



Original/*Pediatría*

## Levels of eicosapentaenoic acid in obese schoolchildren with and without insulin resistance

Karmina Sánchez Meza<sup>1</sup>, Carlos Enrique Tene Pérez<sup>1</sup>, Carmen Alicia Sánchez Ramírez<sup>1</sup>, Roberto Muñoz Valencia<sup>2</sup> and Mario Del Toro Equihua<sup>1</sup>

<sup>1</sup>Universidad de Colima, Facultad de Medicina. Av. Universidad 333, Colonia Las Víboras, Colima, Col., México. CP 28010.

<sup>2</sup>Universidad de Colima, Facultad de Ciencias Químicas. Kilómetro 9 carretera Colima-Coquimatlán, Col. México. CP 28400. México.

### Abstract

**Background:** Obesity in children is now an increasing health risk worldwide in which the insulin-resistance can be present. Studies have linked a diet rich in n-3 fatty acids with a lower prevalence of insulin-resistance.

**Objective:** To compare the levels of eicosapentaenoic acid among obese children with and without insulin-resistance.

**Materials and Methods:** In 56 randomly school-age children with obesity, insulin-resistance was determined by the homeostasis model assessment for insulin-resistance index and the serum levels of eicosapentaenoic acid were determined by gas chromatography. Insulin-resistance was established when the index was >6.0, non-insulin-resistance when that index was <1.4 and as an intermediate group when the index was within the range of 1.4-5.9. The serum levels of eicosapentaenoic acid were compared with the Kruskal-Wallis and Mann-Whitney U tests, as needed.

**Results:** No differences in age or sex were identified among the groups studied. The anthropometric parameters were significantly higher in the group of children with insulin-resistance than in the other two groups. The children with insulin-resistance had significantly lower levels of eicosapentaenoic acid than the non-insulin-resistance group [12.4% area under the curve vs. 37.4%,  $p = 0.031$ ], respectively.

**Conclusion:** Obese primary school-aged children with insulin-resistance had lower plasma levels of eicosapentaenoic acid.

(Nutr Hosp. 2015;31:1102-1108)

DOI:10.3305/nh.2015.31.3.8047

Key words: *Eicosapentaenoic acid. Insulin resistance. Obesity. Polyunsaturated fat. Schoolchildren.*

**Correspondence:** Ph. D. Carlos Enrique Tene Pérez.  
Universidad de Colima. Facultad de Medicina.  
Av. Universidad 333, Colonia Las Víboras,  
Colima, Col., México. CP 28010.  
E-mail: carlostene1@hotmail.com

Recibido: 6-IX-2014.  
Aceptado: 12-X-2014.

### NIVELES DE ÁCIDO EICOSAPENTAENOICO EN ESCOLARES OBESOS CON Y SIN RESISTENCIA A LA INSULINA

#### Resumen

**Introducción:** La obesidad en los niños es un problema de salud pública en todo el mundo y la resistencia a la insulina puede estar presente. Existen estudios publicados que han relacionado una dieta rica en n-3 ácidos grasos con una menor prevalencia de resistencia a la insulina en sujetos obesos.

**Objetivo:** Comparar los niveles de ácido eicosapentaenoico en niños escolares obesos con y sin resistencia a la insulina.

**Métodos:** Se eligieron al azar 56 niños en edad escolar con obesidad, a los cuales se les determinó resistencia a la insulina mediante la evaluación del modelo de homeostasis para el índice de resistencia a la insulina y se determinaron los niveles séricos de ácido eicosapentaenoico por cromatografía de gases. La resistencia a la insulina se estableció cuando el índice fue > 6,0, no resistencia a la insulina cuando ese índice fue <1,4 y como un grupo intermedio cuando el índice estaba dentro del rango de 1.4 a 5.9. Los niveles séricos de ácido eicosapentaenoico se compararon entre los grupos estudiados con las pruebas de Kruskal-Wallis y Mann-Whitney U, según fue necesario.

**Resultados:** No hubo diferencias en la edad o el sexo entre los grupos estudiados. Los parámetros antropométricos fueron significativamente mayores en el grupo de niños con resistencia a la insulina que en los otros dos grupos. Los niños con resistencia a la insulina tenían niveles significativamente más bajos de ácido eicosapentaenoico que el grupo que no tenía resistencia a la insulina [área 12,4% bajo la curva frente a 37,4%,  $p = 0,031$ ], respectivamente.

