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Prostaglandins, Leukotrienes and Essential Fatty Acids

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Docosahexaenoic acid levels in erythrocytes and their association with the University Selection Test Outcomes in Chile

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ARTICLE INFO

Keywords: Docosahexaenoic acid Scholastic achievement Education Nutritional status Brain development Head circumference Socio-economic status

ABSTRACT

The aim of this study was to quantitate the relative impact of DHA and AA levels in erythrocytes, anthropometric parameters and socio-economic status of school-age children, of both genders, graduated from high school in Chile, on the scholastic achievement in the University Selection Test (Prueba de Selección Universitaria, PSU) both language scholastic achievement (LSA) and mathematics scholastic achievement (MSA). A representative sample of 671 school-age young graduated from high school in 2013, 550 and 548 of them took the PSU for LSA and MSA, respectively. Only school-age young with high (n = 91) and low (n = 69) SA in both tests were considered. A total of 122 school-age children agreed to participate in the study and were divided as follows: Group 1: high PSU outcome (n = 70; males n = 48) and Group 2: low PSU outcome (n = 52; males n = 23). Data were analyzed by means of SAS software. Independently of gender, DHA, socio-economic status and head circumference-for-age Z-score were the most relevant parameters explaining both LSA ($R^2 = 0.650$; p < 0.0001). These results can be useful for nutrition, health and education planning, in order to protect children starting from an early age and thus increase their school outcomes.

1. Introduction

Scholastic achievement (SA) is a multifactorial process conditioned by multiple factors dependent on the child, his/her family and from the educational system that also affects enrollment, attendance, and dropout.¹ Our previous findings revealed that SA is more properly explained by child intelligence, parental intelligence and schooling level (especially maternal), the occurrence of undernutrition in the first year of life, nutritional background parameters, head circumference, brain volume as well as some socio-economic, socio-cultural, demographic and educational system variables.¹⁻⁴

Nutrition is one of the many factors that affect children's cognitive development. Poor cognitive performance of school-age children is significantly associated with prenatal and especially with postnatal nutritional history, the environment, and maternal-infant interaction.⁵⁻⁷ In this regard, the evidence indicates that head circumference, the anthropometric indicator of both nutritional background and brain development, is the most relevant anthropometric parameter associated with SA and intelligence.^{1-4,7}

Little research has been carried out in Chile, as in others countries, to explore the effects of fatty acids (FAs) on SA. In this regard, FAs have relevant and specific physiological and biochemical function in the body, especially in the brain, highlighting their role as energy source (9 kcal/g), composition of cell membranes, regulators of cell signaling pathways, control of gene expression and cognitive development.⁸ Some FAs are actively involved in nervous tissue metabolism during all stages of the vital cycle, particularly during brain and retina development, and in the acquisition of cognitive abilities and visual capacities

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https://doi.org/10.1016/j.plefa.2018.11.003

Received 14 August 2018; Received in revised form 6 November 2018; Accepted 7 November 2018 0952-3278/ © 2018 Elsevier Ltd. All rights reserved.

in children.⁹ Docosahexaenoic acid (C22:6n – 3, DHA) and arachidonic acid (C20:4n – 6, AA) play a specific role in these metabolic processes,¹⁰ DHA and AA are long chain polyunsaturated FAs (LCPUFAs) belonging to the n – 3 and n – 6 FAs family respectively. These LCPUFAs are synthesized by the organism through the desaturation and elongation of the respective essential FAs; alpha-linolenic acid (C18:3n – 3, ALA) and linolenic acid (C18:2n – 6, LA) which are the FAs precursors of DHA and AA respectively.¹¹ Besides the endogenous synthesis of DHA and AA, it is also possible to incorporate these LCPUFAs to the body through the diet.^{12,13} For example, DHA is mainly contained in fatty fish (tuna, mackerel, salmon, among others) and seafood whereas AA can be found in eggs and meat of terrestrial animals (pork and beef).^{14,15}

Many contributions has been previously reported the specific impact of DHA and AA in the acquisition and improvement of cognitive capacity in children during the first years of life and their SA.¹⁰ Most nutritional interventions aiming to provide DHA and AA are carried out by: (a) supplementation of infant formulas with LCPUFAs (b) supplementation with DHA to the mother during pregnancy and/or lactation, or (c) supplementation to children.^{10,14,16} However, the impact of these LCPUFAs on the performance of students in university selection tests has not been yet evaluated, therefore, being an aspect that should be investigated. Considering these points, the aim of this study was to quantitate the relative impact of the level of DHA and AA in erythrocytes, anthropometric parameters and socio-economic status of school-age children, of both genders, graduated from high school in Chile, on the University Selection Test (PSU) outcomes both language (LSA) and mathematics (MSA).

2. Material and methods

2.1. Study population

The target population, 96,197 children (39% of the Chilean school population), included all school-age children enrolled in the 1st grade of high school (1HSG) in the Metropolitan Region of Chile in 2010 who took the 2009 Quality Education Measurement System (SIMCE) tests administered by the Agency for Education Quality at the end of 2009 both in language and mathematics. They belonged to public, private-subsidized, and private non-subsidized schools from urban areas. Four years later, they took the PSU, at the end of 2013 when they graduated from high school.¹⁷

2.2. Sample selection

The population sampling included all educational establishments from the urban areas of the Metropolitan Region of Chile. The sampling was carried out as follows: firstly, 33 educational establishments which represented 2.61% of the total population of the 1262 urban schools, were randomly selected by proportional allocation according to their stratification by type of school and level of SA of the educational establishments (EESA) in the 2009 Quality Education Measurement System (SIMCE) tests classified as high, medium or low by de Ministry of Education.¹⁷ Secondly, in each of these 33 schools all students enrolled in the 1HSG as well as their parents, the principals of the establishments and the language and mathematics teachers were invited to participate. A total of 671 school-age children of the 2010 1HSG, as well as their parents, the school principals and teachers agreed to participate and signed the informed consent form. Students' age ranged from 12.7y to 17.6y (mean age = 14.8 ± 0.6 y). At the end of the year 2013, school-age children of the 2010 1HSG graduated from the 4th grade of high school (4HSG) and took the PSU, for admission to university.¹⁸ From the total sample (n = 671), 550 and 548 school-age children took the PSU tests both LSA and MSA, respectively. In the present study, only school-age children with high (n = 91) and low SA (n = 69) in both tests were considered since the present research is part of a major study in which brain structural parameters were assessed

though magnetic resonance imaging (MRI). Due to the higher economical cost of MRIs, students with medium SA were discarded. A total of 122 school-age children agreed to participate in the study and were divided as follows: Group 1: high PSU outcome (n = 70; males n = 48) and Group 2: low PSU outcome (n = 52; males n = 23).

