Total Fatty Acids Content of Erythrocyte Membrane Phospholipids in Healthy Children from Birth to 6 Years Old

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Abstract: *Objective:* Relate the profile of total fatty acids in erythrocyte membrane with the child nutritional status.

Method: It was determined the concentration of total fatty acids in erythrocyte membrane of 138 children under 6 years (69girls, 69children) through a blood sample (2mL) by capillary gas chromatography. Likewise assessed the status through Anthropometry, Dietetics and clinical nutrition.

Results: 33 fatty acids were found in the erythrocyte membrane: 14 saturated, 8 monounsaturated, 11 Polyunsaturated and 2 Trans fatty acids. The SFA had the highest concentration, especially Palmitic acid with 22.9mg/100mL in children. DHA showed a concentration of 6.3mg/100mL and the EPA of 1.2mg/100mL, showing a positive correlation ($r = 0.55$) statistically significant ($p < 0.002$). The n-6 PUFAS showed statistically significant difference ($p < 0.001$) between the age groups, of the total sample BMI/age values showed variable Z score from scratch, according to WHO, but have values indicating malnutrition, with a tendency to overweight and obesity. This trend is higher in boys than in girls. The dietary intake of nutrient show great variability in girls, with statistically significant differences ($p < 0.05$, 95% CI) between the age groups for energy, total fat, SFA and MUFA. In Children, only fiber has statistically significant differences.

Conclusions: The concentration of the measured fatty acids remains constant in the erythrocyte membrane and apparently is not modified with respect to the age. These values are low in Mexican children.

Keywords: n-3 fatty acids, mexican children, erythrocyte membrane, nutritional status, newborns.

1. INTRODUCTION

The composition of the erythrocyte membrane fatty acid has been used frequently as an indicator of the nutrition of essential fatty acids [1]. After birth there is a decrease of Docosahexaenoic acid (DHA) and arachidonic acid (ARA) in infants fed with conventional formulas that contain only precursors of these acids, such as linoleic acid (LA) and alpha-linolenic acid (ALN), respectively. In studies in humans appears to be more consistent composition of the erythrocyte, than the plasma phosphoglycerides. In children without ARA fish oil-fed, proportions of this acid decrease sharply in the plasma, but not in red blood cells; which means that in these subjects is not so necessary preformed ARA. At the same time, that there is deficiency of ARA also there is reduction in growth. This intrauterine growth retardation has increased in recent years in the Mexican population, informed by the detriment in the latest national nutrition surveys.

The correlations are high and significantly positive, between phosphatidylcholine (PC) and phosphatidylethanolamine (PE) and the fractions of DHA and ARA in the erythrocyte membrane in children to term and preterm and with their respective mothers. LA and ALN are inversely associated with ARA and DHA contents in some fractions of phosphoglycerides in mothers and their babies. This is very important, since the composition of the erythrocyte membrane is determined by diet, suggesting a physiological mechanisms that attempt to maintain an appropriate balance between ARA and DHA, which in turn allows stability on membrane, deformability and optimum reception and enzymatic functions [2]. The composition of fatty acids of the phospholipids of red blood cells (ePL), reflects the composition of other tissues and any nutritional deficit which alter its content in the body, affects more pronounced early the membranes of red blood cells [3]. Clinical studies often use DHA in erythrocytes as a biochemical marker of neuronal DHA. The justification for this is mainly based on a study conducted by Carlson *et al*., in which showed that rats weaned and

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fed a diet with a ratio of LA: ALN, 240:1 had lower concentrations of DHA, in PE fraction of erythrocytes, the cerebral cortex, cerebellum [4]. Honstra, *et al.* reveals that the nutritional status of fetal DHA is positively related with the size and weight at birth [5].

It is suggested that erythrocyte has a function as storage potential of ARA and DHA and as a vehicle for the transport of these fatty acids from the maternal circulation to the placenta, the fetus will use them in development [6]. The content and distribution of long chain polyunsaturated fatty acids (PUFA) in the phospholipids of maternal and fetal erythrocytes it has already investigated. The results show that in erythrocytes of term newborn babies, the content of n-6 and n-3; in particular ARA and DHA were, from the statistical point of view, higher than their respective mother. However, in preterm infants all phospholipids in erythrocyte were found to be statistically lower than their mother. To compare the content of ARA and DHA among the groups studied, only the DHA decreases in preterm group. Women with premature babies showed an ARA/DHA proportion in blood higher than those babies at term. By this observation, it is suggested that high concentrations of ARA and proportion of ARA/DHA in the maternal and fetal erythrocytes can be considered a sign early risk of prematurity [7]. The incidence of prematurity in México increased from 6.5% in 2011 to 7.4% in 2012 according to the national surveys (only the Mexican Institute of social security, IMSS, reports 8% incidence of prematurity, interval 2.6-6.6). This prematurity also correlated with a significant increase in perinatal mortality in México, until 2015 reached 2%. For this reason, it is required to determine risk factors to establish preventive measures. Profile of plasma phospholipids fatty acids are comparable patterns with other tissues, like erythrocytes phospholipid composition, indicating that it can be the expression of the nutrition state fatty acids of the individual. The long-chain PUFA are conditionally essential substrates during early life related to the quality of growth and development. Therefore, dietary supply suitable during pregnancy, infancy and early childhood that avoids such fatty acids decrease is desirable [8].

