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Higher levels of C-reactive protein associated with higher adiposity in Mexican schoolchildren

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Abstract

Introduction: The development of chronic-degenerative diseases secondary to obesity in early infancy has alerted health providers to the importance of identifying the risk factors for obesity and assessing preventive treatment to reduce risks. Studies performed on a pediatric population have examined the role of inflammatory biomarkers (specifically CRP and TNF-α) and adiposity with inconsistent results.

Objective: To assess the relationship between the serum levels of C-reactive protein and tumor necrosis factor-alfa with adiposity measured by bioimpedance analysis in schoolchildren.

Methods: Cross sectional design. Data were collected from 74 schoolchildren randomly selected in a local primary school in the city of Colima, Mexico. The mean age was 9.4 years (1.5, SD); 33 (44.6%) were girls. The adiposity (percentage of fat mass) was measured using bioimpedance analysis and anthropometric measurements. Serum C-reactive protein and tumor necrosis factor alpha were determined with enzyme-linked immunosorbent assay. The association between adiposity and serum inflammatory biomarkers was assessed with non parametric tests (Mann Whitney and Kruskall Wallis tests), and parametric tests (Pearson's correlation).

Results: Children with obesity had a significantly higher level of C-reactive protein [2.90 mg/L (0.07-9.37)] compared with children with a normal percentage of fat mass [0.71 mg/L (0.07-5.75)] ($p < 0.001$). No differences between groups were identified regarding serum levels of tumor necrosis factor-alfa. Modest correlations were identified between serum levels of C-reactive protein, adiposity determined by bioimpedance analysis ($r = 0.453$, $p < 0.001$); body mass index ($r = 0.398$, $p = 0.001$); triceps skinfold ($r = 0.369$, $p = 0.002$); and subescapular skinfold ($r = 0.405$, $p < 0.001$). No correlation was found between adiposity and serum tumor necrosis factor-alfa.

Conclusion: Subclinical inflammation manifested by higher serum levels of C-reactive protein was identified in schoolchildren with higher percentage of fat mass as determined by bioimpedance analysis and other anthropometric measurements.

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Keywords: *Schoolchildren adiposity. C-reactive protein. Tumor necrosis factor-alpha. Bioimpedance.*

LOS NIVELES ELEVADOS DE PROTEÍNA C REACTIVA SE ASOCIAN A UNA MAYOR ADIPOSIDAD EN ESCOLARES MEXICANOS

Resumen

Introducción: Las enfermedades crónico-degenerativas, secundarias a la obesidad en niños, ha alertado a los profesionales de la salud sobre la importancia de identificar los factores de riesgo asociados a obesidad y establecer un enfoque preventivo para reducir el riesgo de complicaciones. Algunos estudios realizados en población pediátrica han estudiado el papel de los biomarcadores inflamatorios [específicamente proteína C-reactiva (PCR) y factor de necrosis tumoral alfa (TNF-α)] asociado a adiposidad con resultados inconsistentes.

Objetivo: Evaluar la relación entre los niveles séricos de PCR y TNF-α con adiposidad determinada mediante impedancia bioeléctrica en niños en edad escolar.

Métodos: Diseño transversal analítico. Se recopilaron los datos de 74 alumnos seleccionados al azar en una escuela primaria local en la ciudad de Colima, México. La edad media fue de 9,4 años (1.5 DE), treinta y tres (44.6%) fueron niñas. La adiposidad (porcentaje de grasa) se midió mediante impedancia bioeléctrica y antropometría. La PCR y TNF-α séricos se determinaron con el ensayo por inmunoabsorción ligado a enzimas (ELISA). Las comparaciones de las variables entre los grupos se analizaron con pruebas no paramétricas (U de Mann Whitney y Kruskall Wallis) y la correlación de Pearson.

