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Maternal plasma polyunsaturated fatty acid status in late pregnancy is associated with offspring body composition in childhood

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Abstract

Context—Maternal diet during pregnancy has been linked to offspring adiposity, but it is unclear whether maternal polyunsaturated fatty acid (PUFA) status during pregnancy affects offspring body composition.

Objective—We investigated the associations between maternal plasma n-3 and n-6 PUFA status at 34 weeks gestation and offspring body composition.

Design and setting—A prospective UK population-based mother-offspring cohort: the Southampton Women's Survey (SWS).

Participants—12583 non-pregnant women were recruited into the SWS, of which 1987 delivered a baby before 31st December 2003. 293 mother-child pairs had complete measurements of maternal plasma PUFA concentrations in late pregnancy and offspring body composition at ages 4 and 6 years.

Main Outcomes Measured—Offspring body composition by DXA, yielding fat mass(FM), lean mass(LM), percentage fat mass(%FM) and percentage lean mass(%LM).

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RJM, SMR, JHD and EMD have nothing to declare. KMG has acted as a consultant to Abbott Nutrition and Nestle Nutrition and has received reimbursement for speaking at an Abbott Nutrition Conference on Pregnancy Nutrition and Later Health Outcomes and at a Nestle Nutrition Institute Workshop. KMG, CC and NCH are part of an academic consortium that has received research funding from Abbott Nutrition, Nestec and Danone. PCC serves on Scientific Advisory Boards of the Danone Research Centre in Specialised Nutrition and Aker Biomarine, acts as a consultant to Mead Johnson Nutritionals and Vifor Pharma, has received speaking honoraria from Abbott Nutrition, Nestle, Unilever and DSM, and currently has research funding from Vifor Pharma. HMI has received a consultancy fee from Bayer plc.

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Results—Maternal plasma n-6 PUFA concentration positively predicted offspring fat mass at 4 years (β=0.14 SD/SD, p=0.01) and 6 years (β=0.11 SD/SD, p=0.04), but there was no association with offspring lean mass at either age (β=0.005 SD/SD, p=0.89 & β=0.008 SD/SD, p=0.81, respectively). Maternal plasma n-3 PUFA concentration displayed no associations with offspring fat mass at 4 years (β=0.057 SD/SD, p=0.34) or 6 years (β=0.069 SD/SD, p=0.21). Maternal plasma n-3 PUFA status positively correlated with offspring lean mass on univariate analysis (4yrs β=0.11, p=0.06; 6yrs β=0.14, p=0.02), however this was confounded by a positive association with offspring height.

Conclusions—This observational study suggests that maternal n-6 PUFA status during pregnancy might influence offspring adiposity in childhood.

Keywords

long chain polyunsaturated fatty acids; adiposity; body composition; fetal programming

Introduction

There is increasing evidence that fetal programming by the nutritional environment in utero influences body composition in childhood and adulthood. In both animal and human studies, offspring born to mothers who are obese or diabetic have a higher percentage body fat that persists into adulthood (1). Additionally, there is accumulating evidence that not only the total energy content of maternal diet is important, but also its individual dietary constituents (2;3).

Long chain polyunsaturated fatty acids (PUFA) are an essential component for normal growth and development, and there is evidence that the relative intake of individual PUFA might influence adipose tissue development. The n-6 PUFAs, derived from plant oils, are highly adipogenic (4;5); n-3 PUFA have been proposed as having the converse effect on adipogenesis (4) but the evidence for this is conflicting (6).

