Maternal plasma PUFA concentrations during pregnancy and childhood adiposity: the Generation R Study[1–3]

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ABSTRACT

Background: Maternal polyunsaturated fatty acid (PUFA) concentrations during pregnancy may have persistent effects on growth and adiposity in the offspring. A suboptimal maternal diet during pregnancy might lead to fetal cardiometabolic adaptations with persistent consequences in the offspring.

Objective: We examined the associations of maternal PUFA concentrations during pregnancy with childhood general and abdominal fat–distribution measures.

Design: In a population-based, prospective cohort study of 4830 mothers and their children, we measured maternal second-trimester plasma n–3 (ω-3) and n–6 (ω-6) PUFA concentrations. At the median age of 6.0 y (95% range: 5.6, 7.9 y), we measured childhood body mass index (BMI), the fat mass percentage, and the android:gynoid fat ratio with the use of dual-energy X-ray absorptiometry and measured the preperitoneal abdominal fat area with the use of ultrasound. Analyses were adjusted for maternal and childhood sociodemographic- and lifestyle-related characteristics.

Results: We observed that higher maternal total n–3 PUFA concentrations, and specifically those of eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, were associated with a lower childhood total-body fat percentage and a lower android:gynoid fat mass ratio ($P < 0.05$) but not with childhood BMI and the abdominal preperitoneal fat mass area. Higher maternal total n–6 PUFA concentrations, and specifically those of dihomo-γ-linolenic acid, were associated with a higher childhood total-body fat percentage, android:gynoid fat mass ratio, and abdominal preperitoneal fat mass area ($P < 0.05$) but not with childhood BMI. In line with these findings, a higher maternal n–6: n–3 PUFA ratio was associated with higher childhood total-body and abdominal fat mass.

Conclusions: Lower maternal n–3 PUFA concentrations and higher n–6 PUFA concentrations during pregnancy are associated with higher body fat and abdominal fat in childhood. Additional studies are needed to replicate these observations and to explore the causality, the underlying pathways, and the long-term cardiometabolic consequences. *Am J Clin Nutr* 2016;103:1017–25.

Keywords: adiposity, childhood, fat mass, pregnancy, PUFAs

INTRODUCTION

Fetal life and infancy are critical periods for the development of obesity in later life (1). Maternal and fetal nutrition might affect fetal and childhood growth and risk of obesity in childhood and adulthood. PUFAs are critical nutrients for fetal development (2). Animal studies have suggested that supplementation of the maternal diet during pregnancy and lactation with n–3 PUFAs leads to lower offspring body weight and less fat accumulation (3). In humans, a study in 293 mother-offspring pairs suggested that lower maternal n–3 PUFA and higher n–6 PUFA concentrations during pregnancy were associated with higher BMI and higher fat mass in children aged 4–6 y (4). Furthermore, higher maternal concentrations of dihomo-γ-linolenic acid (DGLA),[10]

[1] Supported by the Erasmus Medical Center, Rotterdam, the Erasmus University Rotterdam, the Netherlands Organization for Health Research and Development, the Netherlands Organisation for Scientific Research, the Ministry of Health, Welfare and Sport, and the Ministry of Youth and Families for the general design of the Generation R Study. Research that led to the results received funding from an unrestricted grant from Nutricia and the European Union's Seventh Framework Programme (FP7/2007–2013) project EarlyNutrition under grant 289346. VWVJ received an additional grant from the Netherlands Organization for Health Research and Development (VIDI 016.136.361) and a consolidator grant from the European Research Council (ERC-2014-CoG-648916). HT received a research grant from the European Community's 7th Framework Programme (FP7/2008–2013) under grant 212652 (NUTRIMENTHE project, "The Effect of Diet on the Mental Performance of Children"). The fatty acid analysis was cofunded by the European Research Council (advanced grant ERC-2012-AdG 322605 META-GROWTH; to BK).

[2] The funders had no role in the design or conduct of the study; the collection, management, analysis, or interpretation of the data; or the preparation, review, or approval of the manuscript.

[3] Supplemental Figures 1 and 2 and Supplemental Tables 1–5 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[10] Abbreviations used: AA, arachidonic acid; ALA, α-linolenic acid; DGLA, dihomo-γ-linolenic acid; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; DXA, dual-energy X-ray absorptiometry; LA, linoleic acid.

Received April 10, 2015. Accepted for publication January 19, 2016.

First published online February 24, 2016; doi: 10.3945/ajcn.115.112847.

which is an n–6 PUFA, were associated with higher childhood BMI, waist circumference, and the sum of skinfold thicknesses in 234 children aged 7 y (5). The majority of previous studies that have investigated the relations between early life fatty acid status and childhood obesity have used BMI. However, BMI does not distinguish lean mass from fat mass and provides no insight about the body fat distribution (6, 7). Studies in adults and children have suggested that higher total-body fat and abdominal fat mass levels, independent from BMI, are associated with cardiovascular disease risk factors, disease, and mortality (8–10). Despite many previous observational and small experimental studies, it is still unclear whether maternal PUFA status influences offspring growth and fat mass development (4, 5, 11–13). Thus far, results from observational studies and randomized controlled trials have not been consistent (14, 15).