Resultados: Los niños con obesidad presentaron un nivel sérico [2,90 mg/L (0,07-9,37)] significativamente más alto de PCR en comparación con los niños con un porcentaje normal de grasa [0,71 mg/L (0,07-5,75)] ($p < 0,001$). Con respecto a los niveles séricos de TNF-α no se identificaron diferencias entre los grupos estudiados. Se obtuvieron correlaciones modestas entre los niveles séricos de PCR con la adiposidad determinado por impedancia bioeléctrica ($r = 0,453$, $p < 0,001$); índice de masa corporal ($r = 0,398$, $p = 0,001$); pliegue cutáneo tricipital ($r = 0,369$, $p = 0,002$), y pliegue cutáneo subescapular ($r = 0,405$, $p < 0,001$). No se encontró correlación entre la adiposidad y el nivel sérico de TNF-α.

Conclusión: La inflamación subclínica determinada por elevación de los niveles séricos de proteína C-reactiva fue identificada en niños en edad escolar con mayor porcentaje de grasa determinado por impedancia bioeléctrica y antropometría.

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Palabras clave: *Adiposidad en escolares. Adiposidad. Proteína C reactiva. Factor de necrosis tumoral alfa. Impendancia bioeléctrica.*

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Abbreviations

BIA: Bioimpedance analysis.
BF: Body fat.
BMI: Body mass index.
CDD: Chronic-degenerative diseases.
CRP: C-reactive protein.
CVD: Cardiovascular disease.
NW: Normal weight.
SSF: Subscapular skin fold.
TNF-α: Tumor necrosis factor alpha.
TSF: Triceps skin fold.
OB: Obese.
OF: Overfat.
ST: Skinfold thickness.
STE: Slaughter equation.
UF: Underfat.
WC: Waist circumference.

Introduction

The development of chronic-degenerative diseases (CDD) such as type 2 diabetes mellitus, hypertension, dyslipidemia, and carotid-artery atherosclerosis secondary to obesity in early infancy has alerted health providers to the importance of identifying the risk factors for obesity and assessing preventive treatment to reduce cardiovascular disease(CVD). It has been reported that an obese adult who was an overweight or obese child, has an increased risk for developing CDD.[1] Also, several studies have described the association between low grade chronic inflammation manifested by the elevation of inflammatory biomarkers including c-reactive protein (CRP) or inflammatory cytokines such as interleukin-6 and tumor necrosis factor alpha (TNF-α) with obesity, insulin resistance, and CVD.[2-8].

Studies performed on a pediatric population have examined the role of inflammatory biomarkers (specifically CRP and TNF-α) and adiposity with inconsistent results. Some of them have identified an association between higher serum concentrations of inflammatory biomarkers with higher measures of adiposity, but others have identified higher concentrations in children with a normal nutritional status compared with those that are obese.[2,4,7-11] In other respects, these studies have measured adiposity mainly by body mass index (BMI) or waist circumference (WC) and there are only a few that have measured adiposity with direct methods.[2,5]

The primary aim of this study was to assess the relationship between the serum levels of CRP and TNF-α with adiposity measured by bioimpedance analysis (BIA) in schoolchildren. The secondary aim was to correlate the percentage of fat by BIA, BMI, WC, triceps skin fold (TSF), and subscapular skin fold (SSF) with the serum concentrations of CRP and TNF-α.

Materials and methods

Patients and study design

Seventy-four schoolchildren randomly selected in a local primary school in Colima, Mexico were recruited in the study between november 2011-march 2012. The mean age was 9.4 years (1.5, SD); thirty-three (44.6%) were girls. A cross-sectional design was used in the study. Children with genetic, chronic, and systemic diseases, or current or recent infection were excluded. The dependent variables were serum concentrations of CRP and TNF-α and the independent variable was the adiposity determined by BIA.