Animal studies have suggested that offspring of mothers fed a diet high in n-3 PUFA during pregnancy and lactation had lower fat mass and reduced adipocyte size compared to offspring of control dams (7). An American mother-child cohort study found greater maternal combined intake of the n-3 PUFAs eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA) at 29 weeks gestation was associated with lower subscapular and triceps skinfold thickness and reduced odds of obesity in their offspring, but no associations between maternal plasma PUFA concentration and measures of adiposity were identified (8). Four randomised controlled trials of n-3 PUFA supplementation during pregnancy and lactation, with anthropometric outcomes, have been reported (9-13); only one found lower BMI at 21 months of age in offspring of mothers who received DHA supplementation from 21 weeks gestation until the end of the third month of lactation (9). In the remaining studies no reduction in BMI and/or skinfold thicknesses were identified in the offspring of supplemented mothers at 12 months (11), 7 years (10) and 19 years (12). However, none of these trials undertook measurements of maternal plasma PUFA concentrations to determine the effectiveness of supplementation and the measures of adiposity used provide little indication of proportionate body composition. We therefore evaluated maternal plasma PUFA concentration in late pregnancy in relation to offspring body composition at 4 and 6 years of age as determined by Dual-Energy Xray Absorptiometry (DXA) in a prospective mother-offspring cohort.

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Materials and Methods

The Southampton Women's Survey

The Southampton Women's Survey (SWS) is a study of 12583 non-pregnant women aged 20 to 34 years, resident in the city of Southampton, UK (14). Assessments of lifestyle, diet and anthropometry were performed at study entry (April 1998 – December 2002), and, for women who became pregnant, again at 11 and 34 weeks gestation.

The SWS was conducted according to the guidelines laid down in the Declaration of Helsinki, and the Southampton and South West Hampshire Research Ethics Committee approved all procedures. Written informed consent was obtained from all participating women and by a parent or guardian with parental responsibility on behalf of their children.

Maternal data

At the pre pregnancy interview details of maternal parity, highest educational attainment and social class were obtained and height and weight were measured. At 34 weeks gestation the women were reweighed; pregnancy weight gain from pre-pregnancy to 34 weeks gestation was categorised as inadequate, adequate or excessive according to the Institute of Medicine (IOM) 2009, as previously described (15). Diet during the preceding 3 months was also assessed using a 100-item validated food-frequency questionnaire (16). Smoking status and walking speed were ascertained by direct interview.

Fatty acid composition of maternal plasma phosphatidylcholine

Venous blood was taken into heparinised tubes at 34 weeks gestation. Plasma was prepared and stored at −80°C until analysis. Dipentadeconoyl phosphatidylcholine was added to thawed plasma as internal standard prior to total lipid extraction with chloroform/methanol (2:1 vol/vol); butylated hydroxytoluene was added to the extraction as antioxidant. Phosphatidycholine (PC), which constitutes about 75% of plasma phospholipid (17), was isolated by solid phase extraction on aminopropylsilica cartridges using chloroform to elute triacylglycerol and cholesteryl ester fractions, which were then discarded, and then chloroform/methanol (60:40 vol/vol) to elute the PC. Purified PC was dissolved in toluene and fatty acid methyl esters generated by reaction with methanol containing 2% (vol/vol) sulphuric acid at 50°C for 2 hours. After cooling and neutralisation, fatty acid and methyl esters were extracted into hexane. Fatty acid methyl esters were separated by chromatography on a BPX-70 column (30m × 220μm; film thickness 0.25μm) fitted to a Hewlett-Packard HP6980 gas chromatograph. Front inlet temperature was 300°C; initial column temperature was 115°C and was programmed to hold this temperature for 2 minutes, and then to increase temperature at 10°C/min to 200°C, to hold at 200°C for 10 minutes, to increase temperature at 10°C/min to 240°C, and then to hold this temperature for 2 minutes. Helium was used as the running gas and fatty acid methyl esters were detected by flame ionisation. Fatty acid methyl esters were identified by comparison with retention times of standards run previously and they were quantified using ChemStation software (18). Data were expressed as both absolute concentration (microgram per millilitre of plasma) and as percentage contribution to the total plasma PC fatty acid pool.

Childhood assessments of diet and body composition

There were 1987 singleton live births before 31st December 2003. The children were followed up at birth and during infancy. Duration of breastfeeding was determined from feeding histories obtained at 6 and 12 months of age. At 3 years, the children's diets were assessed using an administered food frequency questionnaire (19). The key dietary pattern identified by principal component analysis was a 'prudent' pattern, characterised by greater consumption of fruit, vegetables, water, wholemeal bread and fish, and lower consumption

of white bread, crisps, chips and processed meat. A prudent diet score was calculated for each child that indicated their compliance with the pattern, and therefore the quality of their diet (19).

