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Serum polyunsaturated fatty acids in infancy are associated with body composition in adolescence

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Summary

Background: Polyunsaturated fatty acids (PUFA) have been related to the development of adiposity. N-3 PUFA appears to be protective against obesity risk, while n-6 PUFA may be associated with greater adiposity. However, most studies have been conducted among adults. The role of PUFA in infancy is unknown.

Objective: To examine associations of serum PUFA at age 1 year with age- and sex-adjusted body mass index Z score (BMIZ) change through age 16 years and body composition at 16 years.

Methods: We quantified serum PUFA in 636 Chilean infants aged 1 year. We measured BMIZ at ages 1, 5, 10 and 16 years, and body composition by dual energy X-ray absorptiometry at 16 years. We estimated differences in 1- to 16-years BMIZ change between PUFA quartiles from multivariable linear mixed models with restricted cubic splines. At 16 years, we estimated differences in total fat mass (ToFM), truncal fat mass (TrFM), total lean mass (TLM), percent total fat mass (%ToFM) and percent truncal fat mass (%TrFM) between PUFA quartiles using linear regression.

Results: PUFA were not associated with BMIZ change. Alpha-linolenic acid (ALA) was positively associated with TrFM (P = .03) and %TrFM (P < .0001) at 16 years while eicosapentaenoic acid (EPA) was inversely associated with %TrFM (P = .001). Docosapentaenoic acid (DPA) was positively associated with ToFM (P = .01), TrFM (P = .009), %ToFM (P = .02) and %TrFM (P = .02). Gamma-linolenic acid (GLA) and the Δ6-desaturase (D6D) activity index were each positively, linearly associated with ToFM, TrFM and %ToFM. The Δ5-desaturase (D5D) activity index was inversely associated with %TrFM (P = .04).

Abbreviations: %ToFM, percent total fat mass; %TrFM, percent truncal fat mass; AA, arachidonic acid; ALA, alpha-linolenic acid; BMI, body mass index; BMIZ, BMI-for-age Z score; CI, confidence interval; D5D, ⍰5-desaturase; D6D, ⍰6-desaturase; DGLA, dihomo-gamma-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; DXA, dual energy X-ray absorptiometry; EPA, eicosapentaenoic acid; FA, fatty acids; GLA, gamma-linolenic acid; IDA, iron deficiency anaemia; INTA, University of Chile Institute of Nutrition and Food Technology; LA, linoleic acid; PUFA, polyunsaturated fatty acids; SES, socioeconomic status; TLM, total lean mass; ToFM, total fat mass; TrFM, truncal fat mass.

Conclusions: ALA, DPA, GLA and the D6D index at 1 year of age were positively associated with adiposity at age 16 years, while EPA and the D5D index were inversely associated with central adiposity. Our results related to EPA and desaturase indices are in agreement with limited prior studies.

KEYWORDS
body composition, body mass index, children, Chile, infants, polyunsaturated fatty acids

1 | INTRODUCTION

Childhood obesity is the most prevalent early life risk factor for the development of chronic diseases, including type 2 diabetes, hypertension and coronary heart disease.[1] Childhood obesity has increased globally throughout recent decades[2] and represents a significant public health concern. Identifying potentially modifiable nutritional exposures that influence the early development of adiposity is crucial in order to address this epidemic.

Polyunsaturated fatty acids (PUFA) are nutrients that may be related to the etiology of obesity. These fatty acids (FA), primarily classified into the n-3 and n-6 families, are necessary for a variety of physiologic processes. In particular, long-chain PUFA are precursors for eicosanoids, signalling molecules that regulate cardiovascular and immune function. In animal and in vitro studies, both plant- and marine-derived n-3 PUFA reduce or prevent adiposity, through reduction of fat deposition in adipose tissue and appetite suppression.[3,4] Conversely, n-6 PUFA up-regulate adipocyte size and number.[5] Studies of PUFA and adiposity among adult humans have yielded mixed results. Some, but not all, trials of n-3 PUFA supplementation have found an inverse effect on adiposity.[6] While several observational studies (reviewed by Naughton et al[7]) report a positive association between the n-6 PUFA linoleic acid (18:2 n-6; LA) and body weight, a meta-analysis of n-6 PUFA supplementation trials did not find strong evidence for an effect of these PUFA on adiposity.[8]

PUFA status in infancy may be particularly influential on future adiposity. The precursor PUFA alpha-linolenic acid (18:3 n-3; ALA) and LA cannot be synthesized endogenously, and their conversion into long-chain PUFA is inefficient in humans.[9] Thus, infants must largely obtain these FA through diet, initially from breast milk or formula[10] and later through complementary feeding. Nutrition during infancy can have long-term effects on cardiovascular health.[11] Adipose tissue in childhood may be especially sensitive to the effects of PUFA, since this is the time during which the number of adipocytes in the body is determined.[12] Most previous investigations of early-life PUFA and adiposity have focused on exposure in utero.[13] The potential effects of PUFA in infancy may differ from those in utero, but results from previous studies have been inconclusive, and little research exists on measures of long-term adiposity. In a recent review of both observational studies and trials of n-3 PUFA supplementation in infancy, results were a mix of positive, inverse and null associations of specific PUFA with measures of adiposity through middle childhood.[13] In addition to these inconclusive findings, the outcomes in these studies were assessed at ages 6 to 8 years, and thus it is still unclear whether PUFA might have longer-reaching effects on adiposity throughout development. Moreover, very few studies have been conducted in low- and middle-income countries,[14-16] despite the rapidly growing burden of childhood obesity[2] and low availability of dietary PUFA[17] affecting many of these regions.

We aimed to investigate the relations between serum PUFA biomarkers at 1 year of age and measures of adiposity at age 16 years in a cohort of children from Santiago, Chile.

2 | METHODS

2.1 | Study design and population

We conducted a longitudinal investigation among Chilean children who were enrolled as infants in studies on iron status and were followed through adolescence. Details of the study design have been published previously.[18,19] Briefly, participants were recruited at ages 4 to 6 months from low- and middle-income communities in Santiago, Chile between 1991 and 1996. Eligible infants were singleton, born at term weighing at least 3 kg through uncomplicated, vaginal births, and did not have major health complications. Infants without iron deficiency anaemia (IDA) at baseline were enrolled in a trial of iron supplementation. These participants were randomly assigned to high- or low-dose iron supplementation or usual nutrition until 12 months of age. Infants with IDA were treated with iron and enrolled in an observational study of neurodevelopment, along with a group of non-anaemic controls. There were 1798 infants enrolled. The cohort was followed during middle childhood and adolescence: 888 children were assessed at 5 years of age, 1127 at 10 years of age and a subset of 679 were assessed at a median age of 16.8 years of age as part of a study on cardiometabolic risk.[20] The study of FA was conducted among participants who had a stored serum sample available from age 1 year plus at least one more sample from ages 5 or 10 years. For analyses of BMIZ change between 1 and 16 years of age, we included 636 children in the FA study who had BMIZ data available at 1 year of age and at ≥1 subsequent assessment (5, 10 or 16 years of age). For the analysis of PUFA at 1 year of age and DXA measures of body composition at 16 years of age, there were 382 children with available information. A flowchart of the sample size at each inclusion step is provided in Figure S1.

The study procedures were approved by institutional review boards at the University of Chile Institute of Nutrition and Food Technology (INTA), the University of Michigan and the University of California, San Diego. Parents provided written informed consent to participate for their children, and children provided assent beginning at 10 years of age.