2.3. Ethical considerations

This study was approved by the Committee on Ethics in Studies in Humans of the Institute of Nutrition and Food Technology Dr. Fernando Monckeberg Barros (INTA), University of Chile, and ratified by the Committee on Bioethics of the National Fund for Scientific and Technological Development (FONDECYT), Chile. The subjects' consent was obtained according to the norms for Human Experimentation, Code of Ethics of the World Medical Association (Declaration of Helsinki).¹⁹ The field study was carried out during 2015.

2.4. PSU

In Chile, the PSU evaluates the quality of education at the end of the high school cycle for university admission. This standardized test has nationwide coverage.^{17,18} Results from the 2013 PSU outcomes both LSA and MSA tests, were registered for the 2010 1HSG school-age children when they graduated from 4HSG in 2013. The PSU, the baccalaureate examination for university admission, has a maximum total score of 850 and a minimum score of 150 for each test (LSA and MSA tests with 80 items each) and were expressed as mean \pm SD.¹⁸ PSU scores were provided by the Department of Evaluation, Measurement and Educational Registry (DEMRE) of the University of Chile and, by the Studies Centre of the Ministry of Education. In fact, scores below 450 barred students from applying to higher education.¹⁸

2.5. Fatty acid profile

Blood samples were taken from fasted male and female young for erythrocyte fatty acid assessment. Quantitative extraction and separation of total lipids was carried out according to Bligh and Dyer.²⁰ Total lipids were extracted by thin layer chromatography according to Ruiz-Gutierrez et al.²¹ FAs methyl esters for gas-chromatography analysis were prepared according Morrison and Smith.²² Specific conditions for gas-chromatography procedures were previously described by Valenzuela et al.²³

2.6. Anthropometric nutritional parameters

Measurements of weight, height and head circumference were carried out at school using standardized procedures and all the instruments were verified before measuring each subject.²⁴ The postnatal nutritional background was assessed through the head circumference-forage Z-score (Z-HC) using the tables of Ivanovic et al., (1995).²⁵ The current nutritional status was expressed as body mass index (BMI, weight/height²) and compared to the NCHS-CDC tables and expressed as Z-BMI.^{26,27}

2.7. Socio-economic status

Socio-economic status (SES) was measured applying the Graffar's modified scale which considers schooling and occupation of the household head, and characteristics of the housing (building materials, ownership, water supply and ownership of durable goods).²⁸ Graffar's modified scale has been adapted for Chilean urban and rural populations and classified the sample into five socio-economic strata: 1 = high; 2 = medium-high; 3 = medium; 4 = medium-low; and 5 = low. SES was also expressed as quantitative variable, Graffar's score.

Table 1

Scholastic achievement (SA) in the University Selection Test (PSU), demographic, nutritional and socio-economic status measurements of the sample by group and gender.

Measurements PSU SA PSU outcomes	Group 1 High SA (n = 70) Males (n = 48)	Females $(n = 22)$	Group 2 Low SA (n = 52) Males (n = 23)	Females (n = 29)	F	р
LSA	$688.02^{a} \pm 47.51$	$665.04^{a} \pm 51.72$	$405.00^{\rm b}$ ± 73.10	$397.10^{b} \pm 72.74$	214.73	0.0001
MSA	$728.69^{a} \pm 62.62$	$674.22^{\text{D}} \pm 68.70$	$400.30^{\circ} \pm 75.10$	$391.83^{\circ} \pm 68.62$	220.61	0.0001
Demographic						
Age (y)	$18.1^{a} \pm 0.3$	$18.0^{a} \pm 0.3$	$18.6^{b} \pm 0.7$	$18.0^{a} \pm 0.5$	8.31	0.0001
Postnatal nutritional back	rground					
Z-HC	$0.86^{a} \pm 0.97$	$0.21^{\rm a} \pm 1.03$	$0.14^{\rm a} \pm 1.26$	$-0.78^{b} \pm 1.06$	13.24	0.0001
Current nutritional status						
BMI	22.91 ± 3.86	24.66 ± 4.83	23.40 ± 4.47	23.89 ± 5.05	0.76	0.5215
SES						
Graffar`s score	$7.85^{a} \pm 1.95$	$7.45^{a} \pm 2.39$	$9.26^{b} \pm 2.24$	$10.45^{b} \pm 1.50$	13.55	0.0001

Values are shown as mean \pm S.D. Group means with the same letter are not significantly different at the 0.05 level based on Bonferroni's test. LSA, language SA; MSA, mathematics SA; Z-HC, head circumference-for-age Z-score; Z-BMI, body mass index Z-score; SES, socio-economic status.

2.8. Statistical analysis

Data analysis included chi-squared test for ordinal variables and, ANOVA and Bonferroni's test for comparison of means. Pearson correlation coefficients were used for quantitative variables. In the linear regression analysis, the stepwise procedure was used to establish the most important independent variables that could affect PSU outcomes (dependent variable). The determination coefficient (R^2) was calculated to measure the fit of the regression models.²⁹ For all hypothesis tests the level of significance was 0.05. Data were processed using the Statistical Analysis System package (SAS 9.3, SAS Institute Inc. (Cary, NC).

3. Results

3.1. PSU outcomes, nutritional measurements and SES of the sample by group and gender

Table 1 shows PSU outcomes, nutritional measurements and SES of the sample by group and gender. Independently of gender, LSA outcomes were significantly higher in school-age children from Group 1 who achieved high SA than their peers from Group 2 with low SA (F = 214.73; p < 0.0001). On the contrary, males from Group 1 exhibited significantly higher MSA scores than females belonging to the same group and from males and females from Group 2 (F = 220.61; p < 0.0001). Z-HC, indicator of postnatal nutritional background of brain development, was higher in males from the Group 1 but was not significantly different from females of the same group and of males from Group 2. It is noticeable that females from Group 2 presented the lowest Z-HC values of the total sample (F = 13.24; p < 0.0001). Current nutritional status expressed as BMI did not differ significantly in each Group and between both Groups. Independently of gender, schoolage children from Group 1 showed Graffar scores significantly higher compared with their respective peers from Group 2. School-age children from Group 1 belonged mainly to high and medium SES and those from Group 2, mainly to the low SES, especially females (86.2%) (Fig. 1) $(X_0^2 (6 \text{ df}) = 45.0444 > X_t^2 (6 \text{ df}) p < 0.0001 = 22.457).$

3.2. Association between PSU outcomes and FAs levels in erythrocytes by group

The association between *PSU outcomes and* FAs *levels in erythrocytes by group and* gender is shown in Table 2. No significant differences were observed for Σ SFA (saturated FAs) levels as well as for each SFA by group and gender. Σ MUFA (monounsaturated FAs) and C18:1 (oleic acid) levels were significantly higher in males from Group1 compared with females of the same group; however these values did not differ



Fig. 1. Distribution of the sample by socio-economic status (SES), group and gender.