**Conclusión:** los niños en edad escolar obesos con resistencia a la insulina presentaron niveles plasmáticos más bajos de ácido eicosapentaenoico que los niños escolares obesos sin resistencia a la insulina.

(Nutr Hosp. 2015;31:1102-1108)

DOI:10.3305/nh.2015.31.3.8047

Palabras clave: *Acido eicosapentaenoico. Resistencia a insulina. Obesidad. Grasas poliinsaturados. Escolares.*

## Introduction

Obesity in children is now an increasing health risk worldwide. In Mexico, the 2012 National Health and Nutritional Survey reported the prevalence of overweight and obesity in primary school-aged children (5-11 years old) at 34.4% (19.8 and 14.6%, respectively), according to the WHO criteria. It was 32% (20.2 and 11.8%, respectively) for girls and 36.9% (19.5 and 17.4%, respectively) for boys<sup>1</sup>. Insulin resistance (IR), which can be identified in early childhood, is the common denominator found in the association of obesity with chronic degenerative diseases (CDD) such as type 2 diabetes mellitus, hypertension, dyslipidemia, and carotid-artery atherosclerosis<sup>2,3</sup>. IR is characterized by an impaired response of insulin-sensitive organs to physiological circulating levels of the hormone insulin<sup>4</sup>. Insulin signaling has been described as being controlled by nutrient-sensitive pathways and proinflammatory signals. Recently, several published studies have linked a diet rich in n-3 fatty acids (FAs) with a lower prevalence of IR and diabetes, due to the anti-inflammatory properties that influence several key cardio-metabolic parameters; however, the results of the studies have been inconsistent<sup>5,6,7,8,9,10,11</sup>. The n-3 FAs known as essential FAs are  $\alpha$ -linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). These n-3 fatty acids are found mainly in fish, shellfish, sea mammals, plant oils, and several vegetables<sup>12</sup>. The aim of the present study was to compare the levels of EPA in obese schoolchildren with and without IR.

## Material and Methods

### Protocol

**Design:** Cross-sectional. **Inclusion and setting:** The study was performed on children attending the obesity clinic at a primary care hospital of the *Instituto Mexicano del Seguro Social* in Colima, Mexico, during 2012. **Sampling:** The population was comprised of fifty-six randomly selected schoolchildren presenting with obesity. The mean age was 109.2 months (22.2 SD); 21/56 (37.5%) were girls. Children that presented with diabetes or other chronic diseases, or whose anthropometrical data were missing, were excluded from the study.

### Anthropometric and body composition assessments

All measurements were carried out by trained technicians. Before the data were collected, the main author and two collaborators performed an anthropometrical standardization trial evaluating consistency (intra-group individual measurements) and validity (inter-group comparison with a gold standard) through

Pearson's bivariate correlations; when the "r" was below 0.8, the anthropometrical technique was reviewed and corrected until the desired intra and inter-group correlations were achieved. For weight and height measurements a digital scale with a 64-214 cm long stadiometer (Brand TANITA WB-3000) was used. The children were measured according to standard procedures, wearing lightweight clothing and without shoes<sup>13</sup>. BMI was calculated as weight (kg) divided by height squared (m<sup>2</sup>). The definition of obesity for the children was based on the age-specific BMI Z scores established by the WHO and those children that presented with standard deviations  $\geq +2$  were classified as obese<sup>14</sup>. Waist circumference (WC) was measured using a fiberglass tape placed above the uppermost lateral border of the right ilium, at the end of a normal expiration, and was recorded to the nearest millimeter. This measurement was taken with the child standing upright, feet together, and arms hanging freely at the sides<sup>15</sup>.

### IR determination

A 5 mL fasting blood sample was collected in tubes containing EDTA sodium. Plasma was immediately separated by centrifugation at 3000 rpm at 4 °C for 10 min, and serum was collected by centrifugation at 1000 rpm at room temperature for 10 min. The tubes were labeled and stored at -20 °C; they were stored at -75 °C for fatty acid measurement.

Plasma glucose was determined by the glucose oxidase method (Spinreact, SPAIN) and plasma insulin was determined by the enzyme-linked immunosorbent assay (ELISA) (Alpco<sup>MR</sup> USA). IR was estimated by the homeostasis model assessment for insulin resistance (HOMA-IR) index as  $HOMA-IR = (\text{fasting insulin } (\mu\text{U/L}) \times \text{fasting plasma glucose (mg/dL)}) / 22.5$ . IR was established by a HOMA-IR index above 6.0 and non-IR by a HOMA-IR index below 1.4. A third group was the intermediate group (IG) and was made up of children with a HOMA-IR index in the range of 1.4-5.9.