Anthropometric assessment

Standardization: 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.[12,13]

Weight: Study subjects were weighed on a balance beam scale, without shoes and a minimum of clothing. Weight was recorded to the nearest 100 g.[14,15]

Height: Height was measured and recorded to the nearest 0.1 cm using a standiometer with a movable block. The subjects were measured while standing, without shoes, heels together, back as straight as possible, and arms hanging freely; the head was positioned in the Frankfort horizontal plane and the movable block was brought down until touching the head.[14,15]

Body mass index (BMI). Was calculated as weight (kg) divided by height squared (m^2).[12]

Triceps skinfold (TSF). The right arm was previously positioned bent at the elbow at a 90° angle, with the upper arm held parallel to the side of the body. The distance between the acromion and the olecranon was measured with a fiberglass tape and the midpoint between these two points was marked. The TSF was measured with a Lange skinfold caliper at the previously marked midpoint with the arm hanging loosely at the side of the body. The examiner grasped a vertical pinch of skin and subcutaneous fat between the thumb and forefinger about 1 cm above the previously marked midpoint, gently pulling the skin away from the underlying muscle. The skinfold caliper was placed at the marked midpoint while maintaining the skinfold grasp. Readings were taken in millimeters as soon as the caliper came in contact with the skin and the dial reading stabilized. The average of the three readings was recorded in mm.[15]

Subscapular skinfold (SSF). The SSF was lifted on a diagonal and inclined infero-laterally approximately

45 degrees to the horizontal plane of the natural cleavage lines of the skin. The site was just below the inferior angle of the scapula. The subject stood comfortably erect with the hands relaxed at the sides of the body. The examiner palpated the subject's scapula to locate the inferior border of the scapula, grasping a horizontal pinch of skinfold at about 1 cm below the inferior angle of the right scapula. The jaws of the caliper were applied 1 cm infero-lateral to the thumb and finger lifting the skinfold, and three readings were taken. The average of the three readings was recorded in mm.[15]

Waist circumference (WC). The WC was measured using a fiberglass tape above the uppermost lateral border of the right ilium, at the end of a normal expiration, and was recorded at the nearest millimeter. The measurement was made while the subject stood upright, with feet together and arms hanging freely at the sides. The WC was classified in the percentiles according to the pattern published by Fernandez, et al. in Mexican-American children.[17]

Bioimpedance analysis

All subjects that underwent BIA were asked not to eat, drink, or exercise 8 hrs before testing. The subjects were placed in the supine position with arms and legs abducted from the body. Shoes, socks, belts and other metallic pieces were removed and the areas where the electrodes were placed were previously cleaned with alcohol. Source electrodes were placed proximal to the phalangeal-metacarpal joint on the dorsal surface of the right hand and distal to the transverse arch on the superior surface of the right foot. Sensor electrodes were placed at the midpoint between the distal prominence of the radius and ulna of the right wrist and between the medial and lateral malleoli of the right ankle.[18,19] The BIA was performed using the QuadScand 4000 (Bodystat Limited, Great Britain); resistance and reactance values were provided by BIA and the percentage of fat mass was derived using the available BIA software.

The results of the percentage of fat were classified in four groups (underweight, normal, overweight, and obese) based on the body fat curves published by McCarthy et al.[20]

Measurement of CRP and TNF-α

Five milliliters of venous blood samples were collected in tubes without additives after fasting (8 h). The samples were stored on wet ice and the serum was separated by centrifugation. The separated serum was kept frozen at -75° C until assayed for biomarkers of inflammation.

An ultra-sensitive enzyme-linked immunosorbent assay (ELISA) kit was used to determine TNF-α serum concentrations *(Invitrogen Corporation, California USA)* with standards assayed in duplicate. The cytokine determination sensitivity limit was 0.09 pg/mL. For the analysis of CRP, an ELISA kit was used *(Cell Biolabs Inc. California USA)* with a sensitivity limit of 1 ng/mL.

The serum levels of CRP were classified in the cutpoints of low risk (< 1.0 mg/L), average risk (1.0-3.0 mg/L) and high risk (> 3 mg/L).[21] The cases with serum levels of CRP > 10 mg/L were excluded.

