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Original

Inflammatory mediators and immune response in Mexican adolescents

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Abstract

Introduction: **Low-grade inflammation and increased immunity related to cardiovascular diseases have been described in children and adults, however, studies in Mexican adolescents are being done at present.**

Objective: **To evaluate inflammatory proteins and indicators of immunity in adolescents by gender and body mass index.**

Material and methods: **115 Mexican adolescents, 15-18 years old (36 men), were divided into non-overweight, risk of overweight and overweight by CDC pediatric criteria by body mass index. Serum concentrations of ceruloplasmin, C3 and C4 were quantified by nephelometry; IL-6 and TNF-α from stimulated supernatant were analyzed with Human Th1-Th2 cytokine CBA II kit (BD Biosciences Pharmigen, San Diego, CA), and detected by flow cytometry. Data were analysed by Mann-Whitney U.**

Results: **Gender differences were found in C3 (men: median 118.8, mean rank: 41.0; women: median: 143.9, mean rank: 65.7, p = 0.001) and ceruloplasmin (men: median: 31.01, mean rank: 47.06; women: median: 31.0, mean rank: 62.9, p = 0.015). Differences by BMI were found in C3 (women non-overweight: median: 137.00 mena rank: 36.52; women with risk of overweight/overweight: median: 175.80, mean rank: 57.69, p = 0.002) and C4 (men non-overweight: median: 23.40, mean rank: 16.60; men with risk of overweight/overweight: median: 26.40, mean rank: 26.36, p = 0.028; women non-overweight: median: 24.25, mean rank: 37.16 and women with risk of overweight/overweight: median: 32.80, mean rank: 54.42, p = 0.013).**

Conclusion: **Inflammatory proteins are increased in adolescents with risk of overweight and overweight, particularly in women.**

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Key words: *Adolescents. Immunity. Inflammation. Overweight.*

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MEDIADORES INFLAMATORIOS Y RESPUESTA INMUNITARIA EN ADOLESCENTES MEXICANOS

Resumen

Introducción: **La inflamación de bajo grado y el aumento en la inmunidad relaciondas con las enfermedades cardiovasculares se han descrito en niños y adultos, sin embargo, estudios en adolescentes mexicanos se están haciendo en la actualidad.**

Objetivo: **Evaluar las proteínas inflamatorias e indicadores de la inmunidad en los adolescentes por género e índice de masa corporal.**

Material y métodos: **115 adolescentes mexicanos, 15-18 años (36 niños), fueron clasificados como sin sobrepeso, riesgo de sobrepeso y sobrepeso según los criterios pediátricos de la CDC para índice de masa corporal. Las concentraciones séricas de ceruloplasmina, C3 y C4 se cuantificaron por nefelometría, IL-6 y TNF-α a partir de sobrenadante de cultivo de linfocitos estimulados con mitógeno, se analizaron con Human cytokines Th1-Th2 CBA kit II (BD Biosciences Pharmigen, San Diego, CA), y se detectaron por citometría de flujo. Los datos fueron analizados estadísticamente por la prueba de U Mann-Whitney.**

Resultados: **Las diferencias de género se encontraron en C3 (Hombres: mediana de 118,8 rango promedio: 41,0; Hombres: mediana: 143,9, rango promedio: 65,7, p = 0,001) y ceruloplasmina (Hombres: media: 31,01, rango promedio: 47,06; Hombres: mediana: 31,0, rango promedio: 62,9, p = 0,015). Las diferencias por IMC se encontraron en C3 (Mujeres sin sobrepeso: mediana: 137,00 rango promedio: 36,52; Hombres con riesgo de sobrepeso y sobrepeso: mediana: 175,80, rango promedio: 57,69, p = 0,002) y C4 (Hombres sin sobrepeso: mediana: 23,40, rango promedio: 16,60; Hombres con riesgo de sobrepeso y sobrepeso: media: 26,40, rango promedio: 26,36, p = 0,028; Hombres sin sobrepeso: media: 24,25, rango promedio: 37,16 y Mujeres con riesgo de sobrepeso/sobrepeso: media: 32,80, rango promedio: 54,42, p = 0,013).**

Conclusiónes: **Las proteínas inflamatorias aumentan en los adolescentes con riesgo de sobrepeso y sobrepeso, particularmente en mujeres.**

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Palabras clave: *Adolescentes. Inmunidad. Inflamación. Sobrepeso.*

Abbreviations

BMI: Body Mass Index.

AVENA: Alimentación y Valoración del Estado Nutricional de los Adolescentes españoles.

CRP: C-reactive protein.

PAI-1: Plasminogen activator inhibitor.

