European Journal of Clinical Nutrition
https://doi.org/10.1038/s41430-017-0013-x

ARTICLE

Dietary inflammatory index and inflammatory biomarkers in adolescents from LabMed physical activity study

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Received: 23 November 2015 / Revised: 19 April 2017 / Accepted: 29 July 2017

Abstract

Background/objectives The dietary inflammatory index (DII) is a tool to measure the diet's inflammatory potential and has been used with adults to predict low-grade inflammation. The present study aims to assess whether this dietary score predicts low-grade inflammation in adolescents.

Subjects/methods The sample comprises 329 adolescents (55.9% girls), aged 12–18 years, from LabMed Physical Activity Study. DII score was calculated based on a food-frequency questionnaire and categorized into tertiles. We collected blood samples to determine the follow inflammatory biomarkers: C-reactive protein (CRP), interleukin-6 (IL-6), complement component 3 (C3), and 4 (C4). In addition we calculated an overall inflammatory biomarker score. Odds ratios (OR) and 95% confidence intervals (95%CI) were computed from binary logistic regression models.

Results DII score, comparing first with third tertile, was positively associated with IL-6 in crude model (OR = 1.88, 95% CI:1.09–3.24, $p_{trend} = 0.011$) and in fully adjusted (for biological and lifestyle variables) (OR = 3.38, 95%CI:1.24–9.20, $p_{trend} = 0.023$). Also, DII score was positively associated with C4, when fully adjusted (OR = 3.12, 95%CI:1.21–8.10, $p_{trend} = 0.016$). DII score was negatively associated with C3 in crude model, comparing first with second but not with third tertile, and no significant associations in fully adjusted model were observed, although a trend was found (OR = 1.71, 95% CI:0.63–4.66, $p_{trend} = 0.044$). No significant associations were observed between DII score and CRP. However, DII score was positively associated with the overall inflammatory biomarker score, when fully adjusted (OR = 5.61, 95% CI:2.00–15.78, $p_{trend} = 0.002$).

Conclusions DII score can be useful to assess the diet's inflammatory potential and its association with low-grade inflammation in adolescents.

Introduction

Low-grade inflammation correlates with a set of chronic conditions [1, 2] such as obesity [3], diabetes [3, 4], cardiovascular diseases [5, 6] and cancer [7, 8]. This association has also been found in youth for obesity [9–11], central obesity [12, 13], metabolic syndrome [14, 15], atherosclerosis [16], and several other cardiovascular risk factors [10, 13]. In addition, systemic inflammation in childhood and adolescence is known to continue into adulthood [17].

Inflammatory status is heavily reliant on the measurement of the inflammatory biomarkers, such as acute phase proteins and cytokines [1], and a number of modifying factors, including diet, have been shown to influence the inflammatory status in the life cycle [1, 2].

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Published online: 26 December 2017

Studies about nutrition intake and low-grade inflammation in adolescents are scarce. However, evidence suggests that the consumption of fat and antioxidant vitamins (vitamins E, C and beta-carotene) are determinants of low-grade inflammation during adolescence [18]. While antioxidant vitamins [19, 20] and polyunsaturated fatty acids [19] seem to have anti-inflammatory properties, total fat [20] and saturated fat acids [19, 20] seem to be pro-inflammatory. Moreover, a Western dietary pattern is considered to be pro-inflammatory diet [19].

A dietary pattern approach has been considered advantageous because it considers synergistic or antagonistic interactions among nutrients and other food components [21]. The dietary inflammatory index (DII) [22, 23] is a score that attempts to combine the inflammatory property of each nutrient or food component of the diet.

