Plasma phospholipid polyunsaturated fatty acids composition in early pregnancy and fetal growth trajectories throughout pregnancy: Findings from the US fetal growth studies-singletons cohort

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Summary

Background We aimed to investigate plasma phospholipid PUFA levels in early pregnancy and fetal growth trajectories throughout pregnancy.

Methods Within the NICHD Fetal Growth Studies—Singleton Cohort, we enrolled 2,802 pregnant women at gestational weeks 8—13 and randomly assigned them to four ultrasonogram schedules to capture weekly fetal growth throughout pregnancy. Eleven plasma phospholipid PUFAs were measured at early pregnancy using blood samples collected from a subsample of 321 pregnant women. We modeled fetal growth trajectories across tertiles of PUFAs with cubic splines using linear mixed models after adjusting for major confounders. We then compared pairwise weekly fetal growth biometrics referencing the lowest tertile in each PUFA using the Wald test.

Findings Among plasma n-3 PUFAs in early pregnancy, docosahexaenoic acid (DHA, 22:6n3) and alpha-linolenic acid (ALA, 18:3n3) showed positive associations with all fetal growth measurements. For instance, compared with the lowest tertile, the highest tertile of DHA had greater estimated fetal growth (EFW) and abdominal circumference (AC), starting at 13 weeks of gestation and throughout pregnancy (at gestational week 38: 3235.3 vs. 3089.0 g for EFW; 344.6 vs. 339.2 mm for AC). As for plasma n-6 PUFAs, some showed positive associations (e.g., linoleic acid [LA], 18:2n6) while others (e.g., docosatetraenoic acid [DTA], 22:4n6) showed inverse associations with fetal growth measures.

Interpretation Our data suggested that higher plasma levels of DHA and ALA in the first trimester were associated with increased fetal size and weight throughout subsequent pregnancy.

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Keywords: Fatty acid; Nutrition; Epidemiology; Fetal growth; Pregnancy; Plasma phospholipid; Polyunsaturated fatty acid; Cohort

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Introduction

Nutrition during pregnancy is a major intrauterine environmental factor that alters the expression of the fetal genome. It programs setpoints for metabolic and physiological responses in the offspring, which manifest later

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Research in context

Evidence before this study

Polyunsaturated fatty acids (PUFAs) have profound health implications for metabolic control and vascular reactivity during pregnancy and after delivery. For example, n-3 PUFAs have been shown to positively affect fetal brain development and reduce the recurrence of preterm birth. However, most studies have assessed PUFAs via subjective self-reported dietary or supplementation intake, which is prone to measurement errors. Furthermore, most previous studies used birth weight as a proxy for uterine growth, which is less precise and cannot characterize longitudinal fetal growth trajectories. Therefore, it is crucial to examine objectively the association between plasma phospholipid PUFAs, which represent both exogenous and endogenous sources of PUFAs, and longitudinal fetal growth ultrasound measures and identify the relevance of timing. Such data may provide more informative and precise intervention targets and strategies to optimize fetal growth.

We searched PubMed and Scopus for primary research published in English from the inception of each database to December 31, 2020, using the search terms "polyunsaturated fatty acid" OR "PUFA" OR "n-3 PUFA" OR "n-6 PUFA" OR "DHA" OR "EPA" OR "ALA" OR "LA" AND "fetal growth". We excluded non-human research, non-English publications, and reviews. We found two studies that reported fetal weight outcomes: (i) Project Viva, which reported a relationship between higher seafood intake in the first trimester and smaller fetal growth z-score in the second and third trimesters, and (ii) COPSAC2010 cohort, which did not find any association between maternal whole blood PUFAs and estimated fetal weight gain at mid-trimester. Gathered from the current evidence available, we found that the majority of studies only focused on growth after the child is born, examining metrics such as birth weight, birth size, infant growth, and childhood growth trajectory. Data on the association of plasma phospholipid PUFAs with fetal growth and its relevance of timing throughout pregnancy are scarce.

Added value of this study

This study reported associations of a panel of plasma phospholipid PUFAs with longitudinal fetal growth trajectory throughout pregnancy, together with the relevance of timing identified for each significant association. Among 11 circulating PUFAs investigated in this study, most exhibited strong associations with fetal growth, although the direction of associations varied. For example, docosahexaenoic acid (DHA, 22:6n3) and alpha-linolenic acid (ALA, 18:3n3) were positively associated with fetal weight (EFW), AC, femur length (FL), head circumference (HC), biparietal diameters (BPD) and HC/AC ratio. In contrast, a subset of n-6 PUFAs, including dihomo-gamma-linolenic acid (DGLA, 20:3n6), docosatetraenoic acid (DTA, 22:4n6), and docosapentaenoic acid (n-6 DPA, 22:5n6) showed inverse associations with fetal growth. Findings from our longitudinal study provided novel insights into the roles of different plasma phospholipid PUFAs in fetal development throughout pregnancy and the relevance of timing.

Implications of all the available evidence

Our study investigated the relationship between plasma concentrations of individual plasma phospholipid n-3 and n-6 PUFAs in early pregnancy and fetal growth trajectories throughout pregnancy as measured longitudinally using ultrasound. Further, our study is able to identify the relevance of timing for significant associations. Since data on major clinical or metabolic biomarkers (e.g., family history of diabetes, glucose, and total cholesterol) are available, we evaluated the associations of PUFAs with fetal growth, further adjusting them for all the confounders mentioned above. Taken together, findings from the present study are robust and provide unique insights into the potential roles of different PUFAs in fetal growth trajectories. Key significant findings in PUFAs, such as the requirement of DHA in fetal growth, can be addressed via dietary intervention. Thus, our data might offer evidence to support the targeting of plasma phospholipid PUFAs in early pregnancy to optimize subsequent fetal growth.

in childhood and even adult life.[1,2] Among many types of dietary intake, fatty acids provide most of the energy supplied to the fetus via the placenta, which essentially affects energy metabolism and storage.[3,4] Specifically, polyunsaturated fatty acids (PUFAs) have profound health implications during pregnancy and after child delivery, such as benefiting fetal brain development and reducing the recurrence of preterm birth.[5–8] In particular, growing evidence from observational studies and clinical trials suggests that prenatal dietary intake of n-3 PUFAs (docosahexaenoic acid [DHA] and eicosapentaenoic acid [EPA]) is associated with longer gestation and higher birth weight.[9,10] Unlike n-3 PUFAs, n-6 PUFAs are generally considered inflammatory by nature,[11] and interestingly, studies investigating the role of maternal n-6 PUFA dietary intake on neonatal and childhood adiposity outcomes showed inconsistent results.[12–17]

Reported findings on the effects of n-3 and n-6 PUFAs on size-for-gestational-age and neonatal anthropometry are inconsistent[17] and limit interpretability, because using birth weight as a proxy for *in-uterine* growth is inaccurate and may not reflect the relevance of timing in fetal growth.[18] Most observational and clinical studies assessed PUFAs via self-reported dietary or supplementation intake, which is prone to measurement errors because of the subjectivity of self-reporting.[18,19] Therefore, it is crucial to examine the association of plasma phospholipid PUFAs—taking both exogenous and endogenous sources into account—with longitudinal fetal growth ultrasound measures and identify the relevance of timing. Doing so might help us understand the underlying physiology of

nutrition and fetal growth, and provide potential intervention strategies such as supplementation of PUFAs to improve fetal outcomes. Furthermore, since n-3 PUFAs are more anti-inflammatory while n-6 PUFAs are more pro-inflammatory by nature, it is worth investigating the effects of different subgroups of PUFAs on fetal growth. Therefore, using the data collected from a US multi-ethnic birth cohort, we explored associations between different PUFAs in early pregnancy and fetal growth trajectories, and examined the relevance of timing.

Methods

Study population and design

This study was based on data from the Fetal Growth Studies-Singletons cohort (2009–2013) at the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD). The prospective cohort comprised 2802 pregnant women from 12 clinical centers across the United States. They were 18–40 years of age, at 8–13 gestational weeks, and without major pre-existing chronic diseases (i.e., diabetes, cancer). We registered the study in Clinical Trial Registry (NCT00912132) and have published the detailed recruitment and study protocol elsewhere.[20] We included 321 women from a nested case-control study for analysis based on the primary cohort. Among them, 107 had GDM, diagnosed according to the Carpenter and Coustan criteria following the recommendation of the American College of Obstetrics and Gynecologists (ACOG), and 214 were non-GDM controls. The ratio of women with GDM and non-GDM controls was 1:2. They were matched by maternal age (+/−2 years), self-reported race/ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic, Asian/Pacific Islander), and gestational age (GA) at blood collection (+/− 2 weeks).

The institutional review boards at all participating sites approved both the primary and sub-study. All participants provided written informed consent prior to data collection. The participating clinical centers entered study documents and data, including ultrasound measurements and images, into the Clinical Trial Management System, which were then electronically transferred to the Data Coordinating Center.

Assessments of plasma phospholipid polyunsaturated fatty acids

Upon enrolment at 8–13 weeks of gestation (visit 0), we collected blood from all participants and stored biospecimens at −80°C until thawing them just before assay. PUFAs were extracted using the chloroform/methanol method previously described.[21,22] The concentration of individual PUFAs was expressed as percentage (%) of the weight of the total plasma phospholipid FA fraction. We assayed all case-control matched samples in the same analytic run, but in random order. Then, we performed 40 repeats among the in-house pooled control samples from the same cohort to assess the inter-assay coefficients of variation (CVs). The inter-assay CVs in our study were consistent with readings previously reported among pregnant individuals.[23]

We displayed the composition of all plasma phospholipid PUFAs in Supplementary Table 1. We further identified 11 plasma phospholipid PUFAs and 6 PUFAs-derived indices, including those of four n-3 PUFAs: alpha-linolenic acid (ALA; 18:3n3), eicosapentaenoic acid (EPA; 20:5n3), n-3 docosapentaenoic acid (n-3 DPA; 22:5n3), and docosahexaenoic acid (DHA; 22:6n3); and seven n-6 PUFAs: linoleic acid (LA: 18:2n6), gamma-linolenic acid (GLA; 18:3n6), eicosadienoic acid (EDA; 20:2n6), dihomo-gamma-linolenic acid (DGLA; 20:3n6), arachidonic acid (AA; 20:4n6), docosatetraenoic acid (DTA; 22:4n6) and n-6 DPA (22:5n6). We then calculated the sum of n-3 and n-6 PUFAs, respectively. Lastly, we derived the ratio of product to precursor PUFAs to estimate FA elongase or desaturase enzyme activities, which could implicate lipid metabolism and insulin action.[21] These were Δ6-desaturase activity catalyzing conversions of GLA from LA and Δ5-desaturase activity catalyzing conversions of AA from DGLA.

