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Original Article

Association of oxidative stress and inflammation indicators in Cuban adolescents with the inflammatory potential of diet

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SUMMARY

Introduction: Diet influences chronic inflammation and oxidative stress, which is associated with the development of chronic diseases.

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Aim: To identify the association between the inflammatory potential of the diet and indicators of oxidative stress and inflammation in Cuban adolescents.

Method:A cross-sectional study was conducted, including 39 Cuban adolescents from the Faculty of Medical Sciences in the Bayamo municipality, Granma province. The Dietary Inflammatory Index was calculated from a dietary food frequency survey. Among the indicators of oxidative stress, the concentrations of malondialdehyde and 4-hydroxyalkenals, the concentration of advanced protein oxidation products, and ferric reducing power were determined in blood serum, and the concentration of reduced glutathione in erythrocytes. The leukocyte count and differential count were used as indicators of inflammation.

Results: No significant associations were found across the quartiles of the dietary inflammatory index with the leukocyte count and the differential leukocyte count, whereas a negative and significant association was found with the ferric reducing potential and the concentrations of erythrocyte reduced glutathione and a positive and significant association was found with those of malondialdehyde plus 4-hydroxyalkenals and advanced protein oxidation products.

Conclusions: It is concluded that a pro-inflammatory diet was associated with variations in oxidative stress biomarkers, particularly with decreased indicators of antioxidant defenses and increased indicators of inflammatory oxidative damage.

Keywords: Dietary inflammatory index; Food frequency; Oxidative stress indicators; Inflammatory indicators; Adolescents.

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Objective:to identify the association between the dietary inflammatory index and the indicators of oxidative stress and inflammation in Cuban adolescents.

Method:a cross-sectional study was carried out, which included 39 Cuban adolescents from the Faculty of Medical Sciences of Bayamo, Granma. The Dietary Inflammatory Index was carried out through a semi-quantitative food frequency questionnaire taking into account 20 dietary parameters using a previously published protocol. Of the indicators of oxidative stress, the concentration of malondialdehyde plus 4-hydroxyalkenals, the advanced oxidation protein products and the ferric reducing power, were determined in the blood serum and the concentration of reduced glutathione in erythrocytes. The leukocyte count and differential count were used as an indicator of inflammation.

Results:no significant associations were found across the quartiles of the dietary inflammatory index with the leukocyte and the differential leukocyte count, while a negative and significant association was found with the iron-reducing potential and the erythrocyte concentrations of reduced glutathione and positive and significant with those of malondialdehyde plus 4-hydroxyalkenals and the advanced oxidation protein products.

Conclusions: It is concluded that a pro-inflammatory diet was associated with variations in oxidative stress biomarkers, particularly with a decrease in indicators of antioxidant defenses and an increase in those of inflammatory oxidative damage.

Keywords: Inflammatory index of diet; Frequency of food consumption; Oxidative stress indicators; Inflammation indicators; Adolescents.

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Aim: Identify the association between inflammatory potential of diet and indicators of oxidative stress and inflammation in Cuban adolescents.

Methods:A cross-sectional study was carried out, which included 39 Cuban adolescents from the Faculty of Medical Sciences of Bayamo, Granma. The Inflammatory Index of the Diet was calculated from a dietary question of frequency of food consumption. Based on two indicators of oxidative stress, the concentrations of malondialdehyde plus 4-hydroxyalkenes are determined, the concentration of advanced protein oxidation products with ferric-reducing power in blood serum is the concentration of reduced glutathione in erythrocytes. Leukocyte contamination is differential leukocyte contamination foram used as an indicator of inflammation.

Results: No significant associations were found between the quartis of the dietary inflammatory index with leukocyte transmission and differential leukocyte transmission, whereas a negative and significant association was found with the iron-reducing potential and the reduced erythrocyte glutathione concentrations, positive and significant with the most malondialdeído. 4-hydroxyalkenais and advanced protein oxidation products.

Conclusões: It is concluded that a pro-inflammatory diet was associated with variations in biomarkers of oxidative stress, in particular with a decrease in indicators of antioxidant defenses and an increase in inflammatory oxidative damage.

Keywords: Inflammatory index of diet; Frequency of food consumption; Indicators of oxidative stress; Inflammation indicators; Teenagers.