Therefore, we examined, in a population-based prospective cohort study from early pregnancy onward in 4830 mothers and their children, the associations of maternal n–3 and n–6 PUFA concentrations during pregnancy with childhood BMI (in kg/m^2) and specific body fat measures, including the fat mass percentage and android:gynoid fat ratio, which were measured with the use of dual-energy X-ray absorptiometry (DXA), and the preperitoneal abdominal fat area, which was measured with the use of ultrasound. In addition, we examined whether these associations were independent of maternal and childhood sociodemographic- and lifestyle-related characteristics.

METHODS

Study design

The study was embedded in the Generation R Study, which is a population-based prospective cohort study from fetal life to adulthood conducted in Rotterdam, Netherlands (16, 17). The study was approved by the Medical Ethical Committee of the Erasmus MC, University Medical Center, Rotterdam (MEC 198.782/2001/31). All mothers gave written consent. Pregnant women with an expected delivery date from April 2002 to January 2006 were enrolled in the study. In total, 8879 mothers were enrolled during pregnancy; of those, 7072 mothers had information about PUFA concentrations available, and 6925 mothers gave birth to singleton live-born children. Childhood follow-up data were available for 4830 of these children (a flowchart is shown in **Supplemental Figure 1**).

Maternal fatty acid status

Maternal venous samples were drawn at a median gestational age of 20.5 wk (95% range: 16.5, 24.9 wk). To analyze PUFA concentrations, EDTA plasma samples were selected and transported to the Division of Metabolic Diseases and Nutritional Medicine, Dr. von Hauner Children's Hospital, University of Munich Medical Center. After being thawed, the analysis of the composition of plasma glycerophospholipid PUFAs was performed with the use of a sensitive and precise high-throughput method, which was suitable for large epidemiologic studies as previously described (18). PUFA concentrations were expressed as the proportion of total fatty acids that were present in the chromatogram (weight percentage) (19, 20). On the basis of findings from previous studies, we selected maternal PUFAs for our analyses, which have been associated with risk of cardiovascular and metabolic outcomes in adults and maternal and fetal pregnancy outcomes (4, 21–24). Selected maternal PUFAs were total n–3 PUFA concentrations, which included α-linolenic acid (ALA; 18:3n–3), EPA (20:5n–3), docosapentaenoic acid (DPA; 22:5n–3), and DHA (22:6n–3). Total n–6 PUFA concentrations included linoleic acid (LA; 18:2n–6), γ-linolenic acid (18:3n–6), eicosadienoic acid (20:2n–6), DGLA (20:3n–6), arachidonic acid (AA; 20:4n–6), and docosatetraenoic acid (DTA; 22:4n–6). The n–6:n–3 ratio was calculated as the sum of all n–6 PUFAs (LA, γ-linolenic acid, eicosadienoic acid, DGLA, and AA) divided by the sum of all n–3 PUFAs (ALA, EPA, DPA, and DHA).

Childhood fat-distribution outcomes

At a median age of 6 y, height and weight were measured with the subject not wearing shoes or heavy clothing. Height was measured to the nearest 0.1 cm with the use of a stadiometer (Holtain Ltd.). Weight was measured to the nearest gram with the use of an electronic scale (SECA 888; seca). In addition, BMI was calculated (25).

Total body and regional fat masses were measured with the use of a DXA scanner (iDXA, 2008; GE Lunar) and were analyzed with enCORE software (version 12.6; GE Healthcare) (26). DXA can accurately detect whole-body fat mass and regional body fat mass and has been validated against the use of computed tomography (26, 27). Total fat mass (kg) was calculated as a percentage of total body weight (kg) that was measured with the use of DXA. The android:gynoid fat mass ratio was calculated. The android:gynoid fat ratio reflects the central body fat distribution in the abdomen and hip regions, respectively, and was used as a marker of the waist:hip fat distribution (27).

Abdominal ultrasound examinations were used to measure the preperitoneal fat area as a measure of visceral abdominal fat as previously described (28). Briefly, the preperitoneal fat thickness was measured with a linear (L12–5 MHz) transducer (29), which was placed perpendicular to the skin surface on the median upper abdomen. We scanned longitudinally from the xiphoid process to the navel along the midline (linea alba). The preperitoneal fat mass area was measured as an area of 2 cm length along the midline starting from the reference point in the direction of the navel.