Consecutive subsets of children born before the end of 2003 were invited to attend the Osteoporosis Centre at Southampton General Hospital for a detailed assessment of body composition at 4 and 6 years of age. At these visits, the child's height was measured using a Leicester height measurer (Seca Ltd., UK) and weight (in underpants only) measured using calibrated digital scales (Seca Ltd., UK). A whole body DXA scan was obtained using a Hologic Discovery instrument (Hologic Inc., Bedford, MA, USA) in paediatric scan mode, yielding fat mass, lean mass and bone mineral content (BMC). Percentage fat mass and percentage lean mass were subsequently derived from the child's weight using a three compartment model, which included bone mineral content in a separate compartment from lean mass. The coefficient of variation for body composition analysis using the DXA instrument was 1.4-1.9%. The reliability of DXA in small subjects has been demonstrated previously (20).

Statistical analysis

In order to compare the effects of maternal plasma PUFA concentrations on offspring body composition at 4 and 6 years, the dataset was based on those children that had DXA scans at both time points. Differences in demographic characteristics and body composition of the children at 4 and 6 years by gender were explored using t-tests and Mann-Whitney U tests for normally and non-normally distributed variables, respectively. Owing to gender differences in the children's body composition, all analysis was subsequently adjusted for the sex of the child, and owing to a wider age at assessment, the 6 year data was also adjusted for the child's age. For consistency, all offspring body composition and maternal PUFA variables were standardised with Fisher-Yates transformation to a normally distributed variable with a mean of 0 and SD of 1. Results are presented as standardised beta coefficients (SD per SD). In subsequent multivariable analysis we accounted for a number of maternal characteristics associated with offspring body composition (maternal age at delivery, parity, social class and highest educational qualification, pre-pregnancy body mass index, IOM category of gestational weight, smoking status in late pregnancy, walking speed in late pregnancy, maternal mean daily intake of protein, fat and carbohydrate at 34 weeks gestation), the child's height and duration of breastfeeding using linear regression. We then additionally addressed the potential effect of the child's diet by adding the child's prudent diet score in the model. Analysis was repeated for both total n-3 and n-6 PUFA, the individual n-3 PUFAs eicosapentanoeic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), the n-6 PUFAs linoleic acid (LA, 18:2n-6) and arachidonic acid (AA, 20:4n-6) and the ratio of total n-3 PUFA: total n-6 PUFA. A subsequent multiple imputation strategy was employed to account for a small number of missing maternal covariates and to allow the inclusion of an additional 17 mother-child pairs, in whom the children had DXA measurements of body composition at both 4 and 6 years but the maternal PUFA measurements were missing. All analysis was performed using Stata v11.0 (Statacorp, Texas, USA).

Results

Characteristics of study participants

766 children attended for DXA scan at age 4 years and 531 children at 6 years. Complete datasets including maternal late pregnancy measurements, plasma concentrations of PUFA, and offspring DXA scans at both 4 and 6 years of age were available for 293 mother-child

pairs. The characteristics of the mothers and children are presented in tables 1 and 2, respectively.

The mothers included in this study were of similar parity and smoking habits in late pregnancy, but were older (30.6±3.6 years vs 30.0±3.8 years, p=0.015) and had achieved a higher educational level (24.2% vs 20.5% had a degree, p=0.003) compared with all mothers in the SWS who delivered before 31st December 2003. Compared with the children who attended for DXA at 4 years but who were not included in this cohort, the children were of similar age and gender, but were slightly taller (104.4±3.9cm vs 103.7±4.2cm, p=0.02) but of similar weight (17.9kg (IQR 16.7-19.3kg) vs 17.7kg (IQR16.5-19.3kg), p=0.33), with no differences in body composition. Minor differences in height and weight between children included in this study and those that attended for DXA but not included were also observed at 6 years.