The DII was designed by Cavicchia [22] and updated by Shivappa [23], and it is a tool to measure the diet's inflammatory power, scoring individuals' diets from maximal anti-inflammatory to maximal pro-inflammatory. This index was initially (in the first version) correlated to C-reactive protein (CRP) in apparently healthy adults [22] and seniors [24] (in an adapted first version) and also to other inflammatory biomarkers such as interleukin-6 (IL-6) and a combined inflammatory biomarker score in adults and seniors [25]. For the updated version, several studies have found associations between DII score and CRP, in adults and seniors [26], and IL-6, tumour necrosis factor-alpha (TNF-α) and another score of combined inflammatory biomarker in postmenopausal women [27]. The DII score has been used in several studies to predict mortality [28, 29], survival [30], and diseases, especially cancer [30, 31], but also obesity [32], cardiovascular disease [33] and metabolic syndrome [32, 34].

Considering that the published studies using the DII score were predominantly conducted with adults or seniors, and no study was found to focus on adolescents, this paper aims to assess the association between DII score and inflammatory biomarkers in adolescents.

Subjects and methods

Study design and sampling

We used baseline data, collected in 2011, from the Longitudinal Analysis of Biomarkers and Environmental Determinants of Physical Activity Study (LabMed Physical Activity Study). The LabMed Physical Activity Study is a school-based prospective cohort study carried out in five schools from the north of Portugal, which aimed to evaluate the independent and combined associations of dietary intake and fitness levels on blood pressure levels of adolescents.

The LabMed Physical Activity study was conducted in accordance with the World Medical Association's Helsinki Declaration for Human Studies. The Portuguese Data Protection Authority (#1112434/2011), the Portuguese Ministry of Science and Education (0246200001/2011) and Faculty of Sport, University of Porto, approved the study. All participants were informed of the study's goals, and written informed consent was obtained from participating adolescents and their parents or guardians.

Considering combined healthy diet/physical activity pattern prevalence of 14% [35], we calculated a minimum sample size of 1086 subjects to have a power of 80%, to detect a 15% difference between exposed and unexposed, at 5% significance, considering an expected dropout rate of about 20%.

From an initial total sample of 1229 apparently healthy adolescents (12–18 years), 534 accepted to undergo blood collection. Of these, 412 adolescents had completed and accurate data on dietary intake. Of these, 329 adolescents had physical activity and sedentary time assessment with accelerometers. We found no differences in most variables between those who accepted or not to undergo blood sampling. However, boys, older and current smokers reject more undergoing blood sampling.

Inflammatory biomarkers assessment and overall inflammatory biomarker score

Blood samples were collected from the antecubital vein, at least 10 h of fasting conditions, refrigerated (4–8 °C), and sent to laboratory for measure inflammatory biomarkers: CRP by the latex-enhanced turbidimetric assay (Siemens Advia 1600/1800, Erlangen, Germany); IL-6 by the chemiluminescence immunoassay (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA); complement component 3 (C3) and 4 (C4) by the Immunoturbidimetric assay (Siemens Advia 1600/1800, Erlangen, German).

Inflammatory biomarkers were dichotomized, based on sex- and age-adjusted median values, because they have a very skewed distribution, and no cut-offs values are established for adolescents. Categories considered were higher or lower inflammatory state. Medians of each category (lower/higher) were defined as follows: 0.11/0.92 mg/L for CRP, 1.90/4.20 ng/L for IL-6, 107.00/127.00 mg/dL for C3 and 17.00/25.55 mg/dL for C4.

We created an overall inflammatory biomarker score considering each biomarker categories, assigning one point to those who were above the sex, age-adjusted median or zero for those who were below, and summing all points assigned. The overall inflammatory biomarker score varies from zero to four inflammatory biomarkers above the median. We also create two categories: 0–1 (49.9%) or 2–4 (50.1%) biomarkers above the median.

Physical activity and sedentary time assessment

The physical activity and sedentary time were assessed with accelerometers GT1M (ActiGraph, Pensacola, Florida, USA). Participants were instructed to use the accelerometer attached on the right side of hip, with the notch faced upwards, over five consecutive days (3 weekdays, 2 weekend days) during waking hours and remove it during water-based activities. The epoch length was set to 2 s to allow a more detailed estimate of physical activity intensity.

