Serum Polyunsaturated Fatty Acids in Middle Childhood and Body Mass Index Change Through Adolescence

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Keywords

Polyunsaturated fatty acids · Children · Adolescents · Chile · Body mass index · Adiposity · Growth

Abstract

Background: Polyunsaturated fatty acids (PUFA) status in childhood may be associated with adiposity development. **Objective:** To assess associations of serum PUFA biomarkers in childhood with change in body mass index (BMI)-for-age Z scores (BMIZ) through adolescence. **Methods:** We quantified serum PUFA at ages 5 and 10 years among 418 children from Santiago, Chile. BMI was measured at 5, 10, and 16 years. We compared BMIZ change through age 16 years between quartiles of PUFA at 5 and 10 years and PUFA change 5–10 years by fitting growth curves from mixed effects models. **Results:** At age 5 years, serum docosahexaenoic acid was inversely associated with BMIZ change from ages 5 to 16 years. At age 10 years, arachidonic acid (AA) was nonlinearly positively related to BMIZ change from ages 10 to 16 years. Change in AA and the $\Delta 5$ -desaturase (D5D) activity index between 5 and 10 years were each positively associated with

BMIZ change from ages 10 to 16 years. Change in eicosapentaenoic acid was inversely associated with change in BMIZ.

Conclusions: Serum long-chain n-3 PUFA in middle childhood were associated with less BMI gain through adolescence, whereas AA and D5D activity was related to greater BMI gain.

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Introduction

Polyunsaturated fatty acids (PUFA) are potentially relevant nutritional factors in the etiology of obesity. In vitro and animal studies suggest that long-chain n-3 PUFA reduce adiposity and inhibit weight gain [1], whereas n-6 PUFA promote adipogenesis [2]. However, results from studies in adult humans are mixed [3].

While childhood nutrition can affect long-term cardiometabolic health [4], few studies have focused on PUFA and adiposity among children [5–12]. Middle childhood is a developmental period characterized by changes in body composition and adiposity [13]; there-

fore, dietary fat composition during this period might influence the development of obesity. Few studies have been conducted in low- and middle-income countries where childhood obesity is increasing rapidly [14] and dietary PUFA are limited [15].

We conducted a longitudinal study of serum PUFA biomarkers and body mass index (BMI) change in a cohort of children from Santiago, Chile, who were recruited during the beginning of the country's nutrition transition. The aim of the study was to investigate associations of PUFA status in middle childhood with change in BMI-for-age Z scores (BMIZ) through adolescence.

Methods

Study Design

This longitudinal investigation was conducted in a cohort of children followed from infancy through adolescence for studies of iron deficiency anemia. Details of the original studies have been described previously [16, 17]. From 1991 to 1996, 4–6-month-old infants in Santiago, Chile, were enrolled in either a trial of iron supplementation or a neurodevelopment study. Of 1,798 infants enrolled, 1,657 and 135 completed the trial and neurodevelopment study, respectively. During the follow-up, 888, 1,127, and 1,112 children were assessed at ages 5, 10, and 16 years, respectively.

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 for their children's participation; written consent was obtained from children beginning at age 10 years.

Data Collection

Birth weight in g, birth length in cm, and gestational age in weeks were obtained from hospital records. At enrollment, parents provided information on household socioeconomic indicators. The dates of the first bottle feeding (of infant formula or cow's milk) and last breastfeeding were collected from mothers at weekly study visits until 12 months of age. Height and weight were measured at 5, 10, and 16 years of age at INTA by trained personnel using standardized techniques. Researchers obtained a blood sample by venipuncture at the 1, 5, and 10 years assessments. Blood components were separated on the day of collection and serum aliquots were frozen and cryostored at -80°C in Chile. Samples were transported on dry ice to the University of Michigan at the end of each phase of the study; that is, after field activities were completed for each of the 1, 5, and 10 years assessments, and stored at -80°C .

