19.0)%; $P=0.17$, by simple logistic regression) (n 280). Adjustment for confounding variables did not alter this finding ($P=0.25$), nor did comparison of the highest tertile with Q1 and Q2 combined ($P=0.095$, by multiple logistic regression). There was a marginally significant reduction in the incidence of SGA birth with increasing tertiles of the 'low P:S' component (Q1: 22.6 (95% CI 13.9, 31.2)%; Q2: 13.8 (95% CI 6.7, 20.9)%; Q3: 11.8 (95% CI 5.1, 18.5)%; $P=0.054$, by simple logistic regression), although this did not persist after adjustment for confounding variables ($P=0.16$, by multiple logistic regression).

Discussion

The present study assessed the relationships between maternal *n*-3 LCP status, as assessed by the fatty acid composition of plasma lipids, and pregnancy outcome in an inner-city cohort of adolescents. Less than one-third of the participants reported consuming any oily fish during pregnancy and the remainder presumably deriving *n*-3 LCP from other dietary sources and from endogenous conversion of essential fatty acids. Supplements containing marine oils did not materially contribute to *n*-3 LCP intake since only 2% of the participants reported taking them during pregnancy. Although white fish and canned tuna may have made a small but significant contribution to DHA intake (approximately 0.1 g/serving), the present study did not attempt to assess intake from less rich dietary sources of DHA, such as eggs, offal and meat because of a lack of reliable data in the UK food composition database.

Although an absolute requirement for DHA has not been demonstrated during pregnancy, recent guidelines have recommended intakes ranging from 100 to 300 mg/d based upon levels found to be protective across a broad range of infant outcomes in randomised controlled trials^(30,31). The present UK dietary guidelines for pregnant women recommend consumption of one to two portions of fish/week, of which at least one should be oily⁽³²⁾. This amount agrees with other guidelines⁽³³⁾ and is calculated to provide 100–250 mg of pre-formed DHA/d. While an intake of 100 mg/d may be attainable without consumption of oily fish, 200 mg/d is more challenging⁽³²⁾. In this cohort, the mean plasma DHA proportion was 2.4 (SD 0.7)%, similar to healthy Italian pregnant women assessed using similar methods (2.3 (SD 0.6)%; n 19; GA 33.6 (SD 2.1) weeks)⁽³⁴⁾ and Spanish pregnant women (2.3 (SD 0.5)%; n 36)⁽³⁵⁾. These data do not suggest that adolescents are at additional risk of sub-optimal DHA status, as compared with adults.

In the present study, only 14% of the participants met the recommendation to eat at least one portion/week of oily fish, although this proportion was substantially lower in the white subjects (7%) compared with those of black ethnicity (31%). Higher plasma proportions of *n*-3 LCP have been reported previously in black subjects in both the UK⁽²⁸⁾ and the Netherlands⁽³⁶⁾ and the present study indicates that this difference is attributable to a higher consumption of oily fish by the black subjects rather than to any genetic cause.

A previous study found that UK pregnant women of South Asian ethnicity consuming a vegetarian diet had a significantly reduced duration of gestation (−5.6 d) compared with white, omnivorous women, and it was suggested that this may have been due to a lower consumption of *n*-3 LCP and a higher intake of LA⁽³⁷⁾. In the present study, the mean duration of gestation in the black adolescents, mostly of African and Caribbean ethnicity, was slightly shorter than that of the white subjects (−0.7 d), yet in this case, they had a higher *n*-3 LCP status. This finding suggests that *n*-3 LCP is unlikely to mediate these ethnic differences in duration of gestation. Pregnancy in black and South Asian women has been observed previously to be slightly shorter compared with white Europeans, although importantly this has not been associated with a higher risk of adverse clinical outcome⁽³⁸⁾.

We used principal components analysis to identify the patterns of maternal PUFA intake since it provides a means of summarising common patterns of variation among groups of inter-related variables. In agreement with the previous reports^(28,39), two components were clearly identified, the first characterised by a low P:S ratio, which was higher in the white participants, and the other associated with greater intakes of *n*-3 LCP, this being higher in the black participants. No association was

noted between the 'low P:S' component and pregnancy outcome after adjustment for confounding factors.

No associations were found in this cohort between either oily fish intake or maternal *n*-3 LCP status and pregnancy outcome. The lack of association with the duration of gestation agrees with some^(10,40,41), although not all^(42–44), previous observational studies. Insufficient sampling power was unlikely to have caused this finding, since associations between maternal plasma proportions of PUFA and pregnancy outcome have been observed previously in cohort studies using smaller sample sizes^(43,45) and in case-control studies with markedly fewer cases^(46,47) than that of the present study. Moreover, there was no indication of any emergent non-significant trend between maternal *n*-3 LCP status and pregnancy outcome. A recent Danish study reported higher consumption of oily fish to be associated with a greater risk of fetal growth restriction, which in that instance was attributed to the persistent pollutants occurring in fish from the Baltic Sea⁽⁴¹⁾. In randomised controlled trials where a longer gestation has been found, intakes of *n*-3 LCP have tended to be markedly higher than those normally achieved by diet, ranging from 2 to 4 g/d, approximately tenfold the amount in the typical UK diet^(5,6,48). One exception to this is a randomised controlled trial of eggs fortified with approximately 133 mg DHA, which was claimed to increase the duration of gestation by 6 d⁽⁴⁹⁾. Meta-analyses of these trials^(4–6) reported a slightly longer duration of pregnancy and a marginal increase in birth weight of questionable clinical significance. More recently, a randomised controlled trial designed to assess whether supplementation with 1200 mg/d EPA and 800 mg/d DHA prevents recurrence of preterm birth in women with ≥ 1 prior spontaneous preterm delivery already receiving 17- α -hydroxyprogesterone caproate found no additional benefit of supplementation⁽⁵⁰⁾. The present study provides no further evidence to support an association between maternal DHA status and pregnancy outcome in adolescents.

The strong negative association between maternal smoking use and plasma *n*-3 LCP fatty acids shows the strong potential for confounding in observational studies with fetal outcomes, particularly birth weight, since smoking data are often prone to misreporting bias. However, the present study combined self-reported and biochemical data and, while neither in itself provides a truly reliable reflection of maternal smoking behaviour over the entire duration of pregnancy, together they provide a robust indicator of tobacco use.

The present study has some limitations. Our sample was broadly reflective of the pregnant adolescent populations living in inner-city areas of London and Manchester and was not intended to be representative of UK adolescents as a whole. Our subjects were drawn entirely from metropolitan areas with some of the highest rates of adolescent pregnancy and social deprivation in the UK and therefore the results may not be generalisable to those living in

less densely populated areas where demographic, social and cultural influences may differ. Additionally, ethical considerations prevented collection of fasted plasma, which may, in some cases, have led to the fatty acid composition reflecting very recent dietary intake. We took various steps to prevent this by confirmation of recently eaten foods at interview as well as by measurement of plasma TAG concentration. Although plasma TAG rises during late gestation as fatty acids are mobilised from adipose tissue, no subjects in the present study had concentrations indicative of postprandial lipaemia. Furthermore, most plasma LCP are carried on cholesteryl esters and phospholipids, rather than the TAG fraction⁽⁵¹⁾.

Conclusion

Maternal plasma *n*-3 LCP status during the third trimester is not associated with the duration of gestation or fetal growth in pregnant adolescents deriving little or no *n*-3 LCP from oily fish.

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