High SES (white), medium SES (grey), low SES (black). X_o^2 (6 df) = 45.0444 > X_t^2 (6 df) p < 0.0001 = 22.457.

significantly from those observed from Group 2 ((F = 2.83; p < 0.0414) and (F = 3.56; p < 0.0164), respectively). No significant differences were found for Σ PUFA levels but C20:4n-6 (AA) and C22:6n-3 (DHA) exhibited significant differences. AA was significantly higher in females from Group 1 compared with their males peers, and similar for both genders from Group 2 (F = 3.67; p < 0.0144). Regarding DHA, independently of the gender, values were significantly higher in Group 1 than Group 2 (F = 60.83; p < 0.0001).

3.3. Pearson correlation coefficients between FAs profile in erythrocytes and PSU outcomes

Table 3 expresses the Pearson correlation coefficients between LSA and MSA scores and erythrocyte fatty acid profiles, SES scores and postnatal nutritional background expressed as Z-HC. A negative and significant correlation was observed between LSA and C22:0 (p < 0.0208) and C20:3n – 6 (p < 0.0438); instead, a high positive and significant correlation was observed with DHA (r = 0.736;

Table 2

Association between the scholastic achievemen	t (SA) in the University	y Selection Test (PSU)	outcomes and FAs le	evels in erythrocytes by	group and gender

PSU SA Fatty acids	Group 1 High SA $(n = 70)$ Males $(n = 48)$	Females $(n = 22)$	Group 2 Low SA $(n = 52)$ Males $(n = 23)$	Females $(n = 29)$	F	p value
Σ SFA	44 2 + 5 78	45.6 + 6.53	465 + 473	44 9 + 6 26	0.92	0 4328
C12:0	0.11 + 0.10	0.12 ± 0.21	-0.09 ± 0.10	0.15 ± 0.18	0.72	0.4320
C14:0	110 ± 0.10	110 ± 0.53	0.09 ± 0.10	1.18 ± 0.16	1.55	0.2049
C16:0	27.9 ± 3.37	27.8 ± 4.56	281 + 347	27.7 ± 4.57	0.05	0.2049
C18:0	126 ± 294	138 ± 314	14.4 + 2.38	13.4 ± 2.52	2.60	0.0556
C20:0	0.24 ± 0.19	0.28 ± 0.22	0.29 ± 0.19	13.4 ± 2.32 0.30 + 0.19	0.81	0.0330
C22:0	0.24 ± 0.19	0.20 ± 0.22	0.29 ± 0.19	0.68 ± 0.32	2 44	0.4001
C24:0	1.42 ± 0.65	152 ± 0.48	1.68 ± 0.64	151 ± 0.52	0.07	0.0074
S MUEA	1.42 ± 0.03 28.0 ^a + 5.60	1.52 ± 0.48 $24.5^{b} + 4.22$	$25 7^{ab} + 2.8$	1.51 ± 0.52 25.0 ^{ab} + 5.74	2.97	0.4110
2 MOFA	1.67 ± 0.09	24.3 ± 4.22 1 42 + 0 73	23.7 ± 3.0 1 28 ± 0.62	23.9 ± 3.74 1 40 + 0 75	2.03	0.0414
C10.1	$24.2^{a} + 4.02$	1.43 ± 0.73	1.36 ± 0.02 21 $7^{ab} + 2.46$	1.49 ± 0.73 22.2 ^{ab} + 5.18	3 56	0.4342
C20·1	24.3 ± 4.92 0 52 + 0.41	20.9 ± 3.00	21.7 ± 3.40 0.80 + 1.02	22.2 ± 0.10	1.92	0.0104
C20.1	0.32 ± 0.41	0.42 ± 0.33	0.80 ± 1.02	0.38 ± 0.43	0.20	0.1450
C22.1	0.20 ± 0.17	0.28 ± 0.13	0.25 ± 0.14	0.24 ± 0.10	0.20	0.0901
C24:1	1.28 ± 0.54	1.58 ± 0.49	1.56 ± 0.52	1.38 ± 0.47	2.43	0.0087
2 PUFA	25.1 ± 0.52	27.8 ± 7.24	20.0 ± 3.95	28.1 ± 9.58	1.28	0.2849
C18:2n=6	17.8 ± 4.25	17.5 ± 4.08	16.8 ± 3.31	17.4 ± 4.25	0.31	0.8187
C18:3n=6	0.16 ± 0.07	0.19 ± 0.20	0.14 ± 0.07	0.11 ± 0.07	2.28	0.0828
C18:3n - 3	0.32 ± 0.30	0.26 ± 0.21	0.29 ± 0.33	0.27 ± 0.27	0.44	0.7269
C20:2n-6	0.21 ± 0.15	0.23 ± 0.17	0.27 ± 0.17	0.30 ± 0.21	2.13	0.1006
C20:3n-6	0.82 ± 0.42	1.13 ± 0.55	1.08 ± 0.84	1.21 ± 0.86	2.62	0.0540
C20:4n-6 (AA)	$4.37^{a} \pm 2.07$	$6.46^{\circ} \pm 3.00$	$5.40^{ab} \pm 2.58$	$6.08^{ab} \pm 3.86$	3.67	0.0144
C20:5n-3 (EPA)	0.27 ± 0.27	0.46 ± 1.46	0.47 ± 0.97	1.14 ± 3.01	1.71	0.1688
C22:5n-3	0.46 ± 0.30	0.58 ± 0.41	0.38 ± 0.43	0.51 ± 0.48	1.20	0.3133
C22:6n-3 (DHA)	$2.45^{a} \pm 1.21$	$2.32^{a} \pm 0.55$	$0.40^{\text{D}} \pm 0.22$	$0.40^{\text{p}} \pm 0.20$	60.83	0.0001

