Maternal Plasma Polyunsaturated Fatty Acid Status in Late Pregnancy Is Associated with Offspring Body Composition in Childhood

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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 wk gestation and offspring body composition.

Design and Setting: A prospective United Kingdom population-based mother-offspring cohort, the Southampton Women's Survey (SWS), was studied.

Participants: A total of 12,583 nonpregnant women were recruited into the SWS, among whom 1987 delivered a baby before December 31, 2003; 293 mother-child pairs had complete measurements of maternal plasma PUFA concentrations in late pregnancy and offspring body composition at both ages 4 and 6 yr.

Main Outcomes Measured: We measured offspring body composition by dual-energy x-ray absorptiometry, yielding fat mass, lean mass, percentage fat mass, and percentage lean mass. Results are presented as β-coefficients for standardized variables, therefore reflecting the SD change of the outcome for every 1 SD of the predictor.

Results: After adjustment for maternal factors and child factors including height and duration of breast-feeding, maternal plasma n-6 PUFA concentration positively predicted offspring fat mass at 4 yr ($\beta = 0.14$ SD/SD; $P = 0.01$) and 6 yr ($\beta = 0.11$ SD/SD; $P = 0.04$), but there was no association with offspring lean mass at either age ($\beta = 0.005$ SD/SD, $P = 0.89$; and $\beta = 0.008$ SD/SD, $P = 0.81$, respectively). Maternal plasma n-3 PUFA concentration was not associated with offspring fat mass at 4 yr ($\beta = 0.057$ SD/SD; $P = 0.34$) or 6 yr ($\beta = 0.069$ SD/SD; $P = 0.21$). Maternal plasma n-3 PUFA status was positively associated with offspring lean mass on univariate analysis (4 yr, $\beta = 0.11$, $P = 0.06$; 6 yr, $\beta = 0.14$; $P = 0.02$); 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. **(*J Clin Endocrinol Metab* 98: 299–307, 2013)**

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Abbreviations: AA, Arachidonic acid; BMI, body mass index; CI, confidence interval; DHA, docosahexaenoic acid; DXA, dual-energy x-ray absorptiometry; EPA, eicosapentaenoic acid; IQR, interquartile range; LA, linoleic acid; NEFA, nonesterified fatty acid; PC, phosphatidylcholine; PUFA, polyunsaturated fatty acid.

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 is the total energy content of maternal diet important, but also its individual dietary constituents (2, 3).

Long-chain polyunsaturated fatty acids (PUFAs) are an essential component for normal growth and development, and there is evidence that the relative intake of individual PUFAs might influence adipose tissue development. The n-6 PUFAs, derived from plant oils, are highly adipogenic (4, 5); n-3 PUFAs 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 PUFAs during pregnancy and lactation had lower fat mass and reduced adipocyte size compared with offspring of control dams (7). An American mother-child cohort study found greater maternal combined intake of the n-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) at 29 wk 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 randomized controlled trials of n-3 PUFA supplementation during pregnancy and lactation, with anthropometric outcomes, have been reported (9–13); only one found lower body mass index (BMI) at 21 months of age in offspring of mothers who received DHA supplementation from 21 wk gestation until the end of the third month of lactation (9). In the remaining studies, no reduction in BMI and/or skinfold thickness was identified in the offspring of supplemented mothers at 12 months (11), 7 yr (10), and 19 yr (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 concentrations in late pregnancy in relation to offspring body composition at 4 and 6 yr of age as determined by dual-energy x-ray absorptiometry (DXA) in a prospective mother-offspring cohort.