### EPA determination

Total plasma lipids and FAs were extracted applying the Folch method<sup>16</sup>, using a chloroform:methanol solution (2:1, vol:vol). The extracts were analyzed by means of coupled gas chromatography-mass spectrometry, employing a Varian 3900 gas chromatograph equipped with a Saturn 2100T mass spectrometer detector (Varian, Palo Alto, CA, USA.). The Omegawax 320 capillary column (30m x 0.32 mm x 0.25  $\mu\text{m}$ ) was used. FAs were identified by comparison with a FA standard mixture (Sigma-Aldrich, USA). Results were expressed as % of total FA in plasma. Areas under the curve (AUC) of the EPA chromatographic peaks were calculated for each child.

## Other variables

Sports was defined as any form of physical activity, whether casual or organized, that was aimed at expressing or improving physical fitness and mental well-being, at forming social relationships, or at obtaining results in competitions at all levels<sup>17</sup>. It was assessed through a questionnaire by answering “yes” or “no” and classified into 2 groups: fewer than 3 times a week or more than 3 times a week.

N-3 fatty acid consumption was determined by a food frequency questionnaire, in which the schoolchildren and their parents were the respondents. Trained interviewers conducted the food-frequency questionnaire, asking subjects to recall “average intake over the last six months”; the answers were: never, once a month or less, 2-3 times a month, once a week, 2-3 times a week, 4-7 times a week, once a day, and 2-3 times a day. The list of foods included eggs, oils, avocados, almonds, walnuts, fish oil, cod-liver oil, fish (anchovy, sardine, salmon, and herring), flax seeds, and soy. The intake of any of these food items at least 4 times a week was regarded as adequate.

## Statistical analysis

The data were analyzed using the SPSS software, version 20. The variables studied were described as frequencies and percentages or as medians and interquartile ranges; inferential statistics were addressed by the Mann-Whitney U, Kruskal-Wallis, and chi-square tests. Statistical significance was set at a p value < 0.05.

## Ethics

Informed consent was obtained from the children’s parents or guardians. The study protocol was approved by the Ethics Committee of the *Instituto Mexicano del Seguro Social* (# R-2011-601-26), which conforms to the guidelines of the Declaration of Helsinki in regard to the principles of beneficence, non-maleficence, justice, and autonomy of decision. (Ethical Principles for Medical Research Involving Human Subjects. 52nd release, October 2000).

## Results

*Demographic and anthropometric characteristics.* As shown in table I, no differences in age or sex were found among the three groups. When comparing the IR group of children, the intermediate group and the non-IR group of children, the anthropometric parameters of height, weight, BMI, and waist circumference were significantly higher in the IR group than in the other two groups. The comparison of age, height, weight, BMI, and WC between girls and boys showed no significant differences (Table II).

*Biochemical characteristics.* As shown in table III, the serum glucose levels were higher in the IR group of children, without being significant. However, the comparison of the glucose level of the IR children with the non-IR group was significant (p=0.012). In the three groups the glucose levels remained within normal values. Of the total series, 8/56 (14.3%) of the children were diagnosed

**Table I**  
Comparison of demographic and anthropometric characteristics in three groups of children (non-IR group, intermediate group (IG) and IR group)<sup>1</sup>

	Insulin resistance <sup>2</sup>			P-value*
	non-IR (n=8)	IG (n=36)	IR (n=12)	
<b>Age</b> (Months)	<b>124.0</b> (121-133)	<b>110.5</b> (94-125)	<b>101.5</b> (81-114)	0.080*
<b>Sex</b> (Girls/Boys)	5/3	14/22	3/9	0.227**
<b>Height</b> (m)	<b>1.50</b> (1.4-1.6)	<b>1.43</b> (1.3-1.5)	<b>1.39</b> (1.3-1.5)	0.187*
<b>Weight</b> (Kg)	<b>65.0</b> (61-75)	<b>52.1</b> (40-60)	<b>46.0</b> (36-52)	<b>0.010*</b>
<b>Waist circumference</b> (cm)	<b>95.50</b> (92-98)	<b>86.00</b> (78-91)	<b>78.50</b> (72-86)	<b>0.011*</b>
<b>BMI***</b> (kg/m <sup>2</sup> )	<b>29.4</b> (27-32)	<b>25.9</b> (24-29)	<b>23.4</b> (22-24)	<b>0.002*</b>

<sup>1</sup>Data are reported as median and interquartile range.