Accelerometer data were analysed by an automated data reduction program (ActivLive 6.12, ActiGraph, Pensacola, Florida, USA). Periods with 60 min of consecutive zeros were detected and flagged non-wear time. Participants had to have at least 8 h of data to count as a valid day and to have at least 3 valid days (2 weekdays, 1 weekend day) to be included. This combination of hours and days were studied to achieve a reliability of 90% [36].

After screening, the raw activity 'counts' were processed for determination of time spent in the different physical activity intensities. Physical activity was expressed as the time spent in moderate-to-vigorous physical activity. The cut-points recommend by American College of Sports Medicine [37] were used and we identify moderate-to-vigorous physical activity and sedentary time was expressed in minutes.day^{-1}.

Dietary intake assessment

A self-administered semi-quantitative food-frequency questionnaire validated for Portuguese population [38], and adapted to adolescents [39], was used to measure the dietary intake. The food-frequency questionnaire lists 91 options of food and beverage items or categories, and assesses the food habits in previous 12 months. For each option, there are nine response possibilities (from 'never or less than once per month' to 'six or more times per day'), standard portion sizes, and a seasonality choice. In the end, there is still space available for each respondent include any food not listed. Dietary intake estimation was made multiplying the portion size in grams by the multiple/fraction of daily frequency intake and by a seasonality variation factor for each option selected. The conversion, from food to energy and nutrients intake, was performed using The Food Processor Plus program version SQL (ESHA Research, Salem, OR, USA). This database was supplemented with the Portuguese food composition database [40].

To determine the misreporting of dietary assessment, implausible energy intake was calculated using the Goldberg's method, adapted by Black [41]. First, the basal metabolic rate was calculated using Schofield equation, considering sex and age. Second, a ratio energy intake/basal metabolic rate was compared to the 95% confidence limits (cut-offs). The cut-offs were calculated using our sample specific values for: mean of physical activity level, number of days of dietary assessment, within-subject coefficient of variation in energy intake, between-subject variation in physical activity, and variation in basal metabolism rate. The physical activity level was calculated in using counts. minutes^{-1} and time of daily use from accelerometers, as Trost formula [42], reaching a value of 1.23. A number of 21 days of diet assessment was considered, as Black recommendation for food-frequency questionnaire [41]. The within-subject coefficient of variation in energy intake was calculated considering mean and standard deviation of energy intake in our sample. Between-subject variation in physical activity was calculated considering mean and standard deviation of physical activity level in our sample. A figure of 8.5% was used for the coefficient of variation of repeated basal metabolic rate measurements, as Black suggested [41]. The cut-offs calculated were 0.61–2.48; accordingly, a total of 150 adolescents with energy intake/basal metabolic rate below 0.61 and over 2.48 were considered as misreporting of dietary assessment and were excluded.

Energy intake was expressed in kj.day^{-1} and kcal.day^{-1}. Food-frequency questionnaire data was also used to calculate DII score.

Description of the dietary inflammatory index score

The DII is a literature-based tool [23] that measures the diet' inflammatory properties by a score, and it is based on review about the role of food and dietary constituents on the following inflammatory biomarkers: CRP, TNF-α and Interleukin's 1β, 4, 6 and 10. The review pointed 45 food parameters and they were scored with +1, −1 or 0 according to their inflammatory effects: pro, anti or null, respectively. The number of articles and the type of study were also used to weight each one of 45 food parameters and calculate a 'food parameter-specific overall inflammatory effect score', used as multiplying factors, to calculate a DII score. The final score, ranging from −8.87 to 7.98, is interpreted as strongly anti-inflammatory to strongly pro-inflammatory, respectively.