Values are shown as mean \pm S.D. Group means with the same letter are not significantly different at the 0.05 level based on Bonferroni's test. Fas, fatty acid levels; Σ Saturated fatty acids (SFA) correspond to 6:0, 8:0, 10:0, 12:0, 14:0, 16:0, 18:0, 20:0, 22:0 and 24:0. Σ Monounsaturated fatty acids (MUFA) correspond to 14:1, 16:1, 18:1, 20:1, 22:1 and 24:1. Σ Polyunsaturated fatty acids (PUFA) correspond to 18:2 n - 6, 18:3n - 6, 18:3n - 3, 20:2n - 6, 20:3n - 6, 20:4n - 6, 20:5n - 3, 22:5n - 3 and 22:6n - 3.

p<0.0001). MSA outcomes were negative and significantly correlated with C18:0 (p<0.0322), C 20:1 (p<0.0298) and C20:2n-6 (p<0.0293), and positively correlated with C18:3n-6 (p<0.0276) and DHA (r=0.756 p<0.0001). LSA and MSA scores positive and significantly correlated with SES scores (r=0.483 p<0.0001 and r=0.481 p<0.0001, respectively) and Z-HC (r=0.455 p<0.0001 and r=0.435 p<0.0001, respectively)

3.4. Multiple regression analysis between the PSU scores in LSA and MSA (dependent variable) and most relevant parameters (independent variables)

The multiple regression analysis (Proc Glm Error type III) between PSU tests (dependent variables) and most relevant parameters (independent variables) revealed that, independently of gender, DHA, SES and Z-HC were the independent variables with the greatest explanatory power for both LSA ($R^2 = 0.650$; p < 0.0001) and MSA variances ($R^2 = 0.700$; p < 0.0001) (Table 4).

4. Discussion

The results of the present study show that, independently of gender, DHA is the most important FAs highly and significantly associated with PSU outcomes, in Chilean school-age children who graduated from high school. So, only DHA explained LSA and MSA in the statistical regression model. This is especially relevant since, independently of gender, DHA, SES and Z-HC were the most important independent variables in explaining both LSA ($R^2 = 0.650$, p < 0.0001) and MSA outcomes ($R^2 = 700$; p < 0.0001), variables which are significantly interrelated. Although, C22:0 and C20:3n – 6 were significantly correlated with LSA and, C18:1(oleic acid), C20:1 and C18:3n – 6 with MSA, they did not contribute to explain PSU outcomes in these tests.

It is well known that DHA is one of the main constituents of the brain and that it is a fatty acid that is found in high concentrations in the nervous tissue, particularly in the cerebral cortex and in the retina.¹⁴ In addition, DHA has from the embryonic development onwards,

a fundamental role at the cognitive and visual capacities.¹⁴ On the other hand, the findings of several authors indicate that brain volume is positively and significantly associated with cognitive development and with the learning process at the school-age.¹⁻⁴

Z-HC, the anthropometric indicator of both nutritional background and brain development has been described as the most important index associated with SA.¹⁻⁴ This is coincident with the findings of the present study since Z-HC was the only index of postnatal nutrition that contributed to explain PSU outcomes and significantly associated with SES.²⁵ However, PSU outcomes were not significantly associated with Z-BMI, such as recent findings.³⁰

Regarding nutritional interventions with DHA, this fatty acid has been shown to be beneficial, when the mother is supplemented during pregnancy or it is provided in infant formulas, especially for better visual acuity in premature infants.^{31–34} However, when the children of mothers supplemented in pregnancy or infants who received formulas with DHA exceed 2 years, no significant effects of DHA are observed in the acquisition of greater cognitive and/or visual abilities.³⁵ This situation would be reflecting a limited effect, over time, of DHA supplementation in pregnancy and in the first years of life, the effect of direct intake and/or DHA synthesis being more important in the child's cognitive development. In the long-term, it has been described that DHA levels in breast milk positively and significantly correlated with a higher score in mathematics in children.³⁶

In relation to the synthesis process of DHA, this fatty acid is obtained from ALA, its direct precursor. It has been shown that the presence of polymorphisms in genes encoding the $\Delta-5$ and $\Delta-6$ desaturases enzymes, enzymes responsible for the formation of n-3 LCPUFA from the ALA, are associated with significant changes in the levels of these FAs, particularly DHA.³⁷ This fact would be relevant because, for example, the presence of the polymorphism rs174575 in the gene encoding the enzyme $\Delta-6$ desaturase in children, allows a higher concentration of DHA in tissues and, higher scores in intelligence tests.³⁸

Studies carried out about the effects of DHA on cognitive abilities in

Table 3

Pearson correlation coefficients between the scholastic achievement (SA) in the University Selection Test (PSU) scores both language (LSA) and mathematics (MSA) and FAs profile in erythrocytes, socio-economic score and postnatal nutritional background.