Fatty Acid Analysis

Serum fatty acid (FA) were quantified at the University of Michigan Regional Comprehensive Metabolomics Resource Core. Samples from the 3 assessments (ages 1, 5, and 10 years) were analyzed at the same time. Total lipids were extracted from 200 μL of serum using the Bligh and Dyer method [18] with

nonadecanoic acid (C19:0) as an internal standard. FA were derivatized into their methyl esters with BF_3 -methanol [19] and analyzed using an Agilent 6890N gas chromatograph (Agilent, Santa Clara, CA, USA) equipped with a flame ionization detector, a 100 m $\times$ 0.25 mm $\times$ 0.2 μm SP-2560 column (Sigma-Aldrich, Bellefonte, PA, USA), and Chemstation software. C19:0 and other authentic methyl esters were used to create a calibration curve for FA quantification and to identify FA based on their retention times. The long-term stability of FA in serum stored at -80°C is extremely high. In a study of samples stored for 10 years at this temperature, virtually no degradation of PUFA was detected [20].

Definition of Exposures

The main exposures were serum PUFA biomarkers expressed as weight percentages of total FA (FA%). We examined exposures measured at the 5 and 10 years assessments and change in FA% between the 2. Exposures included alpha-linolenic acid (ALA; 18:3 n-3), eicosapentaenoic acid (EPA; 20:5 n-3), docosahexaenoic acid (DHA; 22:6 n-3), linoleic acid (LA; 18:2 n-6), dihomo-gamma-linolenic acid (DGLA; 20:3 n-6), and arachidonic acid (AA; 20:4 n-6). We also considered the gamma-linolenic acid (18:3 n-6)/LA and AA/DGLA ratios, as activity indices of the $\Delta 6$ -desaturase (D6D) and D5D enzymes, respectively. We categorized all exposures into quartiles.

Definition of Outcomes

The primary outcomes were changes in age- and sex-standardized BMIZ. We calculated BMI as kg/m^2 and converted it to BMIZ using the World Health Organization Growth Reference for children ages 5–19 years [21]. We considered BMIZ change from ages 5 to 16 years (with 5 years PUFA as exposures) and 10–16 years (with 10 years PUFA and 5–10 years PUFA change as exposures).

Covariates

Covariates were sociodemographic and anthropometric characteristics measured in infancy and other serum FA. Birth length and weight were categorized as average or large (≥ 90 th percentile) for gestational age using the INTERGROWTH 21st standards [22]. The inclusion criteria for the original study (birth weight > 3 kg) excluded any children who would be defined as being small for their gestational age. Seven children who were ≤ 10 th percentile for birth length were included in the average for gestational age category. Breastfeeding was categorized as < 6 months, ≥ 6 months mixed breastfeeding, or ≥ 6 months exclusive breastfeeding. Iron supplementation was categorized as any versus none. Socioeconomic status (SES) in infancy was measured using a modified Graffar index [23], which comprises 13 items related to family characteristics, living conditions, and assets. Higher values indicate lower SES. We also considered serum total *trans* FA and serum palmitoleic acid (16:1 n-7).

Statistical Analysis

The final analytic samples included children with (a) serum PUFA and BMIZ measured at 5 years and at least one BMIZ measurement at 10 or 16 years of age ($n = 239$) and (b) PUFA measured at 10 years and BMIZ measured at 10 and 16 years ($n = 418$). The analysis of change in PUFA concentrations included 141 children with PUFA measurements at 5 and 10 years and BMIZ at 10 and 16 years.

Predictors of PUFA at 5 and 10 Years of Age

We examined the distribution of PUFA biomarkers at 5 and 10 years by categories of covariates using means $\pm$ SD. For dichotomous covariates we tested the statistical significance of the associations with Wald tests from linear regression models. For categorical variables, we used a χ^2 score statistic. For ordinal covariates, we conducted tests for linear trend by introducing a variable representing category-specific medians into the model as a continuous predictor. Robust estimates of variance were specified in these models.

PUFA at 5 Years of Age and BMIZ Change from Ages 5 to 16 years

In bivariate analysis, we estimated mean $\pm$ SE BMIZ at ages 5 and 16 years and BMIZ change from 5 to 16 years by quartiles of PUFA biomarkers at age 5 years. These estimates were from growth curves calculated using mixed effects linear regression models of BMIZ. Age was represented as a predictor using restricted cubic splines [24]. These functions consist of piecewise cubic polynomials that are smoothly joined at the knots and linear in the tails. They allow for flexible modeling of nonlinear changes in BMIZ through childhood. We fitted these models using all available measurements and placed knots at the median ages at each assessment. Other predictors included indicators for each PUFA quartile, interaction terms between the quartiles and age terms, and random intercepts and age slopes for each child.