In this study, the DII score was calculated considering 31 food parameters. Eugenol, garlic, ginger, saffron, turmeric, flavan-3-ol, flavones, flavonols, flavonones, anthocyanidis, isoflavones, pepper, thyme/oregano, rosemary were not included because no information was available for these components in the Food Processor nutritional database neither those herbs or spices were included in the food-frequency questionnaire.

Briefly describing the DII score calculation according to Shivappa [23], first a mean and standard deviation were

calculated for the 31 food parameters available (Table 1), because no global data for adolescent is available. Second, a *z*-score of each food parameter and for each participant was calculated. Third, the individual z-score were converted to a centred percentile. Fourth, each centred percentile were multiplied by its respective food parameter-specific overall inflammatory effect score, published by Shivappa [23], and the food parameter-specific DII score is obtained. Finally, the 31 food parameter-specific DII score was summed and an individual DII score was obtained. All food parameters are correlated ($p < 0.001$) with DII score; caffeine, alcohol and green/black tea are food parameters with weakest correlations; while magnesium, vitamin B6 and dietary fibre are food parameters with strongest correlations (Table 1).

Our DII score values ranged from −5.36 to 4.24, and it was categorized, based on tertiles values, in accordance with Shivappa [26], considering Low (First tertile: < −1.34), Medium (Second tertile: −1.34 to 1.41) and High (Third tertile: >1.41) pro-inflammatory dietary property.

Anthropometric assessment data

Height and weight were measured with a portable stadiometer (SECA 213, Hamburg, Germany) and a portable scale (TANITA Inner Scan BC532, Tokyo, Japan), respectively. Adolescents should be standing upright, lightly dressed and no shoes. Body mass index was calculated from the weight (kg) to height squared (m^2) ratio and participants were classified as underweight, normal weight, overweight and obese [43].

Pubertal stage

Pubertal stage, from 1 to 5, was self-assessed relatively the secondary sex characteristics, according to Tanner and Whitehouse criteria [44].

Socio-economic status

Socio-economic status was self-assessed with Family Affluence Scale [45], ranking from 0 to 9, considering lower scores as lower socio-economic status.

Smoking habits

Smoking habits were self-reported and participants were classified according World Health Organization criteria [46] as: non-smokers, former smokers, occasional smokers and current smokers.

Table 1 Mean, standard deviation and correlation with final score of food parameters included in the calculation of DII score for the adolescents from LabMed physical activity study

DII food parameters	Mean	SD	r_s[a]
Alcohol (g/day)	1.22	3.94	−0.219
Vitamin B12 (μg/day)	13.60	10.80	−0.551
Vitamin B6 (mg/day)	2.38	0.88	−0.904
β-Carotene (μg/day)	1 011.43	902.78	−0.699
Caffeine (g/day)	33.51	34.71	−0.185
Carbohydrate (g/day)	266.47	97.26	−0.745
Cholesterol (mg/day)	357.56	168.84	−0.595
Energy (kcal/day)	2 127.72	680.98	−0.809
Total fat (g/day)	76.65	27.04	−0.708
Fibre (g/day)	21.85	10.18	−0.903
Folic acid (μg/day)	372.69	182.23	−0.893
Green/black tea (g/day)	17.20	42.51	−0.263
Iron (mg/day)	16.63	6.33	−0.885
Magnesium (mg/day)	331.71	119.47	−0.919
Monounsaturated fatty acids (g/day)	31.49	11.74	−0.717
Niacin (mg/day)	24.91	9.08	−0.840
n-3 Fatty acids (g/day)	1.40	0.61	−0.793
n-6 Fatty acids (g/day)	10.33	4.67	−0.680
Onion (g/day)	13.27	21.63	−0.409
Protein (g/day)	99.92	34.77	−0.794
Polyunsaturated fatty acids (g/day)	13.97	5.73	−0.727
Riboflavin (mg/day)	2.48	1.00	−0.726
Saturated fat (g/day)	24.60	8.84	−0.580
Selenium (mg/day)	102.87	39.99	−0.827
Thiamin (mg/day)	1.79	0.64	−0.859
Trans fat (g/day)	1.06	0.59	−0.455
Vitamin A (RE/day)	1 237.95	1 277.34	−0.735
Vitamin C (mg/day)	148.70	97.01	−0.817
Vitamin D (μg/day)	4.86	2.89	−0.645
Vitamin E (mg/day)	8.91	4.07	−0.860
Zinc (mg/day)	13.10	4.72	−0.703