2.2 | Data collection

Birth weight in g, length in cm and gestational age in weeks were determined via hospital records. The date of the mother's last menstrual period was used to determine gestational age. Study personnel collected information from parents on household socioeconomic indicators at enrollment. Information on breastfeeding habits, including the date of the first bottle feeding and last breastfeeding, were collected from mothers at weekly study visits that took place from enrollment until the infant was 12 months old. For infants who had already been bottle fed at enrollment, mothers were asked to recall the date of the first bottle feeding. Because feeding breast milk by bottle was non-existent in this population, bottle feeding was equivalent to giving infant formula or cow milk.

Anthropometric measurements were collected at INTA by trained study personnel using standardized techniques. At 1 year of age, weight was measured unclothed to the nearest 10 g and length was measured to the nearest millimetre using a recumbent length board. Weight at 5, 10 and 16 years was measured to the nearest 100 g using a Seca scale (Seca, Hamburg, Germany) and height was measured to the nearest millimetre using a Holtain stadiometer (Holtain, Crymych, UK). All measures were obtained in duplicate and a third measurement was taken if the difference between the first two was greater than 0.3 kg or 0.5 cm; the mean of the two closest values was used. At the 16.8-year assessment, total fat mass, truncal fat mass and total lean mass were measured with a dual energy X-ray absorptiometry (DXA) instrument (Lunar Prodigy Corp., Madison, WI, USA). These measures were conducted in the subset of children who participated in the study of cardiometabolic risk.

A blood sample was collected by venipuncture at 1, 5 and 10 years of age. The median (25th, 75th percentile) age at the 1 year blood draw was 1.00 year (0.99, 1.02). Blood components were separated and serum samples from 1 year of age were stored at −80°C before transportation as a single batch to the University of Michigan where they were cryostored at −80°C and analyzed after approximately 20 years from collection. Serum PUFA stored at −80°C are highly stable. A previous study found virtually no degradation of PUFA in samples stored at −80°C for up to 10 years.[21]

2.3 | Fatty acid analyses

FA in serum were quantified at the University of Michigan Regional Comprehensive Metabolomics Resource Core. Total lipids were extracted from 200 μL of serum according to the method described by Bligh and Dyer.[22] 10 μL of 4 mM nonadecanoic acid (C19:0) was used as the internal standard. The FA fraction of the total lipids was derivatized into methyl esters using BF_3-methanol as previously described.[23] These were extracted with a 2:1 hexane-water, dried and resuspended in hexane. FA were measured using gas chromatography. 1 to 2 μL of sample was injected via autosampler onto an Agilent 6890 N chromatograph (Agilent, Santa Clara, CA, USA) with a flame ionization detector, a 100 m x 0.25 mm × 0.2 μm SP-2560 column (Sigma-Aldrich, Bellefonte, PA) and Chemstation software. C19:0 and other authentic methyl esters were used to create a calibration curve for FA quantification. The authentic methyl esters were also used to identify FA in samples based on retention times. The coefficients of variation for quantification of specific FA ranged between 2.5% and 3.6%.

2.4 | Statistical analyses

2.4.1 | Definition of exposures

The main exposures were serum PUFA biomarkers measured at 1 year of age, expressed as percentage relative to the total FA concentration (FA %) quantified in a sample. The n-3 PUFA we considered were ALA, eicosapentaenoic acid (20:5 n-3; EPA), docosapentaenoic acid (22:5 n-3; DPA) and docosahexaenoic acid (22:6 n-3; DHA). N-6 PUFA included LA, gamma-linolenic acid (18:3 n-6; GLA), dihomo-gamma-linolenic acid (20:3 n-6; DGLA) and arachidonic acid (20:4 n-6; AA). We also considered the ratio of total n-6:n-3 PUFA, which may be important for adiposity development.[24] Additionally, we considered activity indices of the Δ6-desaturase (D6D) and Δ5-desaturase (D5D) enzymes, using the GLA/LA and AA/DGLA ratios, respectively. We categorized all exposures into quartiles to allow for possible non-linear associations with the outcomes.

2.4.2 | Definition of outcomes

The primary outcomes were change in age- and sex-adjusted body mass index (BMI) Z scores (BMIZ) between 1 and 16 years of age and DXA measures of body composition at 16 years of age. BMI was calculated as kg/m^2 and BMIZ were calculated according to the World Health Organization Growth Reference for children ages 5 to 19 years.[25] DXA measures included the total fat mass (ToFM), truncal fat mass (TrFM), total lean mass (TLM), percent total fat mass (%ToFM) and percent truncal fat mass (%TrFM).

2.4.3 | Covariates

All covariates were measured in infancy and included sociodemographic and anthropometric characteristics as well as serum FA.

We categorized birth length and weight as average or large for gestational age according to the INTERGROWTH 21st standards for newborn size.[26] We defined large for gestational age as ≥90th percentile. We did not consider a category of small for gestational age because birth weight ≥ 3 kg was an eligibility criterion for recruitment and there were no children ≤10th percentile for birth weight. Seven children with birth length ≤10th percentile were classified in the average for gestational age group. We categorized breastfeeding as <6 months, ≥6 months mixed bottle/breastfeeding, or ≥ 6 months exclusive breastfeeding. Iron supplementation was categorized as any (combining low- and high-dose groups) vs none. We measured socioeconomic status (SES) using a modified Graffar index.[27] The index consists of 13 items related to family structure, education and employment, crowding and housing condition and ownership of assets. The index ranges from 0 to 65; higher values indicate lower SES. The serum FA that we considered as covariates were total *trans* FA, which have been associated with weight gain in adults.[28]

2.4.4 | Correlates of serum PUFA at 1 year of age

We compared distributions of serum PUFA biomarkers by categories of covariates using means ± SD. We tested the significance of associations using linear regression models with each PUFA biomarker as the outcome. For dichotomous covariates, we obtained *P* values from Wald tests. For ordinal variables, we tested for linear trends using Wald tests of a variable representing category-specific medians introduced as a continuous variable. For categorical covariates, we used a χ^2 score statistic.

2.4.5 | Serum PUFA at 1 year of age and change in BMIZ from ages 1 to 16 years

In bivariate analysis, we estimated means ± SE of BMIZ at 1 and 16 years of age and change in BMIZ between 1 and 16 years by quartiles of the PUFA exposures at age 1 year. These were from BMIZ growth curves estimated using mixed effects linear regression models.[29] The outcome was BMIZ and age was included as a predictor using restricted cubic splines.[30] These piecewise cubic polynomials are smoothly joined at each knot and linear in the tails. They allow for modelling of smoothed, non-linear trajectories of BMIZ across different ages. These methods do not require that all children have the same number of measurements or that the measurements be obtained at the same ages. Thus, we fitted the models with data from all assessments (1, 5, 10 and 16 years) and placed knots at the median ages of children measured at each assessment. Other predictors in the model included indicator variables for each PUFA quartile, interaction terms between the PUFA indicators and all age terms and random intercepts and age slopes for each child. We estimated adjusted mean differences and 95% confidence intervals (CI) in 1 to 16 years BMIZ change between quartiles of serum PUFA from these models. We included covariates that were associated with the PUFA biomarkers or that have been related to BMIZ change. The final model included sex, birth weight, breastfeeding, Graffar index and total serum *trans* FA at 1 year of age. Each long-chain PUFA was adjusted for its immediate metabolic precursor: EPA was adjusted for ALA, DPA for EPA, DHA for DPA, GLA for LA, DGLA for GLA and AA for DGLA. In order to adjust for potential confounding by common dietary sources of n-3 and n-6 PUFA, which co-occur as LA and ALA in foods such as plant oils,[31] we adjusted estimates for each n-3 PUFA for LA and total long-chain n-6 PUFA, while estimates for each n-6 PUFA were adjusted for ALA and total long-chain n-3 PUFA. All covariate FA were entered in the model as restricted cubic splines in order to account for possible residual confounding due to non-linear associations with the outcome. When the association between PUFA quartiles and BMIZ change seemed linear, we estimated the difference in change per 1 SD of the PUFA distribution.