The boys and girls were of similar age, height and weight at either time point, but girls had significantly greater total fat mass (p<0.0001) and percentage fat (p<0.0001) than the boys at both 4 and 6 years (table 2).

Maternal plasma n-6 PUFA concentration and offspring body composition

Maternal plasma total n-6 PUFA concentration displayed significant positive associations with offspring weight at 4 and 6 years (β=0.15, p=0.009 and β=0.17, p=0.003); there was no association with offspring height (β=0.08, p=0.18 and r=0.10, p=0.09 at 4 and 6 years, respectively). Total maternal plasma n-6 PUFA concentration was positively associated with offspring fat mass at 4 and 6 years (β =0.18, p=0.002 and β=0.18, p=0.003), but no significant association with offspring lean mass were identified (β=0.08, p=0.19 & r=0.11 p=0.06 at 4 and 6 years, respectively). Additionally, significant positive associations were identified between maternal total n-6 PUFA concentration and offspring percentage fat mass at 4 years (β=0.14, p=0.02) and 6 years (β=0.14, p=0.01), and negative associations with percentage lean mass. Similar associations were identified at 6 years (Table 3). Analysis for the individual n-6 PUFA LA and AA showed similar associations (Table 3).

Maternal plasma n-3 PUFA concentration and offspring body composition

There was a strong correlation between maternal n-6 PUFA concentration and maternal n-3 PUFA concentration (r=0.73, p<0.0001). Statistically significant correlations between maternal plasma total n-3 PUFA concentration and offspring height, but not weight, at 4 years of age (β=0.12, p=0.04 and β=0.11, p=0.06, respectively) were identified. Positive associations with height (β=0.13, p=0.03) and weight (β=0.16, p=0.008) were present at 6 years. The associations with body composition differed to those identified with maternal n-6 PUFA concentration; total maternal n-3 PUFA status showed no association with offspring fat mass at either 4 or 6 years (β=0.05, p=0.40 and β=0.09, p=0.11), but did positively correlate with offspring lean mass at each time point (β=0.11 p=0.06 and β=0.14, p=0.02). Maternal n-3 PUFA concentrations did not correlate with offspring percentage fat or lean mass. Similar associations were identified between maternal EPA and DHA and offspring height, weight and body composition (Table 3).

Maternal plasma n-3 PUFA:n-6 PUFA ratio and offspring body composition

There were no associations between maternal n-3:n-6 ratio and offspring height or weight at 4 or 6 years (Table 3). n-3:n-6 ratio was negatively associated with offspring fat mass at 4 years (β=−0.13, p=0.02), but this did not persist to 6 years (β=−0.05, p=0.39). No association with total lean mass was identified at 4 or 6 years, but a significant positive association with percentage lean mass at 4 years was found (β =0.16, p=0.006). Again, this was not present at 6 years (β=0.09, p=0.14).

Adjustment for potential confounding factors

277 children were included in the multivariable analysis at 4 years and 275 at 6 years (2 children did not have a measurement of height at 6 years, and the other 16 mother-child pairs were missing data for one or more maternal variables).

After adjustment for potential confounding maternal factors and child height, the associations between maternal n-6 PUFA concentrations and offspring weight were attenuated and of marginal statistical significance (β=0.07, p=0.08 at 4 years & β=0.07, p=0.08 at 6 years), except for the association with linoleic acid and weight, which remained robust at 4 years (β=0.09, p=0.04) and just failed to achieve statistical significance at 6 years (β=0.07, p=0.06). The associations between maternal n-6 PUFA concentration and offspring fat mass remained statistically significant after adjustment for potential confounding factors at both 4 years (β=0.14, p=0.01) and 6 years (β=0.11, p=0.04). The addition of the child's prudent diet score at 3 years to the model did not alter these relationships (4yrs: β=0.14, p=0.02; 6yrs: β=0.11, p=0.04). The standardised beta coefficients for the associations between individual n-6 PUFA and offspring body composition after adjustment for potential confounding factors are shown in Table 4.