The gestational and neonatal and preschool stage in Mexico, is a focus of intervention and monitoring for adequate development and growth, as well as late as obesity and diabetes disease prevention. In Mexico, the information on the composition of fatty acids in risk groups such as children, pregnant women and the elderly is limited, but there are clinical signs of deficiencies of these essential fatty acids: delay in growth, difficulty learning, problems in vision, nerve damage and a greater propensity to diseases. Our hypothesis is that all this symptomatology is correlated with the nourishing state and this in turn with the levels of polyunsaturated fatty acids, mainly with omega-3.

For this reason, the objective of the present study was to determine the profile of total fatty acids in erythrocyte membrane of newborn children up to 6 years of age and its relationship with nutritional status, cared for in the Zone General Hospital no. 30 "Iztacalco".

2. MATERIALS AND METHODS

2.1. Design of the Study

The study was cross-sectional, prospective, randomized and observational. Through a leaflet of the project, the parents of the children were invited to the inclusion of his son to the study. Informed once asked, the signing of the consent in writing, and proceeded to the taking of a sample of 2mL of whole blood. Medical history was later made; anthropometric measurements and records of consumption of food for 24h, the Committee of ethics and research of the hospital approved all procedures.

2.2. Sample Selection

They were included in the study, clinically healthy children of both sexes from birth to six years of age, attending pediatric consultation in the Hospital General of zone no. 30 "Iztacalco" of the IMSS. Children was randomized controlled by convenience: they were grouped according to their age: 0 to 6 months (n = 27); 6 to 12 months (n = 12); from 1 to 2 years of age (n = 20); 2 to 3 years of age (n = 24); 3 to 4 years of age (n = 16); from 4 to 5 years of age (n = 22) and 5 to 6 years of age (n = 17) at the time of duration of the study. In the case of newborn, these were term and post healthy term, by normal delivery and caesarean section.

2.3. Methods

The conventional methods were used to perform the anthropometric measurements of children [9, 10]. We used tape measure fiberglass not flexible (brand SECA, model 201) with millimetric precision (1mm), 205cm for measuring perimeters; a skinfold Lange (Beta Technology Incorporated, Cambridge, Maryland) with measuring arms and floating tips with accuracy of

1mm and opening arm of 70mm (PAN model Housing No 3.008.239); scale (BAME, DGN 2412 model) with stadiometer with capacity of 140 kg; health-o-meter baby scale (SECA model 207) with a capacity of 12kg and with 0-99cm, division 1mm, measuring range weight of 810g.

For the nutrition survey: a reminder of 24 hours applied to the person responsible for the feeding and if the mother was breastfeeding, also applied her reminder. This was formed by time and schedule of meals, full name (type, brand) food, ingredients of each dish and quantity consumed approximate in grams. The data obtained from these surveys were captured in the Excel program and processed for the dietary calculation program Mexfoods [11].

2.4. Preparation of the Sample of Erythrocytes

The blood sample was centrifuged for 15 minutes at 3500xg (Beckman, Co), separating plasma from the red blood cells. Then erythrocytes were washed 3 times with isotonic saline (SSI, Baker Co) removing leukocytes present in its entirety, in each washing. Washed erythrocytes, were divided into fractions of 200 µL and kept at -30 °C until analysis.

2.5. Extraction of Total Lipids

Red blood cell samples were thawed and added 3 mL of Isopropanol (HPLC grade), 4 mL of hexane (RA grade) and 0.5 mL of 1% Ascorbic acid (Baker Co), mix with vortex, centrifuged for 15 minutes at 3500xg. The upper stage was transferred to another conical tube and evaporated to dryness with N_2 extra dry (INFRA, Co). (AOAC, 1997) [12, 13].

2.6. Preparation of Methyl Esters of Fatty Acids Extracted

To the tube containing the lipid extract added you 1mL of 14% methanol/boron trifluoride (BF_3/MeOH, SIGMA CHEM) and 50µL of the internal standard (C17:0), 1.3 mg/mL heptane, (LiChrosolv chromatographic grade); It was heated to 90 ° C for 1 hour in Thermoblock (SKS Bio-Medical Instruments LTD, series 00811). Then added 4 mL of hexane (AR grade), 1mL of light petroleum (Fermont Co.) and 1mL of deionized water (Millipore Co); be centrifugal to 3500xg for 5 minutes, separated the organic phase and 50 mg of anhydrous Na_2SO_4 (Baker Co) were added and it was centrifuged at 3500xg for 5 minutes. The dry organic phase was decanted into another tube, and evaporated to dryness with extra dry N_2. Methylated fatty acid extract was re-suspended in 1mL of hexane (HPLC grade) for analysis by capillary gas chromatography. This solution were injected 5uL in triplicate into the gas chromatograph.