In multivariable analysis, we obtained adjusted mean differences and 95% CI in BMIZ change between PUFA quartiles using adjusted mixed effects models. Variables considered for adjustment were known independent predictors of the outcome that could be related to PUFA but were not their consequence. The final models included sex, birth weight, breastfeeding, Graffar index, *trans* FA, and palmitoleic acid – a proxy for carbohydrate intake – measured at 5 years, and the main exposure PUFA measured in infancy. N-3 PUFA were adjusted for LA and total long-chain n-6 PUFA, while n-6 PUFA were adjusted for ALA and total long-chain n-3 PUFA. Long-chain PUFA were adjusted for their immediate precursor. Covariate FA were represented using restricted cubic splines. All models were fitted using empirical variance estimates.

PUFA at 10 Years of Age and BMIZ Change from Ages 10 to 16 Years

In bivariate analysis, we estimated mean $\pm$ SE BMIZ at ages 10 and 16 years and BMIZ changes from 10 to 16 years of age by PUFA quartiles at age 10 years. These estimates were from mixed effects linear regression models of BMIZ fitted using data from the ages 10 and 16 years assessments. Predictors included indicator variables for PUFA quartile, a linear term for age, interaction terms between each quartile and age, and random intercepts for each child.

In multivariable analysis, we obtained adjusted mean differences with 95% CI in BMIZ change from 10 to 16 years of age between PUFA quartiles. Models were adjusted for infancy characteristics and FA measured at age 10 years.

Change in PUFA FA% from Ages 5 to 10 years and Change in BMIZ from Ages 10 to 16 Years

We calculated the difference in PUFA FA% between the 5 and 10 years assessments and estimated mean BMIZ change

from ages 10 to 16 years by quartiles of PUFA change, following an analogous approach to that described for the age 10 years PUFA as main exposures. Mean differences in BMIZ change were adjusted for infancy characteristics and changes in other FA.

We conducted all analyses using Statistical Analysis Software version 9.4 (SAS Institute, Cary, NC, USA).

Results

PUFA at 5 years of age

Among children included in the analysis of 5 years serum PUFA ($n = 239$), 11, 33, and 195 had additional measurements at age 10 years only, 16 years only, and 10 and 16 years, respectively. Mean $\pm$ SD BMIZ at 5 years of age was 0.94 ± 1.17 ; 44% had overweight or obesity (BMIZ >1).

Predictors of PUFA at 5 years of age. Serum ALA was related to shorter breastfeeding duration (online suppl. Table 1; for all online suppl. material, see www.karger.com/doi/10.1159/000502077). SES in infancy was positively associated with EPA and DGLA. Iron supplementation was related to higher DGLA. Serum AA was positively associated with birth length.

BMIZ change from ages 5 to 16 years. After adjustment, DHA at age 5 years was inversely associated with BMIZ change (p trend = 0.03; Table 1). Compared with children in the lowest quartile of serum DHA, BMIZ change among those in the highest quartile was 0.51 Z score lower (95% CI -0.88 to -0.13 ; $p = 0.008$). None of the n-6 PUFA (Table 1) or desaturase indices (online suppl. Table 2) were associated with BMIZ change.

PUFA at 10 years of age

Mean $\pm$ SD BMIZ at 10 and 16 years of age were 1.03 ± 1.15 and 0.70 ± 1.12 ; prevalence of overweight/obesity at 10 and 16 years of age was, respectively, 55 and 39%.

Predictors of PUFA at 10 years of age. At 10 years, ALA and LA were lower among boys than girls (online suppl. Table 3). EPA was inversely associated with birth weight. The D6D activity index was positively associated with birth length and weight.

BMIZ change from ages 10 to 16 years. N-3 PUFA (Table 2) and desaturase indices (online suppl. Table 4) were not significantly associated with change in BMIZ. Children in the lowest quartile of AA had a mean adjusted change in BMIZ 0.17 Z scores lower (95% CI -0.34 to -0.01 ; $p = 0.04$) than did children in the highest 3 quartiles (Table 2).