Covariates

Information on maternal age, educational level, and ethnicity was obtained at enrollment (16). We measured maternal height and blood pressure at enrollment and obtained information about maternal prepregnancy weight with the use of a questionnaire. We calculated BMI. Information on maternal smoking, alcohol consumption, and folic acid supplement use was assessed with the use of questionnaires during pregnancy. Weight gain at $\leq$30 wk of gestation was calculated as the difference between maternal weight measured at 30 wk of gestation (95% range: 28.4, 32.9 wk) and self-reported weight before pregnancy. As previously described, we used gestational weight gain until 30 wk because this measurement was and available for 3895 mothers (30). Information about maximum weight during pregnancy was assessed with the use of a questionnaire 2 mo after delivery and was only available in a subgroup of 2181 mothers. The

maximum weight from the questionnaire and weight measured at 30 wk were strongly correlated ($r = 0.87$, $P < 0.001$). We used food-frequency questionnaires to assess maternal nutritional information during pregnancy. Information about pregnancy complications, sex, gestational age, and weight at birth was obtained from medical records (31, 32). Information about breastfeeding, the timing of the introduction of solid foods, and the television-watching time was obtained in infancy with the use of questionnaires (33). Information about infant PUFA intake at 13 mo, which was measured with the use of a 211-item food-frequency questionnaire, was available in a subgroup of the study ($n = 2313$) (34).

Statistical analysis

We explored the continuous associations of maternal PUFA concentrations with childhood adiposity outcomes at the age of 6 y with the use of linear regression models. Because the abdominal preperitoneal fat area was not normally distributed, we log transformed this variable for additional analyses. To enable the comparison of effect estimates, we constructed SD scores for all childhood adiposity outcomes and for concentrations of all PUFAs as follows: (observed value – mean) ÷ SD.

We constructed the following different models: *1*) a basic model that included gestational age at maternal blood sampling and child age and sex; *2*) a pregnancy-factor–adjusted model, which was the basic model that was further adjusted for maternal age, educational level, ethnicity, parity, prepregnancy BMI, gestational weight gain at ≤30 wk of gestation, blood pressure at enrollment, smoking, folic acid–supplement use, and total caloric intake during pregnancy and pregnancy complications; *3*) a childhood-factor–adjusted model, which was the basic model that was further adjusted for gestational age and weight at birth, breastfeeding, the timing of the introduction of solid foods, and the average television-watching time; and *4*) a fully adjusted model that included all factors of the other models. All fat mass outcomes were further adjusted for child height. Included covariates were selected on the basis of their associations with the outcomes of interest of previous studies (4, 5) or a >10% change in the effect estimate. We performed an additional analysis by further adjusting these analyses for intake of PUFAs in infants at 13 mo of age in a subgroup. We tested for interaction terms between maternal PUFA concentrations and child sex in relation to adiposity outcomes in childhood. Because no significant interactions were observed, no additional stratified analyses were performed. To reduce the potential bias associated with missing data and to maintain the statistical power, we performed multiple imputations of missing covariates by generating 5 independent data sets with the use of the Markov chain Monte Carlo method after which the pooled effect estimates were calculated. All analyses were performed with the use of the Statistical Package for the Social Sciences program (version 21.0 for Windows; SPSS Inc.).

RESULTS

Subject characteristics

Table 1 shows maternal and childhood characteristics. Mean ± SD second-trimester maternal total n–3 and n–6 PUFA concentrations were 105.0 ± 27.5 and 604.2 ± 88.6 mg/L, respectively (**Table 2**). Nonresponse analyses showed that, compared with children with follow-up measurements, children without follow-up measurements had a lower gestational age at birth and lower birth weight (**Supplemental Table 1**). Also, mothers included in the analyses had a higher n–3 PUFA concentration and lower n–6:n–3 PUFA ratio than did those who were not included (**Supplemental Table 2**). Correlation coefficients between all maternal PUFA concentrations and childhood body fat outcomes are shown in **Supplemental Table 3**.

Maternal n–3 PUFA concentrations and childhood body fat outcomes

Table 3 shows that, in the basic models, higher maternal total n–3 PUFA concentrations and concentrations of each individual n–3 fatty acid were associated with lower childhood BMI and a lower total-body fat mass percentage, android:gynoid fat mass ratio, and abdominal preperitoneal fat mass area (all $P < 0.05$). In the fully adjusted models, all n–3 PUFA concentrations except for ALA concentrations were still associated with a lower

TABLE 1
Characteristics of mothers and their children ($n = 4830$)