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Introduction

The relationship between chronic low-grade inflammation and oxidative stress has been documented in the literature. Growing evidence suggests that low levels of chronic inflammation and oxidative stress are associated with the development of several chronic diseases, such as diabetes, obesity, cardiovascular disease, cancer, respiratory and musculoskeletal disorders, and neurodevelopmental impairment. (1)

Dietary nutrition is a key variable influencing chronic inflammation and oxidative stress status, primarily because daily food intake is a good indicator of the inflammatory and oxidative stress potential of an individual's diet. (2,3) In fact, a healthy diet reduces the adverse effects of inflammatory markers and oxidative stress in adolescents. (1)

It has been suggested that a number of markers can be used to assess inflammation in human nutritional studies. Pro-inflammatory biomarkers such as tumor necrosis factor-α (TNF-α), C-reactive protein (CRP), or cell adhesion molecules have been used as indicators of the effect of dietary patterns on low-grade inflammation status. (2,4,5) Indeed, some foods and their components have been found to impact blood concentrations of these inflammatory biomarkers. (6)

The dietary inflammatory index (DII) was designed as a tool to characterize the inflammatory potential of an individual's diet based on 45 nutritional parameters that, due to their anti-inflammatory or pro-inflammatory properties, can be associated with different inflammatory biomarkers, such as C-reactive protein, interleukins IL-1β, IL-4, IL-6, IL-10, and TNF-α. (7) It assesses the inflammatory potential of the diet based on the balance of pro- and anti-inflammatory properties of its components, including macronutrients, vitamins, minerals, flavonoids, and specific foods. IDI scores are standardized to global dietary intakes, allowing its use across cultures and dietary patterns. (8)

The use of the IID to estimate the influence of diet on indicators of oxidative stress and inflammation has not been widely used in nutritional studies in Latin American populations and particularly in the Cuban population, so it is unknownThe relationship between the Inflammatory Diet Index in Cuban adolescents and indicators of oxidative stress and inflammation is of particular interest as it presents different dietary patterns and morbidity and mortality indicators.

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Methods

A cross-sectional analytical observational study was conducted with 39 first-year medical students from the Faculty of Medical Sciences of Bayamo in late adolescence (between 17 and 19 years old).Each patient gave written consent, and the study was approved by the institution's Ethics Committee. Adolescents who had an acute illness or reported any diagnosed chronic illness at the time of the study were excluded.

Each individual's nutritional status was determined using the anthropometric indicators Body Mass Index (BMI) and Waist Circumference (WC), to classify them as non-obese and overweight or obese, without abdominal obesity and with abdominal obesity respectively. Participants were classified as normal weight when the BMI was between 18.5 and 24.9 Kg/m2, overweight between 25 and 29.9 and obese when the BMI was ≥ 30. (9) Abdominal obesity (OA) was admitted when the WC was ≥ 102 cm in men and 88 cm in women. (10)

Dietary assessment was conducted through a semiquantitative food frequency survey conducted during the 30 days prior to the interview, using the subject interview technique. The interview was conducted by qualified personnel using standardized home measurements. Vitamin supplement intake was added to the daily intake of each nutrient. The data were subsequently transformed into energy and nutrient values using the values from the Cuban Food Composition Table for Practical Use, using the CERES computer program. (11) The data on food frequency were expressed in terms of daily intake.

A previously published protocol was followed to calculate the dietary IDI scores of study participants. (7) The individual overall IDI score was the sum of the specific IDIs of 20 dietary parameters (out of 45 possible items) for which intake data were available from the food frequency survey: energy, protein, total fat, polyunsaturated fatty acids, saturated fatty acids, cholesterol, carbohydrate, total fiber, vitamin A, beta-carotene, vitamin E, thiamin, riboflavin, pyridoxine, vitamin B12, niacin, folate, vitamin C, iron, and zinc. All overall mean daily intakes and standard deviations, and the respective

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inflammatory effect score, were derived from the reference. Energy (total caloric intake) was used as the divisor of the IDI score to derive the energy-adjusted IDI score (E-DII). (12,13)

A blood sample was taken from each participant after fasting for approximately 10 hours. In whole blood, hematocrit (Htc) was determined, along with a global leukocyte count and a differential count. In blood serum, the concentration of malondialdehyde plus 4-hydroxyalkenals (MDA + 4HDA) was determined by the spectrophotometric method of Esterbauer and Cheeseman (1990), (14) and the concentration of advanced protein oxidation products (APOP) according to the spectrophotometric technique described by Witko-Sarsat et. al. (15). As indicators of antioxidant defenses, the ferric reducing potential (FRP) was determined by the colorimetric assay of Bahr and Basulto. (16) and in erythrocytes, the concentration of reduced glutathione (eGSH) was determined by the colorimetric method of Beutler (1963). (17)

Categorical variables are presented as absolute frequencies (n) and relative frequencies (%). Normally distributed continuous variables are described as means ± standard deviation, while those that did not follow a normal distribution and were required for statistical analysis were transformed and are described similarly. The Shapiro-Wilk test was used to check for data normality. The association between BMI and WC variables and the IDI quartiles was examined using the Mann-Whitney U test. A multivariate linear regression model was used to examine the association between oxidative stress and inflammation indicators and the IDI values by quartile. The values for ferric reducing potential and erythrocyte reduced glutathione were logarithm-transformed, and those for malondialdehyde and 4-hydroxyalkenals were log-transformed. In all cases, the significance level was set at 95%.Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS), version 23.0.