Statistical analysis

The data were analyzed with the SPSS version 20. The variables studied were described as frequencies, percentages and median (interquartile range); inferential statistics were performed with non parametric tests (Mann Whitney and Kruskall Wallis tests), and parametric tests (Pearson's correlation). Statistical significance was set at a p value < 0.05.

Ethics

The study protocol was approved by the local ethics committee of the University of Colima, Mexico and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Signed informed consent was obtained from the parents or guardians before the children were enrolled in the study.

Results

The percentage of fat determined by BIA and based on body fat curves was classified into underweight (5.3%), normal (44.6%), overweight (13.5%), and obese (36.5%).

The anthropometric parameters (weight, height, BMI, WC, TSF, SSF) according to the percentage of fat mass determined by BIA are described in table I.

The serum levels of CRP and TNF-α are presented in table II. Children with obesity had a significantly higher level of CRP compared with children with a normal percentage of fat (p< 0.001). However, no differences were identified between groups regarding serum levels of TNF-α (p > 0.05).

The levels of CRP were in the low risk range (CRP < 1 mg/L) in 41.9 % of the children, in the average risk (1-3 mg/L) in 28.4% and in the high risk (> 3 mg/L) in 29.7%. Higher levels of CRP were associated with higher percentage of fat by BIA, BMI, WC, TSF, SSF (table III).

The median levels of CRP and TNF-α in girls were 1.4 mg/L (0.15-8.3 mg/L) and 4.1 mg/L (1-6-8.4 mg/L), respectively; in boys were 1.0 mg/L (0.07-9.4 mg/L) and 3.8 (0.58-12.0), respectively. No differences were found in the levels of CRP and TNF-α among girls and boys (p = 0.228 and p = 0.794).

Table I

Anthropometrical measures according to the percentage of fat mass determined by BIA. Dara are reported as median and interquartile ranges

Anthropometrical measures	Underweight (n = 4)	Normal (n = 33)	Overweight (n = 10)	Obesity (n = 27)
Height (cm)	137.8 (128.0-153.0)	132.0 (107.0-157.0)	145.3 (130.5-155.0)	139.6 (120.5-169.0)
Weight (kg)	29.5 (23.3-40.8)	28.2 (17.1-58.7)	42.4 (26.2-63.1)	45.5 (28.2-80.2)
BMI[†] (kg/m²)	15.6 (14.2-17.4)	15.9 (13.7-25.7)	20.2 (15.5-27.0)	23.9 (19.6-32.8)
WC[‡] (cm)	57.7 (54.5-63.5)	57.0 (46.0-85.0)	73.0 (60.5-88.0)	80.0 (65.0-101.0)
TSF[§] (mm)	10.3 (9.6-11.6)	13.6 (7.6-26.6)	21.2 (12.0-25.6)	24.3 (15.3-34.3)
SSF[¶] (mm)	8.3 (6.0-10.3)	10.0 (4.6-27.6)	21.0 (10.3-32.0)	27.0 (18.0-35.0)

[†]Body mass index; [‡]Waist circumference; [§]Triceps skinfold; [¶]SSF subescapular skinfold.

Table II

Biomarkers of inflammation according to the percentage of fat mass determined by BIA. Data are reported as median and interquartile range

Biomarkers of inflammation	Underweight (n = 4)	Normal (n = 33)	Overweight (n = 10)	Obesity (n = 27)
CRP[†] (mg/L)	0.31 (0.15-0.99)*	0.71 (0.07-5.75)**	1.11 (0.46-4.60)***	2.90 (0.07-9.37)
TNFα (pg/mL)	5.29 (2.80-6.44)	3.88 (0.64-12.0)	4.03 (0.98-6.00)	3.88 (0.58-6.74)

[†]C-reactive protein, [‡] tumor necrosis factor-α.
*p = 0.003 (underweight *vs.* obesity); **p = < 0.001 (normal *vs.* obesity); ***p = 0.046 (overweight *vs.* obesity) with Mann-Whitney U test.