	Value
Maternal characteristic	
Age, y	30.9 (19.9, 39.3)[1]
Gestational age at PUFA measures, wk	20.5 (16.5, 24.9)
Prepregnancy BMI, kg/m^2	23.6 ± 4.2[2]
Weight gain at ≤30 wk of gestation, kg	10.4 ± 4.9
Education, higher, *n* (%)	2400 (46.2)[3]
Ethnicity, European, *n* (%)	2917 (61.5)
Parity, nulliparous, *n* (%)	2757 (57.5)
Total energy intake, kcal/d	2048 ± 558
Smoking during pregnancy, yes, *n* (%)	738 (17.1)
Folic acid supplement use, yes, *n* (%)	2794 (75.6)
Preeclampsia, *n* (%)	91 (2.0)
Gestational hypertension, *n* (%)	182 (4.0)
Gestational diabetes, *n* (%)	44 (0.9)
Birth and infant characteristics	
Boys, *n* (%)	2413 (50.0)
Gestational age at birth, wk	40.1 (35.8, 42.3)
Birth weight, g	3433 ± 553
Ever breastfeeding, yes, *n* (%)	3550 (92.7)
Introduction of solid foods at >6 mo of age, *n* (%)	315 (10.4)
n–3 PUFA intake, g/d	0.6 (0.4)
n–6 PUFA intake, g/d	4.7 (2.9)
Childhood adiposity characteristics	
Age at follow-up, y	6.0 (5.6, 7.9)
BMI, kg/m^2	16.2 ± 1.9
Fat mass, %	24.9 ± 5.7
Android:gynoid fat mass ratio	0.3 ± 0.1
Abdominal preperitoneal fat mass, cm^2	0.39 (0.16, 1.2)

[1]Median; 95% range in parentheses (all such values).
[2]Mean ± SD (all such values).
[3]Valid percentage in parentheses (all such values).

TABLE 2
Second-trimester maternal PUFA concentrations (n = 4830)[1]

	Absolute concentrations, mg/L	Percentage by weight of total sum of fatty acids
Total PUFA concentrations	709.3 ± 97.9	43.1 ± 2.0
Total n–3 PUFAs	105.0 ± 27.5	6.4 ± 1.5
ALA	5.1 ± 1.9	0.3 ± 0.1
EPA	8.7 ± 5.4	0.5 ± 0.3
DPA	12.1 ± 4.3	0.7 ± 0.2
DHA	77.6 ± 20.4	4.8 ± 1.1
Total n–6 PUFAs	604.2 ± 88.6	36.8 ± 2.5
LA	361.9 ± 63.1	22.3 ± 2.8
GLA	1.5 ± 0.7	0.1 ± 0.0
EDA	8.5 ± 1.9	0.5 ± 0.1
DGLA	61.0 ± 16.5	3.7 ± 0.7
AA	156.4 ± 32.6	9.6 ± 1.6
DTA	6.9 ± 2.2	0.4 ± 1.1
Total n–6:n–3 PUFA ratio	6.1 ± 1.7	—

[1]All values are means ± SDs. AA, arachidonic acid; ALA, α-linolenic acid; DGLA, dihomo-γ-linolenic acid; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; EDA, eicosadienoic acid; GLA, γ-linolenic acid; LA, linoleic acid.

childhood total-body fat mass percentage and android:gynoid fat mass ratio (all P <0.05) but not with childhood BMI and the preperitoneal fat mass area. The strongest effect estimate was observed for the associations of maternal total n–3 PUFA concentrations with the childhood total-body fat mass percentage [difference: −0.07 (95% CI: −0.10, −0.05) per SD increase of total maternal n–3 PUFA in the fully adjusted model]. Models that were adjusted for pregnancy and childhood factors separately are given in **Supplemental Table 4** and show that pregnancy factors fully explained the associations of all maternal total n–3 PUFAs with childhood BMI and the abdominal preperitoneal fat mass area. Childhood factors did not materially influence the observed associations.

Maternal n–6 PUFA concentrations and childhood body fat outcomes

Table 4 shows that, in the basic models, higher maternal total n–6 PUFA concentrations and LA, DGLA, and DTA concentrations were associated with higher childhood BMI and a higher total-body fat mass percentage, android:gynoid fat mass ratio, and abdominal preperitoneal fat mass area (all P <0.05). After adjustment for maternal and childhood characteristics, higher total maternal n–6 PUFA and DGLA concentrations were associated with a higher childhood total-body fat mass percentage, android:gynoid fat mass ratio, and abdominal preperitoneal fat mass area (all P < 0.05) but not with childhood BMI. The strongest effect estimate was observed for the associations of maternal total n–6 PUFA concentrations with the childhood total-body fat mass percentage [difference: 0.08 (95% CI: 0.05, 0.10) per SD increase of total maternal n–6 PUFA in the fully adjusted model]. Models that were adjusted for pregnancy and childhood factors separately are given in **Supplemental Table 5** and show that pregnancy factors fully explained the associations of maternal total n–6 PUFAs with childhood BMI.

Maternal n–6:n–3 PUFA ratio and childhood body fat outcomes

Figure 1 shows that a higher maternal n–6:n–3 PUFA ratio was associated with higher childhood BMI and a higher total-body fat mass percentage, android:gynoid fat mass ratio, and

TABLE 3
Maternal n–3 PUFA concentrations and childhood body fat outcomes (n = 4830)[1]