TNFα: Tumor necrosis factor α.

IL-6: Interleukin-6.

C3: Complement 3.

C4: Complement 4.

Cp: Ceruloplasmin.

CDC: Center for disease control.

SPSS: Statistical Package for Social Sciences.

Introduction

The intensive process of urbanization experienced by Latin-American countries, together with the generalized adoption of a western lifestyle in all social classes, has caused an increase in the prevalence of overweight, obesity, hypertension, metabolic syndrome, type II diabetes mellitus and cardiovascular diseases.[1]

Changes produced during obesity affect both humoral and cellular immunity. It is known that adipose tissue, together with its role as energy reserve in form of triacylglycerols, has important endocrine functions, producing several hormones and other signal molecules.[2]

Increased inflammatory peptides are being studied as possible modifiable markers of the elevated risk predictors of disease and the underlying link between obesity and the poor clinical outcomes seen with the metabolic syndrome. More specifically, C-reactive protein (CRP), IL-6 (interleukin-6), leptin, TNF-α (tumor necrosis factor α), and others such as plasminogen activator inhibitor-1 (PAI-1), adiponectin and resistin, may play a role in the pathogenesis, and/or serve as markers of risk for metabolic syndrome.[3]. Other commonly used acute-phase proteins include complement factors C3 and C4, serum amyloid A and ceruloplasmin.

TNF-α and IL-6 are important cytokines, which have been associated with obesity and components of the metabolic syndrome.[4-5] Wärnberg et al. in 2006[6] evaluated the relations of selected inflammatory markers with body fat estimates and patterning in a multicenter study conducted in Spain (Alimentación y Valoración del Estado Nutricional de los Adolescentes españoles: Food and Assessment of the Nutritional Status of Spanish Adolescents. AVENA Study).

The aim of this study was to evaluate inflammatory proteins and indicators of immunity in apparently healthy adolescents by gender and body mass index (BMI).

Materials and methods

Subjects

We included 115 adolescents aged 15 to 18 years old, from public high-schools of the urban area in Toluca City in central Mexico. After obtaining permission from school authorities, we invited adolescents and their parents to an informative session, obtaining signed informed consent from the parents and assent from the adolescents. Only included apparently healthy subjects in the study; those with chronic pharmacologic treatment, disabilities or pregnancy were not included. If the subject presented a transient illness such as respiratory or gastrointestinal diseases, they were re-scheduled for determinations.

This research was conducted with the methodology of the multicenter study AVENA (Alimentación y Valoración del Estado Nutricional de los Adolescentes españoles: Food and Assessment of the Nutritional Status of Spanish Adolescents).[7] The protocol was approved by the Committee of Research and Ethics of the Faculty of Medicine, Universidad Autónoma del Estado de México.

Sample

A non-probabilistic sample size of 115 adolescents was calculated, considering an error of 0.25, a bi-lateral alpha of 0.05, and a power of 80%.

Measurements

Body mass index: A TANITA® TBF300 body composition analyzer was used to measure weight with the subject standing in the up-right position wearing shorts and a light t-shirt, no shoes or socks. Height was measured with a portable SECA® 208 stadimeter, with no shoes or socks and the head in the horizontal Frankfort plane.[8]

BMI was calculated as weight in kg/(height in meters).[2] Adolescents were divided by body mass index (BMI), which was classified according to Center for Disease Control (CDC) growth charts for children and adolescents in: a) no risk of overweight < 85th percentile; b) risk of overweight ≥ 85th and < 95th percentiles; and c) overweight ≥ 95th percentile[9].

Blood sample: Five mL of fasting venous blood was drawn by trained personnel with BD Vacutainer® instruments and centrifuged within two hours at 3000 rpm during 15 minutes and stored at 4ºC, serum was deposited in an individually marked Eppendorf tube and frozen at -70ºC, until all samples were collected and processed simultaneously. C3, C4, CRP and Ceruloplasmin were analyzed with a BN-100 DADE BEHRING® Nephelometer, with anti-C3c, antiC4, anti-ceruloplasmin, CardioPhase* hsCRP and Rheumatology Standard SL DADE BEHRING®.[10]

Table I
Serum concentration of inflammatory proteins and in vitro production of IL-6 and TNF-α in Mexican adolescents by gender