2.7. Chromatographic Analysis

Analysis of methylated fatty acids was performed in a capillary (Varian 3380 CX) gas chromatograph with autosampler (Varian 8200CX), with a nozzle split-splitess and a flame ionization detector. The capillary column used was a SPTM-2560 of 100 m x 0.25mm in diameter, with a particle size of 0.25 nm (SUPELCO, cat. No. 2-4056). Used carrier gas was nitrogen (N_2) grade chromatographic and extra dry (INFRA), in the same way as the hydrogen (H_2) and the air used for the detector. Gas pressure the team will adjust to 30mL/min for N_2, 300 mL/min for air, and 30mL/min for the H_2. With these data, a split of 1:100 ratio was obtained. The chromatographic equipment system possesses a workstation for data management chromatographic (Star Cromatographic WorK Station).

Fatty acids data are presented as mean ± standard deviation in mg100mL of red blood cells. Comparisons between age groups were performed by ANOVA simple analysis and non-parametric analysis. A test of multiple Spearman correlation between the nourishing state of the child and the concentration of PUFA was performed. For the classification and tabulation of anthropometric data obtained during the intervention, were determined using the Z-score Tables established by the WHO, with mean ± standard deviation, confidence intervals of 95 and 99% and with a significance between $p < 0.001$ and $p < 0.05$. All of the above were analyzed using the statistical program SigmaStat (version 3.5 for Windows 97-2003, XP, Vista).

3. RESULTS

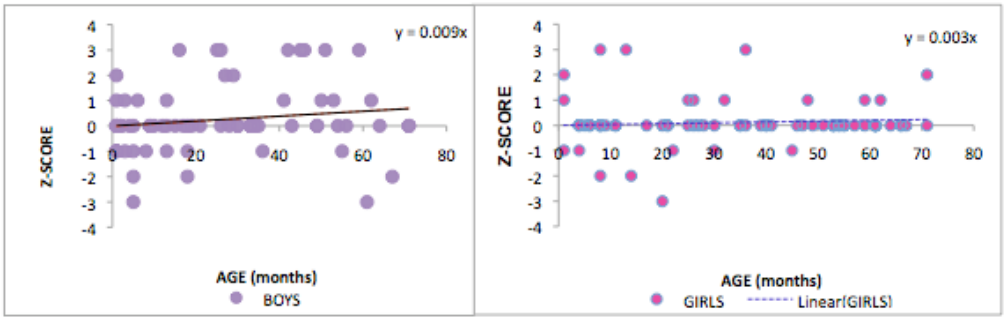
138 children under 6 years were studied. The distribution by sex was 69 girls (50%) and 69 children (50%), with predominance of the group of 0-6 months of age in children with 4-5 years of age in girls. Anthropometric characteristics of children including the study as mean ± standard deviation are shown in Table **1**. The fat and muscle area arm in children (BMA and BFA) under one year is not measurable therefore results are not displayed.

Accordingly, the differences between the two genders can be categorized as normal in the case of all the assessed anthropometric indicators; however, there

Table 1: Anthropometric Characteristics of Child's Studied

Girls							
Age Range	0-6 m	6-12 m	1-2 y	2-3 y	3-4 y	4-5 y	5-6 y
N	9	8	8	10	10	13	11
Age (months)	3.55±2.0	8.87±1.3	18.50±3.4	29.40±3.6	39.50±6.3	53.84±3.3	64.63±3.8
Weight (kg)	5.47±1.1	7.83±0.9	9.86±1.6	11.82±2.2	15.30±2.1	17.91±2.6	19.60±3.6
Size (m)	0.58±0.04	0.68±0.03	0.81±0.1	0.85±0.07	0.97±0.01	1.06±0.04	1.10±0.08
HC (cm)	40.94±5.0	42.71±0.8	45.13±1.4	48.10±3.09	49.03±1.1	48.73±1.4	49.90±2.0
AC (cm)	12.51±1.9	13.48±0.6	13.72±1.3	14.64±1.7	16.82±3.7	16.83±1.7	16.55±1.1
BMI (kg/m^2)	15.78±1.5	16.85±2.7	15.32±3.9	15.99±1.4	16.12±2.4	15.79±1.5	16.15±2.0
AMA (mm)	--	--	907.6±111.1	972.5±195.3	1419.9±1052	1401±307.9	1289.4±183.9
AGA (mm)	--	--	604.3±198.4	755.2±282.3	930.3±477	877.2±332.3	901.1±199.9
Boys							
N	18	4	12	14	6	9	6
Age (months)	2.66±1.8	9.0±0.8	16.08±2.8	30.0±3.3	42.16±3.5	52.88±3.4	66.0±4.3
Weight (kg)	5.22±1.7	8.05±0.9	9.92±1.2	13.82±2.3	17.78±3.9	18.60±3.1	19.16±1.9
Size (m)	0.58±0.07	0.69±0.02	0.77±0.04	0.89±0.04	0.99±0.08	1.04±0.04	1.15±0.08
HC (cm)	39.15±3.5	44.62±1.1	46.40±1.2	49.62±3.2	52.75±3.1	51.50±2.5	51.0±0.8
AC (cm)	11.66±2.3	12.12±2.4	13.40±2.3	15.16±2.0	16.28±2.8	16.08±1.6	15.98±4.9
BMI (kg/m^2)	14.72±2.1	16.72±0.9	16.49±2.2	17.05±2.3	18.15±3.1	16.91±2.6	14.58±2.3
AMA (mm)	--	--	953±308.9	1048.1±324.2	1240.7±307.2	1037.8±364.8	1440.2±751.8
AGA (mm)	--	--	516.5±254.7	812.2±296.7	921.8±511.1	1041.4±593.8	757.4± 634.1