	Differences in childhood adiposity outcomes in SDSs			
Maternal n–3 PUFAs in SDSs	BMI (n = 4830)	Total body fat mass, % (n = 4706)	Android:gynoid fat mass ratio (n = 4706)	Preperitoneal fat mass area (n = 3912)
Total n–3 PUFAs				
Basic model	–0.09 (−0.12, −0.07)*	–0.16 (−0.18, −0.13)*	–0.13 (−0.15, −0.10)*	–0.09 (−0.12, −0.06)*
Full model	−0.02 (−0.05, 0.01)	–0.07 (−0.10, −0.05)*	–0.07 (−0.10, −0.04)*	−0.02 (−0.06, 0.01)
ALA				
Basic model	–0.08 (−0.11, −0.05)*	–0.10 (−0.12, −0.07)*	–0.07 (−0.10, −0.04)*	–0.08 (−0.11, −0.06)*
Full model	0.00 (−0.03, 0.03)	−0.02 (−0.05, 0.00)	−0.02 (−0.05, 0.01)	–0.03 (−0.06, −0.00)*
EPA				
Basic model	–0.08 (−0.10, −0.05)*	–0.13 (−0.15, −0.10)*	–0.09 (−0.12, −0.07)*	–0.07 (−0.10, −0.05)*
Full model	−0.02 (−0.04, 0.01)	–0.06 (−0.08, −0.03)*	–0.04 (−0.07, −0.01)*	−0.02 (−0.05, 0.01)
DPA				
Basic model	–0.05 (−0.08, −0.03)*	–0.11 (−0.14, −0.09)*	–0.08 (−0.11, −0.06)*	–0.04 (−0.07, −0.01)*
Full model	−0.00 (−0.03, 0.03)	–0.05 (−0.08, −0.03)*	–0.05 (−0.08, −0.02)*	0.00 (−0.03, 0.03)
DHA				
Basic model	–0.08 (−0.11, −0.05)*	–0.13 (−0.16, −0.11)*	–0.11 (−0.13, −0.08)*	–0.07 (−0.10, −0.05)*
Full model	−0.02 (−0.04, 0.01)	–0.07 (−0.09, −0.04)*	–0.06 (−0.09, −0.03)*	−0.02 (−0.05, 0.01)

[1]All values are regression coefficients; 95% CIs in parentheses. Values reflect differences in SDSs of childhood BMI, total-body fat mass percentage, Android:gynoid fat mass ratio, and abdominal preperitoneal fat mass area per SD change in maternal n–3 PUFA concentrations, respectively. Basic models were adjusted for gestational age at blood sampling and for child age, sex, and height (fat mass outcomes only). Full models were adjusted for pregnancy factors and childhood factors, which included maternal age, educational level, ethnicity, parity, prepregnancy BMI, weight gain at ≤30 wk of gestation, blood pressure at enrollment, smoking, folic acid–supplement use, total calorie intake during pregnancy, pregnancy complications, gestational age and weight at birth, breastfeeding duration, the timing of the introduction of solid foods, and television-watching time, respectively. *P < 0.05. ALA, α-linolenic acid; DPA, docosapentaenoic acid; SDS, SD score.

TABLE 4
Maternal n–6 PUFA concentrations and childhood body fat outcomes (n = 4830)[1]

	Differences in childhood adiposity outcomes in SDSs			
Maternal n–6 PUFAs in SDSs	BMI (n = 4830)	Total body fat mass, % (n = 4706)	Android:gynoid fat mass ratio (n = 4706)	Preperitoneal fat mass area (n = 3912)
Total n–6 PUFAs				
Basic model	0.11 (0.08, 0.14)*	0.17 (0.15, 0.20)*	0.12 (0.09, 0.15)*	0.14 (0.11, 0.17)*
Full model	0.03 (−0.00, 0.06)	0.08 (0.05, 0.10)*	0.06 (0.03, 0.10)*	0.07 (0.04, 0.11)*
LA				
Basic model	0.03 (0.00, 0.06)*	0.08 (0.06, 0.11)*	0.07 (0.04, 0.10)*	0.06 (0.03, 0.08)*
Full model	0.01 (−0.02, 0.04)	0.05 (0.03, 0.08)*	0.06 (0.03, 0.09)*	0.03 (−0.00, 0.06)
GLA				
Basic model	0.03 (−0.00, 0.05)	0.03 (0.01, 0.06)*	0.02 (−0.01, 0.05)	0.06 (0.03, 0.08)*
Full model	0.01 (−0.02, 0.03)	0.01 (−0.01, 0.04)	−0.00 (−0.03, 0.03)	0.04 (0.01, 0.07)*
EDA				
Basic model	−0.00 (−0.03, 0.03)	0.02 (−0.01, 0.05)	0.02 (−0.01, 0.05)	0.01 (−0.02, 0.04)
Full model	−0.01 (−0.03, 0.02)	0.00 (−0.02, 0.03)	0.02 (−0.01, 0.05)	−0.01 (−0.04, 0.02)
DGLA				
Basic model	0.06 (0.03, 0.09)*	0.06 (0.04, 0.09)*	0.07 (0.04, 0.09)*	0.07 (0.04, 0.10)*
Full model	0.01 (−0.02, 0.03)	0.04 (0.02, 0.07)*	0.05 (0.02, 0.08)*	0.06 (0.03, 0.09)*
AA				
Basic model	0.09 (0.06, 0.11)*	0.08 (0.05, 0.10)*	0.03 (−0.00, 0.05)	0.08 (0.05, 0.11)*
Full model	0.01 (−0.02, 0.04)	−0.01 (−0.04, 0.02)	–0.04 (−0.07, −0.01)*	0.02 (−0.01, 0.05)
DTA				
Basic model	0.08 (0.06, 0.11)*	0.08 (0.06, 0.11)*	0.06 (0.03, 0.09)*	0.10 (0.07, 0.12)*
Full model	0.02 (−0.01, 0.05)	0.01 (−0.02, 0.04)	0.01 (−0.02, 0.04)	0.04 (0.01, 0.07)*