<sup>2</sup>The presence or absence of IR was determined by HOMA-IR (homeostasis model assessment for insulin resistance) as IR >6.0 and non-IR <1.4, respectively).

\*The p values were calculated with the Kruskal-Wallis and \*\* Chi-square tests.

\*\*\*BMI (body mass index).

**Table II**  
Comparison of demographic and anthropometric characteristics between girls and boys

	Girls (n=21)	Boys (n=35)	P-value*
<b>Age (Months)</b>	<b>112.0</b> (88-123)	<b>113.0</b> (99-128)	0.330
<b>Height (m)</b>	<b>1.45</b> (1.3-1.5)	<b>1.44</b> (1.3-1.5)	0.953
<b>Weight (Kg)</b>	<b>49.2</b> (38-66)	<b>52.4</b> (43-61)	0.710
<b>Waist circumference (cm)</b>	<b>86.00</b> (77-90)	<b>87.0</b> (80-92)	0.330
<b>BMI** (kg/m<sup>2</sup>)</b>	<b>24.4</b> (22-29)	<b>27.1</b> (24-28)	0.330

<sup>1</sup>Data are reported as median and interquartile range

<sup>2</sup>\*The p values were calculated with the Mann-Whitney U test.

\*\*BMI: body mass index.

**Table III**  
Comparison of biochemical characteristics and serum levels of EPA between girls and boys<sup>1</sup>

	Girls (n=21)	Boys (n=35)	P-value <sup>3</sup>
<b>Glucose (mg/dl)</b>	<b>84.7</b> (79-92)	<b>85.6</b> (82-90)	0.678
<b>Insulin (μUI/ml)</b>	<b>16.0</b> (10-28)	<b>10.7</b> (6-19)	0.053
<b>Homa-IR<sup>2</sup></b>	<b>3.559</b> (2.07-6.58)	<b>2.360</b> (1.39-3.87)	0.077
<b>Eicosapentaenoic acid (% AUC)*</b>	29.82 (6.3-57.5)	<b>23.75</b> (8.0-48.7)	0.939

<sup>1</sup>Data are reported as median and interquartile range.

<sup>2</sup>The presence or absence of IR was determined by HOMA-IR (homeostasis model assessment for insulin resistance) as IR >6.0 and non-IR <1.4, respectively).

<sup>3</sup>The p values were calculated with the Mann-Whitney U test.

\*%AUC: % of area under the curve.

with IR and the serum levels of insulin were significantly higher in this group compared with the non-IR group or the IG. Insulin levels in the non-IR group of children were within normal values. When comparing the serum biochemical parameters between girls and boys, we identified higher levels of insulin in girls that almost reached statistical significance, as well as a trend towards a higher HOMA-IR index in girls (Table III).

*EPA serum levels.* When comparing the serum levels of EPA among the three study groups, children with IR had significantly lower levels of this FA than the non-IR group (Table IV and Fig. 1).

*Dietary intake and physical activity.* When analyzing other variables such as sports and n-3-FA intake, we found that 16/56 (28.6%) of the children played a sport more than 3 times a week. Ninety-one percent (51/56) of the study children consumed n-3-FAs more than 4

times a week. The comparison of these two variables between the IR group vs. the non-IR group showed no significant difference (p=0.701) in relation to sports and (p=0.213) n-3 FA intake. When comparing the EPA serum levels with the dietary intake of n-3 fatty acids, we found that the levels were statistically higher in the children with an adequate intake (p=0.008)

## Discussion

One fourth of the obese children enrolled in the present study had IR. When we compared the plasma levels of EPA among the three study groups, the IR group had significantly lower levels. The results of studies conducted on children and adults associating n-3 FAs and IR are inconsistent, and in addition, the

**Table IV**  
*Comparison of biochemical characteristics and serum levels of EPA in three groups of children (non-IR group, intermediate group (IG) and IR group)<sup>1</sup>*

	Insulin Resistance <sup>2</sup>			P-value <sup>3</sup>
	IR (n=8)	IG (n=36)	non-IR (n=12)	
<b>Glucose</b> (mg/dL)	<b>93.4</b> (86-102)	<b>85.3</b> (82 - 89)	<b>84.2</b> (79-89)	0.188
<b>Insulin</b> (μUI/mL)	<b>35.6</b> (31-45)	<b>14.3</b> (10-18)	<b>5.1</b> (3-6)	<b>&lt;0.0001</b>
<b>Homa-IR<sup>2</sup></b>	<b>8.240</b> (6.86-10.23)	<b>2.770</b> (2.25-3.84)	<b>1.005</b> (0.61-1.27)	<b>&lt;0.0001</b>
<b>Eicosapentaenoic acid</b> (% Area under the curve)	<b>12.4</b> (4-26)	<b>24.8</b> (8-48)	<b>37.4***</b> (23-53)	<b>0.044**</b>

<sup>1</sup>Data are reported as median and interquartile range.