Table 1. Changes in BMIZ^a by serum biomarkers of PUFA at 5 years of age among children from Santiago, Chile

FA quartile (median, weight % of total FA)	Number	5 Years BMIZ ^b , mean ± SE	16 Years BMIZ, mean ± SE	Change in BMIZ 5–16 years ^b , mean ± SE	Adjusted difference in change ^c , 95% CI
Overall	239	0.93±0.08	0.70±0.07	−0.23±0.06	
<i>N-3 PUFA</i>					
<i>ALA (18:3 n-3)</i>					
Q1 (0.12)	59	0.93±0.16	0.68±0.16	−0.25±0.13	Reference
Q2 (0.25)	60	0.64±0.14	0.59±0.12	−0.05±0.13	0.30 (−0.08 to 0.67)
Q3 (0.34)	60	1.12±0.16	0.76±0.16	−0.36±0.12	0.06 (−0.29 to 0.41)
Q4 (0.45)	60	1.05±0.15	0.78±0.15	−0.26±0.11	0.05 (−0.30 to 0.39)
<i>p</i> trend ^d		0.29	0.54	0.56	0.94
<i>EPA (20:5 n-3)</i>					
Q1 (0.12)	59	0.87±0.14	0.71±0.14	−0.16±0.13	Reference
Q2 (0.22)	60	0.90±0.16	0.60±0.15	−0.30±0.11	−0.16 (−0.50 to 0.18)
Q3 (0.30)	60	1.10±0.18	0.87±0.15	−0.23±0.12	−0.09 (−0.43 to 0.25)
Q4 (0.41)	60	0.86±0.14	0.63±0.15	−0.23±0.13	−0.06 (−0.44 to 0.32)
<i>p</i> trend		0.84	0.95	0.77	0.84
<i>DHA (22:6 n-3)</i>					
Q1 (0.32)	59	0.70±0.15	0.72±0.15	0.02±0.12	Reference
Q2 (0.48)	60	0.97±0.15	0.62±0.13	−0.35±0.13	−0.37 (−0.74 to −0.01)
Q3 (0.62)	60	0.93±0.15	0.87±0.16	−0.07±0.13	0.00 (−0.36 to 0.35)
Q4 (0.90)	60	1.12±0.16	0.60±0.15	−0.51±0.10	−0.51 (−0.88 to −0.13)
<i>p</i> trend		0.09	0.73	0.004	0.03
<i>N-6 PUFA</i>					
<i>LA (18:2 n-6)</i>					
Q1 (21.58)	59	0.92±0.15	0.69±0.15	−0.23±0.10	Reference
Q2 (26.06)	60	1.05±0.15	0.80±0.14	−0.25±0.12	−0.07 (−0.40 to 0.25)
Q3 (28.99)	60	1.08±0.16	0.81±0.14	−0.27±0.12	−0.08 (−0.40 to 0.23)
Q4 (32.02)	60	0.68±0.15	0.50±0.16	−0.18±0.15	0.03 (−0.38 to 0.44)
<i>p</i> trend		0.40	0.49	0.84	0.95
<i>DGLA (20:3 n-6)</i>					
Q1 (0.35)	59	0.73±0.15	0.53±0.15	−0.20±0.12	Reference
Q2 (0.71)	60	1.03±0.15	0.77±0.15	−0.26±0.14	−0.06 (−0.41 to 0.29)
Q3 (0.88)	60	0.77±0.14	0.62±0.13	−0.15±0.10	0.13 (−0.19 to 0.46)
Q4 (1.08)	60	1.20±0.17	0.89±0.16	−0.30±0.13	0.05 (−0.31 to 0.41)
<i>p</i> trend		0.10	0.14	0.71	0.64
<i>AA (20:4 n-6)</i>					
Q1 (1.17)	59	0.81±0.14	0.61±0.15	−0.19±0.13	Reference
Q2 (1.58)	60	0.94±0.15	0.73±0.14	−0.21±0.12	0.00 (−0.41 to 0.40)
Q3 (1.93)	60	0.85±0.17	0.60±0.15	−0.25±0.10	0.01 (−0.35 to 0.38)
Q4 (2.28)	60	1.13±0.16	0.87±0.16	−0.26±0.14	−0.07 (−0.48 to 0.35)
<i>p</i> trend		0.19	0.35	0.68	0.78

^a According to the World Health Organization Growth Reference 2007 [21] for children ages 5–19 years.