[1]All values are regression coefficients; 95% CIs in parentheses. Values reflect differences in SDSs of childhood BMI, total-body fat mass percentage, Android:gynoid fat mass ratio, and abdominal preperitoneal fat mass area per SD change in maternal n–6 PUFA concentrations, respectively. Basic models were adjusted for gestational age at blood sampling and for child age, sex, and height (fat mass outcomes only). Full models were adjusted for pregnancy factors and childhood factors, which included maternal age, educational level, ethnicity, parity, prepregnancy BMI, weight gain at ≤30 wk of gestation, blood pressure at enrollment, smoking, folic acid–supplement use, total calorie intake during pregnancy, pregnancy complications, gestational age and weight at birth, breastfeeding duration, the timing of the introduction of solid foods, and television-watching time, respectively. *$P < 0.05$. AA, arachidonic acid; DGLA, dihomo-γ-linolenic acid; DTA, docosatetraenoic acid; EDA, eicosadienoic acid; GLA, γ-linolenic acid; LA, linoleic acid.

abdominal preperitoneal fat mass area in the basic model (all $P < 0.05$). In the fully adjusted model, a higher maternal n–6:n–3 PUFA ratio was associated with a higher childhood total-body fat mass percentage, android:gynoid fat mass ratio, and abdominal preperitoneal fat mass area [differences: 0.05 (95% CI: 0.03, 0.06), 0.05 (95% CI: 0.03, 0.07), and 0.03 (95% CI: 0.02, 0.05), respectively, per SD increase in the total n–6:n–3 PUFA ratio]. Associations of the maternal n–6:n–3 PUFA ratio with childhood body fat outcomes that were adjusted for pregnancy and childhood factors are presented in **Supplemental Figure 2.**

Additional analysis to take infant PUFA intake into account

We performed an additional analysis in 2313 children to explore whether the associations were explained by infant PUFA intake. We observed that an additional adjustment for PUFA intake in infants did not materially affect the observed associations (data not shown).

DISCUSSION

In this population-based, prospective cohort study, we observed that lower maternal n–3 PUFA concentrations and higher n–6 PUFA concentrations during pregnancy were associated with a higher body fat percentage and an adverse general and abdominal fat distribution in childhood. The associations of maternal n–3 and n–6 PUFA concentrations with detailed childhood fat mass outcomes were only partly explained by maternal and childhood characteristics.

Methodologic considerations

We used a population-based, prospective cohort study design that included a large number of subjects. Of all children whose maternal PUFA concentrations were available, 64% of the children participated in the follow-up studies at the age of 6 y. The nonresponse could have led to biased effect estimates if the associations of maternal PUFA concentrations with childhood body composition were different between children who were included in the analyses and those who were not included in the analyses. A nonresponse analysis showed that birth weight and gestational age at birth were lower in children who were not included in the analyses than in those who were included. Also, the n–6:n–3 PUFA ratio was higher in mothers of children who were not included in the analysis. A selective loss to follow-up may have led to an underestimation of the effect estimates if children with adverse body fat profiles from mothers with higher n–6:n–3 ratios had higher risk of loss to follow-up. We measured a large number of maternal PUFA concentrations in blood samples only once during pregnancy. These concentrations of maternal PUFAs may not have fully reflected the concentrations of PUFAs that the fetus was exposed to during the full