Subjects and Methods

The Southampton Women's Survey (SWS)

The SWS is a study of 12,583 nonpregnant women aged 20 to 34 yr, resident in the city of Southampton, United Kingdom (14). Assessments of lifestyle, diet, and anthropometry were performed at study entry (April 1998 to December 2002) and, for women who became pregnant, again at 11 and 34 wk 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 prepregnancy interview, details of maternal parity, highest educational attainment, and social class were obtained, and height and weight were measured. At 34 wk gestation, the women were reweighed; pregnancy weight gain from prepregnancy to 34 wk gestation was categorized 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 (PC)

Venous blood was taken into heparinized tubes at 34 wk gestation. Plasma was prepared and stored at −80 C until analysis. Dipentadeconoyl PC was added to thawed plasma as internal standard before total lipid extraction with chloroform/methanol (2:1 vol/vol); butylated hydroxytoluene was added to the extraction as antioxidant. PC, which constitutes about 75% of plasma phospholipid (17), was isolated by solid-phase extraction on aminopropyl silica 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) sulfuric acid at 50 C for 2 h. After cooling and neutralization, fatty acid and methyl esters were extracted into hexane. Fatty acid methyl esters were separated by chromatography on a BPX-70 column (30 m × 220 μm; film thickness, 0.25 μm) fitted to a Hewlett-Packard HP6980 gas chromatograph (Hewlett-Packard Co., Palo Alto, CA). Front inlet temperature was 300 C; initial column temperature of 115 C was programmed to be held for 2 min, then to increase at 10 C/min to 200 C, to hold at 200 C for 10 min, to increase at 10 C/min to 240 C, and then to hold this temperature for 2 min. Helium was used as the running gas, and fatty acid methyl esters were detected by flame ionization. Fatty acid methyl esters were identified by comparison with retention times of standards run previously, and they were quantified using ChemStation software (Agilent Technologies, Palo Alto, CA) (18). Data were expressed as absolute concentration (micrograms per milliliter of plasma), which has been shown to be related to fatty acid consumption (19).

Childhood assessments of diet and body composition

There were 1987 singleton live births for enrolled mothers before December 31, 2003. The children were followed up at birth and during infancy. Duration of breast-feeding was determined from feeding histories obtained at 6 and 12 months of age.

At 3 yr, the children's diets were assessed using an administered food-frequency questionnaire (20). The key dietary pattern identified by principal component analysis was a "prudent" pattern, characterized by greater consumption of fruit, vegetables, water, whole-meal bread, and fish, and lower consumption of white bread, crisps, chips, and processed meat. A prudent diet score was calculated for each child, which indicated their compliance with the pattern and therefore the quality of their diet (20).

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 yr of age. At these visits, the child's height was measured using a Leicester height measurer (Seca Ltd., Birmingham, UK) and weight (in underpants only) measured using calibrated digital scales (Seca Ltd.). A whole-body DXA scan was obtained using a Hologic Discovery instrument (Hologic Inc., Bedford, MA) in pediatric scan mode (APEX 3.3 software, Hologic Inc.), yielding fat mass, lean mass, and bone mineral content. 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 (21).

Statistical analysis

To compare the effects of maternal plasma PUFA concentrations on offspring body composition at 4 and 6 yr, the dataset was based on those children who had DXA scans at both time points. Differences in demographic characteristics and body composition of the children at 4 and 6 yr by sex were explored using *t* tests and Mann-Whitney *U* tests for normally and non-normally distributed variables, respectively. Owing to sex differences in the children's body composition, all analysis was subsequently adjusted for the sex of the child, and owing to a wider age range at assessment, the 6-yr data were also adjusted for the child's age. For consistency, all offspring body composition and maternal PUFA variables were standardized with Fisher-Yates transformation to a normally distributed variable with a mean of 0 and SD of 1. Results are presented as standardized β-coefficients and therefore reflect the SD change in outcome for each 1 SD change in predictor (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, prepregnancy body mass index, IOM category of gestational weight gain, smoking status in late pregnancy, walking speed in late pregnancy, maternal estimated daily energy intake at 34 wk gestation), the child's height, and the duration of breast-feeding 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 PUFAs, the individual n-3 PUFAs EPA (20:5n-3), and 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 PUFAs to total n-6 PUFAs. 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 yr but the maternal PUFA measurements were missing. All analysis was performed using Stata version 11.0 (StataCorp, College Station, TX).