Fetal growth measurement throughout pregnancy (10–40 weeks of gestation age)

Women received an ultrasonographic examination at enrolment 8–13 weeks into gestation (visit 0) and another one at of the four following ultrasonography schedules by randomization: weeks 16, 24, 30, 34 and 38 (group A); weeks 18, 26, 31, 35 and 39 (group B); weeks 20, 28, 32, 36 and 40 (group C); weeks 22, 29, 33, 37 and 41 (group D), as stated in Supplementary Table 2 and our prior publication.[24] All study visits allowed ±1 week of the targeted GA to accommodate the subjects' availability. We permuted blocks and generated numbers via a computer-based pseudo-random number generator in terms of randomization. In this way, we captured weekly fetal growth data in a mixed longitudinal randomization scheme without exposing individual women to ultrasound every week.[24]

At each ultrasonographic examination, trained sonographers performed standard operating procedures using identical equipments (Voluson E8; GE Healthcare, Boston, US) and assessed a series of fetal growth biometrics including head circumference (HC, mm), biparietal diameter (BPD, mm), AC, mm), humerus length (HL, mm), femur length (FL, mm) and HC/AC ratio. We then calculated the estimated fetal weight (EFW, g) using a Hadlock formula based on HC, AC and FL.[25] There was high inter- and intra-grader reliability, regardless of maternal obesity status.[24]

Covariates

At enrolment, research coordinators interviewed participants to find out maternal demographics, pregnancy history, and lifestyle behaviors. Further, they obtained clinical examination results, such as blood pressure and anthropometric indices from medical records. We selected maternal pre-pregnancy body mass index (BMI) as a key covariate based on our prior findings on the association between maternal obesity and faster fetal growth in the same cohort.[26] In addition, we collected data on the sex of the infant from medical charts and GA at delivery based on ultrasound-verified last menstrual period (LMP) of mothers and date of delivery. Despite having matched maternal age and race/ethnicity between cases and controls, we continued to control for maternal age and race/ethnicity to obtain conservative estimates. We applied two adjustment models in the following order: Model 1, adjusted for maternal age and race/ethnicity; Model 2, adjusted for covariates in Model 1 and additionally for nulliparity, pre-pregnancy BMI and sex of the infant.

Statistical analysis

Because women with GDM were overrepresented in the analytic sample with biomarkers, the sample was re-weighted to represent the full cohort (e.g., in the re-weighted sample 4% of women had GDM as opposed to 33% in the non-weighted sample). Weights were created following the idea of pseudolikelihood in Samuelsen (1997).[27] Distributions of the characteristics of study participants in the re-weighted data were comparable to those in the overall NICHD Fetal Growth Singleton-Study.[24,28]

We use descriptive statistics to summarize crude and weighted characteristics of women and their neonates in the primary and the nested case-control cohort. Tertiles of individual PUFAs were treated as independent variables, and trajectories of all fetal biometrics were treated as dependent variables and modeled using a cubic spline model estimated using a restricted maximum likelihood approach.[29] Because of the skewed distribution of all fetal growth biometrics, we then log-transformed all assessments to stabilize variances across GA and approximate normal distribution. The model initially included fixed effects of the linear, quadratic and cubic terms and cubic spline terms of GA (3 knots at 25^{th}, 50^{th}, 75^{th} percentiles), as well as a random intercept and random effects of the linear, quadratic, and cubic terms and cubic spline terms of weekly GA. The random effect covariance was unstructured, and the random effect of the cubic spline term of weekly GA was removed to facilitate model convergence.

The steps involved in the analysis are: (i) Step 1 tested the overall difference in fetal growth trajectories across tertiles of PUFAs (i.e., global testing) fitted in Crude Model, Model 1, and Model 2, and then the addition of interaction between PUFAs tertiles and GA in the same models (i.e., log-likelihood ratio test); (ii) Step 2 applied Bonferroni correction[30] in Model 2 with an interaction term between PUFAs tertiles and GA since we were testing multiple outcomes simultaneously; and (iii) Step 3 selected the significant associations from Step 2, and calculated the weekly means (log-transformed fetal biometrics) and compared weekly differences in fetal growth biometrics across tertiles of each PUFA (the lowest tertile as reference) in Model 2, using the Wald tests.

We evaluated potential effect modification by race-ethnicity (non-Hispanic white vs. Hispanic black or Hispanic or Asian/Pacific Islander), pre-pregnancy obesity (yes vs. no), and infant sex (male vs. female) in the global test. In addition, we included family history of diabetes, maternal random glucose level at enrolment, and maternal total cholesterol level at enrolment in the sensitivity analysis. We conducted all the analyses using SAS version 9.4 (SAS Institute, Cary, NC, USA). We reported all estimates with a 95% confidence interval (CI) or p-value. We defined significance as a two-tailed p-value of 0.05.

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Results

The weighted percentage of each maternal characteristic in our cohort nested case-control study showed comparable results with the original cohort (Supplementary Table 3).

All PUFAs and PUFA-derived indices were significantly associated with the ratio of HC/AC, and most of them were significantly associated with HC, BPD, and AC, even after Bonferroni correction (Table 1, Supplementary Tables 4-5). Among all PUFAs, DHA and ALA were consistently and significantly associated with all six fetal growth biometrics ($p < 0.05$).

n-3 PUFAs and fetal growth

Overall, plasma DHA levels were positively associated with the fetal growth curve throughout pregnancy. Compared with the lowest tertile, the highest tertile of plasma DHA levels was significantly associated with an increment in EFW, AC, FL, and BPD, starting in early pregnancy (EFW at week 13: 71.83 vs. 69.08 g, $p = 0.02$; AC at week 13: 69.05 vs. 66.95 mm; $p = 0.003$; FL at week 11: 4.60 vs. 3.46 mm, $p<0.00001$; BPD at week 12: 19.70 vs. 18.89 mm; $p = 0.001$), and attenuating in late pregnancy (EFW at week 39: 3513.91 vs. 3344.39 g; $p = 0.11$; AC at week 38: 344.64 vs. 339.23 mm; $p = 0.20$; FL at week 39: 74.10 vs. 73.52 mm; $p = 0.81$; BPD

	EFW	AC	FL	HC	BPD	HC/AC
PUFAs						
18:2n6 (Linoleic Acid, LA)	↑	↑	n.s.	↑	↑	↓
18:3n3 (Alpha Linolenic acid, ALA)	↑	↑	↑	↓	↓	↓
18:3n6 (Gama Linolenic acid, GLA)	↑	n.s.	↑	↑	n.s.	↑
20:2n6 (Eicosadienoic acid, EDA)	n.s.	↓	↓	↓	↓	↑
20:3n6 (Dihomo-gamma-linolenic acid, DGLA)	↓	↑	n.s.	↓	↓	↑
20:4n6 (Arachidonic acid, AA)	↑	↓	↑	↑	n.s.	↑
20:5n3 (eicosapentaenoic acid, EPA)	n.s.	↑	n.s.	↓	↓	↓
22:4n6 (docosatetraenoic acid, DTA)	↓	↑	n.s.	↓	↓	↓
22:5n3 (docosapentaenoic acid, DPA)	n.s.	↓	n.s.	↑	↓	↑
22:5n6 (n-6 docosapentaenoic acid, n-6 DPA)	↑	↓	n.s.	↓	n.s.	↑
22:6n3 (docosahexaenoic acid, DHA)	↑	↑	↑	↑	↑	↓
Delta 6	↑	n.s.	↑	↑	↑	↑
Delta 5	n.s.	↓	n.s.	↑	↓	↓
PUFA_3n	n.s.	↑	↑	↑	↑	↓
PUFA_6n	n.s.	n.s.	↑	n.s.	↑	↑

***Table 1*: Summary of the significant impact of PUFAs on overall fetal biometry velocity throughout pregnancy (10–40 weeks GA).**
Abbreviation: n.s., non-significant; EFW, estimated fetal weight; AC, abdominal circumference; FL, femur length; HC, head circumference; BPD, biparietal diameter; HC/AC, ratio of head circumference and abdominal circumference.

at week 38: 89.71 vs. 89.38 mm; $p = 0.75$) (Tables 2A & Figure 1). Women in the highest tertile of plasma DHA levels had an increment of both HC and HC/AC ratio, as measured in the 13th week of gestation (HC: 86.14 vs. 83.57 mm, $p = 0.003$; HC/AC ratio: 1.26 vs. 1.25, $p = 0.07$) which was discontinued by mid-late pregnancy compared with the lowest tertile (HC at week 31: 288.35 vs. 288.04, $p = 0.93$; HC/AC ratio at week 27: 1.11 vs. 1.10, $p = 0.62$) (Table 2B & Figure 1). Women in the middle tertile showed a similar magnitude of increment in all fetal growth biometrics as in the highest tertile (Tables 2 & Figure 1).

Even though ALA was also positively associated with the fetal growth curve, the duration was shorter than that of DHA. For instance, compared with the lowest tertile, the highest tertile of ALA plasma levels was associated with an increment of EFW, AC and FL starting in early pregnancy (EFW at week 14: 89.85 vs. 89.20 g, $p = 0.73$; AC at week 12: 60.04 vs. 57.54 mm; $p<0.00001$; FL at week 11: 2.70 vs. 2.19 mm, $p = 0.0002$) and ended in mid-pregnancy (EFW at week 24: 677.70 vs. 675.71 g; $p = 0.88$; AC at week 21: 166.54 vs. 165.66 mm, $p = 0.56$; FL at week 25: 45.34 vs. 45.14 mm, $p = 0.80$) (Tables 3A and 3B & Figure 2). A similar trend was observed in the middle tertile of plasma ALA levels (Tables 3A and 3B & Figure 2).