DII dietary inflammatory index, *RE* retinol equivalents, *SD* standard deviation, r_s correlation

[a] correlation coefficients based on Spearman test

All $p < 0.001$

Statistical analyses

Participants' characteristics are presented as percentages, medians and inter-quartiles range. Mann–Whitney *U* test, Qui-square test and Spearman's correlation were used to assess associations between variables.

To study the association between DII score tertiles and inflammatory biomarkers, fifteen binary logistic regression models were constructed. There were three models (crude, sex-adjusted and fully adjusted) for each inflammation biomarker and for the overall inflammatory biomarker

score, as dependent variables, and DII score tertiles, as predictor. Fully adjusted model were adjusted for sex, age, pubertal stage (Tanner A and B), body mass index, energy intake, socio-economic status, sedentary time, moderate-to-vigorous physical activity and smoking habits. Multicollinearity was tested, and no multicollinearity between independent variables was observed. Post hoc power calculations were performed considering our sample size ($n = 329$), our minimal odds ratio (OR = 3.12), a null hypothesis value of 0.5, and 5% significance, achieving a power of 0.99.

A 0.05 level of significance and 95%CI (confidence interval) were considered. Data was analysed using the statistical package SPSS®, version 21.0 (SPSS Inc., Chicago, IL, USA) and power were calculated using G*power, version 3.1 (Faul, Erdfelder, Lang, & Buchner, 2007).

Results

Girls presented on average a lower CRP than boys and no significant differences were observed for other inflammatory biomarker and DII score (Table 2).

The IL-6 was positively associated with DII score and adolescents within the third tertile of the DII score had higher prevalence of higher IL-6 than adolescents within the first or second tertiles. However, adolescents within first tertile of the DII score had higher prevalence of higher C3. No significant differences were observed for the other biomarkers or for the overall inflammatory biomarker score and DII score, neither significant correlations were observed between any inflammatory biomarkers or the overall inflammatory biomarker and score DII score when this variables were continuously treated (Table 3).

Table 4 shows for fully adjusted models that adolescents within the third tertile of the DII score showed significantly higher odds of having higher IL-6 (OR = 3.38, 95% CI:1.24–9.20, $p_{trend} = 0.023$), C4 (OR = 3.12, 95% CI:1.21–8.10, $p_{trend} = 0.016$), and the overall inflammatory biomarker score (OR = 5.61, 95%CI:2.00-15.78, $p_{trend} = 0.002$).

Discussion

To the best of our knowledge, this is the first study exploring associations between the DII score and inflammatory biomarkers in adolescents.

We showed that DII score predicted low-grade inflammation, specifically IL-6, C4 and the overall inflammatory biomarker score, in adolescents. In our study, the DII score was independently and positively associated with IL-6, and adolescents whose diets showed low- or medium-inflammatory properties had a lower prevalence of higher IL-6. Also, in the fully adjusted regression model, when comparing DII score of the first tertile (low-inflammatory diet) with the third tertile (high-inflammatory diet), the odds of having higher IL-6 was about three times higher. These findings seem to be important since IL-6 is considered a more sensitive indicator of cardiovascular disease than others like CRP [47, 48].