2.4.6 | Serum PUFA at 1 year of age and body composition at 16 years

In bivariate analysis, we compared distributions of ToFM, TrFM, TLM, %ToFM and %TrFM by quartiles of serum PUFA using means ± SD. We conducted tests for linear trend using linear regression models with each body composition measure as the outcome and a variable representing median values of each PUFA quartile introduced as a continuous variable. In multivariable analysis we obtained adjusted mean differences and 95% CI in the body composition measures by quartiles of serum PUFA. These models included indicators for PUFA quartiles and were also adjusted for sex, birth weight, breastfeeding, Graffar index, BMI at 1 year of age and total serum *trans* FA. We also included other PUFA as covariates following the strategy described for the analyses of BMIZ change. Because body composition may differ by sex in adolescence, we conducted supplemental analyses stratified by sex by including interaction terms between sex and all other model predictors and testing for their statistical significance.

We fitted all models with empirical variance estimates, which are robust to heteroskedasticity and deviations from normality.[32] All analyses were conducted using Statistical Analysis Software version 9.4 (SAS Institute, Cary, NC).

3 | RESULTS

The number of children with 2, 3 or 4 measurements was 78, 183 and 375, respectively. Mean ± SD BMIZ at 1, 5, 10 and 16 years of age was 0.81 ± 0.94, 0.98 ± 1.18, 1.02 ± 1.16 and 0.71 ± 1.13, respectively. At age 16 years, mean ± SD ToFM, TrFM and LM was

19.8 ± 10.1, 10.0 ± 5.7 and 44.0 ± 8.9 kg, respectively. Mean ± SD % ToFM and %TrFM was 29.1 ± 10.9 and 49.3 ± 5.9.

3.1 | N-3 PUFA

3.1.1. | Correlates of n-3 PUFA at 1 year

Serum ALA was positively associated with iron supplementation (Table S1). EPA was positively related to exclusive breastfeeding duration and total *trans* FA. DPA was inversely associated with iron supplementation. DHA was related to female sex and exclusive breastfeeding duration, and inversely associated with total serum *trans* FA.

3.1.2. | BMIZ change between ages 1 and 16 years

N-3 PUFA at 1 year of age were not significantly associated with BMIZ change from 1 to 16 years (Table 1 and Table S2).

3.1.3. | Body composition at age 16 years

ALA was positively associated with TrFM and %TrFM in non-linear fashions (Table 2 and Table S2). Adjusted mean differences (95% CI) between children with ALA in quartile (Q) 4 vs <Q4 were 1.5 kg (0.2, 2.8; $P = .03$) for TrFM and 3.3 percentage points (95% CI: 2.0%, 4.6%; $P < .0001$) for %TrFM. EPA was inversely associated with %TrFM

TABLE 1 Changes in BMI-for-age Z scores (BMIZ)[a] from 1 to 16 years of age by serum n-3 polyunsaturated fatty acid biomarkers at 1 year of age among children from Santiago, Chile

Fatty acid quartile (median, weight % of total FA)	N	1 year BMIZ (mean ± SE)[b]	16 years BMIZ (mean ± SE)	Change in BMIZ 1-16 years (mean ± SE)[b]	Adjusted difference in change (95% CI)[c]
Overall	636	0.81 ± 0.04	0.72 ± 0.05	−0.09 ± 0.05	
N-3					
ALA (18:3 n-3)					
Q1 (0.21)	159	0.81 ± 0.07	0.68 ± 0.09	−0.13 ± 0.11	Reference
Q2 (0.28)	159	0.73 ± 0.07	0.61 ± 0.09	−0.12 ± 0.10	−0.01 (−0.30, 0.27)
Q3 (0.39)	159	0.88 ± 0.08	0.76 ± 0.09	−0.12 ± 0.10	−0.04 (−0.33, 0.25)
Q4 (1.32)	159	0.84 ± 0.08	0.83 ± 0.09	−0.01 ± 0.10	0.06 (−0.24, 0.36)
P, trend[d]				.33	.55
EPA (20:5 n-3)					
Q1 (0.07)	159	0.78 ± 0.07	0.65 ± 0.09	−0.12 ± 0.10	Reference
Q2 (0.13)	159	0.80 ± 0.07	0.80 ± 0.09	0.00 ± 0.10	0.10 (−0.18, 0.38)
Q3 (0.22)	159	0.81 ± 0.07	0.77 ± 0.09	−0.05 ± 0.10	0.07 (−0.21, 0.35)
Q4 (0.34)	159	0.86 ± 0.08	0.66 ± 0.09	−0.20 ± 0.11	−0.06 (−0.35, 0.23)
P, trend				.46	.56
DPA (22:5 n-3)					
Q1 (0.08)	160	0.78 ± 0.07	0.48 ± 0.09	−0.30 ± 0.09	Reference
Q2 (0.11)	158	0.78 ± 0.07	0.72 ± 0.09	−0.05 ± 0.10	0.23 (−0.04, 0.50)
Q3 (0.14)	159	0.75 ± 0.07	0.98 ± 0.09	0.23 ± 0.10	0.54 (0.26, 0.82)
Q4 (0.21)	159	0.94 ± 0.08	0.71 ± 0.09	−0.24 ± 0.11	0.07 (−0.21, 0.35)
P, trend				.75	.71
DHA (22:6 n-3)					
Q1 (0.23)	158	0.84 ± 0.08	0.67 ± 0.10	−0.17 ± 0.10	Reference
Q2 (0.43)	160	0.87 ± 0.06	0.69 ± 0.09	−0.17 ± 0.10	0.05 (−0.23, 0.33)
Q3 (0.62)	159	0.71 ± 0.07	0.72 ± 0.09	0.01 ± 0.10	0.20 (−0.11, 0.50)
Q4 (0.90)	159	0.83 ± 0.08	0.80 ± 0.09	−0.03 ± 0.10	0.17 (−0.16, 0.50)
P, trend				.19	.28

[a]According to the World Health Organization Growth Reference for children ages 5 to 19 years.[25]

[b]From growth curves estimated using mixed effects linear regression models with BMIZ as the outcome, and predictors that included indicator variables for each PUFA quartile, linear and cubic spline terms for age, and interaction terms between the quartiles and age terms. All models included random intercepts and age slopes to account for within-child correlations of repeated BMI measurements.

[c]Adjusted for sex, birth weight (large vs average for gestational age), breastfeeding (<6 months, ≥6 months w/ bottle feeding, ≥6 months exclusive), Graffar index (indicator variables for quintiles) and total serum trans fatty acids (FA). All n-3 PUFA are adjusted for LA and total long-chain n-6 PUFA. All long chain PUFA are adjusted for their precursor PUFA. Covariate FA are represented with linear and restricted cubic spline terms.