<sup>2</sup>The presence or absence of IR was determined by HOMA-IR (homeostasis model assessment for insulin resistance) as IR >6.0 and non-IR <1.4, respectively).

<sup>3</sup>The p values were calculated with the Kruskal-Wallis test\*\*.

\*\*\*p=0.031 when comparing the IR and non-IR groups (Mann-Whitney U test).

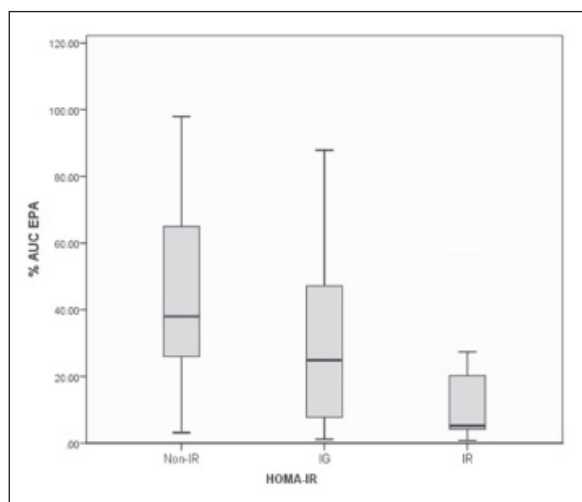
number of studies involving children is much lower. A study done by Saito *et al.* on 32 obese children (12 ± 2.6 years of age) that aimed to identify the relationships between DHA content and the components of metabolic syndrome, among others, found that the DHA content in plasma phospholipids was lower in children with metabolic syndrome than in those that did not present with the disorder; however, the difference was not significant (p=0.076)<sup>18</sup>. Baur *et al.* investigated the interrelationships between the type of infant feeding, skeletal muscle biopsies, and fasting blood samples in children under 2 years of age in whom the muscle phospholipid FA composition was analyzed and found significant inverse correlations between fasting

plasma glucose and the percentage of DHA (r=0.47, p<0.003) and the total levels of polyunsaturated FAs (r=0.38, p<0.05). They concluded that early changes in skeletal muscle membrane phospholipid FA saturation may play a role in the subsequent development of diseases associated with IR<sup>19</sup>.

A randomized controlled trial of DHA supplementation vs. placebo in 60 children with biopsy-confirmed non-alcoholic fatty liver disease (NAFLD) identified the odds of more severe versus less severe liver steatosis after treatment as being lower in children treated with DHA compared with placebo. They also found that the insulin sensitivity index increased and triglycerides decreased to a similar degree in the groups that received DHA as compared with placebo<sup>20</sup>.

A group of researchers in Denmark studied n-3 FAs in children and adolescents that presented with signs of metabolic syndrome and found the interesting phenomenon of beneficial associations between n-3 FAs and HDL. However, DHA was unexpectedly associated with higher mean arterial pressure<sup>21</sup>. A previous study carried out on adolescents by the same Danish group found that DHA status was significantly correlated with higher blood pressure and increased fasting insulin<sup>22</sup>. Another study performed by Rump *et al.* determined the FA concentrations in umbilical cord blood; when those children were seven years old, fasting glucose, insulin, proinsulin, and leptin levels were measured (n=259). The n-3 FA concentrations were negatively related to insulin concentrations, HOMA-IR values, body fat, and proinsulin and leptin levels. The highest insulin concentrations were found in children with a low birth weight and a low gamma-linolenic acid concentration at birth<sup>23</sup>.