Results

Table 1 shows the characteristic distribution of participants across the IDI quartiles according to sex and baseline anthropometric indicators. The mean IDI score was +1.39 (SD = 0.17), with values ranging from a minimum proinflammatory value of +0.84 to a maximum of +1.66, a fairly narrow range. The sample (n = 39) was predominantly female (76.9%). Most study participants, according to BMI, were of normal or underweight (84.6%) and without abdominal obesity (97.4%). No association was observed across the IDI quartiles with sex, nutritional status by BMI, or WC.

Table 1. Distribution across quartiles of the Inflammatory Diet Index of adolescents according to sex and anthropometric indicators.

Characteristics		Total	R1 (x ≤1.30)	R2 (1.30 < x ≤1.39)	R3 (1.39 < x ≤1.55)	R4 (x > 1.55)	p
		Frequency n (%)					
Sex	Male	9 (23.1)	2 (5.1)	2 (5.1)	2 (5.1)	3 (7.7)	0.87
	Female	30 (76.9)	8 (20.5)	8 (20.5)	8 (20.5)	6 (15.4)	
BMI (kg/m2)	Without obesity	33 (84.6)	10 (25.6)	7 (17.9)	8 (20.5)	8 (20.5)	0.07
	Overweight or Obese	6 (15.4)	0 (0)	3 (7.69)	2 (5.1)	1 (2.6)	
CC (cm)	Without abdominal obesity	38 (97.4)	10 (25.6)	9 (23.1)	10 (25.6)	9 (23.1)	0.39
	Abdominal obesity	1 (2.6)	0 (0)	1 (2.6)	0 (0)	0 (0)	

The values of the oxidative stress and inflammation indicators are shown in Table 2. No variations were observed between the means of the leukocyte count and differential count values across the quartiles of the IID. Regarding the oxidative stress indicators, a downward trend was observed in the means of the PRF values and the erythrocyte concentrations of reduced glutathione across the quartiles of the IID, while the means of the concentrations of malondialdehyde and 4-hydroxyalkenals increased, and the concentrations of advanced protein oxidation products increased towards the third and fourth ranges of IID values.

Table 2. Indicators of oxidative stress and inflammation across quartiles of the Dietary Inflammatory Index in adolescents.

	R1 (x ≤ 1.30)	R2 (1.30 < x ≤ 1.39)	R3 (1.39 < x ≤ 1.55)	R4 (x > 1.55)
Indicators	Mean (± SD)			
Total leukocytes	6.23 (1.22)	6.68 (1.22)	6.34 (0.97)	6.57 (0.99)
Neutrophils	0.60 (0.03)	0.56 (0.05)	0.58 (0.05)	0.60 (0.06)
Lymphocytes	0.33 (0.04)	0.40 (0.06)	0.37 (0.05)	0.36 (0.07)
Eosinophils	0.05 (0.05)	0.03 (0.03)	0.03 (0.03)	0.03 (0.04)
Monocytes	0.02 (0.01)	0.01 (0.01)	0.02 (0.02)	0.02 (0.02)
PRF (µmol Fe2+ /L)	159.46 (53.01)	125.55 (22.92)	123.30 (30.76	120.20 (21.56)
GSHe (mmol/L erythrocytes)	5.89 (0.54)	5.09 (0.60)	4.67 (0.43)	4.30 (0.19)
MDA + 4HDA (µmol/L)	1.74 (0.54)	2.77 (0.80)	3.76 (0.68)	4.50 (0.40)
PAOP (µmol/L)	13.34 (0.87)	13.34 (0.87)	14.94 (0.91)	18.51 (1.24)

The results of the estimation of the association between the adjusted IID and the values of the indicators of oxidative stress and inflammation are shown in Table 3. No significant associations were found with the leukocyte count, nor with the differential leukocyte count, as indicators of chronic inflammation ($p>0.05$). Regarding the oxidative stress indicators, negative and significant associations were obtained with PRF ($p<0.05$) and erythrocyte reduced glutathione ($p<0.01$), while they were positive and significant for malondialdehyde plus 4-hydroxyalkenals and PAOPs ($p<0.01$).

Table 3.β values of the Dietary Inflammatory Index in the multivariate linear regression model.