^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 versus average for gestational age), breastfeeding (<6 months, ≥6 months w/bottle feeding, ≥6 months exclusive), Graffar index (indicator variables for quintiles), total serum TFAs, palmitoleic acid, and serum concentrations of the main exposure PUFA measured in infancy. In addition, all n-3 PUFA are adjusted for LA and total long-chain n-6 PUFA. All n-6 PUFA are adjusted for ALA and total long-chain n-3 PUFA. 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 Z score as the outcome and a variable representing medians of PUFA quartiles introduced as a continuous predictor.

BMI, body mass index; BMIZ, BMI-for-age Z score; FA, fatty acid; PUFA, polyunsaturated FA; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; DGLA, dihomo-gamma-LA; AA, arachidonic acid; TFA, trans fatty acid.

Table 2. Changes in BMIZ^a by serum biomarkers of PUFA at 10 years of age among children from Santiago, Chile

FA quartile (median, weight % of total FA)	Number	10 Years BMIZ ^b , mean ± SE	16 Years BMIZ, mean ± SE	Change in BMIZ 10–16 years ^b , mean ± SE	Adjusted difference in change ^c , 95% CI
Overall	418	1.03±0.06	0.73±0.05	−0.31±0.03	
<i>N-3 PUFA</i>					
<i>ALA (18:3 n-3)</i>					
Q1 (0.27)	104	0.64±0.11	0.46±0.09	−0.18±0.07	Reference
Q2 (0.37)	105	1.01±0.10	0.65±0.10	−0.36±0.08	−0.14 (−0.32 to 0.04)
Q3 (0.44)	105	0.96±0.11	0.64±0.11	−0.32±0.06	−0.13 (−0.31 to 0.05)
Q4 (0.57)	104	1.53±0.11	1.16±0.11	−0.37±0.07	−0.13 (−0.31 to 0.05)
<i>p</i> trend ^d		<0.0001	<0.0001	0.07	0.22
<i>EPA (20:5 n-3)</i>					
Q1 (0.11)	104	0.98±0.12	0.71±0.11	−0.26±0.07	Reference
Q2 (0.21)	105	1.00±0.11	0.61±0.11	−0.39±0.06	−0.12 (−0.30 to 0.05)
Q3 (0.28)	105	1.07±0.12	0.80±0.11	−0.27±0.07	0.02 (−0.18 to 0.21)
Q4 (0.42)	104	1.09±0.10	0.78±0.09	−0.31±0.07	−0.11 (−0.29 to 0.07)
<i>p</i> trend		0.42	0.43	0.94	0.43
<i>DHA (22:6 n-3)</i>					
Q1 (0.43)	104	1.13±0.11	0.67±0.11	−0.45±0.07	Reference
Q2 (0.57)	105	0.98±0.10	0.67±0.09	−0.31±0.07	0.06 (−0.14 to 0.26)
Q3 (0.68)	105	0.93±0.11	0.80±0.10	−0.13±0.06	0.25 (0.05 to 0.45)
Q4 (0.90)	104	1.10±0.12	0.76±0.12	−0.34±0.07	0.11 (−0.11 to 0.34)
<i>p</i> trend		0.95	0.51	0.26	0.31
<i>N-6 PUFA</i>					
<i>LA (18:2 n-6)</i>					
Q1 (25.96)	104	1.09±0.11	0.72±0.10	−0.37±0.08	Reference
Q2 (29.92)	105	1.22±0.11	0.90±0.12	−0.32±0.07	0.07 (−0.14 to 0.28)
Q3 (32.59)	105	0.96±0.11	0.68±0.11	−0.27±0.07	0.06 (−0.17 to 0.28)
Q4 (35.77)	104	0.87±0.11	0.60±0.10	−0.27±0.06	−0.08 (−0.32 to 0.17)
<i>p</i> trend		0.09	0.28	0.27	0.58
<i>DGLA (20:3 n-6)</i>					
Q1 (0.85)	104	0.68±0.11	0.43±0.10	−0.25±0.07	Reference
Q2 (1.08)	105	0.94±0.11	0.64±0.11	−0.30±0.06	0.03 (−0.16 to 0.21)
Q3 (1.27)	105	1.10±0.11	0.78±0.10	−0.31±0.06	0.04 (−0.14 to 0.22)
Q4 (1.55)	104	1.42±0.11	1.05±0.11	−0.37±0.08	0.07 (−0.15 to 0.29)
<i>p</i> trend		<0.0001	<0.0001	0.26	0.53
<i>AA (20:4 n-6)</i>					
Q1 (2.23)	104	1.33±0.11	0.88±0.11	−0.44±0.07	Reference
Q2 (2.81)	105	0.89±0.10	0.59±0.11	−0.30±0.07	0.15 (−0.05 to 0.35)
Q3 (3.37)	105	1.03±0.11	0.76±0.10	−0.27±0.07	0.20 (0.01 to 0.39)
Q4 (4.19)	104	0.89±0.12	0.67±0.11	−0.22±0.07	0.22 (0.00 to 0.44)
<i>p</i> trend		0.03	0.37	0.02	0.06