The results are mean ± standard deviation, **N:** Number of Subjects **HC**: Head Circumference, **AC**: Arm circumference, BMI: Body Mass Index, **AMA**: Arm Muscle Area, **AGA**: Arm Grass Area; m = months, y= years.



*According WHO

Figure 1: Body mass index z-score* in from birth to six old.

is greater statistical difference by age group in girls than in boys, indicating that despite having normality, is present a trend of malnutrition, overweight and obesity. The anthropometric results are best interpreted by age group and not between age groups. There is a statistically significant difference ($p < 0.05$, 95% CI) for height/age indicator in children, BMA in girls and BFA in children.

In Figure **1** it is observed that the BMI/Age ratio showed varying values of the Z-score from scratch, according to the WHO, but they present values that indicate some degree of malnutrition, with a tendency to overweight and obesity. This trend is higher in boys than in girls. In children, increases from 10 months and girls are kept constant. There is a 9.7% overweight in children under 5 years old, according to the national survey of health and nutrition (ENSANUT, 2012) figure that has been increasing since the national survey on nutrition (ENN, 1988) with a 7.8%.

On the other hand, Figure **2** shows that head circumference shows a positive correlation ($r = 0.76$) with a statistical significance ($p < 0.001$, 99% CI) with

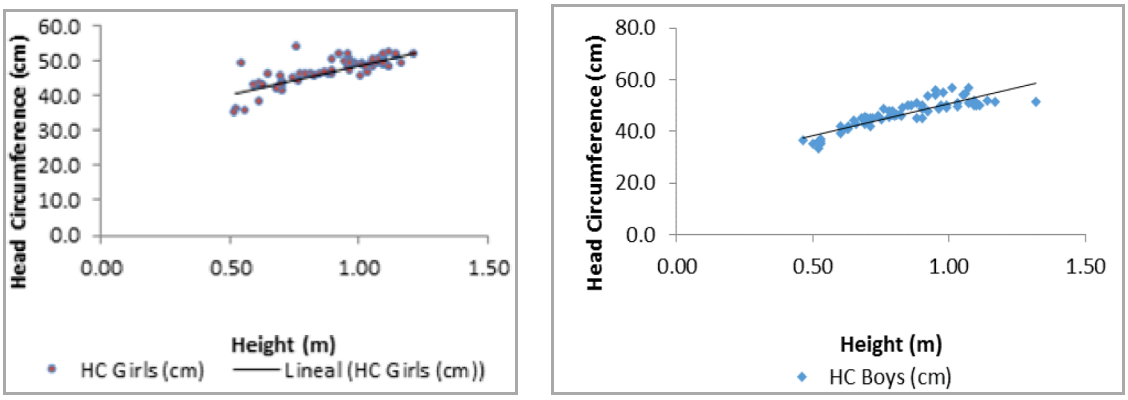


Figure 2: Relationship between Head Circumference (HC) with Height from Birth to Six Years Old.