$\begin{array}{c c c c c c c c c c c c c c c c c c c $	Fatty acids profile	PSU SA			
$\begin{array}{c c c c c c c c c c c c c c c c c c c $		LSA		MSA	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$		R	p value	R	p value
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Σ SFA	-0.112	0.220	-0.137	0.132
C14:0 -0.007 0.940 0.019 0.832 C16:0 -0.015 0.870 -0.077 0.398 C18:0 -0.171 0.060 -0.194 0.032 C20:0 -0.066 0.470 -0.162 0.074 C22:0 -0.210 0.021 -0.166 0.069 C24:0 -0.092 0.314 -0.131 0.149 Σ MUFA 0.071 0.438 0.106 0.244 C16:1 0.061 0.503 0.132 0.147 C18:1 0.101 0.270 0.139 0.127 C20:1 -0.131 0.153 -0.197 0.330 C22:1 0.050 0.587 0.129 0.156 C24:1 -0.092 0.314 -0.141 0.122 Σ PUFA -0.054 0.555 -0.044 0.627 C18:3n - 6 0.153 0.094 0.199 0.28 C18:3n - 6 0.153 0.094 0.199 0.28 C18:3n - 6 -0.172 0.660 -0.197 0.29 C20:2n - 6 -0.172 0.660 -0.197 0.294 C20:3n - 6 -0.184 0.044 -0.152 0.094 C20:5n - 3 (EPA) -0.095 0.302 -0.111 0.224 C22:5n - 3 -0.082 0.372 -0.054 0.554 C22:6n - 3 (DHA) 0.736 0.0001 0.756 0.0001 SesIIIIIC2:4n - 3 (DHA) 0.736 0.0001	C12:0	-0.069	0.452	-0.021	0.819
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C14:0	-0.007	0.940	0.019	0.832
C18:0 -0.171 0.060 -0.194 0.032 C20:0 -0.066 0.470 -0.162 0.074 C22:0 -0.210 0.021 -0.166 0.069 C24:0 -0.092 0.314 -0.131 0.149 Σ MUFA 0.071 0.438 0.106 0.244 C16:1 0.061 0.503 0.132 0.147 C18:1 0.101 0.270 0.139 0.127 C20:1 -0.131 0.153 -0.197 0.030 C22:1 0.050 0.587 0.129 0.156 C24:1 -0.092 0.314 -0.141 0.122 Σ PUFA -0.054 0.555 -0.044 0.627 C18:2n-6 0.072 0.435 0.103 0.260 C18:3n-6 0.153 0.094 0.199 0.028 C18:3n-6 0.153 0.094 0.199 0.228 C20:2n-6 -0.172 0.660 -0.197 0.299 C20:3n-6 -0.184 0.044 -0.152 0.094 C20:4n - 6 (AA) -0.093 0.313 -0.112 0.220 C20:5n - 3 (EPA) -0.095 0.302 -0.111 0.224 C22:5n - 3 -0.082 0.372 -0.054 0.554 C22:5n - 3 -0.082 0.372 -0.054 0.554 C22:5n - 3 0.483 0.0001 0.756 0.0001 SESUUUUUCurrent Nutritional background -0.091	C16:0	-0.015	0.870	-0.077	0.398
$\begin{array}{ccccccc} C20:0 & -0.066 & 0.470 & -0.162 & 0.074 \\ C22:0 & -0.210 & 0.021 & -0.166 & 0.069 \\ C24:0 & -0.092 & 0.314 & -0.131 & 0.149 \\ \Sigma \ MUFA & 0.071 & 0.438 & 0.106 & 0.244 \\ C16:1 & 0.061 & 0.503 & 0.132 & 0.147 \\ C18:1 & 0.101 & 0.270 & 0.139 & 0.127 \\ C20:1 & -0.131 & 0.153 & -0.197 & 0.030 \\ C22:1 & 0.050 & 0.587 & 0.129 & 0.156 \\ C24:1 & -0.092 & 0.314 & -0.141 & 0.122 \\ \Sigma \ PUFA & -0.054 & 0.555 & -0.044 & 0.627 \\ C18:3n-6 & 0.072 & 0.435 & 0.103 & 0.260 \\ C18:3n-6 & 0.153 & 0.094 & 0.199 & 0.028 \\ C18:3n-3 & 0.050 & 0.593 & 0.083 & 0.361 \\ C20:2n-6 & -0.172 & 0.060 & -0.197 & 0.029 \\ C20:3n-6 & -0.184 & 0.044 & -0.152 & 0.094 \\ C20:4n-6 \ (AA) & -0.095 & 0.302 & -0.111 & 0.224 \\ C22:5n-3 & -0.082 & 0.372 & -0.054 & 0.554 \\ C22:6n-3 \ (EPA) & 0.736 & 0.0001 & 0.756 & 0.0001 \\ SES \\ Graffar`s \ score & 0.483 & 0.0001 & 0.481 & 0.0001 \\ Postnatal nutritional \ background \\ Current \ Nutritional \ status \\ BMI & -0.033 & 0.734 & -0.091 & 0.339 \\ \end{array}$	C18:0	-0.171	0.060	-0.194	0.032
$\begin{array}{ccccccc} C22:0 & -0.210 & 0.021 & -0.166 & 0.069 \\ C24:0 & -0.092 & 0.314 & -0.131 & 0.149 \\ \Sigma \ MUFA & 0.071 & 0.438 & 0.106 & 0.244 \\ C16:1 & 0.061 & 0.503 & 0.132 & 0.147 \\ C18:1 & 0.101 & 0.270 & 0.139 & 0.127 \\ C20:1 & -0.131 & 0.153 & -0.197 & 0.030 \\ C22:1 & 0.050 & 0.587 & 0.129 & 0.156 \\ C24:1 & -0.092 & 0.314 & -0.141 & 0.122 \\ \Sigma \ PUFA & -0.054 & 0.555 & -0.044 & 0.627 \\ C18:2n - 6 & 0.072 & 0.435 & 0.103 & 0.260 \\ C18:3n - 6 & 0.153 & 0.094 & 0.199 & 0.028 \\ C18:3n - 6 & 0.050 & 0.593 & 0.083 & 0.361 \\ C20:2n - 6 & -0.172 & 0.060 & -0.197 & 0.029 \\ C20:3n - 6 & -0.184 & 0.044 & -0.152 & 0.094 \\ C20:4n - 6 (AA) & -0.093 & 0.313 & -0.112 & 0.220 \\ C20:5n - 3 \ (EPA) & -0.095 & 0.302 & -0.111 & 0.224 \\ C22:5n - 3 & -0.082 & 0.372 & -0.054 & 0.554 \\ C22:6n - 3 \ (DHA) & 0.736 & 0.0001 & 0.756 & 0.0001 \\ SES \\ Graffar`s \ score & 0.483 & 0.0001 & 0.481 & 0.0001 \\ Postnatal nutritional \ background \\ Z-HC & 0.455 & 0.0001 & 0.435 & 0.0001 \\ Current \ Nutritional \ status \\ BMI & -0.033 & 0.734 & -0.091 & 0.339 \\ \end{array}$	C20:0	-0.066	0.470	-0.162	0.074