Results

Characteristics of study participants

A total of 766 children attended for DXA scan at age 4 yr and 531 children at 6 yr. Complete datasets including maternal late pregnancy measurements, plasma concentrations of PUFAs, and offspring DXA scans at both 4 and 6 yr of age were available for 293 mother-child pairs. The characteristics of the mothers and children are presented in Tables 1 and 2, respectively. A total of 277 children were included in the multivariable analysis at 4 yr and 275 at 6 yr (two children did not have a measurement of height at 6 yr, and the other 16 mother-child pairs were missing data for one or more maternal variables).

The mothers included in this study were of similar parity and smoking habits in late pregnancy, but were older (30.6 ± 3.6 *vs.* 30.0 ± 3.8 yr; $P = 0.015$) 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 December 31, 2003. Plasma n-3 PUFA status in late pregnancy was similar in the mothers included in this study to that in the 1483 mothers whose

TABLE 1. Characteristics of the mothers

Maternal characteristic	
n	293
Age (yr)	30.6 ± 3.6
Height (cm)	164.3 ± 6.6
Prepregnancy BMI (kg/m^2), median (IQR)	24.1 (22.3–27.4)
Smoking in late pregnancy	13.4 (39)
Duration of breast-feeding	
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 PUFAs	70.0 (51.6–92.8)
EPA	5.1 (3.2–7.7)
DHA	51.8 (38.8–70.5)
Total n-6 PUFAs	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 wk gestation (g/d)	
Protein	87.2 ± 22.8
Carbohydrate	312.6 ± 100.8
Fat	93.0 ± 28.3

Data are expressed as percentage (number) or mean ± SD, unless otherwise specified.

TABLE 2. Characteristics of the children at 4 and 6 yr of age

	4 yr		6 yr	
	Boys	**Girls**	**Boys**	**Girls**
n	153	140	153	140
Age (yr)	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)	104.7 (101.7–106.9)	104.2 (101.5–107.3)	120.7 (117.6–124.7)	120.4 (117.0–124.5)
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)[c]	4.8 (3.9–5.6)	6.1 (4.9–7.6)[c]
Lean mass (kg)	13.0 (12.1–14.0)	12.3 (11.1–13.1)[c]	17.7 (16.4–19.4)	17.0 (15.7–18.4)[b]
Fat percentage	24.0 (22.2–26.5)	28.6 (25.9–32.3)[c]	20.1 (18.0–23.5)	25.5 (21.6–29.5)[c]
Lean percentage	72.4 (70.0–74.3)	68.0 (64.4–70.8)[c]	76.2 (73.0–78.4)	71.0 (67.4–74.6)[c]
BMC percentage	3.4 (3.3–3.6)	3.4 (3.2–3.6)[a]	3.5 (3.4–3.7)	3.4 (3.2–3.6)[c]

Data are presented as median (IQR). BMC, Bone mineral content.

[a] $P < 0.05$; [b] $P < 0.001$; [c] $P < 0.0001$ comparing boys and girls of the same age.

children did not have DXA at 4 and 6 yr of age, but plasma n-6 PUFA status was lower in the included mothers [median, 493 μg/ml; interquartile range (IQR), 372–616 μg/ml; *vs.* median, 516 μg/ml; IQR, 402–631 μg/ml; $P = 0.04$]. Compared with the children who attended for DXA at 4 yr but who were not included in this cohort, the children were of similar age and sex, were slightly taller (104.4 ± 3.9 *vs.* 103.7 ± 4.2 cm; $P = 0.02$) but of similar weight [17.9 (IQR, 16.7–19.3) *vs.* 17.7 (IQR, 16.5–19.3) kg; $P = 0.33$], 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 yr. The children included in the study had greater total lean mass at 6 yr [17.5 (IQR, 16.1–18.9) *vs.* 16.7 (IQR, 15.4–17.9) kg; $P < 0.001$], than children of mothers who did not have PUFA measurements, but similar percentage lean mass [73.7 (IQR, 70.3–76.5) *vs.* 73.9 (IQR, 69.9–76.4) %; $P = 0.84$].