Table III

Anthropometric data according to the classification of risk of CRP. Data are reported as median and interquartile range

Anthropometry	Low risk (n = 31)	Average risk (n = 21)	High risk (n = 22)	p*
% of fat by BIA[†]	20.6 (6.0-34.0)	30.4 (14.0-46.0)	33.8 (15.0-43.0)	**< 0.001**
BMI[‡] (kg/m²)	16.5 (13.7-29.0)	20.3 (15.5-31.0)	23.2 (14.9-32.8)	**< 0.001**
WC[§] (cm)	58.3 (51.0-94.0)	70.5 (55.5-101.0)	76.9 (46.0-101.0)	**0.001**
TSF[¶] (mm)	13.5 (7.6-34.3)	21.0 (8.6-32.0)	21.5 (8.6-32.0)	**0.001**
SSF[‡‡] (mm)	10.8 (4.6-28.3)	21.0 (6.0-33.3)	25.8 (5.0-35.0)	**< 0.001**

[†]Bioimpedance analysis BIA; [‡]BMI body mass index; [§]WC waist circumference; [¶]TSF triceps skinfold; [‡‡]SSF subescapular skinfold.
*p determined by Kruskall-Wallis test.

Serum levels of CRP correlated with measures of adiposity (BMI, TSF, and SSF) (table IV) and with the percentage of fat mass determined by BIA (fig. 1).

The WC was not correlated positively with CRP, but when comparing the mean levels between the children with WC > percentile 90th *vs.* percentile 10-90th, the CRP levels were significantly higher in the group of children with WC > percentile 90th *vs.* percentile 10-90th (p = 0.001) (table V).

Discussion

In the present series, adiposity was determined by BIA since this method has shown a positive correlation between BMI and TSF.[16,19] However, BMI is used to make a diagnosis based on the weight and size of individuals, but does not calculate an exact fat percentage. BIA allows for the body fat percentage to be assessed and is relatively simple, quick, although requires technical skill. Some studies have reported the BIA as an alternative approach to dual-energy X ray-absorptiometry, the gold standard method for body composition assessment in children and adults.[22-24] To the best of our knowledge, this is one of the few studies performed on children that determines body fat percentage with BIA and correlates the serum levels of inflammatory biomarkers.

No correlation was found between the percentage of fat mass and serum TNF-α concentration. This finding is similar to results reported in other published studies. In a study on 109 Mexican-American children, McFarlin et al.[4] found no statistical difference in the levels of TNF-α and IL-6 in children presenting with normal nutritional status, overweight, and obesity. In Bulgaria, a study including 137 pre-puberal children that determined abdominal obesity by WC measurement did not

Table IV
Correlation of anthropometric variables with serum levels of TNF-α and CRP

Anthropometrical measures	Serum levels of TNF-α		Serum levels of CRP	
	r	p*	r	p*
Weight (kg)	-0.060	0.612	0.207	0.083
% fat mass (BIA)[†]	-0.014	0.904	0.453	**< 0.001**
BMI[‡] (kg/m²)	-0.030	0.800	0.398	**0.001**
WC[§] (cm)	-0.025	0.833	0.313	**0.008**
TSF[¶] (mm)	0.038	0.748	0.369	**0.002**
SSF[‡‡] (mm)	-0.074	0.529	0.405	**< 0.001**