[d]From mixed effects linear regression models with BMI as the outcome and a variable representing medians of PUFA quartiles introduced as continuous.

TABLE 2 Body composition[a] at 16 years of age by serum n-3 polyunsaturated fatty acid biomarkers at 1 year of age among children from Santiago, Chile

		Total fat mass (kg)		Truncal fat mass (kg)		Total lean mass (kg)		% Total fat mass		% Truncal fat mass	
Fatty acid quartile (median, weight % of total FA)	N	Mean ± SD	Adjusted difference (95% CI)[b]	Mean ± SD	Adjusted difference (95% CI)	Mean ± SD	Adjusted difference (95% CI)	Mean ± SD	Adjusted difference (95% CI)	Mean ± SD	Adjusted difference (95% CI)
Overall	382	19.8 ± 10.1		10.0 ± 5.7		44.0 ± 8.9		29.1 ± 10.9		49.3 ± 5.9	
ALA (18:3 n-3)											
Q1 (0.21)	98	19.5 ± 9.9	Reference	9.8 ± 5.8	Reference	42.8 ± 8.5	Reference	29.4 ± 11.1	Reference	48.7 ± 6.3	Reference
Q2 (0.28)	98	18.4 ± 8.9	−0.4 (−2.7, 2.0)	9.0 ± 4.9	−0.5 (−1.9, 0.9)	43.8 ± 8.4	−0.1 (−1.6, 1.3)	27.8 ± 9.8	−0.2 (−2.3, 1.9)	47.6 ± 5.3	−1.1 (−2.8, 0.5)
Q3 (0.39)	90	19.6 ± 9.3	0.4 (−2.2, 2.9)	9.8 ± 5.3	0.1 (−1.4, 1.6)	45.0 ± 9.8	1.1 (−0.5, 2.7)	28.6 ± 10.2	0.1 (−2.2, 2.4)	49.0 ± 5.9	0.1 (−1.7, 1.9)
Q4 (1.32)	96	21.8 ± 12.0	1.9 (−1.0, 4.7)	11.5 ± 6.5	1.3 (−0.3, 2.9)	44.3 ± 8.8	0.6 (−1.0, 2.2)	30.5 ± 12.3	1.1 (−1.4, 3.5)	51.9 ± 5.0	2.9 (1.2, 4.7)
P, trend[c]		.05	.12	.008	.03	.47	.55	.21	.28	<.0001	<.0001
EPA (20:5 n-3)											
Q1 (0.07)	95	19.3 ± 11.2	Reference	9.9 ± 6.1	Reference	43.0 ± 8.6	Reference	28.6 ± 11.7	Reference	50.3 ± 5.5	Reference
Q2 (0.13)	95	20.5 ± 9.3	1.1 (−1.4, 3.7)	10.2 ± 5.1	0.1 (−1.3, 1.5)	43.4 ± 8.8	−0.9 (−2.4, 0.6)	30.3 ± 10.0	2.2 (0.1, 4.4)	48.6 ± 5.8	−2.4 (−3.9, −0.8)
Q3 (0.22)	99	19.6 ± 10.3	0.5 (−2.3, 3.3)	10.0 ± 5.9	0.1 (−1.5, 1.7)	45.0 ± 9.4	0.6 (−1.0, 2.2)	28.5 ± 11.2	0.8 (−1.6, 3.2)	49.7 ± 5.7	−1.6 (−3.2, 0.0)
Q4 (0.34)	93	19.8 ± 9.8	−0.1 (−2.7, 2.6)	9.9 ± 5.7	−0.3 (−1.8, 1.2)	44.4 ± 8.7	0.1 (−1.5, 1.6)	28.9 ± 10.7	0.2 (−2.1, 2.5)	48.6 ± 6.3	−2.5 (−4.2, −0.7)
P, trend		.98	.74	.95	.64	.19	.42	.79	.62	.17	.04
DPA (22:5 n-3)											
Q1 (0.08)	91	17.1 ± 9.0	Reference	8.5 ± 5.1	Reference	43.5 ± 9.0	Reference	26.7 ± 10.7	Reference	48.1 ± 5.9	Reference
Q2 (0.11)	96	20.1 ± 9.8	1.8 (−0.6, 4.2)	10.2 ± 5.5	1.1 (−0.3, 2.5)	42.8 ± 8.5	0.6 (−1.0, 2.1)	30.0 ± 11.0	1.6 (−0.6, 3.8)	49.5 ± 5.5	1.6 (−0.1, 3.3)
Q3 (0.14)	98	21.2 ± 10.7	4.1 (1.4, 6.8)	10.9 ± 6.0	2.4 (0.9, 4.0)	45.7 ± 9.5	1.6 (−0.1, 3.3)	29.7 ± 10.7	3.5 (1.1, 5.9)	50.3 ± 5.7	2.6 (0.8, 4.3)
Q4 (0.21)	97	20.6 ± 10.6	2.3 (−0.2, 4.8)	10.4 ± 5.8	1.3 (−0.2, 2.7)	43.8 ± 8.3	0.9 (−0.8, 2.5)	29.9 ± 11.0	1.8 (−0.5, 4.1)	49.2 ± 6.1	1.1 (−0.6, 2.9)
P, trend		.04	.10	.04	.13	.48	.29	.10	.15	.30	.33
DHA (22:6 n-3)											
Q1 (0.23)	92	19.1 ± 10.5	Reference	9.7 ± 6.0	Reference	45.3 ± 8.8	Reference	27.7 ± 11.6	Reference	49.2 ± 6.3	Reference
Q2 (0.43)	100	19.7 ± 9.5	1.1 (−1.6, 3.7)	10.2 ± 5.4	0.7 (−0.8, 2.3)	43.3 ± 8.5	−1.5 (−3.0, 0.0)	29.3 ± 10.7	2.1 (−0.3, 4.6)	50.8 ± 5.3	1.5 (−0.1, 3.1)
Q3 (0.62)	95	20.0 ± 10.6	0.1 (−3.0, 3.2)	10.0 ± 5.9	−0.2 (−2.0, 1.5)	43.7 ± 8.7	0.1 (−1.5, 1.8)	29.4 ± 10.8	0.0 (−2.8, 2.8)	48.8 ± 6.2	−1.1 (−3.1, 0.9)
Q4 (0.90)	95	20.3 ± 10.1	−0.4 (−3.9, 3.2)	10.1 ± 5.5	−0.6 (−2.6, 1.4)	43.6 ± 9.5	−1.0 (−2.8, 0.9)	29.9 ± 10.5	0.2 (−2.8, 3.3)	48.3 ± 5.4	−1.8 (−3.9, 0.3)
P, trend		.42	.72	.77	.42	.31	.54	.19	.84	.06	.03

[a]Measured by dual-energy X-ray absorptiometry.

[b]From linear regression models with a given body composition measure as the outcome and indicator variables for PUFA quartiles as predictors. In addition, all models were adjusted for sex, birth weight (large vs average for gestational age), breastfeeding (<6 months, ≥6 months w/ bottle feeding, ≥6 mo exclusive), Graffar index (indicator variables for quintiles), BMI at 1 year (continuous) and total serum trans fatty acids. All n-3 PUFA are adjusted for LA and total long-chain n-6 PUFA. All long chain PUFA are adjusted for their precursor PUFA. Covariate fatty acids are represented with linear and restricted cubic spline terms.