C24:0 -0.092 0.314 -0.131 0.149 Σ MUFA 0.071 0.438 0.106 0.244 C16:1 0.061 0.503 0.132 0.147 C18:1 0.101 0.270 0.139 0.127 C20:1 -0.131 0.153 -0.197 0.300 C22:1 0.050 0.587 0.129 0.156 C24:1 -0.092 0.314 -0.141 0.122 Σ PUFA -0.054 0.555 -0.044 0.627 C18:2n - 6 0.072 0.435 0.103 0.260 C18:3n - 6 0.153 0.094 0.199 0.028 C18:3n - 6 0.153 0.094 0.029 $C20:3n - 6$ -0.172 0.660 -0.197 0.029 C20:2n - 6 -0.172 0.660 -0.197 0.220 $C20:3n - 6$ -0.082 0.372 -0.054 0.554 C20:5n - 3 (EPA) -0.095 <	C22:0	-0.210	0.021	-0.166	0.069
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C24:0	-0.092	0.314	-0.131	0.149
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Σ MUFA	0.071	0.438	0.106	0.244
C18:10.1010.2700.1390.127C20:1 -0.131 0.153 -0.197 0.030C22:10.0500.5870.1290.156C24:1 -0.092 0.314 -0.141 0.122 Σ PUFA -0.054 0.555 -0.044 0.627C18:2n-60.0720.4350.1030.260C18:3n-60.1530.0940.1990.028C18:3n-6 -0.172 0.660 -0.197 0.029C20:2n-6 -0.172 0.060 -0.197 0.029C20:3n-6 -0.184 0.044 -0.152 0.094C20:4n - 6 (AA) -0.093 0.313 -0.112 0.220C20:5n - 3 (EPA) -0.095 0.302 -0.111 0.224C22:5n - 3 -0.082 0.372 -0.054 0.554C22:6n - 3 (DHA)0.7360.00010.7560.0001SESUUUUUC-HC0.4550.00010.4810.0001SesUUUUUZ-HC0.4550.00010.4350.0001BMI -0.033 0.734 -0.091 0.339	C16:1	0.061	0.503	0.132	0.147
$\begin{array}{cccccc} C20:1 & -0.131 & 0.153 & -0.197 & 0.030 \\ C22:1 & 0.050 & 0.587 & 0.129 & 0.156 \\ C24:1 & -0.092 & 0.314 & -0.141 & 0.122 \\ \Sigma \ PUFA & -0.054 & 0.555 & -0.044 & 0.627 \\ C18:2n-6 & 0.072 & 0.435 & 0.103 & 0.260 \\ C18:3n-6 & 0.153 & 0.094 & 0.199 & 0.028 \\ C18:3n-3 & 0.050 & 0.593 & 0.083 & 0.361 \\ C20:2n-6 & -0.172 & 0.060 & -0.197 & 0.029 \\ C20:3n-6 & -0.184 & 0.044 & -0.152 & 0.094 \\ C20:4n-6 (AA) & -0.093 & 0.313 & -0.112 & 0.220 \\ C20:5n-3 (EPA) & -0.095 & 0.302 & -0.111 & 0.224 \\ C22:5n-3 & -0.082 & 0.372 & -0.054 & 0.554 \\ C22:6n-3 (DHA) & 0.736 & 0.0001 & 0.756 & 0.0001 \\ SES & & & & \\ Graffar`s \ score & 0.483 & 0.0001 & 0.481 & 0.0001 \\ Postnatal nutritional \ background \\ Z-HC & 0.455 & 0.0001 & 0.435 & 0.0001 \\ Current \ Nutritional \ status \\ BMI & -0.033 & 0.734 & -0.091 & 0.339 \\ \end{array}$	C18:1	0.101	0.270	0.139	0.127
$\begin{array}{ccccccc} C22:1 & 0.050 & 0.587 & 0.129 & 0.156 \\ C24:1 & -0.092 & 0.314 & -0.141 & 0.122 \\ \Sigma \ PUFA & -0.054 & 0.555 & -0.044 & 0.627 \\ C18:2n-6 & 0.072 & 0.435 & 0.103 & 0.260 \\ C18:3n-6 & 0.153 & 0.094 & 0.199 & 0.028 \\ C18:3n-3 & 0.050 & 0.593 & 0.083 & 0.361 \\ C20:2n-6 & -0.172 & 0.060 & -0.197 & 0.029 \\ C20:3n-6 & -0.184 & 0.044 & -0.152 & 0.094 \\ C20:4n-6 (AA) & -0.093 & 0.313 & -0.112 & 0.220 \\ C20:5n-3 & -0.082 & 0.372 & -0.054 & 0.554 \\ C22:5n-3 & -0.082 & 0.372 & -0.054 & 0.554 \\ C22:5n-3 & -0.082 & 0.372 & -0.054 & 0.554 \\ C22:6n-3 (DHA) & 0.736 & 0.0001 & 0.756 & 0.0001 \\ SES \\ Graffar's \ score & 0.483 & 0.0001 & 0.481 & 0.0001 \\ Postnatal nutritional \ background \\ Z-HC & 0.455 & 0.0001 & 0.435 & 0.0001 \\ Current \ Nutritional \ status \\ BMI & -0.033 & 0.734 & -0.091 & 0.339 \\ \end{array}$	C20:1	-0.131	0.153	-0.197	0.030
$\begin{array}{cccccc} C24:1 & -0.092 & 0.314 & -0.141 & 0.122 \\ \Sigma \mbox{ PUFA} & -0.054 & 0.555 & -0.044 & 0.627 \\ C18:2n-6 & 0.072 & 0.435 & 0.103 & 0.260 \\ C18:3n-6 & 0.153 & 0.094 & 0.199 & 0.028 \\ C18:3n-3 & 0.050 & 0.593 & 0.083 & 0.361 \\ C20:2n-6 & -0.172 & 0.060 & -0.197 & 0.029 \\ C20:3n-6 & -0.184 & 0.044 & -0.152 & 0.094 \\ C20:4n-6 (AA) & -0.093 & 0.313 & -0.112 & 0.220 \\ C20:5n-3 & -0.082 & 0.372 & -0.054 & 0.554 \\ C22:5n-3 & -0.082 & 0.372 & -0.054 & 0.554 \\ C22:5n-3 & 0.736 & 0.0001 & 0.756 & 0.0001 \\ SES & & & & \\ Graffar's score & 0.483 & 0.0001 & 0.481 & 0.0001 \\ Postnatal nutritional background & & & \\ Z-HC & 0.455 & 0.0001 & 0.435 & 0.0001 \\ Current Nutritional status & & & \\ BMI & -0.033 & 0.734 & -0.091 & 0.339 \\ \end{array}$	C22:1	0.050	0.587	0.129	0.156
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	C24:1	-0.092	0.314	-0.141	0.122
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	Σ PUFA	-0.054	0.555	-0.044	0.627
$\begin{array}{cccccccc} {\rm C18:3n-6} & 0.153 & 0.094 & 0.199 & 0.028 \\ {\rm C18:3n-3} & 0.050 & 0.593 & 0.083 & 0.361 \\ {\rm C20:2n-6} & -0.172 & 0.060 & -0.197 & 0.029 \\ {\rm C20:3n-6} & -0.184 & 0.044 & -0.152 & 0.094 \\ {\rm C20:4n-6} \left({\rm AA} \right) & -0.093 & 0.313 & -0.112 & 0.220 \\ {\rm C20:5n-3} \left({\rm EPA} \right) & -0.095 & 0.302 & -0.111 & 0.224 \\ {\rm C22:5n-3} & -0.082 & 0.372 & -0.054 & 0.554 \\ {\rm C22:6n-3} \left({\rm DHA} \right) & 0.736 & 0.0001 & 0.756 & 0.0001 \\ {\rm SES} & & & & \\ {\rm Graffar's score} & 0.483 & 0.0001 & 0.481 & 0.0001 \\ {\rm Postnatal nutritional background} & & & \\ {\rm Z-HC} & 0.455 & 0.0001 & 0.435 & 0.0001 \\ {\rm Current Nutritional status} & & & \\ {\rm BMI} & -0.033 & 0.734 & -0.091 & 0.339 \\ \end{array}$	C18:2n-6	0.072	0.435	0.103	0.260