[†]Bioimpedance analysis; [‡]BMI body mass index; [§]WC waist circumference; [¶]TSF triceps skinfold; [‡‡]SSF subescapular skinfold.
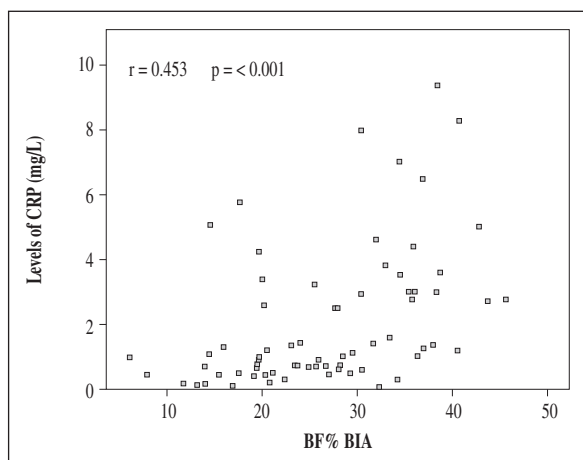*p determined by Pearson's correlation coefficient.

Fig. 1.—Correlation between serum levels of CRP (mg/L) and percentage of fat measured by bioimpedance analysis.

find cytokine elevation, including TNF-α, in the children that presented with abdominal obesity.[7] A study published by Dixon et al.,[11] conducted on 112 Latino schoolchildren, found a higher circulating TNF-α level in thinner girls, and no differences for boys. These findings clearly differ from other authors that have identified significantly higher serum levels of TNF-α and IL-6 in obese children compared with non-obese children.[2,8,9]

This discrepancy in the relationship between TNF-α concentrations and adiposity has been explained by differences in age, sex, body fat mass, mixed pubertal stages, mixed ethnic groups, and physical fitness level

Table V
Serum levels of CRP according to WC in percentiles

WC (percentiles)	Serum levels of CRP (mg/L)	
	Median	Interquartile range
Percentile < 10th	0.69	(0.078-5.75
Percentile 10-90th	0.82	(0.122-7.99
Percentile > 90th	2.90	(0.070-9.37)

Comparing the levels between percentile > 90th *vs.* percentile 10-90th, p = 0.001 (Mann-Whitney U test).

among the studies.[9,11,25-28] In the present study we asked the children not to exercise 8 hours before BIA body fat determination and venous blood extraction, and the children were at school when these were carried out; the ethnicities of the children were similar and they were school-aged children (less than 12 years old), although the Tanner-stage was not assessed.

Another important fact regarding TNF-α is the lack of a reference value in healthy children, which has already been reported by several authors.[2,11]

Regarding CRP, the present study identified a modest correlation with the percentage of fat mass determined by BIA and levels of CRP. These results are consistent with other studies performed in pediatric populations. In 2007, McFarlin et al.[4] determined the effect of weight on inflammatory biomarkers in Mexican-American children. They found significantly higher concentrations of plasma CRP in overweight children compared with children at risk for overweight (p = 0,003). In 2003, Wu, et al.[10] evaluated the relationship of serum levels of CRP with anthropometrics in 835 children (12-16 years of age), and they found significantly higher concentrations of CRP in children with higher BMIs. In the study done by Galcheva[7] on 137 pre-puberal children (6-10 years of age), they reported that CRP concentrations increased in proportion to the degree of abdominal obesity. Retnakaran et al, also performed a study in 228 children in Canada, aged 10-19 years identifying higher levels of CRP in subjects with greater adiposity measured by BMI, WC and % of body fat.[5] Other studies performed on children and adolescents have described relationships between inflammatory biomarkers with insulin resistance (measured by fasting insulin and the homeostasis model of insulin resistance), abnormal lipid profile (higher levels of LDL and lower levels of HDL), and hypertension and arterial changes.[3,5-7,10,29] It is even thought to be a relatively moderate predictor of CVD risk in adults.[30] And although data regarding high CRP and obesity are correlated, there is currently no consensus that high serum levels of CRP can be regarded as a CVD risk marker in children and adolescents since there are not sufficient data linking increased CRP levels in childhood to adult disease outcomes.[31,32]

Conclusion

Subclinical inflammation manifested by higher serum levels of C-reactive protein was identified in schoolchildren with higher percentage of fat mass determined by BIA and other anthropometric measurements. No relationship between the serum levels of TNF-α with adiposity measured by BIA and anthropometry was identified.

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