Studies conducted on adults have also produced controversial results. Zhu *et al.* determined the rela-



*Fig. 1.—Percentage of area under the curve (% AUC) of eicosapentaenoic acid in non-IR (HOMA <1.4), IG (HOMA 1.5-5.9), and IR (HOMA >6.0) obese children.*



relationship between serum omega-3 PUFAs (polyunsaturated fatty acids) and IR in 51 patients with type 2 diabetes mellitus and NAFLD and found a modest negative correlation between HOMA-IR values and n-3-FA serum levels ( $r = -0.491$ ,  $p < 0.05$ ). In an Inuit population of individuals 18 years of age or older (452 participants)<sup>24</sup>, Thorseng *et al.* determined the contents of n-3 FAs and the n-3/n-6 ratio in erythrocyte membrane phospholipids and found a significant inverse association between n-3 FAs and the n-3/n-6 ratio and HOMA-IR values. Other authors have found the contrary; in a randomized trial of 258 subjects 45-70 years of age<sup>25</sup>, Griffin *et al.* compared the effects on insulin sensitivity of modifying the dietary n-3 and n-6 fatty acid ratio and found that dietary intervention had no effect on insulin sensitivity<sup>7</sup>. Another study performed on 162 healthy adults that were fed two different iso-energetic diets supplemented with 3.6 g/d of n-3 FA or olive oil (placebo) for 3 months, found no improvement in insulin sensitivity, insulin secretion, beta cell function, or glucose tolerance<sup>8</sup>.

Various methods have been established to define insulin sensitivity. The euglycemic clamp and modified minimal model are considered the gold standards, but they are complex and invasive tests and are applied mainly for research purposes<sup>26</sup>. The HOMA-IR index is another method for determining insulin resistance and is frequently used in the field of clinical research<sup>27</sup>. In addition, there is no consensus in the literature on the definition of IR in children. The cut-off values of the HOMA-IR index and plasma insulin levels in children vary according to the authors. In the present series, IR in children was defined as a HOMA-IR value above 6. Previous studies conducted on Mexican schoolchildren have defined IR as a HOMA-IR value higher than 3.88<sup>28</sup>. Juarez *et al.* carried out a study on 466 obese children between the ages of 11 and 13 years in Campeche, Mexico, and defined IR using the HOMA-IR with a cut-off point above 3.4. When classifying four categories for HOMA-IR values, they also found that a cut-off point higher than 5.5, which is above the 75th percentile, increased the risk of metabolic syndrome, compared with the lowest HOMA-IR percentile, which was a cut-off point below 2.4<sup>29</sup>. Kurtoglu *et al.* defined IR values according to a HOMA-IR value of 2.7 in boys and 2.2 in girls in the pre-pubertal period, and 5.2 in boys and 3.8 in girls in the pubertal period. Other criteria that can be considered are the fasting insulin levels; at above 15  $\mu\text{U/mL}$  in the pre-pubertal period, 30  $\mu\text{U/mL}$  in the pubertal period, and 20  $\mu\text{U/mL}$  in the post-pubertal period, they are regarded as hyperinsulinism and consequently, IR. According to these latter criteria, in the present study, 23/56 (41.1%) of the children could be regarded as presenting with IR, which is a higher frequency than the one we reported with the HOMA-IR index cut-off point above 6<sup>30</sup>.

To the best of our knowledge, this study is the first to assess the association between serum levels of EPA in plasma and IR determined by the HOMA-IR index

in Mexican schoolchildren. Our study has two weak points: the first is that the EPA was measured in serum, and previous studies have reported that n-3 FA determination in serum reflects the intake of the past two weeks, whereas the measurement of n-3 FA in adipose tissue and erythrocyte membranes enables the intake over the past weeks and months to be estimated<sup>31,32</sup>. The second weak point is that the Tanner stage was not evaluated and even though the study was only conducted on primary school-aged children, it is well known that transient insulin resistance develops in children during puberty, a condition that is accepted to be physiological<sup>30</sup>.

The data of the present study suggest the significant roles that the n-3 FAs play, specifically the relation of EPA serum levels to insulin sensitivity. These findings highlight the importance of the nutrient intake goal of representing 1-2% of the total energy intake, within a n6/n3 ratio from 5-10 to 1<sup>33</sup>, in order to reduce the development of IR and all its associated co-morbidities<sup>34,35,36</sup>. Further studies on children are required to determine the role that variables such as energy intake, dietary habits, and socio-economic status, among others, play in the association of n-3 FAs with IR.

In conclusion, the results of the present series showed that obese schoolchildren with IR had lower plasma levels of EPA, and therefore nutritional intervention should encourage the consumption of n-3 FAs in the diet of both obese and non-obese children as a preventive care measure.