$\begin{array}{ccccccc} C18:3n-3 & 0.050 & 0.593 & 0.083 & 0.361 \\ C20:2n-6 & -0.172 & 0.060 & -0.197 & 0.029 \\ C20:3n-6 & -0.184 & 0.044 & -0.152 & 0.094 \\ C20:4n-6 (AA) & -0.093 & 0.313 & -0.112 & 0.220 \\ C20:5n-3 (EPA) & -0.095 & 0.302 & -0.111 & 0.224 \\ C22:5n-3 & -0.082 & 0.372 & -0.054 & 0.554 \\ C22:6n-3 (DHA) & 0.736 & 0.0001 & 0.756 & 0.0001 \\ SES \\ Graffar's score & 0.483 & 0.0001 & 0.481 & 0.0001 \\ Postnatal nutritional background \\ Z-HC & 0.455 & 0.0001 & 0.435 & 0.0001 \\ Current Nutritional status \\ BMI & -0.033 & 0.734 & -0.091 & 0.339 \\ \end{array}$	C18:3n-6	0.153	0.094	0.199	0.028
$\begin{array}{ccccccc} C20:2n-6 & -0.172 & 0.060 & -0.197 & 0.029 \\ C20:3n-6 & -0.184 & 0.044 & -0.152 & 0.094 \\ C20:4n-6 (AA) & -0.093 & 0.313 & -0.112 & 0.220 \\ C20:5n-3 (EPA) & -0.095 & 0.302 & -0.111 & 0.224 \\ C22:5n-3 & -0.082 & 0.372 & -0.054 & 0.554 \\ C22:6n-3 (DHA) & 0.736 & 0.0001 & 0.756 & 0.0001 \\ SES & & & & & \\ Graffar's score & 0.483 & 0.0001 & 0.481 & 0.0001 \\ Postnatal nutritional background & & & \\ Z-HC & 0.455 & 0.0001 & 0.435 & 0.0001 \\ Current Nutritional status & & & \\ BMI & -0.033 & 0.734 & -0.091 & 0.339 \\ \end{array}$	C18:3n-3	0.050	0.593	0.083	0.361
$\begin{array}{ccccccc} C20:3n-6 & -0.184 & 0.044 & -0.152 & 0.094 \\ C20:4n-6 (AA) & -0.093 & 0.313 & -0.112 & 0.220 \\ C20:5n-3 (EPA) & -0.095 & 0.302 & -0.111 & 0.224 \\ C22:5n-3 & -0.082 & 0.372 & -0.054 & 0.554 \\ C22:6n-3 (DHA) & 0.736 & 0.0001 & 0.756 & 0.0001 \\ SES & & & & & & \\ Graffar's score & 0.483 & 0.0001 & 0.481 & 0.0001 \\ Postnatal nutritional background & & & & \\ Z-HC & 0.455 & 0.0001 & 0.435 & 0.0001 \\ Current Nutritional status & & & \\ BMI & -0.033 & 0.734 & -0.091 & 0.339 \\ \end{array}$	C20:2n-6	-0.172	0.060	-0.197	0.029
$\begin{array}{cccccc} C20:4n-6 \ (AA) & -0.093 & 0.313 & -0.112 & 0.220 \\ C20:5n-3 \ (EPA) & -0.095 & 0.302 & -0.111 & 0.224 \\ C22:5n-3 & -0.082 & 0.372 & -0.054 & 0.554 \\ C22:6n-3 \ (DHA) & 0.736 & 0.0001 & 0.756 & 0.0001 \\ SES & & & & & & \\ Graffar's score & 0.483 & 0.0001 & 0.481 & 0.0001 \\ Postnatal nutritional background & & & & \\ Z-HC & 0.455 & 0.0001 & 0.435 & 0.0001 \\ Current Nutritional status & & & \\ BMI & -0.033 & 0.734 & -0.091 & 0.339 \\ \end{array}$	C20:3n-6	-0.184	0.044	-0.152	0.094
C20:5n - 3 (EPA) -0.095 0.302 -0.111 0.224 C22:5n - 3 -0.082 0.372 -0.054 0.554 C22:5n - 3 0.736 0.0001 0.756 0.0001 SES - - 0.001 0.481 0.0001 Ses - - 0.455 0.0001 0.481 0.0001 Postnatal nutritional background - - - 0.001 0.435 0.0001 Current Nutritional status - - 0.734 - 0.091 0.339	C20:4n-6 (AA)	-0.093	0.313	-0.112	0.220
C22:5n - 3 -0.082 0.372 -0.054 0.554 C22:6n - 3 (DHA) 0.736 0.0001 0.756 0.0001 SES - - 0.483 0.0001 0.481 0.0001 Postnatal nutritional background - - 0.455 0.0001 0.435 0.0001 Current Nutritional status - 0.033 0.734 -0.091 0.339	C20:5n-3 (EPA)	-0.095	0.302	-0.111	0.224
C22:6n – 3 (DHA) 0.736 0.0001 0.756 0.0001 SES Graffar`s score 0.483 0.0001 0.481 0.0001 Postnatal nutritional background Z-HC 0.455 0.0001 0.435 0.0001 Current Nutritional status BMI -0.033 0.734 -0.091 0.339	C22:5n-3	-0.082	0.372	-0.054	0.554
SES 0.483 0.0001 0.481 0.0001 Postnatal nutritional background 0.455 0.0001 0.435 0.0001 Z-HC 0.455 0.0001 0.435 0.0001 Current Nutritional status -0.033 0.734 -0.091 0.339	C22:6n-3 (DHA)	0.736	0.0001	0.756	0.0001
Graffar's score 0.483 0.0001 0.481 0.0001 Postnatal nutritional background	SES				
Postnatal nutritional background Comparison Comparison <td>Graffar`s score</td> <td>0.483</td> <td>0.0001</td> <td>0.481</td> <td>0.0001</td>	Graffar`s score	0.483	0.0001	0.481	0.0001
Z-HC 0.455 0.0001 0.435 0.0001 Current Nutritional status -0.033 0.734 -0.091 0.339	Postnatal nutritional background				
Current Nutritional status BMI -0.033 0.734 -0.091 0.339	Z-HC	0.455	0.0001	0.435	0.0001
BMI -0.033 0.734 -0.091 0.339	Current Nutritional status	5			
	BMI	-0.033	0.734	-0.091	0.339