The previously observed relationships between maternal n-3 PUFA concentrations and offspring weight no longer reached statistical significance (β=0.05, p=0.22 at 4 years & β=0.07, p=0.09 at 6 years). The association with offspring height was attenuated (β=0.11, p=0.09 at 4 years & β=0.12, p=0.06 at 6 years), and the associations with lean mass was no longer observed (Table 4).

There were no statistically significant associations between n-3:n-6 PUFA ratio and offspring body composition after adjustment for potential confounding factors (Table 4).

The multiple imputation strategy allowed for the inclusion of an additional 17 mother-child pairs for whom DXA measurements of offspring body composition were available but maternal PUFA concentrations were missing, and was used to account for missing covariates in the adjusted models. This dataset contained 310 mother-child pairs. The relationships were similar to the original associations.

Discussion

In this prospective mother-offspring cohort study, we identified a number of key associations between maternal PUFA status and offspring body composition. Firstly, a key finding was that association maternal plasma phosphatidylcholine n-6 PUFA concentration was positively associated with offspring adiposity. This association was evident at 4 years, and persisted at 6 years; it was robust to adjustment for a number of potential confounding factors including maternal weight gain and diet in pregnancy and the quality of the child's diet, suggesting that pre-natal PUFA exposure could be linked to risk of offspring obesity. Secondly, we observed an association between maternal plasma n-3 PUFA concentration and offspring lean mass, although this was confounded by offspring height. After adjustment for potential confounding factors, there was a trend towards a positive relationship between maternal n-3 PUFA concentration and offspring height suggesting that maternal n-3 PUFA status might affect offspring linear growth in childhood. Thirdly, after adjustment for potential confounding factors, there were no associations between maternal plasma n-3:n-6 PUFA ratio and offspring body composition. This finding might be important in determining potential interventions with regards to maternal diet in pregnancy to reduce the burden of obesity, and provides an explanation as to why previous supplementation studies using n-3 PUFA have been of limited success in altering offspring BMI.

The strength of our study is the detailed phenotyping of mother-offspring pairs, including comprehensive assessments in pregnancy and at multiple time points in childhood. These detailed assessments are unique to the Southampton Women's Survey and although the children who were included in this study were a subset of the SWS cohort who tended to be slightly taller and heavier and to have mothers with higher levels of educational attainment, they do represent the full spectrum of offspring height, weight and family backgrounds and therefore selection bias is unlikely. However, there are a number of limitations to this study. Firstly, we measured body composition using DXA, a technique that has not previously been utilised in studies investigating the relationships between maternal PUFA status and offspring fat and lean masses. DXA-measured body composition has been previously validated chemically in small animals (21), and we used specific paediatric software, movement artefact was minimal and the small numbers of children with excess movement artefact were excluded from the analysis. Secondly, it is not possible to determine that the developing fetus was exposed to the same concentrations of PUFA as were measured in the maternal samples. The transfer of PUFA from the maternal to fetal circulation by the placenta occurs via several mechanisms including passive and facilitated diffusion of non-esterified fatty acids (NEFA). NEFA may be released from multiple fatty acid sources including triglycerides and phospholipids following liberation by placental lipoprotein lipase and endothelial lipase. It has previously been demonstrated that in late pregnancy PUFA preferentially incorporate into maternal plasma phospholipids and trigylcerides over NEFA and cholesterol esters, and the highest concentration of PUFA is found in phospholipids (22). However, the relative transfer of PUFA from each fraction to the fetus has not been determined and may differ. Therefore the association between PUFA derived from alternative maternal lipid fractions not analysed in this study, and offspring body composition cannot be fully determined from our results. Additionally, it has previously been demonstrated that biomagnification of PUFA occurs from the maternal to the fetal circulation due to preferential placental transfer. DHA and AA in particular have preferential accretion in the fetal circulation (22), and this might explain the lack of associations between maternal plasma phosphatidylcholine DHA and AA status and offspring body composition in this study. However, despite this, DHA supplementation in pregnancy does increase cord blood DHA, and maternal and cord or neonatal blood PUFA concentrations are moderately correlated (23;24); current thinking is that whilst fetal PUFA exposure is dependent on maternal plasma PUFA status other additional maternal or placental factors might also influence fetal levels. Thirdly, it has previously been proposed that the critical period for adipogenesis is between 14 and 16 weeks gestation (25); we cannot be certain that the PUFA concentrations measured at 34 weeks are reflective of that in earlier pregnancy. Fourthly, we did not have the opportunity to adjust for multiple comparisons as most of the outcomes were highly related, limiting the use of conventional multi-comparison processing (26). Finally, it is not possible in this observational study to determine whether the associations are causal. Maternal PUFA status might reflect other contributing dietary components or lifestyle factors and the findings could be confounded by similarities between maternal diet in pregnancy and the child's postnatal dietary exposures. Nonetheless, the associations between maternal n-6 PUFA concentrations and offspring adiposity were robust to adjustment to maternal diet in pregnancy. We did not measure the child's plasma fatty acids, but we did obtain detailed breastfeeding histories and used childhood dietary questionnaires in an attempt to control for differences in postnatal diet.