[c]From linear regression models with a variable representing medians of PUFA quartiles introduced as continuous.

(adjusted mean difference >Q1 vs Q1: −2.1%; 95% CI: −3.5%, −0.9%; *P* = .001). DPA was positively associated with fat mass indices in non-linear manners. Adjusted mean differences (95% CI) between DPA > Q1 vs Q1 for ToFM, TrFM, %ToFM and %TrFM were, respectively, 2.7 kg (95% CI: 0.7, 4.8; *P* = .01), 1.6 kg (95% CI: 0.4, 2.8; *P* = .009), 2.3% (95% CI: 0.4, 4.2; *P* = .02) and 1.8% (95% CI: 0.3, 3.2; *P* = .02). Most associations did not differ by sex (Tables S3 and S4). However, the positive association of ALA with %TrFM was stronger among boys (*P*, interaction = .02), as was the inverse association of EPA with %TrFM (*P*, interaction = .004).

3.2 | N-6 PUFA

3.2.1 | Correlates of n-6 PUFA at 1 year

LA was positively associated with exclusive breastfeeding ≥6 months and with iron supplementation (Table S5). It was inversely associated with total *trans* FA. Serum GLA was inversely related to length-for-gestational age and socioeconomic status. DGLA was positively associated with length- and weight-for-gestational age and inversely related to iron supplementation. AA was positively associated with length- and weight-for-gestational age, and exclusive breastfeeding ≥6 months, and inversely related to iron supplementation and total *trans* FA. The n-6:n-3 ratio was inversely associated with total *trans* FA. The D6D index was inversely associated with length-for-gestational age and socioeconomic status.

3.2.2 | BMIZ change between ages 1 and 16 years

Neither n-6 PUFA, the n-6:n-3 ratio, nor desaturase indices at 1 year of age were significantly associated with change in BMIZ (Table 3 and Table S6).

3.2.3 | Body composition at 16 years of age

Without adjustment for n-3 PUFA, GLA (*P*, trend = .02) and the D6D index (*P*, trend = .01) were each positively associated with %ToFM (Table S6). In fully adjusted models, GLA and the D6D index were each positively, linearly associated with ToFM, TrFM and %ToFM (Table 4). Every 1 SD (0.36) difference in GLA was associated with an adjusted higher mean ToFM (1.0 kg per SD; 95% CI: 0.1, 1.9; *P* = .03), TrFM (0.6 kg per SD; 95% CI: 0.1, 1.1; *P* = .03) and %ToFM (1.0 percentage points per SD; 95% CI: 0.2%, 1.7%; *P* = .01). A 1 SD difference (0.02) in the D6D index was related to a higher mean ToFM (1.2 kg per SD; 95% CI: 0.0, 2.3; *P* = .04), TrFM (0.7 kg per SD; 95% CI: 0.0, 1.4; *P* = .05) and %ToFM (1.1 percentage points per SD; 95% CI: 0.2%, 2.1%; *P* = .02). The D5D index was inversely associated with %TrFM. Children in the highest quartile had a mean %TrFM 1.9 percentage points lower than did children in the lowest quartile (95% CI: −3.8%, −0.1%; *P* = .04). A slight inverse association between DGLA and %TrFM was present only among girls (*P*, interaction = .02); no other associations differed by sex (Tables S7 and S8).

4 | DISCUSSION

In this longitudinal study of Chilean children, PUFA at 1 year of age were not associated with BMIZ changes through childhood and adolescence. Nevertheless, they were related to body composition at age 16 years. Serum ALA at 1 year of age was positively associated with TrFM and %TrFM at 16 years. EPA was inversely associated with %TrFM, while DPA was positively associated with ToFM, TrFM, %ToFM and %TrFM. GLA and the D6D index were each positively associated with ToFM, TrFM and %ToFM. The D5D index was inversely associated with %TrFM.

Serum ALA was positively associated with truncal fat. This is in contrast to a previous study in which maternal plasma ALA during pregnancy was inversely associated with the android/gynoid fat mass ratio and pre-peritoneal fat mass area in offspring at 6 years of age.[33] One potential explanation for this discrepancy is the difference in ages at which body composition was measured in the two studies. Body composition may not track completely between childhood and adolescence. Thus, the relation between early-life ALA and body composition at 6 years of age may be different than its relation with body composition at 16 years. Other possible explanations include the differences in timing of exposure assessment, measures of central adiposity, or ALA intake between the study populations. Four other studies of early-life ALA exposure and measures of adiposity have used BMI as an outcome, which does not distinguish between fat compartments. None of these found associations between ALA in cord blood[34,35] or breastmilk[15,36] with BMI during infancy or childhood, consistent with our finding of no association with BMI change. The mechanism underlying a potential effect of ALA on central adiposity is unclear. One possibility is that much of the serum ALA measured in these children could have been *trans* ALA. In areas where hydrogenated oils are present in the food supply, *trans* ALA represents a large proportion of total dietary ALA.[37] Chile had not banned *trans* FA from its food supply at the time of FA measurement in this study.[38] Although few studies have investigated the health effects of *trans* ALA, other *trans* FA have been positively associated with central adiposity.[39] We did not measure *trans* isomers of ALA and could not differentiate its effects from those of *cis* ALA. The association could also be due to confounding by unmeasured aspects of diet.

We found that EPA was inversely associated with %TrFM. In a randomized trial, supplementation with EPA + DHA from birth to 6 months resulted in lower waist circumference at 5 years of age.[40] In another study, maternal plasma EPA during pregnancy was inversely associated with the android/gynoid fat mass ratio and pre-peritoneal fat mass area at 6 years.[33] Previous studies of EPA during pregnancy, in cord blood, or in breastmilk have not consistently found protective associations with measures of overall adiposity such as BMI and %ToFM.[13] However, our results and others suggest that EPA may be specifically protective against central adiposity, which is related to

TABLE 3 Changes in BMI-for-age Z scores (BMIZ)[a] from 1 to 16 years of age by serum n-6 polyunsaturated fatty acid biomarkers and desaturase activity indices at 1 year of age among children from Santiago, Chile