Table 2: Child Dietary Intake Calculated by 24 Hours Recall

GIRLS								
Age Range	**0-6 m**	**6-12 m**	**1-2 y**	**2-3 y**	**3-4 y**	**4-5 y**	**5-6 y**	***p*Value**
Caloric Intake (kcal)	1830 ± 1203	765 ± 384	1095 ± 320	1379 ± 394	1273 ± 245	*1564 ± 605	1537 ± 383	*0.012
Dietetic Fiber(g)	8.91 ± 8.2	2.41 ± 2.0	5.335 ± 3.8	4.62 ± 3.6	4.97 ± 2.4	7.28 ± 5.5	6.36 ± 3.2	NS
Carbohydrates(g)	242 ± 198	114 ± 54.7	149 ± 70.1	198 ± 78.0	150 ± 45.9	209 ± 119.0	199 ± 67.6	NS
Proteins (g)	**19.4 ± 32	*19.23 ± 13.7	38.2 ± 9.7	49.62 ± 30.6	47.45 ± 12.1	48.76 ± 13.1	47.39± 12.7	*0.004
Total Lipids (g)	60.1 ± 37.9	25.2 ± 13.8	38.3 ± 7.6	52.9 ± 17.0	53.7 ± 12.8	58.1 ± 24.4	58.5 ± 15.1	*0.005
SFA (g)	23.8 ± 11.1	7.85 ± 5.0	12.01 ± 6.6	15.58 ± 7.3	19.53 ± 3.9	20.75 ± 7.8	19.95 ± 5.9	**0.001
MUFA (g)	17.2 ± 12.7	3.93 ± 5.0	7.94 ± 4.0	12.84 ± 8.7	17.48 ± 5.3	18.92 ± 7.0	22.11 ± 7.2	**0.001
PUFA (g)	13.3 ± 7.6	3.10 ± 4.1	8.23 ± 4.7	10.18 ± 5.8	9.96 ± 6.5	12.09 ± 9.0	10.59 ± 3.5	NS
BOYS								
Caloric Intake (kcal)	1841 ± 955	1475 ± 762	1688 ± 638	1803 ± 669	1473 ± 645	*1611 ± 567	1636 ± 443	NS
Dietetic Fiber (g)	6.11 ± 4.5	7.53 ± 6.7	6.31 ± 3.8	7.88 ± 3.7	*5.38 ± 4.3	6.09 ± 2.3	5.0 ± 2.6	NS
Carbohydrates(g)	241 ± 160	238 ± 110	255. ± 115	265. ± 134	183 ± 106.2	215. ± 98.3	235 ± 110	NS
Proteins (g)	55.22 ± 32	43.2 ± 42.2	49.5 ± 31.8	54.51 ± 17.9	52.04 ± 21.5	51.10 ± 17.5	47.84 ± 14.4	NS
Total Lipids (g)	70.4 ± 39	37.2 ± 22.7	52.6 ± 31.2	61.17 ± 17.6	59.44 ± 18.5	58.24 ± 21.8	56.96 ± 15.8	NS
SFA (g)	22.3 ± 13	14.44 ± 10.3	14.8 ± 10.1	21.72 ± 7.4	21.49 ± 7.6	20.98 ± 8.2	19.32 ± 7.8	NS
MUFA (g)	18.8 ± 12	6.80 ± 10.7	12.18 ± 9.4	20.85 ± 9.6	17.45 ± 6.3	23.64 ± 11.4	18.0 ± 6.2	NS
PUFA (g)	16.4 ± 14	6.93 ± 7.3	13.01 ± 8.1	10.97 ± 6.4	12.90 ± 8.6	10.09 ± 2.8	9.89 ± 3.9	NS

The results are mean ± standard deviation, *p < 0.05 test Kruskal-Wallis ANOVA; **p <0.001 test Kolmogorov-Smirnov, IC 99%; **SFA:** Saturated Fatty Acids; **MUFA:** Monounsaturated Fatty Acids; **PUFA:** Polyunsaturated Fatty Acids.

respect to the age for girls and for children in the same way shows a positive correlation (r = 0.91) with a statistical significance (p < 0.001, 99% CI) regarding the age. Referring to that a discrepancy there is between the head circumference and the size, reducing the likelihood of a pathological basis of this. However, its usefulness is limited, because the proportion between the head size and length you can be normal, unless you mean a proper head size; serve as an example dwarfism and microcephaly birth syndromes.

The dietary intake of lipids was calculated using the "MEXFOODS" software from the Mexican Tables Nutritional Value of Food [11]. The results are presented in Table **2**, where the total consumption of energy intake show intervals 765-1830 kcal/d in girls and from 1473 to 1871 kcal/d in boys. Girls consumed 2.4-8.9 g dietary fiber/d and children 5-3-7.8 g/d.

Ingested fat (Total Lipids) show intervals from 25-60g/d for girls and 37-70g/d for children. They consumed 7-23g SFA/d in girls and 14 to 22g SFA/d in children; 3 to 18g MUFA/d in girls and 6-23g MUFA/d in children and 3-13g PUFA/d in girls and 6-16g PUFA/d in children. Because the data show great variability, no statistically significant differences were observed in children. However, in girls, statistically significant differences (p < 0.05, 95% CI) between age groups in terms of energy intake, Total Protein and Lipids was observed. In the first year of life is observed a higher intake of protein in children compared with girls, becoming more than double their consumption. In

relation to the SFA, MUFA and PUFA consumption is observed in the first half of life there are values but higher than in the second. But these values are normalized after the first year. We observed significant statistical differences ($p < 0.001$, 99% CI) in the consumption of SFA and MUFA.

In Table **3** shows the results obtained by analyzing the concentration of total fatty acids (mg) in erythrocyte membrane of children birth to 6 years of age. The results are better interpreted when they are by age group and not by gender. There is a difference with statistical significance ($p < 0.05$, 95% CI) in SFA of 1-2 years in girls; MUFA from 6 months to 2 years in girls; PUFA from 1-3 years and 4-5 years in children; SFA from 6 months to 2 years in girls, n-3 totals from 0-6 months, 2-3 years and 4-6 years in girls and children in 4-6 years; n-6 total of 6 months to 3 years in girls, relationship n-6/n-3 from 0-6 months and 3-4 years and children from 0-6 months and EPA/DHA in 3-4 years from girls and children from 0-6 months.