The statistically significant positive association between maternal n-6 PUFA status and offspring fat mass observed in our cohort is in contrast to that reported by Donahue et al, who did not identify an association between maternal percentage plasma n-6 PUFA concentration and BMI z score or sum of subscapular and triceps skinfold thickness at 3 years (8). However percentage plasma n-6 concentration is dependent on total fatty acid intake, and the fatty acid characteristics of their population differed to ours: in their

population mean percentage n-3 PUFA was lower and mean percentage n-6 PUFA higher than in our cohort.

Prostacyclin is a key metabolic intermediate implicated in adipogenesis (27), and might represent a mechanism through which differences in the PUFA could alter body fatness. The n-6 PUFA arachidonic acid, derived directly from the diet, or through linoleic acid metabolism, is a precursor of prostacyclin which enhances the differentiation of preadipocytes into functional adipocytes (27). Prostacyclin receptor deficient mice do not gain weight in response to a high linoleic acid diet (4). n-3 PUFAs, particularly EPA, can inhibit this process through inhibition of the activity of the cyclooxygenase enzymes which are necessary for the generation of prostacylin (28). This is consistent with our finding that maternal total n-6 PUFA concentration was associated with offspring fat mass. Although we observed no negative association between n-3 PUFA and offspring adiposity to support an inhibitory effect of n-3 PUFA on this process, this might be partly confounded by the high correlation between maternal n-3 and n-6 PUFA. Maternal n-3:n-6 PUFA ratio did display a negative association with fat mass, thus suggesting a high relative n-3 PUFA concentration might have beneficial effects on adipogenesis.

We found no association, positive or negative, between maternal n-3 PUFA concentration and offspring adiposity. Although we identified unadjusted relationships between maternal n-3 PUFA concentrations and offspring height and lean mass, the relationships with lean mass were confounded by an association between maternal n-3 PUFA concentration and offspring height. Four randomised controlled trials of maternal n-3 PUFA supplementation in pregnancy have been reported (9-12), of which only one found a reduction in BMI in children born to mothers supplemented with DHA (9). These inconsistencies might in part reflect variation in the type and level of supplementation (200-2700 mg/day n-3 PUFA as DHA alone or as a combination of DHA and EPA) and the gestational age at which supplementation was commenced. The limitation of supplementation studies is that usually the dietary fat content is not controlled, which may well influence the possible effect of the fatty acid supplement. Additionally, as plasma PUFA concentrations were not determined, it is unknown if supplementation increased maternal PUFA concentrations. Despite of the limitations of the previous randomised trials, the findings of this study would also not support n-3 supplementation as a likely effective approach to reducing offspring adiposity.