Fatty acid quartile (median, weight % of total FA)	N	1 year BMIZ (mean ± SE)[b]	16 y BMIZ (mean ± SE)	Change in BMIZ 1–16 years (mean ± SE)[b]	Adjusted difference in change (95% CI)[c]
LA (18:2 n-6)					
Q1 (16.53)	159	0.78 ± 0.07	0.56 ± 0.10	−0.23 ± 0.11	Reference
Q2 (21.31)	159	0.72 ± 0.08	0.79 ± 0.09	0.07 ± 0.10	0.34 (0.03, 0.64)
Q3 (25.87)	159	0.80 ± 0.07	0.74 ± 0.09	−0.06 ± 0.09	0.22 (−0.11, 0.54)
Q4 (30.97)	159	0.95 ± 0.08	0.80 ± 0.09	−0.16 ± 0.10	0.11 (−0.27, 0.49)
P, trend[d]				.88	.73
GLA (18:3 n-6)					
Q1 (0.00)	159	0.83 ± 0.07	0.64 ± 0.09	−0.18 ± 0.10	Reference
Q2 (0.07)	159	0.87 ± 0.07	0.79 ± 0.09	−0.07 ± 0.10	0.10 (−0.18, 0.37)
Q3 (0.11)	159	0.81 ± 0.08	0.69 ± 0.10	−0.12 ± 0.11	0.05 (−0.25, 0.35)
Q4 (0.42)	159	0.75 ± 0.08	0.75 ± 0.09	0.00 ± 0.09	0.21 (−0.08, 0.49)
P, trend				.21	.18
DGLA (20:3 n-6)					
Q1 (0.44)	159	0.84 ± 0.08	0.68 ± 0.09	−0.16 ± 0.10	Reference
Q2 (0.61)	159	0.74 ± 0.07	0.84 ± 0.09	0.10 ± 0.10	0.25 (−0.03, 0.53)
Q3 (0.76)	159	0.76 ± 0.07	0.73 ± 0.09	−0.03 ± 0.10	0.13 (−0.15, 0.40)
Q4 (0.96)	159	0.90 ± 0.08	0.63 ± 0.10	−0.27 ± 0.11	−0.12 (−0.41, 0.17)
P, trend				.29	.28
AA (20:4 n-6)					
Q1 (0.94)	159	0.90 ± 0.07	0.66 ± 0.09	−0.24 ± 0.10	Reference
Q2 (1.31)	159	0.68 ± 0.07	0.68 ± 0.09	0.00 ± 0.10	0.26 (−0.04, 0.56)
Q3 (1.65)	159	0.78 ± 0.07	0.68 ± 0.09	−0.09 ± 0.10	0.22 (−0.10, 0.55)
Q4 (2.27)	159	0.90 ± 0.08	0.86 ± 0.10	−0.04 ± 0.11	0.32 (−0.04, 0.68)
P, trend				.29	.13
N-6:N-3 ratio					
Q1 (12.7:1)	159	0.88 ± 0.08	0.78 ± 0.09	−0.10 ± 0.11	Reference
Q2 (18.3:1)	159	0.71 ± 0.08	0.66 ± 0.09	−0.04 ± 0.10	0.06 (−0.22, 0.35)
Q3 (22.9:1)	159	0.81 ± 0.07	0.74 ± 0.10	−0.07 ± 0.10	0.06 (−0.23, 0.35)
Q4 (32.0:1)	159	0.85 ± 0.07	0.70 ± 0.09	−0.15 ± 0.09	0.00 (−0.28, 0.29)
P, trend				.63	.96
Desaturase activity indices					
Δ6-Desaturase index (GLA/LA)					
Q1 (0.000)	159	0.85 ± 0.08	0.63 ± 0.08	−0.22 ± 0.10	Reference
Q2 (0.003)	159	0.81 ± 0.07	0.84 ± 0.10	0.03 ± 0.11	0.22 (−0.06, 0.50)
Q3 (0.005)	159	0.85 ± 0.07	0.66 ± 0.09	−0.19 ± 0.11	0.02 (−0.27, 0.31)
Q4 (0.017)	159	0.74 ± 0.08	0.76 ± 0.09	0.01 ± 0.09	0.24 (−0.04, 0.52)
P, trend				.19	.18
Δ5-Desaturase index (AA/DGLA)					
Q1 (1.57)	159	0.83 ± 0.07	0.63 ± 0.09	−0.20 ± 0.10	Reference
Q2 (1.94)	159	0.76 ± 0.07	0.66 ± 0.09	−0.10 ± 0.10	0.05 (−0.22, 0.33)
Q3 (2.42)	159	0.84 ± 0.07	0.88 ± 0.09	0.04 ± 0.11	0.13 (−0.17, 0.43)
Q4 (3.21)	159	0.83 ± 0.08	0.71 ± 0.09	−0.11 ± 0.10	0.01 (−0.31, 0.32)
P, trend				.54	.99

[a]According to the World Health Organization Growth Reference for children ages 5 to 19 years.[25]

[b]From growth curves estimated using mixed effects linear regression models with BMI as the outcome, and predictors that included indicator variables for each PUFA quartile, linear and cubic spline terms for age and interaction terms between the quartiles and age terms. All models included random intercepts and age slopes to account for within-child correlations of repeated BMI measurements.

[c]Adjusted for sex, birth weight (large vs average for gestational age), breastfeeding (<6 mo, ≥6 mo w/ bottle feeding, ≥6 mo exclusive), Graffar index (indicator variables for quintiles) and total serum trans fatty acids. All n-6 PUFA are adjusted for ALA and total long-chain n-3 PUFA. All long chain PUFA are adjusted for their precursor PUFA. Covariate fatty acids are represented with linear and restricted cubic spline terms.

[d]From mixed effects linear regression models with BMI as the outcome and a variable representing medians of PUFA quartiles introduced as continuous.

TABLE 4 Body composition[a] at 16 years of age by serum n-6 polyunsaturated fatty acid biomarkers and desaturase activity indices at 1 year of age among children from Santiago, Chile