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 yr (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 yr ($\beta = 0.15$, $P = 0.009$; and $\beta = 0.17$, $P = 0.003$, respectively), although these were attenuated and of marginal statistical significance after adjustment for confounding factors ($\beta = 0.08$, $P = 0.08$; and $\beta = 0.07$, $P = 0.08$, respectively); there was no association with offspring height ($\beta = 0.08$, $P = 0.18$; and $\beta = 0.10$, $P = 0.09$ at 4 and 6 yr, respectively). Total maternal plasma n-6 PUFA concentration was positively associated with offspring fat mass at 4 and 6 yr ($\beta = 0.18$, $P = 0.002$; and $\beta =$

TABLE 3. Associations between maternal plasma PUFAs at 34 wk gestation and offspring body composition at 4 and 6 yr

	4 yr					
	Height	**Weight**	**Fat mass**	**Lean mass**	**% Fat mass**	**% Lean mass**
n-3 PUFAs						
Total n-3 PUFAs	0.12* (0.01, 0.24)	0.11 (−0.00, 0.23)	0.05 (−0.07, 0.17)	0.11 (−0.00, 0.23)	−0.01 (−0.12, 0.11)	0.00 (−0.11, 0.12)
Adjusted[a]	0.11 (−0.11, 0.23)	0.06 (−0.03, 0.14)	0.05 (−0.06, 0.17)	0.03 (−0.04, 0.11)	0.04 (−0.08, 0.16)	−0.04 (−0.16, 0.07)
EPA	0.15* (0.03, 0.26)	−0.02 (−0.02, 0.21)	−0.02 (−0.14, 0.10)	0.13* (0.02, 0.25)	−0.08 (−0.19, 0.04)	0.07 (−0.05, 0.19)
Adjusted[a]	0.12 (−0.00, 0.24)	0.01 (−0.08, 0.09)	−0.04 (−0.15, 0.08)	0.02 (−0.05, 0.10)	−0.04 (−0.16, 0.08)	0.03 (−0.09, 0.15)
DHA	0.11 (−0.01, 0.22)	0.10 (−0.01, 0.22)	0.04 (−0.08, 0.15)	0.10 (−0.01, 0.22)	−0.01 (−0.13, 0.10)	0.01 (−0.11, 0.13)
Adjusted[a]	0.10 (−0.02, 0.22)	0.07 (−0.02, 0.15)	0.06 (−0.06, 0.17)	0.04 (−0.03, 0.12)	0.04 (−0.08, 0.15)	−0.04 (−0.16, 0.08)
n-6 PUFAs						
Total n-6 PUFAs	0.08 (−0.04, 0.19)	0.15** (0.04, 0.27)	0.18** (0.07, 0.30)	0.08 (−0.04, 0.19)	0.14* (0.02, 0.25)	−0.14* (−0.26, −0.03)
Adjusted[a]	0.07 (−0.05, 0.19)	0.08 (−0.01, 0.16)	0.14* (0.03, 0.26)	−0.00 (−0.08, 0.07)	0.14* (0.02, 0.25)	−0.14* (−0.25, −0.03)
LA	0.06 (−0.06, 0.18)	0.15** (0.03, 0.26)	0.19** (0.08, 0.31)	0.06 (−0.05, 0.18)	0.15** (0.04, 0.27)	−0.16** (−0.27, −0.04)
Adjusted[a]	0.06 (−0.06, 0.18)	0.05 (−0.04, 0.13)	0.16** (0.05, 0.27)	0.00 (−0.07, 0.08)	0.15** (0.04, 0.27)	−0.15* (−0.27, −0.04)
AA	0.08 (−0.03, 0.20)	0.13* (0.01, 0.24)	0.13* (0.02, 0.25)	0.08 (−0.04, 0.19)	0.09 (−0.03, 0.20)	−0.09 (−0.21, 0.02)
Adjusted[a]	0.08 (−0.04, 0.20)	0.05 (−0.04, 0.13)	0.09 (−0.03, 0.20)	−0.00 (0.08, 0.07)	0.08 (−0.04, .19)	−0.08 (−0.20, 0.03)
n-3:n-6 PUFA ratio	0.09 (−0.02, 0.21)	−0.01 (−0.13, 0.11)	−0.13* (−0.25, −0.02)	0.07 (−0.04, 0.19)	−0.16** (−0.28, −0.05)	0.16** (0.05, 0.27)
Adjusted[a]	0.09 (−0.04, 0.21)	−0.01 (−0.10, 0.08)	−0.10 (−0.21, 0.02)	0.04 (−0.04, 0.12)	−0.10 (−0.22, 0.02)	0.10 (−0.02, 0.22)