Marker	*n*	*Gender*	*Median*	*Mean Rank*	*Sum of Ranks*	*U*	*p*
C3	79	Women	143.90	65.75	5,194.00	810.00	0.001*
mg/dL	36	Men	118.80	41.00	1,476.00		
C4	79	Women	25.00	60.05	4,744.00	1,260.00	0.329
mg/dL	36	Men	24.40	53.50	1,926.00		
Cp	79	Women	31.00	62.99	4,976.00	1,028.00	0.015*
mg/dL	36	Men	31.01	47.06	1,694.00		
CRP	79	Women	0.30	57.48	4,541.00	1,381.00	0.628
mg/L	36	Men	0.30	59.14	2,129.00		
IL-6	79	Women	320.20	61.61	4,867.50	1,136.50	0.084
pg/mL	36	Men	182.75	50.07	1,802.50		
TNF-α	79	Women	54.40	59.65	4,712.50	1,291.50	0.428
pg/mL	36	Men	48.50	54.38	1,957.50		

C3 = complement 3; C4 = complement 4; Cp = ceruloplasmin; CRP = C-reactive protein; IL-6 = interleukin 6; TNF-α = tumor necrosis factor alfa; U = Mann Whitney-U. p* = statistically significant.

Cytokine production was assessed in cultured mitogen-stimulated peripheral blood mononuclear cells (PBMCs). Mononuclear cells were isolated from heparinized peripheral blood with Ficoll-Hypaque Plus (GE Healthcare Bio-Sciences AB, Sweden) and washed twice in RPMI-1640 medium (SIGMA-Aldrich, USA). The PBMC were resuspended in RPMI-1640 containing 10% fetal bovine serum and 1% penicillin/streptomycin. The concentration was adjusted to 10^6 cells/mL, stimulating with mitogens, phytohemaglutinin (3.5 µl/mL) and lipopolysaccharide (1.5 µl/mL) per well incubated for 48 h, at 37° C and 5% CO_2. Following incubation the cells were removed by centrifugation and supernatant stored at -70° C prior to analysis. Cytokine (IL-6 and TNF-α) content of the supernatant was assessed using the Human Th1/Th2 cytokine CBA II kit (BD Biosciences Pharmingen, San Diego, CA), and analyzed by flow cytometry (Facs Diva, BD®).[11]

Statistical analysis

Data were analyzed with SPSS 13.0 for Windows by descriptive statistics (mean ± sd, median, mean rank) and Mann Whitney-U.

Results

As shown in table I, we found higher values of inflammatory proteins in women participants than in men, however differences were statistically significant only for C3. With respect to interleukins, girls presented non-significantly higher medians of IL-6 and TNF-α than boys.

As can be observed in table II, adolescents with non-overweight and risk of overweight/overweight had higher concentrations of serum inflammatory proteins than those with normal weight. Statistically significant differences were found in C3 women non-overweight and risk of overweight/overweight ($p \leq 0.002$) and C4 men ($p \leq 0.028$) and women ($p \leq 0.013$) non-overweight and risk of overweight/overweight.

In terms of IL-6 and TNF-α, as indicators of immune response in adolescents with risk of overweight and overweight, cytokine secretion was found lower compared with normal weight participants, however differences were non-statistically significant.

Discussion

Complement C3 and C4 are the major plasma proteins of the immune system complement pathways. The synthesis of these proteins is increased in response to inflammation and infection but at a slower rate than traditional acute phase proteins.[12,13]

Our results differ from what Wärnberg et al. in 2004[14] found, with respect to gender differences in their study of apparently healthy Spanish adolescents, where C3, C4 and CRP were higher in men than women; while ceruloplasmin was higher in women. In our study, all three values were higher in women. A recent study in newborns Mexican where compared C3 and C4 concentrations from other ethnic groups and geographic regions, finding that in the Mexican population is typical of this ethnicity.[15]

Yilmazer et al.[16] described the effect of estrogens and progesterone on C3 and C4, with a study of hormonal replacement therapy for post-menopausal women, where they found a increase in serum concentrations of these proteins after 3 to 9 months of treatment. On the other hand, Carranza et al.[17] showed no change after a similar replacement therapy. Thus it is not possible to

Table II
Serum concentration of inflammatory proteins and in vitro production of IL-6 and TNF-α in Mexican adolescents classified as non-overweight and risk of overweight/overweight