Unlike DHA and ALA, other n-3 PUFAs such as EPA and n-3 DPA did not significantly affect fetal weight but fetal size (Supplementary Tables 4-5). Interestingly, women in the highest tertile of n-3 DPA plasma levels exhibited a decrement in fetal size in early and mid-pregnancy (AC at week 12: 56.38 vs. 58.41 mm, $p = 0.01$; HC at week 11: 58.45 vs. 61.05 mm, $p = 0.03$), but an increment in fetal size in the late pregnancy (AC at week 39: 362.50 vs. 349.34, $p = 0.002$; HC at week 38: 330.06 vs. 322.87 mm, $p = 0.009$), compared with the lowest tertile (Supplementary Table 6).

n-6 PUFAs and fetal growth

Overall, higher levels of LA were associated with larger fetal weight, HC, and BPD. Even though there is a transient increment in fetal weight from early to mid-pregnancy with higher levels of LA, the magnitude of increase is only significant in the middle tertile of plasma LA levels, compared with the lowest tertile. For instance, compared with the lowest tertile, a significant increment of EFW was observed in week 17 in the middle tertile (186.99 vs. 179.71 g, $p = 0.039$) and waned off quickly by week 24 (663.23 vs. 665.22 g, $p = 0.874$) (Supplementary Table 7).

Besides LA, other n-6 PUFAs were also significantly associated with fetal weight in the global test. GLA and AA showed a positive relationship with fetal weight and size, whereas DGLA, DTA, and n-6 DPA showed an inverse relationship with fetal weight and size. For example, women in the highest tertile of AA plasma levels had significant increments of fetal weight and fetal size in early pregnancy, which waned off slowly in late pregnancy (EFW: at week 12: 57.65 vs. 54.24, $p = 0.008$; at week 37: 2972.67 vs. 2920.20, $p = 0.50$; FL: at week 11: 4.31 vs. 3.74 mm, $p = 0.001$; at week 37: 68.81 vs. 68.10 mm, $p = 0.65$), compared with the lowest tertile (Supplementary Table 8). Conversely, compared with women in the lowest tertile of plasma DGLA levels, those in the highest tertile had consistent significant

GW	EFW					AC, mm					FL				
	Back-transformed geometric mean, gram			Pairwise ratio (95% CI)		Back-transformed geometric mean, mm			Pairwise ratio (95% CI)		Back-transformed geometric mean, mm			Pairwise ratio (95% CI)	
	1st tertile	2nd tertile	3rd tertile	2nd vs 1st tertile	3rd vs 1st tertile	1st tertile	2nd tertile	3rd tertile	2nd vs 1st tertile	3rd vs 1st tertile	1st tertile	2nd tertile	3rd tertile	2nd vs 1st tertile	3rd vs 1st tertile
10	41.24	36.22	33.99	0.88 (0.74, 1.04)	***0.82 (0.70, 0.98)***	36.66	40.62	36.93	***1.11 (1.00, 1.23)***	1.01 (0.89, 1.14)	1.81	2.31	2.89	***1.28 (1.12, 1.46)***	***1.60 (1.37, 1.87)***
11	47.46	45.42	43.75	0.96 (0.87, 1.05)	0.92 (0.84, 1.01)	45.83	49.08	46.79	***1.07 (1.01, 1.13)***	1.02 (0.96, 1.09)	3.46	4.11	4.60	***1.19 (1.06, 1.33)***	***1.33 (1.17, 1.51)***
12	56.50	57.46	56.16	1.02 (0.97, 1.06)	0.99 (0.95, 1.04)	55.98	58.58	57.58	***1.05 (1.02, 1.07)***	***1.03 (1.00, 1.06)***	5.86	6.59	6.82	***1.13 (1.01, 1.25)***	***1.16 (1.04, 1.31)***
13	69.08	73.10	71.83	***1.06 (1.02, 1.09)***	***1.04 (1.01, 1.07)***	66.95	69.08	69.05	***1.03 (1.01, 1.05)***	***1.03 (1.01, 1.05)***	8.90	9.64	9.50	1.08 (0.98, 1.20)	1.07 (0.96, 1.19)
14	86.13	93.21	91.44	***1.08 (1.04, 1.12)***	***1.06 (1.02, 1.10)***	78.52	80.49	80.90	***1.03 (1.00, 1.05)***	***1.03 (1.01, 1.05)***	12.34	13.02	12.54	1.05 (0.96, 1.16)	1.02 (0.91, 1.13)
15	108.71	118.75	115.74	***1.09 (1.05, 1.14)***	***1.06 (1.02, 1.11)***	90.49	92.65	92.90	***1.02 (1.00, 1.05)***	***1.03 (1.01, 1.06)***	15.88	16.46	15.78	1.04 (0.94, 1.14)	0.99 (0.90, 1.10)
16	137.88	150.64	145.50	***1.09 (1.05, 1.13)***	***1.06 (1.01, 1.10)***	102.69	105.38	104.84	***1.03 (1.00, 1.05)***	***1.02 (1.00, 1.05)***	19.27	19.77	19.09	1.03 (0.94, 1.14)	0.99 (0.90, 1.10)
17	174.49	189.70	181.48	***1.09 (1.04, 1.12)***	***1.04 (1.00, 1.08)***	114.96	118.43	116.62	***1.03 (1.01, 1.05)***	***1.01 (1.00, 1.05)***	22.42	22.86	22.36	1.02 (0.93, 1.11)	1.00 (0.90, 1.09)
18	218.72	236.34	224.34	***1.08 (1.04, 1.11)***	1.03 (0.99, 1.07)	127.19	131.50	128.24	***1.03 (1.01, 1.06)***	1.01 (0.99, 1.04)	25.42	25.78	25.55	1.01 (0.93, 1.10)	1.01 (0.92, 1.10)
19	270.38	290.60	274.57	***1.07 (1.03, 1.11)***	1.02 (0.98, 1.06)	139.31	144.31	139.79	***1.04 (1.01, 1.06)***	1.00 (0.99, 1.03)	28.39	28.70	28.71	1.01 (0.93, 1.10)	1.01 (0.93, 1.10)
20	329.84	352.76	332.71	***1.07 (1.03, 1.10)***	1.01 (0.97, 1.05)	151.21	156.68	151.32	***1.04 (1.01, 1.06)***	1.00 (0.98, 1.03)	31.32	31.63	31.83	1.01 (0.93, 1.09)	1.02 (0.93, 1.10)
21	397.54	423.25	399.37	***1.06 (1.03, 1.09)***	1.00 (0.97, 1.04)	162.79	168.52	162.75	***1.04 (1.01, 1.06)***	1.00 (0.98, 1.02)	34.16	34.53	34.87	1.01 (0.94, 1.09)	1.02 (0.94, 1.11)
22	473.91	502.49	475.23	***1.06 (1.03, 1.10)***	1.00 (0.96, 1.04)	174.03	179.79	174.03	***1.03 (1.01, 1.05)***	1.00 (0.98, 1.02)	36.88	37.36	37.80	1.01 (0.94, 1.09)	1.02 (0.95, 1.11)
23	559.41	590.94	560.94	***1.06 (1.03, 1.09)***	1.00 (0.96, 1.04)	184.92	190.51	185.13	***1.03 (1.01, 1.05)***	1.00 (0.98, 1.02)	39.48	40.12	40.58	1.02 (0.95, 1.09)	1.03 (0.96, 1.10)
24	654.62	689.20	657.21	***1.05 (1.02, 1.09)***	1.00 (0.96, 1.05)	195.49	200.75	196.02	***1.03 (1.01, 1.05)***	1.00 (0.98, 1.02)	41.95	42.77	43.20	1.02 (0.96, 1.09)	1.03 (0.96, 1.10)
25	760.25	798.01	764.82	***1.05 (1.02, 1.08)***	1.01 (0.96, 1.05)	205.83	210.63	206.73	***1.02 (1.00, 1.04)***	1.00 (0.98, 1.02)	44.30	45.31	45.66	1.02 (0.96, 1.09)	1.03 (0.97, 1.10)
26	877.25	918.37	884.64	***1.05 (1.01, 1.08)***	1.01 (0.97, 1.05)	216.05	220.34	217.28	***1.02 (1.00, 1.04)***	1.01 (0.98, 1.03)	46.57	47.74	47.97	1.03 (0.97, 1.08)	1.03 (0.97, 1.09)
27	1006.90	1051.62	1017.68	***1.04 (1.01, 1.08)***	1.01 (0.97, 1.06)	226.32	230.11	227.74	***1.02 (1.00, 1.04)***	1.01 (0.98, 1.03)	48.78	50.06	50.15	1.03 (0.97, 1.08)	1.03 (0.97, 1.09)
28	1150.86	1199.54	1165.15	***1.04 (1.01, 1.08)***	1.01 (0.97, 1.06)	236.83	240.21	238.18	***1.01 (1.00, 1.03)***	1.01 (0.99, 1.03)	50.99	52.28	52.23	1.03 (0.98, 1.08)	1.02 (0.97, 1.08)
29	1310.03	1363.99	1328.39	***1.04 (1.01, 1.07)***	1.01 (0.97, 1.06)	247.67	250.88	248.70	***1.01 (1.00, 1.03)***	1.00 (0.99, 1.02)	53.22	54.43	54.26	1.02 (0.98, 1.07)	1.02 (0.97, 1.07)
30	1483.52	1545.10	1507.48	***1.04 (1.01, 1.07)***	1.02 (0.97, 1.06)	258.72	262.06	259.34	***1.01 (1.00, 1.03)***	1.00 (0.99, 1.02)	55.44	56.51	56.26	1.02 (0.98, 1.06)	1.01 (0.97, 1.06)
31	1669.39	1741.78	1701.10	***1.04 (1.01, 1.07)***	1.02 (0.97, 1.06)	269.84	273.60	270.13	***1.01 (1.00, 1.03)***	1.00 (0.99, 1.02)	57.61	58.54	58.23	1.02 (0.98, 1.05)	1.01 (0.97, 1.06)
32	1864.56	1951.96	1906.88	***1.05 (1.01, 1.08)***	1.02 (0.97, 1.06)	280.85	285.29	281.07	***1.02 (1.00, 1.04)***	1.00 (0.99, 1.02)	59.68	60.51	60.17	1.01 (0.98, 1.05)	1.01 (0.97, 1.05)
33	2064.65	2172.37	2121.27	***1.05 (1.02, 1.09)***	1.03 (0.98, 1.08)	291.55	296.91	292.17	***1.02 (1.00, 1.04)***	1.00 (0.99, 1.02)	61.59	62.46	62.08	1.01 (0.98, 1.05)	1.01 (0.97, 1.05)
34	2264.07	2398.45	2339.46	***1.06 (1.03, 1.09)***	1.03 (0.98, 1.09)	301.73	308.20	303.46	***1.02 (1.00, 1.04)***	1.01 (0.99, 1.03)	63.30	64.39	63.97	1.02 (0.98, 1.06)	1.01 (0.97, 1.05)
35	2460.48	2624.58	2555.71	***1.07 (1.03, 1.10)***	1.04 (0.99, 1.09)	311.25	318.88	314.94	***1.02 (1.01, 1.04)***	1.01 (0.99, 1.03)	64.79	66.31	65.83	1.02 (0.98, 1.07)	1.02 (0.97, 1.06)
36	2658.26	2845.93	2770.69	***1.07 (1.03, 1.11)***	1.04 (0.99, 1.10)	320.38	328.85	326.19	***1.03 (1.01, 1.05)***	***1.02 (1.00, 1.04)***	66.27	68.16	67.71	1.03 (0.98, 1.07)	1.02 (0.98, 1.07)
37	2864.52	3058.06	2993.11	***1.07 (1.03, 1.11)***	1.04 (0.99, 1.11)	329.56	338.08	336.40	***1.03 (1.00, 1.05)***	***1.02 (1.00, 1.05)***	68.00	69.80	69.67	1.03 (0.98, 1.08)	1.02 (0.97, 1.08)
38	3088.97	3256.49	3235.35	***1.05 (1.02, 1.09)***	1.05 (0.99, 1.11)	339.23	346.54	344.64	***1.02 (1.00, 1.04)***	1.02 (0.99, 1.04)	70.30	71.09	71.77	1.01 (0.96, 1.07)	1.02 (0.96, 1.08)
39	3344.39	3436.82	3513.91	1.03 (0.98, 1.07)	1.05 (0.99, 1.12)	349.92	354.23	349.95	1.01 (0.99, 1.04)	1.00 (0.98, 1.03)	73.52	71.90	74.10	0.98 (0.92, 1.04)	1.01 (0.95, 1.07)
40	3647.45	3594.92	3850.68	0.99 (0.90, 1.07)	1.06 (0.97, 1.15)	362.23	361.13	351.36	1.00 (0.95, 1.05)	0.97 (0.92, 1.02)	78.10	72.08	76.75	***0.92 (0.86, 0.99)***	0.98 (0.91, 1.06)