In our study, DII score was not associated with CRP, contrary to what we were expecting considering the DII scores conception (a literature-based tool about the role of diet on inflammatory biomarkers, including CRP) [22, 23] and validation [22, 26], but consistent with other studies in adults [25, 27]. However, some studies have found this relationship, particularly those conducted with apparently healthy adults [22, 26, 34], or seniors [24]. In this regard, it is important to notice that in these studies, the CRP mean levels were much higher than in our sample. For example, in the SEASONS cohort [26], CRP mean ranged from 2.2 ± 5.1 to 2.2 ± 5.7 mg/L in women and from 2.3 ± 4.4 to 2.4 ± 4.6 mg/L in men, whereas in our study, the corresponding values were 0.83 ± 2.24 mg/L (girls) and 1.62 ± 4.54 mg/L (boys). Moreover, only 7% of the participants in our study presented CRP levels between 3 and 10 mg/L, while in the SEASONS cohort, this prevalence reached 18%. Another important concept to be noted is the number of modifying factors related to the inflammatory biomarkers such as age or body fatness [1, 2]. Again comparing our study to the SEASONS cohort [26], our age range is 12–18 years, while the SEASONS cohort is 20–70 years; by contrast, our prevalence of normal BMI is about 65% (girls) and 68% (boys), while the SEASONS cohort was about 44% (women) and 30% (men). Thus, with all of these parameters described, differences in the prevalence of CRP inadequacy and the presence of modifying factors of inflammatory biomarkers between samples may help to explain the differences in the association between DII score and CRP across the studies.

We also found an association between the DII score with C4 and the overall inflammatory biomarker score, in line with some authors [25, 27] who found a relationship between DII score and a different inflammatory biomarker scores only for the fully adjusted model. This means that adolescents with a high pro-inflammatory diet have an odds ratio five times higher of having two to four biomarkers above the median. However, these associations are true only for the fully adjusted model. Calder et al. [1] discussed how modifying factors can affect the concentration of inflammatory biomarkers, and we tried to control the effect of most of the possible variables in the fully adjusted models, such as age and pubertal stage; body mass index as a measurement of body fatness; sedentary time and moderate-to-vigorous physical activity as measurements of physical

Table 2 Participants' characteristics according to sex in adolescents from the LabMed physical activity study

		All[a] ($n = 329$)	Girls[a] ($n = 184$)	Boys[a] ($n = 145$)	p[b]
DII score		0.57 (−0.92–2.07)	0.63 (−0.82–2.24)	0.37 (−1.26–1.78)	0.120
Age (years)		15.0 (13.0–16.0)	15.0 (13.0–16.0)	14.0 (13.0–15.0)	0.447
Pubertal stage: Tanner A[c]	2	8.2%	3.3%	14.5%	<0.001
	3	34.3%	28.3%	42.1%	
	4	45.3%	55.4%	32.4%	
	5	12.2%	13.00%	11.00%	
Pubertal stage: Tanner B[c]	2	7.9%	2.7%	14.5%	<0.001
	3	21.3%	19.6%	23.4%	
	4	50.2%	48.9%	51.7%	
	5	20.7%	28.8%	10.3%	
Body mass index	underweight	3.00%	2.2%	4.1%	0.715
	normal weight	66.3%	66.3%	66.2%	
	overweight	22.8%	22.8%	22.8%	
	obese	7.9%	8.7%	6.9%	
Socio-economic status		6.0 (5.0–8.0)	6.5 (6.0–8.0)	6.0 (5.0–8.0)	0.479
Energy intake	($kj.day^{-1}$)	8 648 (6 734–10 609)	8 501 (6 567–10 061)	8 818 (6 916–11 190)	0.038
	($kcal.day^{-1}$)	2 059 (1 603–2 526)	2024 (1 564–2 395)	2 100 (1 647–2 664)	
Sedentary behaviour ($minutes.day^{-1}$)		667.4 (619.4–725.3)	678.4 (632.8–734.1)	645.9 (607.5–713.2)	0.003
Moderate-to-vigorous physical activity ($minutes.day^{-1}$)		51.0 (39.1–65.3)	45.5 (35.1–59.5)	56.7 (43.0–71.5)	<0.001
Smoking habits[d]	Current smokers	1.2%	1.1%	1.4%	
	Occasional smokers	0.9%	1.1%	0.7%	
	Former smokers	5.8%	3.8%	8.3%	
	Non-smokers	92.1%	94.0%	89.7%	
CRP (mg/L)		0.20 (0.11–0.77)	0.11 (0.11–0.49)	0.34 (0.11–1.26)	<0.001
IL-6 (ng/L)		1.90 (1.90–3.00)	1.90 (1.90–3.40)	1.90 (1.90–3.35)	0.268
C3 (mg/dL)		116.0 (107.0–126.0)	119.0 (107.0–127.0)	115.0 (106.5–126.0)	0.179
C4 (mg/dL)		20.0 (16.0–24.0)	20.0 (16.0–25.0)	20.0 (17.0–24.0)	0.587
Overall inflammatory biomarkers score[e]		0.57 (−0.92–2.07)	0.63 (−0.82–2.24)	0.37 (−1.26–1.78)	0.476