Fas, fatty acids. Σ Saturated fatty acids (SFA) correspond to 6:0, 8:0, 10:0, 12:0, 14:0, 16:0, 18:0, 20:0, 22:0 and 24:0. Σ Monounsaturated fatty acids (MUFA) correspond to 14:1, 16:1, 18:1, 20:1, 22:1 and 24:1. Σ Polyunsaturated fatty acids (PUFA) correspond to 18:2 n-6, 18:3n-6, 18:3n-3, 20:2n-6, 20:3n-6, 20:4n-6, 20:5n-3, 22:5n-3 and 22:6n-3. SES, socio-economic status; Z-HC, head circumference-for-age Z-score; BMI, body mass index.

children and young people are few in the world. However, dietary supplementation with DHA in 4-year-old children (400 mg/day for 4 months) allowed a better learning capacity (acquisition of vocabulary) and listening comprehension in pre-school children.³⁹ In turn, the DOLAB study reported that children between 7 and 9 years old, with low levels of DHA in erythrocytes, have a lower performance in language, together with a greater opposition to parents and teachers (misconduct).⁴⁰ The same authors showed that supplementation of the diet with DHA (600 mg/day) for 6 months allowed to the children significantly increase their SA in language.¹⁶

Another study of supplementation with n-3 LCPUFAs (EPA + DHA) for 4 months, conducted in children between 7 and 12 years of age, showed that the increase in the levels of DHA in the erythrocyte were positively and significantly correlated, with a better capacity of learning and better behavior, especially in those children with attention deficit and hyperactivity.⁴¹ In addition, in subjects between 17 and 45 years of age with a low intake of DHA, dietary supplementation with this fatty acid improved memory capacity and reaction time, particularly in men.⁴²

However, over 12 years of age there is no important evidence regarding the impact of DHA on SA, since studies are scarce.¹⁶ Therefore, the findings of the present study would be the first to establish the significant and high association between the levels of DHA in erythrocytes and the performance in PSU tests, the baccalaureate examinations for university admission, of national coverage in Chile.

Table 4

Multiple regression analysis between the University Selection Test (PSU) scores in language (LSA) and mathematics scholastic achievement (MSA) (dependent variables) and most relevant parameters (independent variables).

Most relevant parameters LSA	Estimate	Standard error of estimate	T for H0: parameter = 0	p > T	
Intercept	546.54	37.93	14.41	0.0001	
Gender					
Females	-18.70	20.01	-0.93	0.35	
Males	0.00				
SES					
High	57.51	25.23	2.28	0.03	
Low	-45.44	22.96	-1.98	0.05	
Medium	0.00				
Z-HC					
< 0 (mean)	-43.91	20.84	-2.11	0.04	
≥ 0	0.00				
FAs					
C22:0	-59.46	31.03	-1.92	0.06	
C20:3n-6	-17.33	13.53	-1.28	0.20	
C22:6n-3 (DHA)	64.84	7.95	8.16	0.0001	
Model $R^2 = 0.650$; Root MSE (Root mean squared error (standard deviation of the dependent variable LSA in the PSU) = 93.87708; Model <i>F</i> value = 27.05, $p < 0.0001$.					

MSA				
Intercept	574.79	62.11	9.25	0.0001
Gender				
Females	-23.23	21.20	-1.10	0.28
Males	0.00			
SES				
High	35.85	26.96	1.33	0.19
Low	-52.13	24.12	-2.16	0.03
Medium	0.00			
Z-HC				
<0 (below mean)	-52.45	21.67	-2.42	0.02
≥0	0.00			
FAs				
C18:0	-6.48	3.51	-1.84	0.07
C20:1	-6.51	16.62	-0.39	0.70
C18:3n-6	158.11	91.01	1.74	0.09
C20:2n-6	-46.72	59.09	-0.79	0.43
C22:6n-3 (DHA)	76.94	8.60	8.95	0.0001

Model $R^2 = 0.700$; Root MSE (root mean squared error) standard deviation of the dependent variable MSA in the PSU) = 99.66987; Model *F* value = 25.64, p < 0.0001.

Note. DHA, docosahexaenoic acid; SES, socio-economic status expressed as Graffar score; Z-HC, head circumference-for-age Z-score; FAs, fatty acids;C18:0, octadecanoic acid; C18:3n – 6, gamma-linolenic acid; C20:1, eicosenoic acid; C20:3n – 6, dihomo-gamma-linolenic acid; C22:0, docosanoic acid.

5. Conclusions

The results of this study highlight the importance of DHA in their association with the learning process at the school level, specially, with the PSU. Therefore, these findings can be useful for nutrition of FAs, health and educational planning, in order to protect children starting from an early age and so increase their school outcomes.

Acknowledgments

The authors are grateful to the Agency for Quality Education and to the Studies Center of the Ministry of Education of Chile and, to the Department of Evaluation, Measurement and Educational Registry (DEMRE) of the University of Chile, for all the facilities given to carry out this research which reviewed, approved and authorized this study, providing 2013 PSU outcomes. Presented in the 13th Congress of the International Society for the Study of Fatty Acids and Lipids (2018 ISSFAL) in Las Vegas, May 27–31, 2018.

Conflict of interest

The authors declare no conflict of interest.

Funding

This work was supported by Grants 1100431 and 1150524 from the National Fund for Scientific and Technologic Development (FONDECYT)(PI: Daniza Ivanovic).

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