Indicators	**β values**	**IC (95%)**	**p**
Total leukocytes	0.015	-0.047 / 0.077	0.638
Neutrophils	0.001	-0.013 / 0.015	0.860
Lymphocytes	0.006	-0.012 / 0.024	0.493
Eosinophils	-0.023	-0.058 / 0.013	0.200
Monocytes	0.006	-0.019 / 0.031	0.618
PRF (µmol Fe2+ /L)	-0.033	-0.061 / -0.005	0.022

GSHe (µmol/L)	-0.044	-0.056 / -0.033	0.000
MDA + 4HDA (µmol/L)	8,785	7.08 / 10.50	0.000
PAOP (µmol/L)	2,204	1.87 / 2.54	0.000

Discussion

Systemic inflammation is associated with adverse health outcomes. Diet is a critical associated factor, and the IID is proposed as a promising tool to identify the association between diet and health outcomes. (18)

The association between more proinflammatory diets with overweight and obesity, both general and abdominal obesity, has been clearly substantiated. For adolescents, excess visceral adipose tissue is a cause of chronic inflammation because adipose tissue is the main source of circulating proinflammatory cytokines that increase in response to chronic stress, leading to increases in inflammatory oxidative stress biomarkers. (19) Its relationship with sex has also been reported. Chuang Zhang et. al. (1) found significant differences between quartiles of IDI, sex, and BMI; the association between IDI and markers of oxidative stress and inflammation was more pronounced in the subgroup of overweight and obese adolescents. Another study reported that younger, male participants with higher BMI and waist circumference were more likely to have more proinflammatory IDIs. (20)

Parvin Dehghan et al, (21) examined the association between the IID, the dietary antioxidant index (IAD) and mental health in adolescent girls, finding no association between the nutritional status assessed by the BMI and the IID tertiles, coinciding with the results of the present study, whose results we attribute to the anthropometric characteristics of the caseload.

In this study, the total leukocyte count and the differential count were used as indicators of inflammation. White blood cells are considered a reliable biomarker of inflammation, and elevated levels are associated with several chronic conditions (22), and diet is a strong moderator of inflammation and white blood cell counts. (20)

Chuang Zhang et. al, (1) found significant associations between IDI in children and lymphocyte count in an adjusted model and in others adjusted for different covariates. However, Wirth Michael D. et. al. (20) when comparing the 4th quartile of the IDI with the 1st quartile, there was no statistically significant difference in the total white blood cell count, the same occurred with lymphocytes, monocytes and neutrophils, however, when the IDI was analyzed as a continuous variable, there was an association with the total leukocyte and neutrophil counts. They also found that as the IDI increased, lymphocyte and monocyte percentages decreased, while neutrophil percentages increased. These results do not agree with those obtained in the present investigation, which is attributed to differences in the characteristics of the sample, in terms of variables that may be related to inflammation.

It is widely recognized that diet affects inflammation levels, (1,4,5) but few relevant studies have evaluated how diet affects inflammatory markers in adolescents. One theory is that a pro-inflammatory diet may increase inflammatory cytokine levels by affecting oxidative stress and immunological mechanisms. (1) Phagocytes produce oxygen free radicals and release them into structures after ingesting a pro-inflammatory meal. Oxygen free radicals that drive somatic inflammatory factors are often associated with increased inflammation, suggesting that a pro-inflammatory diet may lead to plasma inflammation. (23)

This study shows that IID-A scores reflected a variation in oxidative stress biomarkers, with increased indicators of oxidative damage to lipids and proteins and decreased indicators of exogenous and endogenous antioxidant defenses across IID-A quartiles. Consistent with the results of this work, a case-control study examining the relationship between IID and oxidative stress markers in patients with non-alcoholic liver disease found a significant negative association between total serum antioxidant capacity and IID, and a significant positive association between MDA and IID in the healthy patient group. (24)

In the case of PAOPs, it has been reported that their levels reflect a state of oxidative stress in extracellular fluids, and that they may contribute to the inflammatory response. (25,26) For this reason, it is not unreasonable to think that they are also related to proinflammatory diets.

Although no studies have examined the effects of IID on GSH levels, there is considerable evidence showing the effect of diet on them. ω-3 PUFAs, polyphenols, carotenoids, and zinc, with anti-inflammatory scores, inhibit inducible NO synthase expression and NO production, which can prevent or attenuate cellular GSH depletion. Meanwhile, several vitamins, also with anti-inflammatory scores, also increase GSH levels and decrease concentrations of lipid peroxidation biomarkers. Conversely, high levels of dietary fat, long-chain saturated fatty acids, low-density lipoproteins, and iron, which enhance inducible NO synthase expression and NO production, can exacerbate GSH loss from cells. (27) This may explain the decrease in GSH concentrations across IID-A quartiles in this study.

Conclusions

The results of this study allow us to conclude that a pro-inflammatory diet was associated with variations in oxidative stress biomarkers, particularly with decreased indicators of antioxidant defenses and increased indicators of inflammatory oxidative damage, despite no changes in inflammatory biomarkers such as total leukocyte levels and their types.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Authorship contribution

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