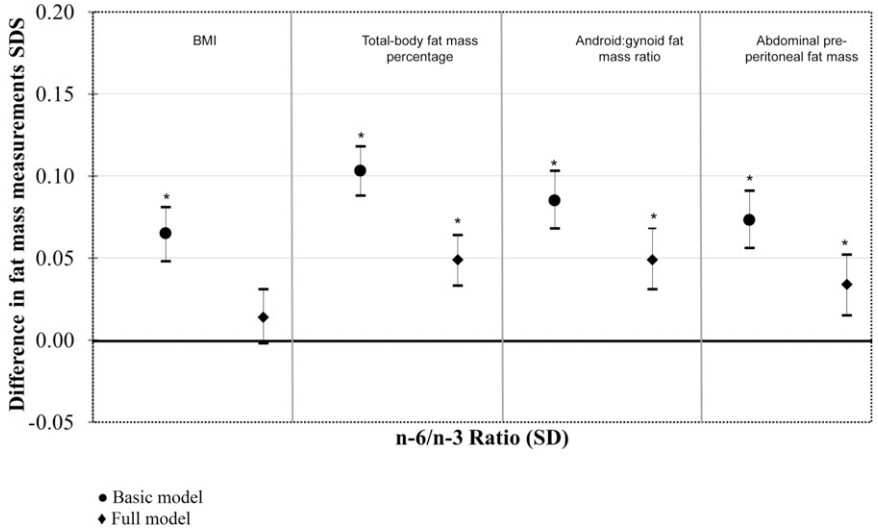

FIGURE 1 Regression coefficients (95% CIs) of the maternal n–6:n–3 PUFA ratio and childhood body fat outcomes (n = 4830). Values reflect differences in SDSs of childhood BMI, the total-body fat mass percentage, the Android:gynoid fat mass ratio, and the abdominal preperitoneal fat mass area per SD change in the maternal n–6:n–3 PUFA ratio. Basic models were adjusted for gestational age at blood sampling and for child age, sex, and height (fat mass outcomes only). Full models were adjusted for the following pregnancy factors and childhood factors: maternal age, educational level, ethnicity, parity, prepregnancy BMI, weight gain at ≤30 wk of gestation, blood pressure at enrollment, smoking, folic acid–supplement use, total calorie intake during pregnancy, pregnancy complications, gestational age and weight at birth, breastfeeding duration, the timing of the introduction of solid foods, and television-watching time, respectively. *$P < 0.05$. SDS, SD score.

pregnancy because this exposure also depends on placental transfer (35). Also, PUFAs measured in plasma may reflect a time frame of dietary intake of ∼2 wk and seem to be reasonable indicators for the recent intake (36). Unfortunately, no information was available about erythrocyte lipid concentrations, which reflect a longer intake period. We performed detailed measurements of the outcomes of childhood body fat distribution. DXA quantifies the fat content with a high precision and has the capacity for a regional analysis but cannot differentiate between the 2 abdominal fat compartments (26, 27). Ultrasound is a reliable method to differentiate between abdominal visceral and subcutaneous fat compartments with the use of an area measurement as a proxy for these fat compartments (37). Both DXA and abdominal ultrasound have been validated against computed tomography (26, 27, 37). We presented the associations of plasma PUFA concentrations with childhood adiposity outcomes in different regression models (i.e., a basic model, pregnancy model, childhood model, and fully adjusted model) to enable interpretation of the results with and without different adjustment approaches. We observed the largest changes in effect estimates when we further adjusted the basic model for maternal prepregnancy BMI. Additional adjustment for childhood factors did not change the effect estimates. We included various possible confounders because of the potential of residual confounding in the observational study. However, we may have overadjusted the fully adjusted models by including various covariates that were potentially involved in causal pathways. Despite extensive adjustment, residual confounding may still have been an issue. Most importantly, we did not have detailed information available about childhood PUFA blood concentrations or dietary intake in the full cohort. However, one previous study showed that an adjustment for child PUFA concentrations did not change the association between maternal PUFA concentrations and childhood body composition (5). Additional studies are needed to further explore the potential role of confounding by maternal and childhood dietary factors in these observed associations.

Interpretation of main findings

An adequate PUFA supply is important for optimal fetal development (38). Previous studies have suggested that lower maternal n–3 PUFA and higher n–6 PUFA concentrations are associated with increased risk of adverse birth outcomes (4, 21). Alterations in maternal n–3 and n–6 PUFA concentrations might also have long-term offspring consequences (35). Several studies have suggested that lower maternal n–3 PUFA and higher n–6 PUFA concentrations are associated with higher BMI in the offspring, but results have not been not consistent (5, 15, 39, 40). A study in 234 mother-child pairs of the Maastricht Essential Fatty Acid Birth cohort in the Netherlands showed that higher maternal DGLA, which is an n–6 PUFA, during pregnancy was associated with increased childhood BMI at age 7 y (5). In another study in 388 German mother-child pairs, a higher n–6:n–3 PUFA ratio in cord blood was associated with higher BMI at 10 y of age (41). Another study in 208 pregnant women in Germany showed that higher concentrations of maternal AA, which is an n–6 PUFA, were associated with lower offspring BMI at 1 y of age (42). Thus, results from previous observational studies have suggested that maternal PUFA concentrations during pregnancy have a persistent effect on childhood growth and adiposity. Such observational studies may have suffered from residual confounding. To take confounding into account in the associations of maternal PUFA concentrations and childhood adiposity outcomes, several randomized trials have been performed. A randomized, double-blind, clinical trial in 144 pregnant women showed that infants of mothers who received 200 mg DHA (which is an n–3 PUFA)/d during pregnancy had lower BMI at 21 mo of age, but there was no difference at age 6 y (43). A meta-analysis of 6 randomized controlled trials showed no

effect of maternal n–3 PUFA supplementation during pregnancy on BMI in preschool children (14). Thus, results from these trials have not supported the results obtained in observational studies. However, because of the subject selection and specific composition of the supplements, results from these trials are difficult to generalize to larger population-based samples.