Similarly, there is significant difference ($p < 0.001$, 99% CI) in n-3 totals of 6-12 months and 3-4 years in girls and children from 0-6 months and 3-4 years and EPA/DHA of 6-12 months in girls and children of 2-3 years. Until this moment, there are no studies that show comparable results. There were no statistically significant differences by gender, for each age group. Multiple comparisons between age groups were performed by Dunn's test (ANOVA), which is used when treatment groups are unequal. Multiple comparisons statistically significant differences between groups of 0-12 months and 1-2 years against groups of 2-5 years were observed. The concentration of EPA + DHA in the erythrocyte membrane by age group showed no statistical difference ($p = 0.771$). Pearson correlation between EPA and DHA ($r = 0.55$) of the 138 children included in the study yielding with a statistical significance ($p = 0.002$) was performed. Therefore, the data are best interpreted by gender that by age group for these variables. It is noted that in the case of the erythrocyte membrane, in the n-3 PUFA, concentrates more DHA than EPA and ALA, in that order, and then containing more arachidonic acid than AL as part of the n-6. Indeed cis-11, 14-eicosadienoic acid (C20: 2) is more concentrated in the PUFA erythrocyte membrane after oleic and palmitic acid.

Table 3: Total Fatty Acids Concentration (mg/%) in Erythrocyte Membrane in Child's from 0-6 Years

Age Range	0-6mo	6-12mo	1-2y	2-3y	3-4y	4-5y	5-6y
FA Total[£]	4288 ± 7778	3507 ± 7581	490.8 ± 899.1	2376 ± 5288	13432 ± 16614	4043 ± 7073	4354 ± 5757
SFA Total	36.5 ± 18	44.8 ± 11.3	*47.5 ± 9.9	38.8 ± 16.2	40.1 ± 9.7	40.6 ± 14.3	45.7 ± 13.5
MUFA Total	36.5 ± 17	*36.9 ± 5.2	*38.4 ± 3.4	39.14 ± 5.6	44.40 ± 9.7	37.70 ± 8.1	36.19 ± 6.9
PUFA Total	27 ± 22.6	22.8 ± 13.2	13.99 ± 9.9	22.1 ± 12.5	15.45 ± 12.6	23.5 ± 12.6	18.1 ± 13.4
t FA	11.2 ± 20	*6.9 ± 14	*1.21 ± 0.9	1.14 ± 1.7	1.33 ± 2.0	3.46 ± 4.8	4.82 ± 7.9
n-3 Total	*10.2 ± 6	**12.0 ± 9	8.65 ± 3.8	*7.84 ± 4.0	**7.14 ± 5.0	*12.7 ± 6.9	*9.41 ± 4.9
n-6 Total	2.25 ± 2,2	*9.3± 12	*2.24 ± 1.4	*2.10 ± 0.5	2.45 ± 1.9	1.96 ± 1.7	3.61 ± 2.0
n-6/n-3	*4.60 ± 5	19.5 ± 16.9	4.78 ± 1.4	6.85 ± 8.89	*13.1 ± 13.4	9.6 ± 9.4	0.38 ± 0.5
EPA + DHA	12.4 ± 22	**6.5 ± 15	1.05 ± 0.9	3.22 ± 1.5	*1.43 ± 2.1	*3.66 ± 5.1	8.01 ± 5.8
FA Total[£]	4022 ± 6986	8912 ± 11099	9543 ± 17975	5713 ± 7144	3626 ± 8579	5704 ± 8276	12550 ± 11561
SFA Total	46.6 ± 12	39.05 ± 19	43.0 ± 15	43.25 ± 11	36.17 ± 17	43.96 ± 10	42.06 ± 15
MUFA Total	38.19 ± 9	38.75 ± 3.1	39.11 ± 4.4	36.4 ± 11.7	41.81 ± 3.4	38.65 ± 5.9	39.99 ± 6.6
PUFA Total	15.2 ± 11	22.2 ± 16	*17.9 ± 14	*20.4 ± 18	22 ± 14	*17 ± 10	17.9 ± 14
t FA	2.30 ± 4.3	ND	1.25 ± 1.4	7.85 ± 17.8	1.87 ± 1.4	0.72 ± 1.1	1.45 ± 2.1
n-3 Total	**6.9 ± 5	8.32 ± 1.9	6.07 ± 3.64	**5.58 ± 4	7.59 ± 6.1	*6.13 ± 5.2	*8.31 ± 5.1
n-6 Total	4.31 ± 1.3	ND	3.37 ± 1.7	3.62 ± 3.0	ND	5.0 ± 3.6	2.63 ± 2.3
n-6/n-3	*7.8 ± 7.3	ND	6.98 ± 6.5	8.21 ± 8.6	5.14 ± 3.5	11.11 ± 9.1	0.17 ± 0.2
EPA + DHA	*2.9 ± 4.5	ND	1.46 ± 1.5	**10.3 ± 20	1.63 ± 1.2	0.85 ± 1.2	13.37 ± 8.6