***Table 2A*: Back-transformed and pairwise ratio of weekly fetal growth biometrics (EFW, AC & FL) across DHA (22:6n3) tertiles in the NICHD Fetal Growth Studies-Singletons.**

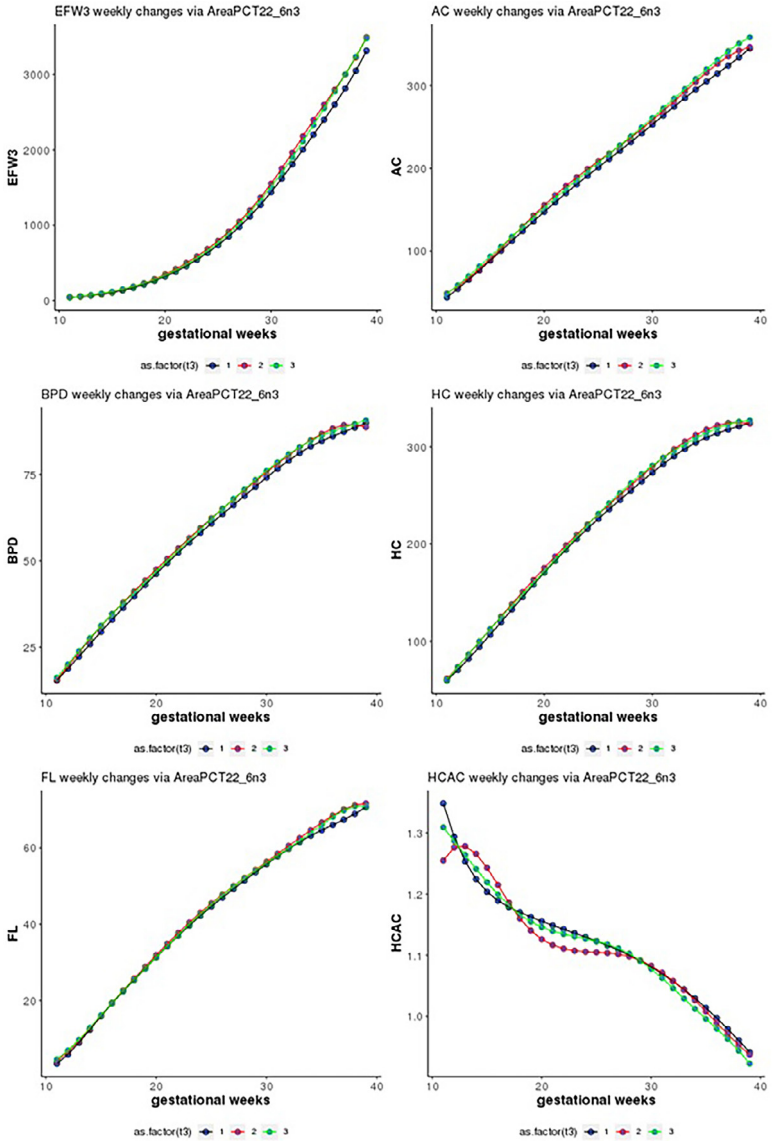

Figure 1. Back-transformed geometric means of EFW by gestational weeks to tertiles of DHA within the NICHD Fetal Growth Studies-Singletons, from 11-39 weeks gestational age. 1st tertile curve is in blue, 2nd tertile curve is in red and 3rd tertile is in green. The shaded area indicated a significant increment of EFW in the 3rd tertile compared with the 1st tertile of DHA.

reductions in fetal weight and size in early pregnancy, which waned off gradually in late pregnancy (EFW: at week 13: 69.29 vs. 73.07 g, p = 0.005; at week 34: 2333.11 vs. 2344.53 g, p = 0.0.83; AC: at week 11: 40.25 vs. 42.64 mm, p<0.00001; at week 29: 249.61 vs. 250.07 mm, p = 0.83) (Supplementary Table 9).

PUFA-derived indices and fetal growth

Compared with women in the lowest tertile, those in the highest tertile of estimated Δ6-desaturase activity had significant increments in fetal weight and size from early pregnancy on, waning off only in late pregnancy (EFW: at week 12: 57.43 vs. 54.75 g, p = 0.02; at week 38: 2962.17 vs. 2946.28, p = 0.83; FL: at week 11: 4.10 vs. 3.67 mm, p = 0.004; at week 34: 62.06 vs. 61.94, p = 0.93) (Supplementary Table 10).

The sensitivity analysis of additional adjustment on family history of diabetes, maternal plasma random glucose level, and total cholesterol level at visit 0 in the global test did not attenuate any significant associations in the relationship between PUFAs or PUFA-derived indices and fetal growth biometrics identified in Model 2 (Supplementary Tables 11–13).