		Total fat mass (kg)		Truncal fat mass (kg)		Total lean mass (kg)		% Total fat mass		% Truncal fat mass	
Fatty acid quartile (median, weight % of total FA)	N	Mean ± SD	Adjusted difference (95% CI)[b]	Mean ± SD	Adjusted difference (95% CI)	Mean ± SD	Adjusted difference (95% CI)	Mean ± SD	Adjusted difference (95% CI)	Mean ± SD	Adjusted difference (95% CI)
LA (18:2 n-6)											
Q1 (16.53)	98	19.0 ± 11.3	Reference	9.5 ± 6.1	Reference	43.0 ± 8.4	Reference	28.3 ± 12.1	Reference	49.0 ± 5.8	Reference
Q2 (21.31)	95	20.3 ± 9.2	1.4 (−1.3, 4.1)	10.4 ± 5.4	0.9 (−0.6, 2.5)	43.9 ± 9.0	0.2 (−1.5, 1.9)	29.9 ± 9.7	1.9 (−0.5, 4.2)	49.6 ± 6.1	0.9 (−0.8, 2.6)
Q3 (25.87)	89	19.6 ± 9.8	1.1 (−1.7, 4.0)	9.9 ± 5.5	0.8 (−0.8, 2.3)	45.6 ± 9.0	1.1 (−0.6, 2.8)	28.4 ± 10.8	1.3 (−1.2, 3.8)	49.5 ± 5.8	1.3 (−0.4, 3.1)
Q4 (30.97)	100	20.2 ± 10.2	0.7 (−2.7, 4.1)	10.2 ± 5.7	0.6 (−1.3, 2.5)	43.5 ± 9.0	0.1 (−1.9, 2.1)	29.7 ± 10.8	1.1 (−1.8, 3.9)	49.1 ± 5.8	0.9 (−1.0, 2.9)
P, trend[c]		.52	.74	.54	.61	.47	.73	.57	.56	>.99	.31
GLA (18:3 n-6)											
Q1 (0.00)	86	18.2 ± 9.0	Reference	9.2 ± 5.1	Reference	44.4 ± 9.2	Reference	27.5 ± 10.5	Reference	49.3 ± 5.9	Reference
Q2 (0.07)	89	19.7 ± 11.1	0.9 (−1.7, 3.5)	10.1 ± 6.2	0.6 (−0.9, 2.0)	44.7 ± 8.7	0.1 (−1.5, 1.7)	28.3 ± 11.8	0.6 (−1.8, 2.9)	50.2 ± 5.7	0.6 (−1.1, 2.3)
Q3 (0.11)	96	20.3 ± 11.0	1.2 (−1.5, 3.8)	10.2 ± 6.1	0.5 (−1.0, 2.1)	43.7 ± 9.3	0.4 (−1.3, 2.1)	29.6 ± 11.3	0.9 (−1.5, 3.3)	49.0 ± 6.4	−0.6 (−2.3, 1.2)
Q4 (0.42)	111	20.7 ± 9.4	2.8 (0.4, 5.2)	10.4 ± 5.3	1.5 (0.2, 2.9)	43.3 ± 8.5	−0.3 (−1.8, 1.3)	30.5 ± 9.9	2.9 (0.7, 5.1)	48.8 ± 5.4	0.7 (−1.0, 2.3)
P, trend		.13	.03	.25	.03	.28	.58	.04	.005	.30	.38
DGLA (20:3 n-6)											
Q1 (0.44)	89	19.5 ± 10.2	Reference	9.8 ± 5.6	Reference	43.0 ± 9.2	Reference	29.2 ± 11.3	Reference	49.1 ± 5.8	Reference
Q2 (0.61)	98	20.6 ± 9.6	2.0 (−0.7, 4.6)	10.6 ± 5.6	1.3 (−0.2, 2.8)	44.7 ± 8.5	0.8 (−0.6, 2.3)	29.6 ± 10.3	1.9 (−0.4, 4.2)	50.1 ± 5.5	1.3 (−0.3, 2.9)
Q3 (0.76)	94	19.3 ± 10.1	0.5 (−2.0, 3.1)	9.7 ± 5.4	0.4 (−1.0, 1.9)	44.1 ± 8.9	1.1 (−0.5, 2.6)	28.6 ± 10.8	0.4 (−1.8, 2.7)	49.6 ± 5.4	1.3 (−0.4, 2.9)
Q4 (0.96)	101	19.8 ± 10.7	0.6 (−2.2, 3.4)	9.9 ± 6.1	0.4 (−1.2, 1.9)	44.0 ± 9.0	0.5 (−1.2, 2.3)	28.9 ± 11.3	0.3 (−2.1, 2.8)	48.4 ± 6.5	0.1 (−1.7, 1.9)
P, trend		.97	.99	.82	.98	.63	.58	.73	.83	.32	.89
AA (20:4 n-6)											
Q1 (0.94)	87	19.5 ± 9.8	Reference	10.0 ± 5.6	Reference	42.6 ± 8.2	Reference	29.4 ± 10.9	Reference	49.9 ± 5.2	Reference
Q2 (1.31)	107	19.3 ± 9.3	0.2 (−2.6, 3.0)	9.8 ± 5.4	0.1 (−1.5, 1.7)	44.1 ± 9.4	1.3 (−0.4, 2.9)	28.7 ± 10.0	−0.1 (−2.5, 2.4)	49.7 ± 5.8	−0.2 (−1.9, 1.5)
Q3 (1.65)	98	19.0 ± 10.6	−0.2 (−3.3, 2.9)	9.6 ± 5.8	−0.3 (−2.0, 1.5)	44.3 ± 8.8	1.2 (−0.6, 2.9)	28.0 ± 11.8	−0.7 (−3.3, 1.9)	49.0 ± 6.3	−1.0 (−2.8, 0.8)
Q4 (2.27)	90	21.5 ± 10.8	1.1 (−2.7, 5.0)	10.7 ± 6.0	0.4 (−1.6, 2.5)	44.7 ± 8.9	0.2 (−1.9, 2.3)	30.4 ± 10.9	1.6 (−1.5, 4.7)	48.6 ± 6.0	−1.1 (−3.2, 1.0)
P, trend		.18	.57	.37	.72	.12	.87	.47	.31	.09	.23
N-6:N-3 ratio											
Q1 (12.7:1)	91	20.8 ± 10.9	Reference	10.9 ± 6.1	Reference	43.5 ± 8.6	Reference	30.1 ± 11.4	Reference	51.4 ± 5.5	Reference
Q2 (18.3:1)	98	18.6 ± 9.5	−0.9 (−3.4, 1.7)	9.2 ± 5.2	−1.0 (−2.5, 0.4)	44.5 ± 8.4	−0.1 (−1.6, 1.3)	27.8 ± 10.5	−0.3 (−2.5, 1.8)	47.8 ± 5.5	−3.4 (−5.0, −1.8)
Q3 (22.9:1)	94	20.6 ± 10.6	0.6 (−2.2, 3.4)	10.4 ± 5.9	0.0 (−1.7, 1.6)	44.9 ± 10.2	1.5 (−0.1, 3.0)	29.5 ± 10.8	0.1 (−2.2, 2.5)	49.2 ± 5.8	−2.1 (−3.7, −0.4)
Q4 (32.0:1)	99	19.3 ± 9.5	−0.1 (−2.6, 2.5)	9.7 ± 5.4	−0.3 (−1.8, 1.2)	42.9 ± 8.2	−0.9 (−2.5, 0.7)	29.1 ± 10.9	0.6 (−1.7, 2.9)	49.0 ± 6.0	−1.8 (−3.4, −0.2)
P, trend		.52	.81	.38	>.99	.46	.40	.85	.51	.06	.20
Desaturase activity indices											

(Continues)

TABLE 4 (Continued)

Fatty acid quartile (median, weight % of total FA)	N	Total fat mass (kg)		Truncal fat mass (kg)		Total lean mass (kg)		% Total fat mass		% Truncal fat mass	
		Mean ± SD	Adjusted difference (95% CI)[b]	Mean ± SD	Adjusted difference (95% CI)	Mean ± SD	Adjusted difference (95% CI)	Mean ± SD	Adjusted difference (95% CI)	Mean ± SD	Adjusted difference (95% CI)
Δ6-Desaturase index (GLA/LA)											
Q1 (0.000)	92	18.2 ± 8.8	Reference	9.2 ± 5.0	Reference	44.0 ± 9.3	Reference	27.7 ± 10.3	Reference	49.5 ± 5.8	Reference
Q2 (0.003)	82	20.6 ± 11.2	1.2 (−1.4, 3.9)	10.5 ± 6.2	0.7 (−0.8, 2.2)	44.7 ± 9.2	0.2 (−1.4, 1.8)	29.4 ± 11.7	1.1 (−1.3, 3.5)	49.8 ± 5.9	0.1 (−1.6, 1.8)
Q3 (0.005)	96	19.0 ± 10.9	0.8 (−1.8, 3.4)	9.5 ± 5.9	0.3 (−1.2, 1.8)	44.5 ± 8.5	0.3 (−1.2, 1.8)	27.8 ± 11.5	0.4 (−1.9, 2.8)	48.7 ± 6.4	−0.9 (−2.6, 0.9)
Q4 (0.017)	112	21.2 ± 9.6	3.1 (0.7, 5.5)	10.7 ± 5.5	1.7 (0.3, 3.1)	42.8 ± 8.6	−0.2 (−1.8, 1.4)	31.2 ± 10.0	3.1 (1.0, 5.3)	49.2 ± 5.4	0.7 (−0.8, 2.3)
P, trend		.06	.01	.10	.02	.16	.64	.01	.003	.71	.23
Δ5-Desaturase index (AA/DGLA)											
Q1 (1.57)	102	19.0 ± 10.1	Reference	9.7 ± 5.7	Reference	43.9 ± 8.3	Reference	28.1 ± 10.9	Reference	49.9 ± 5.5	Reference
Q2 (1.94)	97	18.9 ± 9.0	−0.6 (−3.1, 1.8)	9.6 ± 5.3	−0.5 (−1.9, 1.0)	44.0 ± 9.5	0.5 (−1.1, 2.0)	28.4 ± 10.5	−0.4 (−2.7, 1.9)	49.3 ± 6.2	−1.2 (−2.8, 0.4)
Q3 (2.42)	96	21.4 ± 11.0	0.4 (−2.3, 3.1)	10.9 ± 6.1	0.2 (−1.4, 1.7)	44.0 ± 9.4	0.3 (−1.3, 1.9)	30.7 ± 11.3	0.3 (−2.1, 2.7)	49.4 ± 5.8	−1.0 (−2.7, 0.7)
Q4 (3.21)	87	19.9 ± 10.3	−0.7 (−3.8, 2.4)	9.8 ± 5.6	−0.6 (−2.4, 1.1)	43.8 ± 8.4	−0.6 (−2.3, 1.1)	29.2 ± 10.7	−0.3 (−2.9, 2.4)	48.4 ± 5.8	−1.9 (−3.8, −0.1)
P, trend		.35	.78	.61	.57	.93	.38	.32	.93	.08	.07