DII dietary inflammatory index, *CRP* C-reactive protein, *IL-6* interleukin-6, C3 complement component 3, *C4* complement component 4

[a] The data shown in percentage for categorical variables and median (interquartile range) for continuous variables

[b] *P*-value was calculated based on Qui-squared test for categorical variables and Mann–Whitney U test for continuous variables

[c] Tanner A indicates development stages of breast in girls and genitalia (penis size and testicular volume) in boys; Tanner B indicates development stages of public hair distribution (Tanner B)

[d] Qui-squared test performed with 'Current smokers' and 'Occasional smokers' together to improve power of test

[e] Overall inflammatory biomarkers score were designed calculating an age and gender adjusted z-score for each inflammatory biomarker (CRP, IL-6, C3 and C4) and summing them

Table 3 Differences and correlations between inflammatory biomarkers and DII in adolescents from the LabMed physical activity study

			DII score					
			Continuous		Tertiles—inflammatory property			p^b
			r_s	p^a	First −Low (< −1.34)	Second −Medium (−1.34 to 1.41)	Third −High (>1.41)	
CRP	Continuos		−0.089	0.109				
	Categories	Lower			54.3%	51.7%	54.3%	0.900
		Higher			45.7%	48.3%	45.7%	
IL-6	Continuos		0.096	0.083				
	Categories	Lower			71.4%	69.9%	56.9%	0.046
		Higher			28.6%	30.1%	43.1%	
C3	Continuos		0.004	0.939				
	Categories	Lower			40.0%	58.0%	51.7%	0.047
		Higher			60.0%	42.0%	48.3%	
C4	Continuos		0.006	0.919				
	Categories	Lower			52.9%	57.3%	45.7%	0.174
		Higher			47.1%	42.7%	54.3%	
Overall inflammatory biomarkers score[c]	Continuos		0.016	0.528				
	Categories	Lower			44.3%	47.6%	37.9%	0.296
		Higher			55.7%	52.4%	62.1%	

DII dietary inflammatory index, *CRP* C-reactive protein, *IL-6* interleukin-6, *C3* complement component 3, *C4* complement component 4

[a] *P*-values and r_s coefficients were based on Spearman test

[b] *P*-values were based on Qui-square test

[c] Overall inflammatory biomarkers score were designed summing the inflammatory biomarkers (CRP, IL-6, C3 and C4) categories, wherein for each category was assigned one point if the biomarker was above the median adjusted by age and sex or zero if below the median

(in)activity; sex, smoking habits and socio-economic status. These factors together must have a significant impact on inflammatory biomarkers, masking the association between DII score with C4 and with the overall inflammatory biomarker score in the crude models. However, when we control those variables, the association between DII score with C4 and the overall inflammatory biomarker score can be observed.