GW	HC					BPD					HC/AC ratio				
	Back-transformed geometric mean, gram			Pairwise ratio (95% CI)		Back-transformed geometric mean, mm			Pairwise ratio (95% CI)		Back-transformed geometric mean			Pairwise ratio (95% CI)	
	1st tertile	2nd tertile	3rd tertile	2nd vs 1st tertile	3rd vs 1st tertile	1st tertile	2nd tertile	3rd tertile	2nd vs 1st tertile	3rd vs 1st tertile	1st tertile	2nd tertile	3rd tertile	2nd vs 1st tertile	3rd vs 1st tertile
10	50.43	48.74	48.20	0.97 (0.89, 1.05)	0.96 (0.88, 1.04)	12.19	11.74	12.54	0.96 (0.88, 1.06)	1.03 (0.93, 1.14)	1.38	1.20	1.34	0.87 (0.80, 0.94)	0.97 (0.89, 1.06)
11	60.62	60.95	60.26	1.01 (0.96, 1.05)	0.99 (0.95, 1.04)	15.41	15.50	16.00	1.01 (0.96, 1.06)	1.04 (0.98, 1.10)	1.33	1.24	1.32	0.94 (0.90, 0.97)	0.99 (0.95, 1.04)
12	71.71	73.95	73.04	***1.03 (1.01, 1.05)***	1.02 (0.99, 1.04)	18.89	19.54	19.70	***1.03 (1.01, 1.06)***	***1.04 (1.02, 1.07)***	1.28	1.26	1.29	0.98 (0.97, 1.00)	1.01 (0.99, 1.03)
13	83.57	87.34	86.14	***1.05 (1.03, 1.06)***	***1.03 (1.01, 1.05)***	22.51	23.65	23.47	***1.05 (1.03, 1.07)***	***1.04 (1.02, 1.06)***	1.25	1.26	1.26	1.01 (1.00, 1.03)	***1.01 (1.00, 1.03)***
14	96.04	100.77	99.20	***1.05 (1.03, 1.07)***	***1.03 (1.01, 1.06)***	26.18	27.66	27.20	***1.06 (1.04, 1.08)***	***1.04 (1.02, 1.06)***	1.22	1.25	1.24	1.02 (1.10, 1.04)	***1.01 (1.00, 1.03)***
15	108.94	113.95	111.97	***1.05 (1.03, 1.07)***	***1.03 (1.00, 1.05)***	29.81	31.43	30.80	***1.05 (1.03, 1.08)***	***1.03 (1.01, 1.06)***	1.20	1.23	1.22	1.02 (1.00, 1.04)	1.01 (0.99, 1.03)
16	122.09	126.72	124.32	***1.04 (1.02, 1.06)***	1.02 (1.00, 1.04)	33.36	34.91	34.21	***1.05 (1.03, 1.07)***	***1.03 (1.00, 1.05)***	1.19	1.20	1.20	1.01 (1.00, 1.03)	1.01 (0.99, 1.02)
17	135.32	139.06	136.27	***1.03 (1.01, 1.04)***	1.01 (0.99, 1.03)	36.80	38.13	37.43	***1.04 (1.02, 1.06)***	***1.02 (1.00, 1.04)***	1.18	1.17	1.18	1.00 (0.98, 1.01)	1.00 (0.99, 1.02)
18	148.47	151.08	147.99	***1.02(1.00, 1.03)***	1.00 (0.98, 1.02)	40.16	41.18	40.53	***1.03 (1.01, 1.04)***	1.01 (0.99, 1.03)	1.17	1.15	1.16	0.98 (0.97, 1.00)	1.00 (0.98, 1.01)
19	161.39	162.99	159.79	1.01 (0.99, 1.03)	0.99 (0.97, 1.01)	43.46	44.22	43.61	***1.02 (1.00, 1.04)***	1.00 (0.98, 1.02)	1.16	1.13	1.15	0.97 (0.96, 0.99)	0.99 (0.98, 1.01)
20	173.98	174.78	171.76	1.00 (0.99, 1.02)	0.99 (0.97, 1.01)	46.69	47.25	46.70	1.01 (0.99, 1.03)	1.00 (0.98, 1.02)	1.15	1.11	1.14	0.97 (0.95, 0.98)	0.99 (0.98, 1.01)
21	186.14	186.37	183.83	1.00(0.99, 1.02)	0.99 (0.97, 1.01)	49.82	50.27	49.79	1.01 (0.99, 1.03)	1.00 (0.98, 1.02)	1.14	1.11	1.14	0.97 (0.95, 0.98)	0.99 (0.98, 1.01)
22	197.82	197.74	195.90	1.00 (0.98, 1.01)	0.99 (0.97, 1.01)	52.84	53.27	52.86	1.01 (0.99, 1.03)	1.00 (0.98, 1.02)	1.14	1.10	1.13	0.97 (0.95, 0.98)	0.99 (0.98, 1.01)
23	209.01	208.84	207.88	1.00 (0.98, 1.01)	0.99 (0.97, 1.02)	55.77	56.23	55.90	1.01 (0.99, 1.03)	1.00 (0.99, 1.02)	1.13	1.10	1.13	0.97 (0.96, 0.98)	1.00 (0.98, 1.01)
24	219.74	219.68	219.65	1.00 (0.98, 1.01)	1.00 (0.98, 1.02)	58.60	59.16	58.91	1.01 (0.99, 1.03)	1.01 (0.99, 1.02)	1.12	1.09	1.12	0.97 (0.956 0.99)	1.00 (0.99, 1.01)
25	230.06	230.27	231.12	1.00 (0.99, 1.02)	1.00 (0.98, 1.03)	61.36	62.05	61.87	1.01 (0.99, 1.03)	1.01 (0.99, 1.03)	1.12	1.09	1.12	0.98 (0.96, 0.99)	1.00 (0.99, 1.02)
26	240.07	240.63	242.19	1.00 (0.99, 1.02)	1.01 (0.99, 1.03)	64.07	64.90	64.77	1.01 (0.99, 1.03)	1.01 (0.99, 1.03)	1.11	1.09	1.12	0.98 (0.97, 1.00)	1.00 (0.99, 1.02)
27	249.91	250.84	252.75	1.00 (0.99, 1.02)	1.01 (0.99, 1.03)	66.78	67.73	67.62	***1.01 (1.00, 1.03)***	1.01 (0.99, 1.03)	1.10	1.09	1.11	0.99 (0.97, 1.00)	1.00 (0.99, 1.02)
28	259.72	260.95	262.71	1.00 (0.99, 1.02)	1.01 (0.99, 1.03)	69.51	70.53	70.40	***1.01 (1.00, 1.03)***	1.01 (0.99, 1.03)	1.10	1.09	1.10	0.99 (0.98, 1.01)	1.00 (0.99, 1.02)
29	269.48	271.01	271.97	1.01 (0.99, 1.02)	1.01 (0.99, 1.03)	72.27	73.32	73.13	***1.01 (1.00, 1.03)***	1.01 (0.99, 1.03)	1.09	1.08	1.09	0.99 (0.98, 1.01)	1.00 (0.99, 1.01)
30	279.00	280.85	280.51	1.01 (0.99, 1.02)	1.01 (0.98, 1.03)	74.99	76.05	75.77	***1.01 (1.00, 1.03)***	1.01 (0.99, 1.03)	1.08	1.07	1.08	0.99 (0.98, 1.01)	1.00 (0.99, 1.01)
31	288.04	290.23	288.35	1.01 (0.99, 1.02)	1.00 (0.98, 1.02)	77.60	78.65	78.29	***1.01 (1.00, 1.03)***	1.01 (0.99, 1.03)	1.07	1.06	1.06	0.99 (0.98, 1.01)	0.99 (0.98, 1.00)
32	296.37	298.89	295.53	1.01 (0.99, 1.02)	1.00 (0.97, 1.02)	80.02	81.09	80.64	***1.01 (1.00, 1.03)***	1.01 (0.99, 1.03)	1.05	1.05	1.04	0.99 (0.98, 1.01)	0.99 (0.97, 1.00)
33	303.72	306.54	302.10	1.01 (0.99, 1.03)	0.99 (0.97, 1.02)	82.19	83.27	82.78	***1.01 (1.00, 1.03)***	1.01 (0.99, 1.03)	1.04	1.03	1.03	0.99 (0.98, 1.00)	0.99 (0.97, 1.00)
34	309.83	312.89	308.14	1.01 (0.99, 1.03)	0.99 (0.97, 1.02)	84.00	85.15	84.65	***1.01 (1.00, 1.03)***	1.01 (0.99, 1.03)	1.03	1.02	1.01	0.99 (0.97, 1.00)	0.98 (0.97, 1.00)
35	314.74	317.72	313.72	1.01 (0.99, 1.02)	1.00 (0.97, 1.02)	85.48	86.66	86.22	***1.01 (1.00, 1.03)***	1.01 (0.99, 1.03)	1.01	1.00	1.00	0.99 (0.97, 1.00)	0.98 (0.97, 1.00)
36	318.80	321.31	318.64	1.01 (0.99, 1.03)	1.00 (0.97, 1.02)	86.77	87.88	87.52	1.01 (0.99, 1.03)	1.01 (0.99, 1.03)	0.99	0.98	0.98	0.98 (0.97, 1.00)	0.99 (0.97, 1.00)
37	322.41	324.16	322.52	1.01 (0.99, 1.02)	1.00 (0.97, 1.03)	88.02	88.93	88.65	1.01 (0.99, 1.03)	1.01 (0.98, 1.03)	0.98	0.96	0.97	0.98 (0.97, 1.00)	0.99 (0.97, 1.00)
38	325.99	326.81	324.92	1.00 (0.98, 1.02)	1.00 (0.97, 1.02)	89.38	89.96	89.71	1.01 (0.99, 1.03)	1.00 (0.98, 1.03)	0.96	0.95	0.95	0.98 (0.97, 1.00)	0.99 (0.97, 1.00)
39	329.99	329.79	325.44	1.00 (0.98, 1.02)	0.99 (0,96, 1.01)	91.02	91.12	90.82	1.00 (0.98, 1.02)	1.00 (0.97, 1.02)	0.94	0.93	0.93	0.99 (0.97, 1.01)	0.98 (0.97, 1.00)
40	334.86	333.69	323.67	1.00 (0.96, 1.04)	0.97 (0.93, 1.01)	93.13	92.54	92.11	0.99 (0.95, 1.04)	0.99 (0.95, 1.03)	0.93	0.92	0.91	0.99 (0.95, 1.03)	0.98 (0.94, 1.01)

***Table 2B*: Back-transformed and pairwise ratio of weekly fetal growth biometrics (HC, BPD & HC/AC ratio) across DHA (22:6n3) tertiles in the NICHD Fetal Growth Studies-Singletons.**

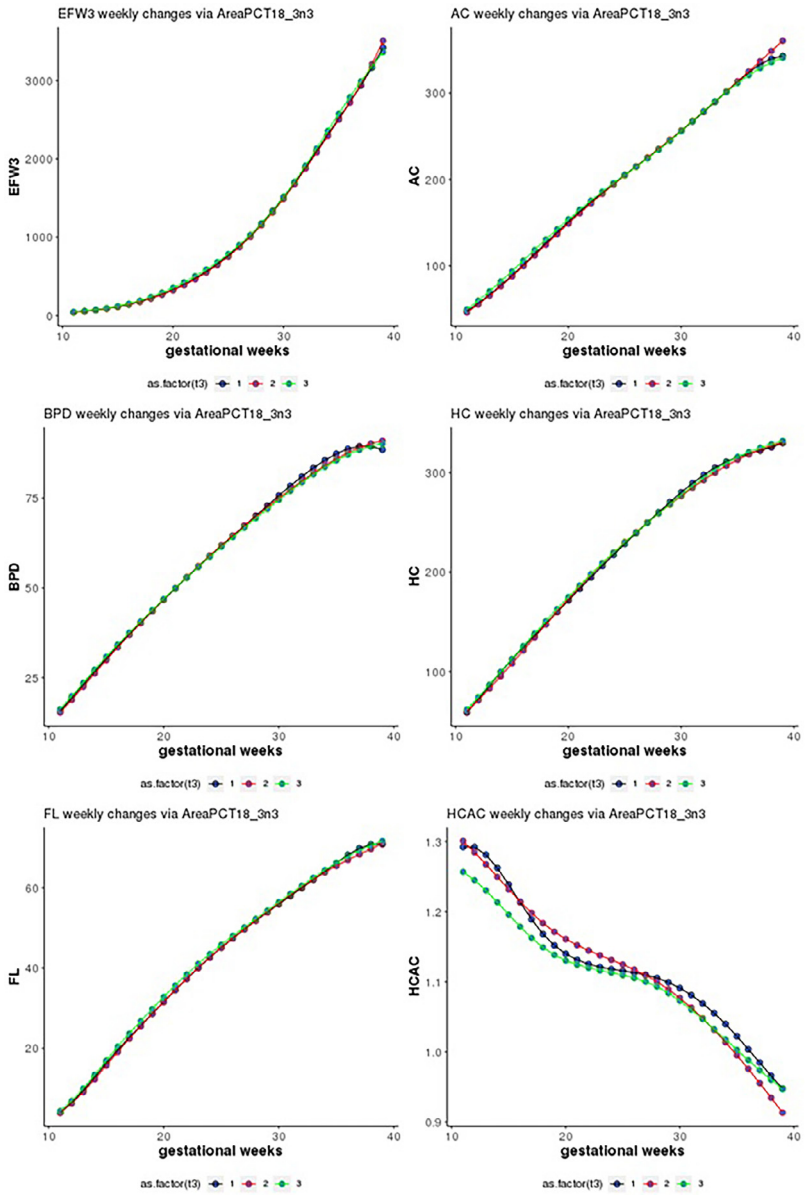

Figure 2. Back-transformed geometric means of EFW by gestational weeks to tertiles of ALA within the NICHD Fetal Growth Studies-Singletons, from 11-39 weeks gestational age. 1st tertile curve is in blue, 2nd tertile curve is in red and 3rd tertile is in green. The shaded area indicated a significant increment of EFW in the 3rd tertile compared with the 1st tertile of ALA.