The contribution of in utero n-6 PUFA exposure to long term adiposity is likely to be small. However the variance in offspring fat mass attributable to maternal n-6 PUFA concentration determined in this study is of similar magnitude (2%) to other maternal and pregnancy-related factors associated with offspring body composition, including maternal walking spend in late pregnancy, measures of maternal fat stores (29), 25-hydroxy-vitamin D status (30) and pregnancy weight gain (15). Although the effect size is considerably smaller than postnatal habitual physical activity (31), it is likely that multiple factors of relatively small effect contribute to fat development. Identifying and targeting many of these small-effect modifiable factors will be necessary to have a positive impact on obesity at the population level. Our findings suggest that approaches to reducing maternal n-6 PUFA intake are more likely to be effective in reducing offspring adiposity than antenatal n-3 PUFA supplementation. Hauner et al did randomise women to n-3 PUFA supplementation with concomitant advice on reducing dietary arachidonic acid intake, but found no significant differences in offspring skinfold thicknesses at 1 year (11). However, the response to the maternal dietary advice was measured by 7 day dietary recall rather than measurements of plasma PUFA and therefore it is possible that poor compliance with dietary advice contributed to the lack of effect. Randomised trials of dietary advice in pregnancy with plasma PUFA measurements before randomisation and at subsequent stages of pregnancy are therefore required.

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In summary, in this observational study, maternal n-6 PUFA was positively associated with offspring fat mass, whereas no significant effects of maternal n-3 PUFA and body composition were identified, suggesting that a low n-6 PUFA intake during pregnancy might reduce offspring adiposity. Intervention studies of dietary advice early in pregnancy with confirmatory measurements of maternal plasma PUFA status are required to confirm this hypothesis and inform appropriate nutritional advice during pregnancy.

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Table 1

Characteristics of the mothers

Maternal Characteristic	
n	293
Age (years), mean±SD	30.6 ± 3.6
Height (cm), mean±SD	164.3 ± 6.6
Pre-pregnancy body mass index (kg/m^2), median (IQR)	24.1 (22.3 – 27.4)
Smoking in late pregnancy, % (n)	13.4 (39)
Duration of breastfeeding, % (n)	
Never tried	13.6 (39)
<1 month	19.4 (56)
1 to 3 months	18.8 (54)
4 to 6 months	18.4 (53)
7 to 11 months	17.7 (51)
12 or more months	12.2 (35)
Plasma PC fatty acid concentration (μg/ml), median (IQR)	
Total n-3 PUFA	70.0 (51.6-92.8)
EPA	5.1 (3.2-7.7)
DHA	51.8 (38.8-70.5)
Total n-6 PUFA	492.9 (372.2-615.8)
LA	324.5 (240.3-400.1)
AA	103.2 (79.7-132.3)
Total n-3:Total n-6 ratio	0.14 (0.12-0.17)
Maternal dietary intake at 34 weeks gestation (g/day), mean±SD	
Protein	87.2 ± 22.8
Carbohydrate	312.6 ± 100.8
Fat	93.0 ± 28.3

PC = phosphatidylcholine; PUFA = long chain polyunsaturated fatty acid; EPA = eicosapentanoic acid; DHA = docosahexaenoic acid; LA = linoleic acid; AA = arachidonic acid

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Table 2

Characteristics of the children at 4 and 6 years of age. Data presented as median (IQR) unless otherwise stated

	4 years		6 years	
	Boys	**Girls**	**Boys**	**Girls**
n	153	140	153	140
Age (years)	4.10 (4.08-4.15)	4.11 (4.08-4.14)	6.60 (6.46-6.75)	6.52 (6.41-6.78)
Height (cm), mean (SD)	104.4 (3.7)	104.4 (4.1)	120.9 (4.9)	121.0 (5.1)
Weight (kg)	17.9 (16.7-19.4)	17.9 (16.9-19.3)	23.6 (21.5-25.5)	23.7 (21.8-26.5)
Fat mass (kg)	4.3 (3.8-5.0)	5.1 (4.5-6.0) ****	4.8 (3.9-5.6)	6.1 (4.9-7.6) ****
Lean mass (kg), mean (SD)	13.1 (1.4)	12.2 (1.4) ****	18.0 (2.1)	17.1 (2.0) ***
Fat percentage	24.0 (22.2-26.5)	28.6 (25.9-32.3) ****	20.1 (18.0-23.5)	25.5 (21.6-29.5) ****
Lean percentage	72.4 (70.0-74.3)	68.0 (64.4-70.8) ****	76.2 (73.0-78.4)	71.0 (67.4-74.6) ****
BMC percentage	3.4 (3.3-3.6)	3.4 (3.2-3.6) *	3.5 (3.4-3.7)	3.4 (3.2-3.6) ****