The results are mean ± standard deviation, £= mg/100mL erythrocytes; **FA:** Fatty Acids; **SFA:** Saturated Fatty Acids; **MUFA:** Monounsaturated Fatty Acids; **PUFA:** Polyunsaturated Fatty Acids; ***t* FA:** *trans* Fatty Acids; **n-3:** omega 3; **n-6:** omega 6; **EPA:** Eicosapentaenoic Acid; **DHA:** Docosahexaenoic Acid; **ND:** Not Determined; *p < 0.05 test Kruskal-Wallis ANOVA, IC 95%, **p <0.001 test Kolmogorov-Smirnov, IC 99%, **NS**= non-significant.

The significance of this is unknown but it correlates well with the high concentration of other monounsaturated fatty acid, like cis-11-eicosenoic acid (r = 0.79) (data not showed).

The Pearson correlation between nutritional status (BMI/Z-score and percentile AGA) and fatty acids (n-3, n-6, ratio n-6/n-3, EPA + DHA and *trans* fatty acids) are shown in Table **4**. Pair (s) of variables with positive correlation coefficients and p < 0.05 valuestend to increase together. For couples with negative correlation coefficients and P values < 0.05, one variable tends to decrease while the other increases. For pairs with P values > 0.05, there is no significant relationship between the two variables. Consequent to this, the only variables that show a positive relationship are Z score of BMI percentile against AGA (r = 0.5), (significance p < 0.001, 99% CI). That indicates that BMI and AGA relate directly and if BMI increases thereby increasing the Pearson correlation calculated between fat from the diet and the concentration of total fatty acids, showed a positive correlation between dietary PUFA and erythrocyte membrane PUFA (r = 0.20 and p = 0.02). The variables tend to increase together, so if PUFA are ingested in the diet these will be reflected in blood erythrocytes. The hypothesis that was raised at the beginning of this work is verified, the nutritional status of children is inadequate by the presence of malnutrition, overweight and obesity. The lipid intake is deficient in PUFA and PUFA concentration is low in erythrocyte membrane, even in the group of children 0-6 months their concentration was not determined. This confirms the poor PUFA intake from breast milk before reported for this age group [14]. SFAs dominate erythrocyte membrane and diet, followed finally MUFA and PUFA raising the prospect of overweight and obesity in children of this age group. Considering the average life of an erythrocyte is 120 days, these data are indicative of a poor dietary intake of PUFA even with a length of intake of 3 to 4 months, which is prevalent in all samples at any age and there are no gender difference. The lack of statistical difference in the fatty acids measured in the phospholipids of red cell membrane in this population can recognize the importance of the composition of erythrocyte membrane is genetically defined, rather than ingestion of the type of fatty acid. This is indicated by this constant concentration through the years, as membrane phospholipids in the present study. However, these finding are low as compared with other studies showing poor with respect to PUFA composition and mainly DHA. The composition of fatty acids, on the other hand, neither is influenced apparently by the same concentration of fatty acids in the plasma fraction of each individual.

Table 4: Obtained Correlation between the Nutrition Status (BMI z-score) and Polyunsaturated Fatty Acids Measured in Red Blood of Children from Newborn to 6Years of Age

BMI z-Score	n-3 Total	n-6 Total	n-6-/n-3	EPA+DHA	TRANS	AGA Percentile
R value	0.151	0.0927	0.186	0.140	0.00	0
p value	0.118	0.296	0.084	0.194	0.9	*0.0000
N	108	129	87	88	72	99

Correlation of Pearson [-1, 1] positive correlation, Value of **p<0.001, IC 99%; *p<0.05, IC 95%.

BMI: Body Mass Index, **n-3:** omega 3, **n-6:** omega 6, **EPA:** Eicosapentaenoic Acid, **DHA:** Docosahexaenoic Acid, **AGA:** Arm Grass Area.

N= Sample number analyzed SigmaStat versión 3.5 Windows 97-2003, XP, Vista.

4. DISCUSSION

In México between 1988 and 2012, the prevalence of malnutrition in children under 6years have had notable declines. The prevalence of wasting and low weight for age decreased to a quarter of the prevalence of 1988, to reach values compatible with populations without malnutrition, while chronic malnutrition (stunting and wasting), but halved the prevalence 1988 remains high (13.6%), representing nearly 1.5 million children under 6 years in this condition [15]. In 2006, the ENSANUT documented the growing importance of overweight and obesity as an epidemic in the Mexican population. In that year the combined prevalence of both categories of nutritional status was presented in 34.8% of all children aged between 5 and 11 years of age in México (20.2% overweight and 14.6% obese), about 8 percentage points (pp) higher than the figure that was reported in the 1999 NNS (26.9%: 17.9% overweight and 9.0% obese). Currently, 34.4% of children in that age range are overweight [15-17]. This study confirms the continuing presence of two chronic degenerative diseases (malnutrition and obesity). Malnutrition occurs with more emphasis the first two years of life and obesity and overweight occurs from age [4]. México is definitely an epidemic of obesity that has not been controlled or decrease; socioeconomic status and poor diet largely influence this effect. The nutrient requirements necessary for the proper functioning of the body, are met from the amount and variety of food eaten [18], which is why the quality of the daily diet is