^a According the World Health Organization Growth Reference 2007 [21] for children ages 5–19 years.

^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, a linear term for age, and interaction terms between the quartiles and age. All models included random intercepts to account for within-child correlations of repeated BMI measurements.

^c Adjusted for sex, birth weight (large versus average for gestational age), breastfeeding (<6 months, ≥6 months w/bottle feeding, ≥6 months exclusive), Graffar index (indicator variables for quintiles), total serum trans fatty acids, palmitoleic acid, and serum concentrations of the main exposure PUFA measured in infancy. In addition, all n-3 PUFA are adjusted for LA and total long-chain n-6 PUFA. All n-6 PUFA are adjusted for ALA and total long-chain n-3 PUFA. 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 Z score as the outcome and a variable representing medians of PUFA quartiles introduced as a continuous predictor.

BMI, body mass index; BMIZ, BMI-for-age Z score; FA, fatty acid; PUFA, polyunsaturated FA; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; DGLA, dihomogamma-LA; AA, arachidonic acid.

Change in PUFA FA% between 5 and 10 years of age

After adjustment, change in EPA was inversely associated with change in BMIZ (Table 3). Children in the highest quartile of EPA change had a 0.32 Z score lower change in BMIZ compared with children in the lowest quartile (95% CI -0.64 to 0.00; p trend = 0.04). Children in the highest quartile of D5D activity change had a mean adjusted change in BMIZ 0.64 Z score higher than did children in the lowest quartile (95% CI 0.27–1.02; p = 0.001; online suppl. Table 5). Mean adjusted BMIZ change among children in the highest AA quartile was 0.51 Z scores higher than among those in the lowest quartile (95% CI 0.11–0.90; p = 0.01; Table 3).

Discussion

In this study, some serum long-chain n-3 PUFA in childhood were inversely associated with BMIZ change up to age 16 years, while AA and the D5D index were positively associated with BMIZ change.

BMIZ in this population was high, and large proportions of children had overweight or obesity. At high percentiles, childhood BMI is correlated with fat mass [25]. Thus, in this study, greater changes in BMIZ likely represent increases in adiposity above levels associated with adverse cardiometabolic health outcomes [26].

DHA at age 5 years was inversely associated with change in BMIZ through 16 years. Cross-sectional studies of DHA and BMI in middle childhood have been inconclusive [6–9, 11, 12]. Serum DHA was positively associated with relative body weight in one longitudinal study [5] and not associated with BMIZ change in another [10]. Two randomized trials of supplementation with EPA + DHA for 1 month [27] or DHA for 6 months [28] found no effect on BMI. Discrepancies between ours and previous results could be explained by reverse causation bias in the cross-sectional studies or differences in the age ranges of participating children. The intervention periods of the trials may have been too short to observe effects on BMI. A protective effect of DHA could be explained through regulation of lipid metabolism by long-chain n-3 PUFA [29].

We found an inverse association between 5 and 10 years EPA change and 10–16 years BMIZ change. Most previous investigations have found null [6, 7, 9–11] or positive [5, 12] associations between EPA and BMI or obesity in children. These discrepancies might be explained by methodological problems or differences in ages and EPA intake among study populations.