BMC = bone mineral content

* p<0.05

** p<0.01

*** p<0.001

**** p<0.0001 comparing boys and girls of the same age

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Table 3

Univariate associations between maternal plasma polyunsaturated fatty acids at 34 weeks gestation and offspring body composition at 4 and 6 years Associations are displayed as beta coefficients for standardised variables (SD/SD).

	4 years						6 years					
	Height	**Weight**	**Fat Mass**	**Lean Mass**	**% Fat Mass**	**% Lean Mass**	**Height**	**Weight**	**Fat Mass**	**Lean Mass**	**% Fat Mass**	**% Lean Mass**
n-3 PUFA												
Total n-3 PUFA	0.120*	0.112	0.050	0.110	−0.006	0.002	0.132*	0.155**	0.094	0.138*	0.048	−0.045
EPA	0.147*	0.094	−0.018	0.132*	−0.076	0.071	0.143*	0.147*	0.047	0.164**	−0.009	0.010
DHA	0.107	0.101	0.039	0.101	−0.014	0.010	0.116*	0.131*	0.074	0.117*	0.033	−0.030
n-6 PUFA												
Total n-6 PUFA	0.079	0.153**	0.184**	0.077	0.138*	−0.141*	0.100	0.173**	0.177**	0.109	0.144*	−0.145*
LA	0.061	0.146**	0.193**	0.064	0.153**	−0.157**	0.082	0.156**	0.175**	0.089	0.148*	−0.149*
AA	0.082	0.126*	0.132*	0.078	0.087	−0.092	0.097	0.170**	0.152**	0.123*	0.116*	−0.116*
n-3:n-6 PUFA ratio	0.094	−0.011	−0.134*	0.072	−0.162**	0.159**	0.087	0.041	−0.051	0.083	−0.081	0.086

* p<0.05

** p<0.01

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Table 4

Relationships between maternal plasma polyunsaturated fatty acids at 34 weeks gestation and offspring body composition at 4 and 6 years after adjustment for maternal age at delivery, parity, social class and highest educational qualification, pre-pregnancy body mass index, gestational weight, smoking status in late pregnancy, walking speed in late pregnancy, maternal mean daily intake of protein, fat and carbohydrate at 34 weeks gestation, the child's height and duration of breastfeeding. Associations are displayed as beta coefficients for standardised variables (SD/SD).

	4 years						6 years					
	Height	**Weight**	**Fat Mass**	**Lean Mass**	**% Fat Mass**	**% Lean Mass**	**Height**	**Weight**	**Fat Mass**	**Lean Mass**	**% Fat Mass**	**% Lean Mass**
n-3 PUFA												
Total n-3 PUFA	0.105	0.053	0.057	0.024	0.045	−0.050	0.117	0.067	0.069	0.030	0.065	−0.062
EPA	0.116	0.004	−0.032	0.019	−0.032	0.026	0.112	0.035	−0.003	−0.041	−0.010	0.010
DHA	0.099	0.064	0.061	0.035	0.042	−0.046	0.109	0.067	0.067	0.031	0.061	−0.059
n-6 PUFA												
Total n-6 PUFA	0.072	0.073	0.142*	−0.001	0.135*	−0.137*	0.094	0.068	0.112*	−0.009	0.106	−0.105
LA	0.062	0.087*	0.161**	0.005	0.151*	−0.153**	0.085	0.074	0.126*	−0.009	0.118*	−0.118*
AA	0.073	0.038	0.085	−0.009	0.080	−0.085	0.089	0.059	0.070	0.019	0.063	−0.061
n-3:n-6 PUFA ratio	0.079	−0.023	−0.096	0.025	−0.096	0.091	0.069	0.019	−0.024	0.030	−0.019	0.022

* p<0.05

** p<0.01