## References

- Gutierrez JP, Rivera DJ, Shamah LT, et al. Encuesta Nacional de Salud y Nutricion 2012. Resultados Nacionales. Cuernavaca, Mexico: Instituto Nacional de Salud Publica (MX), 2012.
- Abeywardena MY, Patten GS. Role of  $\omega 3$  Long-chain Polyunsaturated Fatty Acids in Reducing Cardio-metabolic Risk Factors. *Endocr Metab Immune Disord Drug Targets*. 2011; 11: 232-246.
- Pinel A, Morio LB, Capel F. n-3 polyunsaturated fatty Acids modulate metabolism of insulin-sensitive tissues: implication for the prevention of type 2 diabetes. *J Physiol Biochem*. 2013; 2: 647-58.
- Schenk S, Saberi M, Olefsky JM. Insulin sensitivity: modulation by nutrients and inflammation. *J Clin Invest*. 2008;118: 2992-3002.
- Fedor D and Kelley DS. Prevention of insulin resistance atubey n-3 polyunsaturated fatty acids. *Curr Opin Clin Nutr Metab Care*. 2009;12:138-146.
- Lombardo YB and Chicco AG. Effects of dietary polyunsaturated n-3 fatty acids on dyslipidemia and insulin resistance in rodents and humans. A review. *J Nutr Biochem*. 2006;17:1-13.
- Griffin MD, Sanders TA, Davies IG, et al. effects of altering the ratio of dietary n-6 to n-3 fatty acids on insulin sensitivity, lipoprotein size, and postprandial lipemia in men and postmenopausal women aged 45-70 y y: the OPTILIP Study. *Am J Clin Nutr*. 2006; 86:1670-1679.
- Giacco R, Cuomo V, Vessby B, et al. Fish oil, insulin sensitivity, insulin secretion and glucose tolerance in healthy people: is there any effect of fish oil supplementation in relation to the type of background diet and habitual dietary intake of n-6 and n-3 fatty acids? *Nutr Metab Cardiovasc Dis*. 2007; 17: 572-580.