Serum AA at age 10 years and change in AA between 5 and 10 years were positively associated with BMIZ change between 10 and 16 years. AA may promote adiposity through production of prostacyclin, which up-regulates adipogenesis [30]. However, cross-sectional [5, 7, 9, 12] and longitudinal [5, 10] studies have not found associations between circulating AA and BMI. Mean serum AA weight percentages in the longitudinal studies ranged from 6.0 [10] to 9.1 [5] compared with 3.2 among the 10-year-old Chilean children. This may reflect differences in intake or metabolism of AA, which may affect the association with BMIZ change.

Change in the D5D activity index between 5 and 10 years of age was positively associated with BMIZ change from 10 to 16 years. This is generally inconsistent with previous evidence [5, 31]. We noted that change in the D5D index was inversely associated with BMIZ at 10 years of age, but independent of BMIZ at age 16 years. It is possible that D5D activity is inversely related to BMIZ but only in the short term.

One of the strengths of this study is the prospective design, which limits the possibility of reverse causation bias. PUFA biomarkers are not subject to recall bias or errors from the use of food composition tables. A weakness of the study is that serum PUFA reflect short-term status and may misclassify some participants with respect to long-term intake. For example, switching from a saturated FA to an n-6 PUFA rich diet significantly increases plasma LA concentrations after only 2 days [32]; the maximum increase is reached after 2 weeks. The correlations between plasma PUFA concentrations from a single assessment and data from a food frequency questionnaire that measured average intake during the previous year were weak, 0.23 for n-3 and 0.38 for n-6 [33]. Some of our results may be driven by associations between PUFA and BMIZ at baseline, which could reflect reverse causation. Potential confounding by unmeasured dietary or lifestyle factors, including total energy intake and physical activity levels, limit the possibility of making causal inference from the results. The associations of PUFA status with BMI varied at different ages; while this might provide insights into differing effects of exposure at varying life stages, it could also be the result of chance and the associations may not represent biologically significant effects.

In conclusion, serum concentrations of some long-chain n-3 PUFA in middle childhood are associated with less BMI gain through adolescence, whereas AA and D5D activity is related to higher weight gain. The effect of di-

Table 3. Changes in BMIZ^a according to changes in serum biomarkers of PUFA at 5 and 10 years of age among children from Santiago, Chile