[a]Measured by dual-energy X-ray absorptiometry.

[b]From linear regression models with a given body composition measure as the outcome and indicator variables for PUFA quartiles as predictors. In addition, all models were adjusted for sex, birth weight (large vs average for gestational age), breastfeeding (<6 mo, ≥6 mo w/ bottle feeding, ≥6 mo exclusive), Graffar index (indicator variables for quintiles), BMI at 1 year (continuous) and total serum trans fatty acids. All n-6 PUFA are adjusted for ALA and total long-chain n-3 PUFA. All long chain PUFA are adjusted for their precursor PUFA. Covariate fatty acids are represented with linear and restricted cubic spline terms.

[c]From linear regression models with a variable representing medians of PUFA quartiles introduced as continuous.

development of the metabolic syndrome[41] and cardiovascular disease.[42] Whether early-life EPA decreases risk of future chronic disease remains to be investigated.

DPA at age 1 year was positively associated with ToFM, TrFM, % ToFM and %TrFM at 16 years. In a pooled analysis of two large cohorts, cord blood DPA was not associated with childhood BMI.[43] Maternal DPA during pregnancy was inversely associated with % ToFM and the android/gynoid fat mass ratio at 6 years in a Dutch study.[33] Compared with EPA, DPA is found at lower levels in the diet[44] and may be less bioavailable.[45] Thus, DPA in serum could more closely reflect endogenous activity of D6D than it does DPA intake. Associations between DPA and fat mass might reflect associations with D6D activity rather than an effect of DPA itself.

GLA was positively associated with ToFM, TrFM and %ToFM. This is in agreement with an investigation that reported a positive association between maternal plasma GLA during pregnancy and % ToFM and pre-peritoneal fat mass area in offspring at age 6 years.[33] However, two other studies found no association between cord blood GLA and BMI or %ToFM in childhood.[43,46] A trial of formula supplemented with GLA + DHA until 9 months of age found no effect on BMI or %ToFM at age 10 years.[47] Differences among studies may be the result of differences in GLA levels between populations, or effect modification by desaturase gene variations.[48]

Estimated D6D enzyme activity was positively associated with ToFM, TrFM and %ToFM, while the D5D index was inversely associated with %TrFM. Our results are consistent with two longitudinal studies conducted among children aged 2 to 10 years[49] and 10 years,[50] and evidence that cardiometabolic risk factors are positively related to D6D activity and inversely associated with D5D activity among adults.[51]

Although PUFA in infancy were related to body composition in adolescence, they were not associated with BMIZ trajectories. These results are unexpected since BMI and BMIZ correlate with fat mass throughout childhood. One possible explanation is that the differences in body composition at 16 years by PUFA concentrations may already have been present at 1 year. We tried to overcome this possibility by adjusting the analyses of body composition measures at age 16 years for BMI at 1 year, but BMI in infancy is an imperfect proxy for fat mass. Another possibility is that the outcomes measured by DXA are more precise measures of adiposity than BMIZ, which reflects both fat and lean mass, or that the effects of PUFA differ between body fat compartments, which are not separated by using BMIZ.

One of the primary strengths of this study is its longitudinal design, which limits the possibility of reverse causation. We had an opportunity to examine associations of early-life exposures with outcomes at older ages, which provides evidence for the relevance of PUFA status in infancy for long-term health. PUFA biomarkers are not subject to errors in recall or food composition tables, as opposed to dietary assessment data. Furthermore, we assessed body composition using DXA, which provides reliable and valid measures of fat and lean mass.[52]

One of the limitations of this study is that serum PUFA biomarkers reflect status over a period of weeks and may misclassify participants with respect to long-term intake.[53] Analyses of multiple exposures could have increased type I error. Body composition measures differ by sex in adolescence[54]; although interactions by sex were not statistically significant, effect modification by sex cannot be completely ruled out. The lack of available body composition measurements in infancy prevented us from analyzing change in these outcomes or adjusting for their baseline values. Adiposity at 1 year of age could have influenced serum PUFA levels at 1 year of age, and this reverse causation may have influenced the results related to BMIZ trajectories. However, we chose not to adjust for baseline BMIZ in these analyses because this adjustment can cause bias.[55] Measured baseline BMIZ is the result of both true baseline BMIZ and its measurement error, which in turn contributes to measured BMIZ change from baseline to 16 years, the outcome of interest. As a common effect of two variables (a "collider"), baseline BMIZ naturally blocks the flow of a non-causal statistical association between the exposure (PUFA) and the outcome. Adjusted for baseline BMIZ could unblock such a non-causal path and induce a spurious association between PUFA and BMIZ change from baseline to later ages. Finally, because we only had relative measures of serum PUFA expressed as a percentage of total FA, we were unable to determine the extent to which differences in PUFA FA percentage reflect differences in absolute serum PUFA content or serum levels of saturated, monounsaturated and *trans* FA.

In conclusion, serum ALA and DPA at 1 year of age were positively associated with measures of fat mass at age 16 years, whereas EPA was inversely associated with %TrFM. GLA and the D6D index were also positively associated with adiposity in adolescence, while D5D activity was inversely related to this outcome. PUFA status in infancy might affect long-term body composition and adiposity. Our results could be generalizable to other populations with similar dietary intake and behaviour, which in this Chilean population have been characterized as low in fruits and vegetables, high in snack foods with saturated fat and refined carbohydrates and low in physical activity levels.[56] Our results may also be applicable to populations undergoing a similar nutrition transition. In Chile, economic growth in the 1990s resulted in one of the most rapid transitions in Latin America, including a shift towards Western diets and sedentary lifestyles.[57] By the time of the 16 years follow-up in our study, the transition was advanced with a very low prevalence of stunting and high prevalence of obesity.[58] Intervention studies are warranted to elucidate the potential protective effect of EPA against the development of central adiposity in children and adolescents.

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EV, SG and BL designed the research. SG, BL and AD conducted the research. KF performed the data analysis. KF and EV wrote the paper and had primary responsibility for the final content. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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