Discussion

In this prospective study, we found that higher circulating levels of overall n-3 PUFAs and half of n-6 PUFAs were significantly associated with increase in fetal growth even after controlling for a comprehensive panel of major clinical and metabolic factors, including maternal age, ethnicity/race, nulliparity, pre-pregnancy BMI, fetal sex, family history of diabetes, and plasma levels of glucose and total cholesterol. Among them, n-3 PUFAs (i.e., DHA and ALA) and n-6 PUFAs (i.e., LA, GLA, EDA and AA) exhibited the relevance of timing in fetal growth increment, which was apparent as early as 11-13 weeks of gestation until mid-late pregnancy.

n-3 PUFAs and fetal growth

Traditional evidence suggested that n-3 PUFAs ALA, EPA and DHA all play important roles in the central nervous system. They are indispensable structural components of cellular membranes and essential for early cerebral and retinal development.[6,31,32] However, their effects on growth, especially birth weight and

GW	EFW					AC, mm					FL				
	Back-transformed geometric mean, gram			Pairwise ratio (95% CI)		Back-transformed geometric mean, mm			Pairwise ratio (95% CI)		Back-transformed geometric mean, mm			Pairwise ratio (95% CI)	
	1st tertile	2nd tertile	3rd tertile	2nd vs 1st tertile	3rd vs 1st tertile	1st tertile	2nd tertile	3rd tertile	2nd vs 1st tertile	3rd vs 1st tertile	1st tertile	2nd tertile	3rd tertile	2nd vs 1st tertile	3rd vs 1st tertile
10	39.71	35.45	37.23	0.89 (0.75, 1.07)	0.94 (0.82, 1.07)	39.85	38.30	40.25	0.96 (0.87, 1.07)	1.01 (0.93, 1.10)	2.19	2.39	2.70	1.09 (0.97, 1.23)	***1.23 (1.11, 1.38)***
11	47.24	43.83	45.31	0.93 (0.84, 1.02)	0.96 (0.89, 1.03)	48.20	46.64	49.67	0.97 (0.92, 1.02)	1.03 (0.99, 1.10)	3.99	4.05	4.51	1.02 (0.94, 1.09)	***1.13 (1.06, 1.21)***
12	57.48	54.74	56.22	0.95 (0.91, 1.00)	0.98 (0.94, 1.02)	57.54	55.93	60.04	0.97 (0.95, 1.00)	***1.04 (1.02, 1.07)***	6.48	6.31	6.90	0.97 (0.92, 1.03)	***1.07 (1.02, 1.11)***
13	71.17	68.85	70.75	0.97 (0.93, 1.00)	0.99 (0.96, 1.03)	67.82	66.12	71.20	0.97 (0.96, 1.00)	***1.05 (1.03, 1.07)***	9.55	9.10	9.81	0.95 (0.91, 1.00)	1.03 (0.99, 1.07)
14	89.20	86.96	89.85	0.97 (0.93, 1.02)	1.01 (0.97, 1.05)	78.95	77.09	82.95	0.98 (0.95, 1.00)	***1.05 (1.03, 1.07)***	12.93	12.29	13.07	0.95 (0.90, 1.00)	1.01 (0.97, 1.05)
15	112.57	109.94	114.59	0.98 (0.93, 1.02)	1.02 (0.98, 1.06)	90.79	88.71	95.08	0.98 (0.95, 1.00)	***1.05 (1.03, 1.07)***	16.37	15.67	16.48	0.96 (0.91, 1.01)	1.01 (0.97, 1.05)
16	142.31	138.77	146.00	0.98 (0.94, 1.02)	1.03 (0.99, 1.07)	103.16	100.82	107.40	0.98 (0.95, 1.00)	***1.04 (1.02, 1.06)***	19.64	19.07	19.87	0.97 (0.92, 1.02)	1.01 (0.97, 1.05)
17	179.28	174.33	184.93	0.97 (0.94, 1.01)	1.03 (0.99, 1.07)	115.85	113.24	119.72	0.98 (0.96, 1.00)	***1.03 (1.01, 1.05)***	22.69	22.35	23.14	0.98 (0.94, 1.03)	1.02 (0.98, 1.06)
18	223.90	217.35	231.67	0.97 (0.94, 1.01)	***1.03 (1.00, 1.08)***	128.63	125.77	131.89	0.98 (0.96, 1.00)	***1.03 (1.01, 1.04)***	25.63	25.47	26.29	0.99 (0.95, 1.04)	1.03 (0.99, 1.06)
19	276.35	268.17	285.93	0.97 (0.94, 1.01)	1.03 (0.99, 1.08)	141.29	138.23	143.81	0.98 (0.96, 1.00)	***1.02 (1.00, 1.04)***	28.58	28.50	29.38	1.00 (0.95, 1.04)	1.03 (0.99, 1.07)
20	337.18	327.07	347.80	0.97 (0.93, 1.01)	1.03 (0.99, 1.07)	153.66	150.47	155.38	0.98 (0.96, 1.00)	1.01 (0.99, 1.03)	31.53	31.49	32.39	1.00 (0.96, 1.04)	1.03 (0.99, 1.06)
21	406.99	394.60	417.54	0.97 (0.93, 1.01)	1.03 (0.99, 1.07)	165.66	162.40	166.54	0.98 (0.96, 1.00)	1.01 (0.99, 1.02)	34.44	34.41	35.29	1.00 (0.96, 1.04)	1.02 (0.99, 1.06)
22	486.33	471.30	495.47	0.97 (0.93, 1.01)	1.02 (0.98, 1.06)	177.22	173.96	177.26	0.98 (0.96, 1.00)	1.00 (0.98, 1.02)	37.28	37.23	38.04	1.00 (0.96, 1.04)	1.02 (0.99, 1.06)
23	575.73	557.68	581.99	0.97 (0.94, 1.00)	1.01 (0.97, 1.05)	188.32	185.13	187.57	0.98 (0.96, 1.00)	1.00 (0.98, 1.01)	40.02	39.92	40.63	1.00 (0.96, 1.04)	1.02 (0.98, 1.05)
24	675.71	654.24	677.70	0.97 (0.94, 1.00)	1.00 (0.97, 1.04)	199.00	195.95	197.54	0.98 (0.97, 1.00)	0.99 (0.98, 1.01)	42.64	42.47	43.06	1.00 (0.95, 1.04)	1.01 (0.98, 1.04)
25	786.77	761.55	783.44	0.97 (0.94, 1.00)	1.00 (0.96, 1.04)	209.32	206.49	207.27	0.99 (0.97, 1.01)	0.99 (0.97, 1.01)	45.14	44.89	45.34	0.99 (0.95, 1.04)	1.00 (0.97, 1.04)
26	909.50	880.22	900.45	0.97 (0.93, 1.00)	0.99 (0.95, 1.03)	219.39	216.85	216.94	0.99 (0.97, 1.01)	0.99 (0.97, 1.01)	47.52	47.20	47.52	0.99 (0.95, 1.04)	1.00 (0.97, 1.04)
27	1044.56	1010.98	1030.46	0.97 (0.93, 1.00)	0.99 (0.95, 1.03)	229.39	227.21	226.72	0.99 (0.97, 1.01)	0.99 (0.97, 1.01)	49.78	49.40	49.62	0.99 (0.95, 1.04)	1.00 (0.96, 1.03)
28	1192.72	1154.75	1175.88	0.97 (0.93, 1.00)	0.99 (0.95, 1.03)	239.49	237.75	236.85	0.99 (0.97, 1.01)	0.99 (0.97, 1.01)	51.96	51.55	51.72	0.99 (0.95, 1.04)	1.00 (0.96, 1.03)
29	1354.84	1312.45	1339.25	0.97 (0.93, 1.00)	0.99 (0.95, 1.03)	249.90	248.65	247.55	1.00 (0.98, 1.01)	0.99 (0.97, 1.01)	54.05	53.68	53.85	0.99 (0.95, 1.04)	1.00 (0.96, 1.03)
30	1531.19	1484.33	1520.83	0.97 (0.94, 1.00)	0.99 (0.96, 1.03)	260.61	259.87	258.72	1.00 (0.98, 1.01)	0.99 (0.98, 1.01)	56.10	55.78	56.02	0.99 (0.95, 1.04)	1.00 (0.96, 1.03)
31	1721.75	1670.16	1719.25	0.97 (0.94, 1.00)	1.00 (0.96, 1.04)	271.56	271.30	270.18	1.00 (0.98, 1.02)	0.99 (0.98, 1.01)	58.11	57.82	58.19	1.00 (0.95, 1.04)	1.00 (0.97, 1.04)
32	1926.27	1869.38	1931.81	0.97 (0.94, 1.00)	1.00 (0.96, 1.05)	282.70	282.80	281.70	1.00 (0.98, 1.02)	1.00 (0.98, 1.01)	60.11	59.80	60.32	0.99 (0.95, 1.04)	1.00 (0.97, 1.04)
33	2144.25	2081.03	2154.19	0.97 (0.94, 1.01)	1.00 (0.96, 1.05)	293.95	294.21	293.03	1.00 (0.98, 1.02)	1.00 (0.98, 1.01)	62.14	61.66	62.37	0.99 (0.94, 1.04)	1.00 (0.97, 1.04)
34	2374.92	2303.72	2380.25	0.97 (0.94, 1.00)	1.00 (0.96, 1.05)	305.22	305.36	303.88	1.00 (0.98, 1.02)	1.00 (0.98, 1.01)	64.21	63.40	64.29	0.99 (0.94, 1.04)	1.00 (0.96, 1.04)
35	2616.36	2536.88	2602.88	0.97 (0.94, 1.00)	0.99 (0.95, 1.04)	316.40	316.15	313.94	1.00 (0.98, 1.02)	0.99 (0.98, 1.01)	66.36	64.98	66.04	0.98 (0.93, 1.03)	1.00 (0.96, 1.04)
36	2856.37	2783.62	2819.32	0.97 (0.94, 1.01)	0.99 (0.94, 1.03)	327.03	326.97	323.07	1.00 (0.98, 1.02)	0.99 (0.97, 1.01)	68.42	66.43	67.63	0.97 (0.92, 1.02)	0.99 (0.95, 1.03)
37	3072.91	3049.08	3029.73	0.99 (0.95, 1.04)	0.99 (0.94, 1.04)	336.47	338.40	331.22	1.01 (0.98, 1.03)	0.98 (0.96, 1.01)	70.11	67.79	69.12	0.97 (0.91, 1.02)	0.99 (0.94, 1.03)
38	3239.27	3340.35	3235.49	1.03 (0.99, 1.08)	1.00 (0.95, 1.05)	343.96	351.06	338.34	***1.02 (1.00, 1.05)***	0.98 (0.96, 1.00)	71.12	69.12	70.58	0.97 (0.92, 1.03)	0.99 (0.95, 1.04)
39	3326.96	3666.82	3439.24	***1.10 (1.05, 1.15)***	1.03 (0.98, 1.09)	348.77	365.71	344.37	***1.05 (1.02, 1.08)***	0.99 (0.97, 1.01)	71.14	70.45	72.06	0.99 (0.93, 1.05)	1.01 (0.96, 1.06)
40	3310.49	4040.87	3644.84	***1.22 (1.12, 1.33)***	***1.10 (1.01, 1.20)***	350.16	383.21	349.31	***1.09 (1.04, 1.15)***	1.00 (0.95, 1.05)	69.89	71.86	73.65	1.03 (0.96, 1.11)	1.05 (0.98, 1.14)