Both univariate and multivariate associations after inclusion of potential confounding factors are shown. Associations are displayed as β-coefficient (95% CI) for standardized variables (relative change in SD of outcome for each SD change in predictor). * $P < 0.05$; ** $P < 0.01$.

[a] Adjusted for maternal age at delivery, parity, social class, and highest educational qualification, prepregnancy BMI, gestational weight gain, smoking status in late pregnancy, walking speed in late pregnancy, maternal daily energy intake at 34 wk gestation, the child's height (except for model predicting height as outcome), and duration of breast-feeding.

0.18, $P = 0.003$, respectively), which persisted after adjustment for confounding factors ($\beta = 0.14$, $P = 0.01$; and $\beta = 0.11$, $P = 0.04$, respectively), but no significant associations with offspring lean mass were identified ($\beta = 0.08$, $P = 0.19$; and $\beta = 0.11$, $P = 0.06$ at 4 and 6 yr, respectively). Thus, for each SD increase in maternal total n-6 PUFA concentration, total fat mass at 4 yr increased by 194 g [95% confidence interval (CI), 60, 327 g] and at 6 yr by 324 g (95% CI, 81, 568 g). Additionally, statistically significant positive associations were identified between maternal total n-6 PUFA concentration and offspring percentage fat mass at 4 yr ($\beta = 0.14$; $P = 0.02$) and 6 yr ($\beta = 0.14$; $P = 0.01$), and negative associations of similar magnitude with percentage lean mass. These were robust to adjustment for confounding factors (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 yr of age ($\beta = 0.12$, $P = 0.04$; and $\beta = 0.11$, $P = 0.06$, respectively) were identified. Positive associations with height ($\beta = 0.13$; $P = 0.03$) and weight ($\beta = 0.16$; $P = 0.008$) were present at 6 yr. These findings were not robust to adjustment for confounding factors (Table 3). 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 yr ($\beta = 0.05$, $P = 0.40$; and $\beta = 0.09$, $P = 0.11$, respectively), but did positively correlate with offspring lean mass at each time point ($\beta = 0.11$, $P = 0.06$; and $\beta = 0.14$, $P = 0.02$, respectively), although this was attenuated by the inclusion of offspring height in the multivariable model ($\beta = 0.03$, $P = 0.39$; and $\beta = 0.04$, $P = 0.31$, respectively). 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 yr (Table 3). The n-3:n-6 ratio was negatively associated with offspring fat mass at 4 yr ($\beta = -0.13$; $P = 0.02$), but this did not persist to 6 yr ($\beta = -0.05$; $P = 0.39$). Thus, for each SD increase in n-3:n-6 ratio, percentage fat mass at 4 yr decreased by 0.72 percentage points (95% CI, −1.23, −0.22) and percentage lean mass increased by 0.69 percentage points (95% CI, 0.21, 1.18). No association with total lean mass was identified at 4 or 6 yr, but a significant positive association with percentage lean mass at 4 yr was found ($\beta = 0.16$; $P = 0.006$). Again, this was not present at 6 yr ($\beta = 0.09$; $P = 0.14$), and no associations between n-3:n-6 ratio and offspring body composition were observed after the addition of confounding factors to the model.