Quartile of FA change (median, weight % of total FA)	Number	10 Years BMIZ ^b , mean ± SE	16 Years BMIZ, mean ± SE	Change in BMIZ 10–16 years ^b , mean ± SE	Adjusted difference in change ^c , 95% CI
Overall	141	0.97±0.10	0.67±0.10	−0.30±0.06	
<i>N-3 PUFA</i>					
<i>ALA (18:3 n-3)</i>					
Q1 (−0.10)	35	0.69±0.20	0.48±0.18	−0.21±0.13	Reference
Q2 (0.07)	35	0.64±0.17	0.43±0.16	−0.21±0.11	−0.03 (−0.35 to 0.29)
Q3 (0.18)	36	1.01±0.19	0.61±0.21	−0.41±0.13	−0.18 (−0.51 to 0.16)
Q4 (0.37)	35	1.55±0.18	1.17±0.21	−0.38±0.11	−0.21 (−0.55 to 0.14)
<i>p</i> trend ^d		0.0007	0.01	0.20	0.18
<i>EPA (20:5 n-3)</i>					
Q1 (−0.20)	35	0.96±0.20	0.71±0.20	−0.26±0.10	Reference
Q2 (−0.07)	35	0.83±0.20	0.68±0.22	−0.15±0.11	0.18 (−0.11 to 0.46)
Q3 (0.05)	36	1.14±0.19	0.89±0.18	−0.26±0.13	0.00 (−0.32 to 0.31)
Q4 (0.16)	35	0.95±0.17	0.41±0.19	−0.53±0.13	−0.32 (−0.64 to 0.00)
<i>p</i> trend		0.75	0.47	0.08	0.04
<i>DHA (22:6 n-3)</i>					
Q1 (−0.38)	35	0.85±0.20	0.61±0.18	−0.24±0.11	Reference
Q2 (−0.08)	35	1.02±0.20	0.51±0.23	−0.51±0.13	−0.14 (−0.49 to 0.21)
Q3 (0.14)	36	0.86±0.19	0.69±0.17	−0.17±0.12	0.24 (−0.11 to 0.59)
Q4 (0.41)	35	1.16±0.18	0.87±0.21	−0.29±0.11	−0.07 (−0.43 to 0.29)
<i>p</i> trend		0.34	0.28	0.81	0.87
<i>N-6 PUFA</i>					
<i>LA (18:2 n-6)</i>					
Q1 (−1.64)	35	1.05±0.16	0.61±0.18	−0.44±0.13	Reference
Q2 (1.65)	35	1.11±0.19	0.78±0.19	−0.33±0.13	−0.04 (−0.39 to 0.31)
Q3 (5.22)	36	0.96±0.19	0.75±0.20	−0.22±0.10	0.04 (−0.33 to 0.41)
Q4 (9.67)	35	0.78±0.22	0.55±0.22	−0.23±0.11	−0.03 (−0.43 to 0.37)
<i>p</i> trend		0.26	0.79	0.17	0.98
<i>DGLA (20:3 n-6)</i>					
Q1 (−0.06)	35	0.55±0.20	0.39±0.21	−0.16±0.12	Reference
Q2 (0.24)	35	1.12±0.17	0.83±0.16	−0.28±0.11	−0.10 (−0.41 to 0.21)
Q3 (0.46)	36	1.06±0.19	0.77±0.21	−0.30±0.10	−0.06 (−0.35 to 0.24)
Q4 (0.93)	35	1.16±0.19	0.69±0.21	−0.47±0.15	−0.29 (−0.62 to 0.04)
<i>p</i> trend		0.06	0.43	0.11	0.09
<i>AA (20:4 n-6)</i>					
Q1 (0.45)	35	1.38±0.20	0.79±0.21	−0.59±0.12	Reference
Q2 (1.17)	35	0.94±0.19	0.81±0.20	−0.13±0.12	0.56 (0.22 to 0.90)
Q3 (1.53)	36	0.73±0.16	0.49±0.15	−0.24±0.10	0.40 (0.10 to 0.70)
Q4 (2.17)	35	0.85±0.21	0.61±0.23	−0.24±0.13	0.51 (0.11 to 0.90)
<i>p</i> trend		0.05	0.42	0.06	0.03

^a According to the World Health Organization Growth Reference 2007 [21] for children ages 5–19 years.

^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 change quartile, a linear term for age, and interaction terms between the quartiles and age. All models included random intercepts to account for within-child correlations of repeated BMI measurements.

^c Adjusted for sex, birth weight (large versus average for gestational age), breastfeeding (<6 months, ≥6 months w/bottle feeding, ≥6 months exclusive), Graffar index (indicator variables for quintiles), change in total serum trans fatty acids, change in palmitoleic acid, and serum concentrations of the main exposure PUFA measured in infancy. In addition, all n-3 PUFA are adjusted for change in LA and total long-chain n-6 PUFA. All n-6 PUFA are adjusted for change in ALA and total long-chain n-3 PUFA. Long-chain PUFA are adjusted for change in their precursor PUFA. Covariate fatty acids are represented with linear and restricted cubic spline terms.

^d From mixed effects linear regression models with Z score as the outcome and a variable representing medians of PUFA change quartiles introduced as a continuous predictor.

BMI, body mass index; BMIZ, BMI-for-age Z score; FA, fatty acid; PUFA, polyunsaturated FA; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; DGLA, dihomo-gamma-LA; AA, arachidonic acid.

etary interventions involving long-chain n-3 PUFA in childhood on adiposity development warrants investigation in randomized trials.

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Statement of Ethics

Study procedures were approved by institutional review boards at the University of Chile INTA, the University of Michigan, and the University of California, San Diego. Parents provided written informed consent for their children's participation; written assent was obtained from children beginning at age 10 years.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

S.G., B.L., and E.V. designed the research. A.K.D. and R.A.B. conducted the research. K.S.F. performed the data analysis. K.S.F. and E.V. wrote the paper and had primary responsibility for the final content. All authors have read and approved the final manuscript.

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