Marker	Gender	n	Non overweight		Risk of overweight/overweight		p
			Mean ± sd	Median	Mean ± sd	Median	
C3	Women	36	123.07 ± 28.4	117.20	139.81 ± 29.5	139.30	0.069
mg/dL	Men	79	140.44 ± 25.9	137.00	169.73 ± 33.2	175.80	0.002*
C4	Women	36	24.78 ± 10.5	23.40	30.97 ± 7.21	26.40	0.028*
mg/dL	Men	79	26.04 ± 8.7	24.25	32.11 ± 9.4	32.80	0.013*
Cp	Women	36	28.79 ± 3.90	30.00	29.85 ± 6.74	31.00	0.454
mg/dL	Men	79	30.90 ± 5.28	31.00	35.38 ± 8.5	33.00	0.085
CRP	Women	36	0.43 ± 0.52	0.30	0.61 ± 0.84	0.30	0.742
mg/L	Men	79	0.33 ± 0.15	0.30	0.35 ± 0.11	0.30	0.085
IL-6	Women	36	576.19 ± 750.3	402.30	112.25 ± 67.0	86.50	0.173
pg/mL	Men	79	740.27 ± 1,070.4	395.45	621.12 ± 859.9	210.30	0.667
TNF-α	Women	36	58.91 ± 39.0	75.58	44.97 ± 6.26	43.50	0.506
pg/mL	Men	79	89.83 ± 116.4	54.95	56.89 ± 34.9	51.00	0.456

C3 = complement 3; C4 = complement 4; Cp = ceruloplasmin; CRP = C-reactive protein; IL-6 = interleukin 6; TNF-α = tumor necrosis factor alfa; p* = statistically significant by Mann-Whitney.

presume that women hormonal changes may influence the secretion of complement proteins in women at any age, particulary in adolescents.

Wärnberg[18] in her doctoral thesis reports higher values of CRP in men, with a mean of 1.32 ± 1.60 mg/mL, whereas we found much lower values with no significant differences by gender. Acevedo et al[19] in a study where they correlated CRP with adiposity and other cardiovascular disease risk factors in healthy Chilean children (mean age 11.3 ± 1.9 years), found a mean of 0.9 ± 1.5 mg/L, slightly higher than our values, but lower than those of the Spanish sample.

The mechanism by which ceruloplasmin (Cp) may be contributing to the development of chronic diseases is still under study, however, it is generally accepted that any situation that favours the production of oxidative stress, can cause the release of copper from its molecule, thus reacting with pro-oxidant factors that promote free-radical production. So, it is possible that high Cp concentrations may not necessarily be abnormal, but the oxidative state in which they are produced may determine if it is pathological or not. Taking into account these results it is possible that Cp acts as an antioxidant or a pro-oxidant, depending on its structural integrity. It is necessary to do more research to establish the role of Cp as a marker for inflammatory state.[20]

TNF-α is not usually detectable in healthy individuals, but elevated serum and tissue levels are commonly found in inflammatory conditions,[21] it is explained because the population of this study was healthy.

Our higher values of IL-6 in men than women, may be explained according to Aeckerman[22] and León Nava & Morales Montor[23] by hormonal differences in both genders, as estrogens are capable of stimulating the production of these cytokines, however, we did not measure hormones in our group of adolescents.

When analyzing inflammatory proteins production by BMI, where adolescents were classified as non-overweight or at risk of overweight/overweight, the last two groups showed significantly higher values of C3 and C4. CRP and Cp values were similar in both groups.

Non-overweight adolescents have a better response to stimulation with mitogens, than adolescents with risk of overweight/overweight who had a poor response to stimulation, those indicating that they have a lower immune response; these results are consistent with those reported by Muñoz and Chandra[2,24] who obtained low response of cultured lymphocytes stimulated by mitogen of obese patient.

Overweight and risk of overweight are a consequence of an energy unbalance caused by high energy intakes and low expenses, which has exponentially increased in the last decades. These entities have been associated to serious health problems such as hypertension, dyslipidemia, sleep apnea, arthritis, insulin resistance, type 2 diabetes mellitus and cardiovascular disease.[25]

Obesity has also been related to abnormalities of the immune system, although the mechanisms have not been clearly established. However, it could be due to negative effects of the obesity associated metabolic processes altering defensive mechanisms.

International literature shows controversial results that could be a consequence of the study populations, and the diverse effects of eating patterns, health conditions, as well as cultural, social and economic factors, together with individual host responses.[26,27] We have not found scientific publications similar in Mexican

population adolescent to our study with which to compare our results.

Our data suggest that immunologic alterations found in risk of overweight and overweight adolescents show the beginning of a disease, which could well allow for the establishment of real preventive measures to avoid further adult complications.

Conclusion

Taking into account our findings on the increase of C3 and decrease in IL-6 and TNF-α values in risk of overweight/overweight adolescents, we can conclude that these are in the initial phases of a low grade inflammatory response to their weight status. We must implement urgent measures to improve their diets and increase their physical activity levels in order to help them control their weight avoiding further damage. On the other hand it is necessary to continue research in this area, because in México currently holds the top spot obesity in the world and this work shows the status of risk of overweight/overweight in Mexican adolescents.

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