TABLE 3. Continued

6 yr					
Height	Weight	Fat mass	Lean mass	% Fat mass	% Lean mass
0.13* (0.02, 0.25)	0.16** (0.04, 0.27)	0.09 (−0.02, 0.21)	0.14* (0.03, 0.25)	0.05 (−0.07, 0.16)	−0.05 (−0.16, 0.07)
0.12* (0.00, 0.24)	0.07 (−0.01, 0.15)	0.07 (−0.04, 0.18)	0.04 (−0.04, 0.11)	0.07 (−0.04, 0.19)	−0.07 (−0.18, 0.44)
0.14* (0.03, 0.26)	0.15* (0.03, 0.26)	0.05 (−0.07, 0.16)	0.16** (0.05, 0.28)	−0.01 (−0.13, 0.11)	−0.01 (−0.11, 0.13)
0.12 (−0.01, 0.24)	0.04 (−0.04, 0.12)	0.00 (−0.11, 0.11)	0.05 (−0.03, 0.12)	−0.00 (−0.12, 0.12)	0.00 (−0.11, 0.12)
0.12* (0.00, 0.23)	0.13* (0.02, 0.25)	0.07 (−0.04, 0.19)	0.12* (0.00, 0.23)	0.03 (−0.08, 0.15)	−0.03 (−0.15, 0.09)
0.11 (−0.01, 0.24)	0.07 (−0.01, 0.15)	0.07 (−0.04, 0.18)	0.04 (−0.03, 0.11)	0.07 (−0.05, 0.18)	−0.07 (−0.18, 0.05)
0.10 (−0.02, 0.22)	0.17** (0.06, 0.29)	0.18** (0.06, 0.29)	0.11 (−0.01, 0.22)	0.14* (0.03, 0.26)	−0.15* (−0.26, −0.03)
0.09 (−0.03, 0.22)	0.07 (−0.01, 0.15)	0.11* (0.01, 0.22)	0.01 (−0.06, 0.08)	0.11 (−0.00, 0.22)	−0.11 (−0.22, 0.00)
0.08 (−0.03, 0.20)	0.16** (0.04, 0.27)	0.18** (0.06, 0.29)	0.09 (−0.03, 0.20)	0.15* (0.03, 0.26)	−0.15* (−0.26, −0.04)
0.08 (−0.04, 0.20)	0.07 (−0.01, 0.15)	0.13* (0.02, 0.23)	0.01 (−0.07, 0.08)	0.12* (0.01, 0.24)	−0.12* (−0.24, −0.01)
0.10 (−0.02, 0.21)	0.17** (0.06, 0.28)	0.15** (0.04, 0.27)	0.12* (0.01, 0.24)	0.12* (0.00, 0.23)	−0.12* (−0.23, −0.00)
0.09 (−0.03, 0.21)	0.06 (−0.02, 0.14)	0.07 (−0.03, 0.18)	0.03 (−0.05, 0.10)	0.07 (−0.05, 0.18)	−0.07 (−0.18, 0.05)
0.09 (−0.03, 0.20)	0.04 (−0.08, 0.16)	−0.05 (−0.17, 0.06)	0.08 (−0.03, 0.20)	−0.08 (−0.20, 0.03)	−0.09 (−0.03, 0.20)
0.08 (−0.05, 0.20)	0.03 (−0.05, 0.11)	−0.02 (−0.13, 0.09)	0.04 (−0.03, 0.12)	−0.01 (−0.13, 0.11)	0.01 (−0.10, 0.13)

Multiple imputation strategy

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 it 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 (data not shown).