Only a few studies have assessed the associations of maternal PUFA concentrations with more-detailed offspring body fat measures. A study in 1250 mother-child pairs in Massachusetts showed that higher concentrations of DHA and EPA, which are n–3 PUFAs, in the maternal diet and umbilical cord were associated with lower subcutaneous fat mass measured by the sum of subscapular and triceps skinfold thicknesses at the age of 3 y (21). In the same study, a higher maternal concentration of n–6 PUFAs was associated with a higher sum of childhood subscapular and triceps skinfold thicknesses (21). In this study, a food-frequency questionnaire was used to assess maternal fatty acid intake. A prospective United Kingdom cohort study in 293 mother-child pairs showed that higher maternal n–6 PUFA concentrations were positively associated with offspring body fat mass that was measured with the use of DXA at 6 y of age. The maternal n–3:n–6 PUFA ratio was negatively associated with offspring fat mass at 4 y of age but not at 6 y of age (4). Findings from this latter study suggested that a reduction in the maternal n–6 PUFA concentration is more protective than increasing the concentration of n–3 PUFAs for children. A randomized trial in 208 pregnant women showed no effect of n–3 PUFA supplementation on fat mass as assessed with the use of skinfold thickness and abdominal ultrasonography in the offspring during the first year of life (12).

In the current study, we observed in the basic models that higher maternal n–3 PUFAs and lower total n–6 PUFAs were associated with lower BMI in childhood. However, these associations were fully explained by maternal sociodemographic- and lifestyle-related characteristics. In particular, maternal BMI seemed to largely explain the associations of maternal PUFA concentrations and childhood BMI. In contrast with the associations with childhood BMI, we observed that higher maternal total n–3 PUFA concentrations were associated with a lower childhood total-body fat mass, android:gynoid fat mass ratio, and abdominal preperitoneal fat mass area. Concentrations of the n–3 PUFAs, ALA, EPA, DPA, and DHA, were all associated with these childhood outcomes. Higher maternal total n–6 PUFA concentrations, specifically of LA, DGLA, AA, and DTA, were associated with a higher childhood total-body fat mass, android:gynoid fat mass ratio, and abdominal preperitoneal fat mass area. In line with these findings, a higher maternal n–6:n–3 PUFA ratio was also associated with higher childhood total-body and abdominal fat mass concentrations. These associations were not explained by the maternal and childhood factors of which we had information available. Thus, our results suggest that both lower maternal n–3 PUFA concentrations and higher maternal n–6 PUFA concentrations during pregnancy affect childhood total-body fat mass and abdominal fat mass concentrations. An adverse body fat distribution seems to be associated, independent of BMI, with an adverse cardiovascular disease risk profile (10, 44, 45). Whether the observed body fat differences that were related to maternal PUFA concentrations have adverse health consequences should be further studied.

Because of the observational design of the study, additional studies are needed for replication and to explore causality. If the associations are indeed causal, the underlying biological mechanisms may involve adaptations in fetal fat cell development and adipose tissue growth (4, 46). n–6 PUFAs form a precursor of prostacyclin, which promotes the differentiation of preadipocytes into mature and functional adipocytes (47). On the contrary, n–3 PUFAs inhibit this process of differentiation through the inhibition of the activity of the cyclooxygenase enzymes, which enhance the prostaglandin biosynthesis (48). Thus, n–3 PUFAs inhibit the process of lipid storage and accumulation in the fetuses, thereby reducing both the hyperplasia as well as hypertrophy of growing fat depots (48). Our results are important from an etiological perspective. Although the observed effect estimates were small and without clinical relevance for individuals, the results may be relevant for population concentrations. Unfortunately, causality could not be established by this observational study. Experimental and Mendelian randomization studies may help to assess the causality of the observed associations (49).

In conclusion, we observed that higher maternal n–3 PUFA concentrations and lower maternal n–6 PUFA concentrations during pregnancy are associated with lower childhood total-body fat and abdominal fat. These associations are only partly explained by maternal and childhood sociodemographic- and lifestyle-related characteristics. Additional observational and experimental studies are needed for replication and to explore the causality and long-term cardiometabolic consequences.

The authors' responsibilities were as follows—AJV, OG, VWVJ, and RG: wrote the manuscript; AJV, OG, and RG: conducted the analyses; AJV, VWVJ, and RG: designed the research project and had primary responsibility for the final content of the manuscript; AH, HT, and VWVJ: were involved in the design and planning of the study and the data collection; and TV, JFF, MAW, HD, BK, and HT: critically reviewed and approved the final manuscript. TV works in ErasmusAGE, which is a research center funded by Nestlé Nutrition (Nestec Ltd.), Metagenics Inc., and AXA. None of the other authors reported a conflict of interest related to the study.

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