9. Tsitouras PD, Gucciardo F, Salbe AD, Heward C, and Harman SM. High omega-3 fat intake improves insulin sensitivity and reduces CPR and IL6, but does not affect other endocrine axes in healthy older adults. *Horm. Metab. Res.* 2008; 40: 199-205.
10. Bloedon LT, Balikai S, Chittams J, Cunnane SC, Berlin JA, Rader DJ et al. Flaxseed and cardiovascular risk factors: results from a double blind, randomized, controlled clinical trial. *J. Am. Coll. Nutr.* 2008; 27: 65-74.
11. Browning LM, Krebs JD, Moore CS, Mishra GD, O'Connell MA, and Jebb SA. The impact of long chain n-3 polyunsaturated fatty acid supplementation on inflammation, insulin sensitivity and CVD risk in a group of overweight women with an inflammatory phenotype. *Diabetes Obes. Metab.* 2007; 9: 70-80.
12. Nichols PD, Petrie J, and Singh S. Long-Chain Omega-3 Oils—An Update on Sustainable Sources. *Nutrients.* 2010; 2: 572-585.
13. Frisancho, AR, Tracer SP. Standards of arm muscle by stature for the assessment of nutritional status of children. *Am J Phys Anthropol.* 1987; 73: 459-465.
14. World Health Organization. Global Database on Child Growth and Malnutrition. Cut-off points and summary statistics. WHO, Geneva 2007 [Internet]. Available from: <http://www.who.int/nutgrowthdb/about/introduction/en/index5.html>
15. Fernandez J, Redden D, Pietrobelli A, Allison D. Waist circumference percentiles in nationally representative samples of African-American, European-American and Mexican-American children and adolescents. *J Pediatr.* 2004; 145: 439-44.
16. Folch J, Lees M, Sloane SG. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem.* 1957; 226: 497-509.
17. World Health Organization. Promoting sport and enhancing health in European Union countries: a policy content analysis to support action. WHO, Geneva 2011 [Internet]. Available from: <http://www.euro.who.int/pubrequest>.
18. Saito E, Okada T, Abe Y, Kuromori Y, Miyashita M, Iwata F, Hara M, Ayusawa M, et al. Docosahexaenoic Acid Content in plasma phospholipids and Desaturase Indexes in obese children. *J Atheroscler Thromb.* 2011; 18: 345-350.
19. Baur LA, O'Connor J, Pan DA, Kriketos AD, Storlien LH. The fatty acid composition of skeletal muscle membrane phospholipid: its relationship with the type of feeding and plasma glucose levels in Young children. *Metabolism.* 1998; 47: 106-12.
20. Nobili V, Bedogni G, Alisi A, Pietrobattista A, Risé P, Galli C, et al. Docosahexaenoic acid supplementation decreases liver fat content in children with non-alcoholic fatty liver disease: double-blind randomized controlled clinical trial. *Arch Dis Child.* 2011; 96: 350-3.
21. Damsgaard CT, Stark KD, Hjorth MF, Biloft JA, Astrup A, Michaelsen KF, et al. n-3 PUFA status in school children is associated with beneficial lipid profile, reduced physical activity and increased blood pressure in boys. *Br J Nutr.* 2013;110:1304-12.
22. Lauritzen L, Harsløf LB, Hellgren LI, Pedersen MH, Mølgaard C, Michaelsen KF. Fish intake, erythrocyte n-3 fatty acid status and metabolic health in Danish adolescent girls and boys. *Br J Nutr.* 2012; 107:697-704.
23. Rump P, Popp-Snijders C, Heine RJ, Hornstra G. Components of the insulin resistance syndrome in seven-year-old children: relations with birth weight and the polyunsaturated fatty acid content of umbilical cord plasma phospholipids. *Diabetologia.* 2002; 45: 349-55.
24. Zhu QQ, Lou DJ, Si XW, Guan LL, You QY, Yu ZM, et al. Serum omega-3 polyunsaturated fatty acid and insulin resistance in type 2 diabetes mellitus and non-alcoholic fatty liver disease. *Zhonghua Za Zhi Ke Nei. China.* 2010; 4:305-8.
25. Thorseng T, Witte DR, Vistisen D, Borch-Johnsen K, Bjerregaard P, Jørgensen ME. The association between n-3 fatty acids in erythrocyte membranes and insulin resistance: the Inuit Health in Transition Study. *Int J Circumpolar Health.* 2009; 68: 327-36.
26. Beguinot F. y Nigro C. Measurement of glucose homeostasis in vivo: glucose and insulin tolerance tests. *Methods Mol Biol.* 2012; 933: 219-28.
27. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 1985; 28: 412-419.
28. Valdés RR, Moncada GB, Rivera RS, Aradillas GC, Hernández RH and Torres ÁB. Acrocordones y Acanthosis nigricans: correlación con resistencia a la insulina y sobrepeso en niños mexicanos. Piel y resistencia a la insulina en niños. *Gac Med Mex.* 2011; 147: 297-302.
29. Juárez LC, Klünder KM, Medina BP, Madrigal AA, Mass DE, and Flores HS. Insulin resistance and its association with the components of the metabolic syndrome among obese children and adolescents. *BMC Public Health.* 2010; 10: 318-325.
30. Kurtoglu S., Hatipoglu N., Mazıcioglu M., Kendirici M., Keskin M., and Kondolot M. Resistencia a la insulina en niños y adolescentes obesos: HOMA-IR niveles límites en los períodos prepúberes y púberes. *J Pediatr Clin Res Endocrinol.* 2010; 2: 100-106.
31. Glatz JF, Soffers AE, Katan MB. Fatty acid composition of serum cholesteryl esters and erythrocyte membranes as indicators of linoleic acid intake in man. *Am J Clin Nutr.* 1989; 49: 269-276.
32. Katan MB, Deslypere JP, Birgelen AP, Penders M, Zegwaard M. Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18 month controlled study. *J Lipid Res.* 1997;38: 2012-2022.
33. Azab SF, Saleh SH, Elsaeed WF, Elshafie MA, Sherief LM, Esh AM. Serum trace elements in obese Egyptian children: a case-control study. *Ital J Pediatr.* 2014; 40:20.
34. Yamakawa K, Shimabukuro M, Higa N, Asahi T, Ohba K, Osamu A, et al. Eicosapentaenoic Acid Supplementation Changes Fatty Acid Composition and Corrects Endothelial Dysfunction in Hyperlipidemic Patients. *Cardiol Res Pract.* 2012; 10: 1155-1164.
35. Gillingham LG, Harding SV, Rideout TC, Yurkova N, Cunnane SC, Eck PK, Jones PJ. Dietary oils and FADS1-FADS2 genetic variants modulate [<sup>13</sup>C]α-linolenic acid metabolism and plasma fatty acid composition. *Am J Clin Nutr.* 2013; 97:195-207.
36. Tarlton JF, Wilkins LJ, Toscano MJ, Avery NC, Knott L. Reduced bone breakage and increased bone strength in free range laying hens fed omega-3 polyunsaturated fatty acid supplemented diets. *Bone.* 2013; 52: 578-86.