Discussion

In this prospective mother-offspring cohort study, we identified a number of key associations between maternal PUFA status and offspring body composition. First, a key finding was that maternal plasma PC n-6 PUFA concentration was positively associated with offspring adiposity. This association was evident at 4 yr, and persisted at 6 yr; 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 prenatal PUFA exposure could be linked to risk of offspring obesity. Second, 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 toward 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. Third, 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 regard to maternal diet in pregnancy to reduce the burden of obesity, and it provides an explanation as to why previous supplementation studies using n-3 PUFAs 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 SWS, 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 all comparisons are internal. The included mothers did have marginally lower late pregnancy n-6 PUFA concentrations than other mothers in the cohort. However, relationships between maternal fatty acid concentration and offspring body composition were similar when restricted to participants either above or below the median fatty acid concentration (data not shown), suggesting that this difference is unlikely to have materially influenced the results. There are a number of other limitations to this study. First, we measured body composition using DXA, a technique that has not previously been used 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 (22), and we used specific pediatric software, movement artifact was minimal, and the small numbers of children with excess movement artifact were excluded from the analysis. Second, it is not possible to determine whether the developing fetus was exposed to the same concentrations of PUFAs as were measured in the maternal samples. The transfer of PUFAs from the maternal to fetal circulation by the placenta occurs via several mechanisms, including passive and facilitated diffusion of nonesterified fatty acids (NEFAs). NEFAs may be released from multiple fatty acid sources including triglycerides and phospholipids after liberation by placental lipoprotein lipase and endothelial lipase. It has previously been demonstrated that in late pregnancy PUFAs preferentially incorporate into maternal plasma phospholipids and triglycerides over NEFAs and cholesterol esters, and the highest concentration of PUFAs is found in phospholipids (23). However, the relative transfer of PUFAs from each fraction to the fetus has not been determined and may differ. Therefore, the association between PUFAs derived from alternative maternal lipid fractions not analyzed in this study and offspring body composition cannot be fully determined from our results. Additionally, it has previously been demonstrated that biomagnification of PUFAs 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 (23), and this might explain the lack of associations between maternal plasma PC 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 (24, 25); current thinking is that whereas fetal PUFA exposure is dependent on maternal plasma PUFA status, other additional maternal or placental factors might also influence fetal levels. Third, it has previously been proposed that the critical period for adipogenesis is between 14 and 16 wk gestation (26); we cannot be certain those the PUFA concentrations measured at 34 wk are reflective of those in earlier pregnancy. Fourth, we did not have the opportunity to adjust for multiple comparisons because most of the outcomes

were highly related, limiting the use of conventional multicomparison processing (27). 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 breast-feeding 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.* (8) 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 yr. However, percentage plasma n-6 concentration is dependent on total fatty acid intake, and the fatty acid characteristics of their population differed from ours; in their population, mean percentage n-3 PUFAs was lower and mean percentage n-6 PUFAs was higher than in our cohort.

Prostacyclin is a key metabolic intermediate implicated in adipogenesis (28) and might represent a mechanism through which differences in the PUFAs could alter body fatness. The n-6 PUFA AA, derived directly from the diet or through LA metabolism, is a precursor of prostacyclin that enhances the differentiation of preadipocytes into functional adipocytes (28). Prostacyclin receptor-deficient mice do not gain weight in response to a high-LA 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 prostacyclin (29). 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 PUFAs and offspring adiposity to support an inhibitory effect of n-3 PUFAs on this process, this might be partly confounded by the high correlation between maternal n-3 and n-6 PUFAs. Maternal n-3:n-6 PUFA ratio did display a negative association with fat mass, thus suggesting that 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 concentration 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 randomized 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/d 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, because plasma PUFA concentrations were not determined, it is unknown whether supplementation increased maternal PUFA concentrations. Despite the limitations of the previous randomized 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 speed in late pregnancy, measures of maternal fat stores (30), 25-hydroxyvitamin D status (31), and pregnancy weight gain (15). Furthermore, the 0.18 SD change in total fat mass at 6 yr for each 1 SD change in n-6 maternal PUFA concentration might equate to an 8–15% increased risk of developing type 2 diabetes as an adult (32), suggesting that these results are clinically meaningful. Although the effect size is considerably smaller than postnatal habitual physical activity (33), 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.* (11) did randomize women to n-3 PUFA supplementation with concomitant advice on reducing dietary AA intake but found no significant differences in offspring skinfold thicknesses at 1 yr. However, the response to the maternal dietary advice was measured by 7-d dietary recall rather than measurements of plasma PUFAs, and therefore it is possible that poor compliance with dietary advice contributed to the lack of effect. Randomized trials of dietary advice in pregnancy with plasma PUFA measurements before random-

ization and at subsequent stages of pregnancy are therefore required.

In summary, in this observational study, maternal n-6 PUFA were positively associated with offspring fat mass, whereas no significant effects of maternal n-3 PUFAs 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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