***Table 3A*: Back-transformed and pairwise ratio of weekly fetal growth biometrics (EFW, AC & FL) across ALA (18:3n3) tertiles in the NICHD Fetal Growth Studies-Singletons.**

GW	HC					BPD					HC/AC ratio				
	Back-transformed geometric mean, gram			Pairwise ratio (95% CI)		Back-transformed geometric mean, mm			Pairwise ratio (95% CI)		Back-transformed geometric mean, mm			Pairwise ratio (95% CI)	
	1st tertile	2nd tertile	3rd tertile	2nd vs 1st tertile	3rd vs 1st tertile	1st tertile	2nd tertile	3rd tertile	2nd vs 1st tertile	3rd vs 1st tertile	1st tertile	2nd tertile	3rd tertile	2nd vs 1st tertile	3rd vs 1st tertile
10	44.85	50.00	49.65	***1.11 (1.04, 1.20)***	***1.11 (1.05, 1.17)***	12.37	12.11	12.28	0.98 (0.89, 1.08)	0.99 (0.91, 1.08)	1.27	1.31	1.23	1.04 (0.96, 1.12)	***0.97 (0.91, 1.04)***
11	57.83	60.29	61.65	***1.04 (1.00, 1.09)***	***1.07 (1.03, 1.10)***	15.79	15.34	15.93	0.97 (0.92, 1.02)	1.01 (0.97, 1.05)	1.28	1.30	1.24	1.01 (0.97, 1.06)	***0.97 (0.94, 1.00)***
12	71.59	71.48	74.45	1.00 (0.97, 1.02)	***1.04 (1.02, 1.06)***	19.44	18.83	19.82	0.97 (0.95, 0.99)	***1.02 (1.00, 1.04)***	1.28	1.28	1.24	1.00 (0.98, 1.02)	***0.97 (0.95, 0.99)***
13	85.56	83.41	87.68	0.97 (0.95, 1.00)	***1.02 (1.00, 1.05)***	23.18	22.50	23.78	0.97 (0.95, 0.99)	***1.03 (1.01, 1.04)***	1.27	1.26	1.23	1.00 (0.98, 1.01)	***0.97 (0.96, 0.99)***
14	99.22	95.91	101.02	0.97 (0.94, 0.99)	***1.02 (1.00, 1.04)***	26.88	26.22	27.65	0.98 (0.95, 1.00)	***1.03 (1.01, 1.04)***	1.25	1.24	1.22	1.00 (0.98, 1.02)	***0.98 (0.96, 0.99)***
15	112.26	108.81	114.21	0.97 (0.95, 0.99)	***1.02 (1.00, 1.04)***	30.47	29.91	31.33	0.98 (0.96, 1.01)	***1.03 (1.01, 1.04)***	1.22	1.23	1.20	1.00 (0.98, 1.02)	0.98 (0.97, 1.00)
16	124.55	121.93	127.07	0.98 (0.96, 1.00)	***1.02 (1.00, 1.04)***	33.90	33.50	34.76	0.99 (0.97, 1.01)	***1.03 (1.01, 1.04)***	1.20	1.21	1.18	1.01 (0.99, 1.03)	0.99 (0.97, 1.00)
17	136.25	135.12	139.55	0.99 (0.97, 1.01)	***1.02 (1.00, 1.05)***	37.19	36.97	37.98	0.99 (0.97, 1.01)	***1.02 (1.00, 1.04)***	1.17	1.19	1.17	***1.02 (1.00, 1.03)***	0.99 (0.98, 1.01)
18	147.73	148.24	151.74	1.00 (0.98, 1.03)	***1.03 (1.01, 1.05)***	40.41	40.33	41.07	1.00 (0.98, 1.02)	***1.02 (1.00, 1.04)***	1.15	1.18	1.15	***1.02 (1.01, 1.04)***	1.00 (0.98, 1.01)
19	159.32	161.20	163.78	1.01 (0.99, 1.04)	***1.03 (1.01, 1.05)***	43.62	43.61	44.15	1.00 (0.98, 1.02)	1.01 (0.99, 1.03)	1.14	1.17	1.14	***1.03 (1.01, 1.04)***	1.00 (0.99, 1.02)
20	171.00	173.90	175.65	1.02 (0.99, 1.04)	***1.03 (1.01, 1.05)***	46.82	46.84	47.22	1.00 (0.98, 1.02)	1.01 (0.99, 1.03)	1.13	1.16	1.13	***1.03 (1.01, 1.04)***	1.00 (0.99, 1.02)
21	182.70	186.25	187.25	***1.02 (1.00, 1.04)***	***1.02 (1.00, 1.05)***	49.98	50.01	50.27	1.00 (0.98, 1.02)	1.01 (0.99, 1.02)	1.12	1.15	1.12	***1.03 (1.01, 1.04)***	1.01 (0.99, 1.02)
22	194.34	198.18	198.56	***1.02 (1.00, 1.04)***	***1.02 (1.00, 1.04)***	53.09	53.10	53.29	1.00 (0.98, 1.02)	1.00 (0.99, 1.02)	1.11	1.14	1.12	***1.03 (1.01, 1.04)***	1.01 (0.99, 1.02)
23	205.88	209.62	209.52	1.02 (0.99, 1.04)	***1.02 (1.00, 1.04)***	56.14	56.10	56.27	1.00 (0.98, 1.02)	1.00 (0.99, 1.02)	1.11	1.13	1.12	***1.02 (1.01, 1.04)***	***1.01 (1.00, 1.02)***
24	217.26	220.58	220.12	1.02 (0.99, 1.04)	1.01 (0.99, 1.04)	59.13	59.01	59.18	1.00 (0.98, 1.02)	1.00 (0.98, 1.02)	1.11	1.13	1.11	***1.02 (1.01, 1.03)***	***1.01 (1.00, 1.02)***
25	228.44	231.05	230.39	1.01 (0.99, 1.04)	1.01 (0.99, 1.03)	62.06	61.84	62.03	1.00 (0.98, 1.02)	1.00 (0.98, 1.02)	1.10	1.12	1.11	1.01 (0.99, 1.02)	1.01 (0.99, 1.02)
26	239.36	241.09	240.33	1.01 (0.98, 1.03)	1.00 (0.98, 1.02)	64.93	64.59	64.81	0.99 (0.97, 1.02)	1.00 (0.98, 1.02)	1.10	1.11	1.11	1.01 (0.99, 1.02)	1.01 (0.99, 1.02)
27	250.01	250.77	250.02	1.00 (0.98, 1.03)	1.00 (0.98, 1.02)	67.75	67.28	67.52	0.99 (0.97, 1.01)	1.00 (0.98, 1.02)	1.10	1.10	1.10	1.01 (0.99, 1.02)	1.00 (0.99, 1.02)
28	260.35	260.19	259.51	1.00 (0.97, 1.02)	1.00 (0.97, 1.02)	70.55	69.94	70.15	0.99 (0.97, 1.01)	0.99 (0.98, 1.01)	1.09	1.09	1.10	1.00 (0.99, 1.02)	1.00 (0.99, 1.01)
29	270.36	269.42	268.86	1.00 (0.97, 1.02)	0.99 (0.97, 1.02)	73.31	72.58	72.71	0.99 (0.97, 1.01)	0.99 (0.97, 1.00)	1.09	1.08	1.09	1.00 (0.98, 1.01)	1.00 (0.99, 1.01)
30	279.91	278.38	277.99	0.99 (0.97, 1.02)	0.99 (0.97, 1.02)	76.03	75.18	75.19	0.99 (0.97, 1.01)	0.99 (0.97, 1.00)	1.08	1.07	1.07	0.99 (0.98, 1.01)	1.00 (0.98, 1.01)
31	288.86	286.92	286.74	0.99 (0.97, 1.02)	0.99 (0.97, 1.02)	78.66	77.68	77.57	0.99 (0.97, 1.00)	0.99 (0.97, 1.00)	1.07	1.06	1.06	0.99 (0.98, 1.00)	0.99 (0.98, 1.00)
32	297.06	294.89	294.98	0.99 (0.97, 1.02)	0.99 (0.97, 1.02)	81.15	80.04	79.85	0.99 (0.97, 1.01)	0.98 (0.96, 1.00)	1.06	1.04	1.05	0.99 (0.97, 1.00)	0.99 (0.98, 1.00)
33	304.35	302.13	302.55	0.99 (0.97, 1.02)	0.99 (0.97, 1.02)	83.47	82.22	82.02	0.98 (0.97, 1.00)	0.98 (0.97, 1.00)	1.04	1.03	1.03	0.98 (0.97, 1.00)	0.99 (0.98, 1.00)
34	310.59	308.47	309.28	0.99 (0.97, 1.02)	1.00 (0.97, 1.02)	85.58	84.16	84.06	0.98 (0.96, 1.00)	0.98 (0.97, 1.00)	1.03	1.01	1.02	0.98 (0.97, 1.00)	0.99 (0.98, 1.00)
35	315.65	313.84	315.03	0.99 (0.97, 1.02)	1.00 (0.97, 1.03)	87.41	85.82	85.96	0.98 (0.96, 1.00)	0.98 (0.97, 1.00)	1.01	0.99	1.00	***0.98 (0.97, 0.99)***	0.99 (0.98, 1.00)
36	319.56	318.55	319.70	1.00 (0.97, 1.03)	1.00 (0.97, 1.03)	88.84	87.29	87.66	0.98 (0.96, 1.00)	0.99 (0.97, 1.01)	1.00	0.97	0.99	***0.98 (0.96, 0.99)***	1.00 (0.98, 1.01)
37	322.47	323.04	323.27	1.00 (0.97, 1.03)	1.00 (0.97, 1.03)	89.72	88.67	89.09	0.99 (0.97, 1.01)	0.99 (0.97, 1.01)	0.98	0.95	0.98	***0.98 (0.96, 0.99)***	1.00 (0.98, 1.02)
38	324.56	327.79	325.69	1.01 (0.98, 1.04)	1.00 (0.97, 1.03)	89.89	90.07	90.18	1.00 (0.98, 1.03)	1.00 (0.98, 1.02)	0.96	0.93	0.96	***0.97 (0.96, 0.99)***	1.00 (0.98, 1.02)
39	325.99	333.29	326.94	1.02 (0.99, 1.06)	1.00 (0.97, 1.03)	89.21	91.62	90.85	***1.03 (1.00, 1.05)***	***1.02 (1.00, 1.04)***	0.94	0.91	0.95	***0.97 (0.95, 0.99)***	1.01 (0.99, 1.03)
40	326.95	340.07	327.02	***1.04 (1.00, 1.08)***	1.00 (0.96, 1.04)	87.55	93.44	91.02	***1.07 (1.02, 1.12)***	1.04 (0.99, 1.09)	0.92	0.88	0.93	0.96 (0.93, 1.00)	1.02 (0.98, 1.06)