an essential element that contributes to the health and nutritional status the individual, especially in the first years of life [19]. The erythrocyte membrane has a total of 33 total fatty acids of which 14 are SFA, 8 MUFA and 11 PUFA, the latter comprise the principal n-3 and n-6 fatty acids. Not to mention that there is a presence of these two trans FA. The presence of a higher concentration of saturated fatty acids in the erythrocyte membrane of this study are consistent with studies in obese adolescents, wherein the concentration of SFA determine development of alterations in erythrocyte membrane, mainly affecting their fluidity and rigidity [20, 21]. Generally, the absorption of fatty acids in the sn-2 position of the tracilgliceroles is favored; yet specialty fatty acids placed in the positions sn-1 and sn-3 [14]. Palmitic acid concentrations in red cell membrane is determined are the highest of all the fatty acids which are palmitic acid in the sn-2 position of the triacylglycerol. This is an important component of breast milk accounts for about 25% of the lipid composition, of which 60-85 % is in position 2 triacylglycerol [22]. n-6 PUFAs originating from linoleic acid (LA), containing 18 carbon atoms and 2 double bonds (C18: 2), from this fatty acid gamma-linolenic acid and ARA are derived. The ARA is released from membrane phospholipids by the action of phospholipase A_2 who receives the action of cyclo-oxygenase, lipoxygenase and epoxygenase or cytochrome P_{450}, and becomes, respectively, prostaglandins (E_2, F_{2alpha}, D_2, H_2 and I_2) and thromboxane series 2 (A_2 and B_2), the 4-series leukotrienes (LTB_4) and epoxy-eicosatrienoic acids, which act as cellular messengers and increase platelet aggregation, heart rate and have vasoconstrictor properties and pro-inflammatory [23]. In the present study, ARA is very low, with a concentration of 0.007mg/100mL, which apparently individually modulate the composition of the membrane, which is determined, genetically [24]. However, the concentration of AL is the highest in PUFA fraction, which somehow correlates with the high consumption of meat and vegetable oils in the Mexican population and the very low power consumption of n-3 fatty acids (fish and vegetables). The presence of trans FA in the membranes affects elongase activity of desaturases and affecting the synthesis of fatty acids of long chain [25]. Such is the case of the ARA, which is the precursor of prostaglandins (PG). Cook and Emken studies [26] demonstrate that the presence trans FA decreases synthesis of PG_1 and PG_3, which have anticoagulant activity. PUFA n-3 and n-6 also compete for incorporation (esterification) in membrane lipid fractions (phospholipids and triglycerides) and n-6 PUFA may counteract the potential cardiovascular benefits of PUFA n-3 [27]. n-6 has a higher concentration in erythrocyte membrane, but the n-6/n-3 ratio remains high with respect to the recommendations of the WHO, the greatest contribution of n-3 with children under 1 year comes from breast milk and supplementation that occurs through consuming artificial formulas. The results showed here *trans* FA correlate not significantly with the presence of n-6 PUFA. Moreover, the results obtained *trans* FA (0.7 mg/100 mL) are higher than those found by Cortés E, *et al.* [27] who found the elaidic acid at concentrations of 0.3 % in the erythrocyte membrane. *Trans* FA are incorporated into membrane phospholipids; increase their melting point, which affects membrane fluidity. This parameter is crucial for the regulation of the activity of molecules that are embedded in the membrane (enzymes, receptors, transporters, ion channels, etc.). *Trans* FA, have a higher melting point and its counterpart *cis* similar to saturated fatty acids spatial orientation, thereby lowering membrane fluidity, modulating the activity differently from proteins embedded in it, causing alterations in cell structure and its functions. *Trans* FA, increase the osmotic fragility of the erythrocytes, producing swelling and reduced consumption of oxygen and ATP production in mitochondria, favor the development of experimental arrhythmias in myocites cells, inhibit the Na/K ATPase, and modify the activity adenylate cyclase enzyme, which forms cyclic AMP [28].

5. CONCLUSIONS

We have found wide variation across this study in the nutrient intake in this child group. However, although the study shows constant values in the concentration of fatty acids in erythrocyte membrane in the age groups studied, the diet influences the increase or decrease of certain fatty acids in erythrocyte membrane. Food is the basis of good performance organism [2, 29]. The study shows, however, low concentrations of n-3 PUFAs in the erythrocyte membrane, which apparently is highly correlated, with low intakes of vegetables, edible oil and fish as major sources of these fatty acids.

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ETHICAL APPROVAL

The informed consent of the subjects parents was obtained according "The Mexican Health General Law" before obtains any sample or data.

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