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.

Objectives: 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.

Conclusions: 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.

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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.

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 stadiometer 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 (m²).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 fibreglass 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.\textsuperscript{15}

\textbf{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.\textsuperscript{17}

\textbf{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.\textsuperscript{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.\textsuperscript{20}

\textbf{Measurement of CRP and TNF-\(\alpha\)}

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-\(\alpha\) 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).\textsuperscript{21} The cases with serum levels of CRP >10 mg/L were excluded.

\textbf{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.

\textbf{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 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.\textsuperscript{20}

\textbf{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.
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. 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. 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. 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
find cytokine elevation, including TNF-α, in the children that presented with abdominal obesity. A study published by Dixon et al., 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. 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.

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

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. 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. 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 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. 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. It is even thought to be a relatively moderate predictor of CVD risk in adults. 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.

### Table IV

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

<table>
<thead>
<tr>
<th>Anthropometrical measures</th>
<th>Serum levels of TNF-α</th>
<th>Serum levels of CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p*</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.060</td>
<td>0.612</td>
</tr>
<tr>
<td>% fat mass (BIA)</td>
<td>-0.014</td>
<td>0.904</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>-0.030</td>
<td>0.800</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>-0.025</td>
<td>0.833</td>
</tr>
<tr>
<td>TSF (mm)</td>
<td>0.038</td>
<td>0.748</td>
</tr>
<tr>
<td>SSF (mm)</td>
<td>-0.074</td>
<td>0.529</td>
</tr>
</tbody>
</table>

* p determined by Pearson’s correlation coefficient.

### Table V

**Serum levels of CRP according to WC in percentiles**

<table>
<thead>
<tr>
<th>WC (percentiles)</th>
<th>Serum levels of CRP (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentile &lt; 10th</td>
<td>0.69 (0.078-5.75)</td>
</tr>
<tr>
<td>Percentile 10-90th</td>
<td>0.82 (0.122-7.99)</td>
</tr>
<tr>
<td>Percentile &gt; 90th</td>
<td>2.90 (0.070-9.37)</td>
</tr>
</tbody>
</table>

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

Acknowledgements

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