***Table 3B*: Back-transformed and pairwise ratio of weekly fetal growth biometrics (HC, BPD & HC/AC ratio) across ALA (18:3n3) tertiles in the NICHD Fetal Growth Studies-Singletons.**

subsequent infant and child growth,[33–36] have been equivocal in the past decades. For example, the Danish prospective cohort (n = 8729)[37] and the Norwegian Mother and Child Cohort Study (n = 62,099)[38] observed greater neonatal parameters at birth among pregnant women with higher dietary intakes of fish during pregnancy, while the US Project Viva reported a smaller fetal growth z-score in the second and third trimesters among pregnant women with higher seafood intakes in the first trimester.[39] Most evidence from observational studies and trials stemmed from dietary intake or supplementation, an approach based on a self-reported food recall assessment tool subject to measurement error[18] and without accounting for the endogenous source of n-3 PUFAs.

ALA is essential to the human diet because of the body's inability to synthesize it endogenously. It accounts for 9–11% of total PUFA dietary intake in the Western diet.[40] Our study found that higher circulating levels of ALA and DHA in early pregnancy were associated with subsequent fetal growth throughout gestation. Moreover, emerging evidence suggests that both ALA and DHA accumulate in the fetus via placenta transfer,[5,6,41] and Δ6-desaturase activity was present in the fetal liver from early gestation in rat models.[42,43] Thus, we speculated that high Δ6-desaturase activity might help increase conversion of ALA and subsequently increase DHA levels that could enhance fetal cell growth and replication.[5] In addition, long-chain n-3 PUFAs are known to not only reduce placental inflammation and oxidative stress and inhibit placental angiogenesis,[4] but also assist the peroxisome proliferator-activated receptors (PPARs)—family of the nuclear receptors—in exerting a fundamental role in embryonic and fetal development.[44]

n-6 PUFAs and fetal growth

Unlike n-3 PUFAs which produce anti-inflammatory lipids mediators beneficial for various clinical outcomes, n-6 PUFAs are generally considered pro-inflammatory by nature.[11] Experimental models in rats reported that diets containing higher LA levels, regardless of whether it was in the trans- or cis-form, exhibited an increased expression of lipogenic genes, increased fat mass, and greater adipocyte size and adipocyte number.[45,46] Thus, n-6 PUFAs promoted the expansion of fat depots by upregulating both hyperplasia and hypertrophy, and ultimately increased fat deposition at higher levels of dietary LA intake.[47] However, only a few studies have investigated the role of maternal n-6 PUFA levels on neonatal and childhood adiposity outcomes assessed either by dietary intake or blood concentration.[12–16] And the limitations of such evidence were similar to that found in n-3 PUFAs studies mentioned above.

LA is also an essential fatty acid obtained through the diet, and it accounts for 84–89% of total PUFA dietary intake in the Western diet.[40] Although AA is partially synthesized *in vivo* from its precursor LA via desaturase and elongase activities, it is still primarily obtained via diet.[48,49] It has been widely suggested that LA and AA are critical for membrane phospholipids in the lymphoid system, even though excessive intake of such pro-inflammatory and pro-thrombotic fatty acids might lead to metabolic diseases.[50] Long-chain n-6 PUFAs like LA and AA are also the endogenous ligands that activate PPARs, which are known to attenuate or inhibit the production of vascular damage, inflammation, lipotoxicity, and endothelial dysfunction, which are suboptimal for fetal growth.[44] On the contrary, DGLA—derived predominantly from endogenous sources—has demonstrated the ability to modulate cellular lipid metabolism and eicosanoid synthesis, which prompts inflammatory-induced insulin resistance β-cell destruction.[51] Our study found that higher circulating LA, GLA, and AA levels were associated with increased fetal growth, whereas higher circulating DGLA, DTA, and n-6 DPA levels were associated with reduced fetal growth throughout pregnancy. Such opposite directional changes in fetal growth implied different roles of n-6 PUFAs underlying oxidative stress and inflammatory *in vivo*, which might differentiate subsequent fetal growth trajectories.[6]

Strength and limitations

Our study has a few notable strengths. The prospective and longitudinal data collection included plasma phospholipid PUFAs which represent both exogenous and endogenous sources of PUFAs and longitudinal fetal growth ultrasound measures. With such thorough information, we could examine the temporal relationship between plasma phospholipid PUFAs in early pregnancy and subsequent fetal growth throughout gestation. Also, we identified the relevance of timing for significant associations. In addition, such a comprehensive panel of plasma phospholipid PUFAs may further help elucidate the alleged roles of PUFAs underlying the physiology of fetal development. Our study was not without limitations. Even though this is one of the largest prospective pregnancy cohorts investigating fetal growth throughout pregnancy, the relatively small sample size of 321 subjects may limit the statistical power of identifying more PUFAs with fetal growth. Second, we cannot eliminate residual confounding due to the study's observational nature, even though we controlled for known major confounders in our modeling. However, the availability of major clinical factors and metabolic biomarkers for sensitivity analysis (i.e., family history of diabetes, glucose levels, and total cholesterol) helped improve our findings' robustness. Third, since the early pregnancy blood collection was random, the levels of plasma phospholipid PUFAs could be biased by a meal enriched with fish oil or marine oil prior to

the blood collection. Therefore, this measurement error might lead to non-differential misclassification and type II error.

Conclusion

Our study investigated individual plasma phospholipid n-3 and n-6 PUFAs in early pregnancy and fetal growth trajectories throughout pregnancy based on longitudinal ultrasound measured fetal growth metrics, and also identified the relevance of timing for significant associations. We found that higher circulating levels of plasma phospholipid DHA and ALA were associated with larger fetal weight and size, which started in early pregnancy (weeks 11–13) and waned off in late pregnancy (weeks 37–39). On the other hand, higher circulating levels of plasma phospholipid DGLA, DTA, and n-6 DPA were inversely associated with fetal size, which started in early pregnancy (weeks 11–13) and waned off in mid-late pregnancy (weeks 29–35). Further studies with a larger sample size are still warranted to verify our findings. Considering the modifiable nature of plasma PUFAs and the strong associations between PUFAs (e.g., DHA) and fetal growth independent of maternal obesity, our data imply that optimizing plasma phospholipid PUFAs in early pregnancy might benefit fetal growth.

Contributors

All authors have read and approved the final version of the manuscript and ensured it is the case. L-JL conceptualized the study hypothesis, participated in data analyses, wrote up and edited the manuscript. WJ performed data analyses, assessed and verified the data. CZ contributed in methodology and edited the manuscript. NLW performed fatty acids examination, contributed to methodology and edited the manuscript. MYT performed fatty acids examination, contributed to methodology and edited the manuscript. PA contributed in methodology and edited the manuscript. CZ designed the study, obtained funding, supervised the analysis, reviewed and edited the manuscript, and also assessed and verified the data.

Data sharing statement

Data described in the manuscript, code book, and analytic code will be available upon request pending application and approval of a data-sharing agreement.

Declaration of interests

All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.104180.

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