The association between C3 biomarker and DII score is unclear. While C3 was independently and negatively associated with the DII score and adolescents whose diets showed medium-inflammatory properties had a lower prevalence of higher C3 then adolescents whose diets showed low-inflammatory properties; in the fully adjusted regression model, no association was found although the trend was significant. Confounding factors are probably the source of controversy. It is known that higher concentrations of C3 in adolescents have been linked to high body fatness [12, 49] and, in fact, we found an association between body mass index with C3 (OR = 1.31, 95%: 1.21–1.42, $p < 0.0001$; data not shown) in our fully adjusted model.

Moreover we found an association between DII score and the overall inflammatory biomarker score in fully adjusted model, but not for CRP or C3, although the trend is significant for C3. Furthermore, the overall inflammatory biomarker score had showed a higher odds ratio (OR = 5.61) than IL-6 (OR = 3.38) or C3 (OR = 3.12). In fact, the inflammatory biomarkers in general are considered non-specific pro-inflammatory response markers in healthy people, and the biomarkers' signatures that best represent low-grade inflammation are yet to be fully understood [2]. Our overall inflammatory biomarker score is a more complex and integrated assessment of low-grade inflammation, rather than just an inflammatory biomarker alone. This score takes into account the sums of the effects of all inflammatory biomarkers, that is, those that were shown to have a relationship with the DII (IL-6 and C4) and those that did not (CRP and C3), and it seems to represent better low-grade inflammation in this group of adolescents.

Table 4 Association between DII score tertiles and inflammatory biomarkers categories among adolescents from LabMed physical activity study

	DII score: OR (95% CI)			
	First tertile Low (<−1.34)	Second tertile Medium (−1.34 to 1.41)	Third Tertile High (>1.41)	p_{trend}
CRP models				
Crude	1.00	1.11 (0.62–1.96)	1.00 (0.55–1.81)	0.900
Sex-adjusted	1.00	1.11 (0.63–1.97)	1.02 (0.56–1.86)	0.914
Fully-adjusted[a]	1.00	1.71 (0.83–3.51)	2.33 (0.88–6.20)	0.230
IL-6 models				
Crude	1.00	1.08 (0.57–2.02)	1.89 (1.00–3.58)	0.048
Sex-adjusted	1.00	1.07 (0.57–2.01)	1.82 (0.96–3.45)	0.071
Fully-adjusted[a]	1.00	1.44 (0.68–3.08)	3.38 (1.24–9.20)	0.023
C3 models				
Crude	1.00	0.48 (0.27–0.86)	0.62 (0.34–1.14)	0.049
Sex-adjusted	1.00	0.48 (0.27–0.86)	0.61 (0.33–1.12)	0.047
Fully-adjusted[a]	1.00	0.75 (0.36–1.57)	1.71 (0.63–4.66)	0.044
C4 models				
Crude	1.00	0.83 (0.47–1.48)	1.33 (0.74–2.42)	0.175
Sex-adjusted	1.00	0.83 (0.47–1.45)	1.32 (0.73–2.40)	0.189
Fully-adjusted[a]	1.00	1.13 (0.57–2.28)	3.12 (1.21–8.10)	0.016
Overall inflammatory biomarkers score models[b]				
Crude	1.00	0.88 (0.49–1.56)	1.30 (0.71–2.38)	0.297
Sex-adjusted	1.00	0.88 (0.49–1.56)	1.30 (0.71–2.38)	0.304
Fully-adjusted[a]	1.00	1.75 (0.84–3.66)	5.61 (2.00–15.78)	0.002

OR odds ratio, *CI* confidence interval, *DII* dietary inflammatory index, *CRP* C-reactive protein, *IL-6* interleukin-6, *C3* complement component 3, *C4* complement component 4

[a] All fully adjusted model were adjusted for sex, age, pubertal stage—Tanner A and B, body mass index, energy intake, socio-economic status, sedentary behaviour, moderate-to-vigorous physical activity and smoking habits

[b] Overall inflammatory biomarkers score were designed summing the inflammatory biomarkers (CRP, IL-6, C3 and C4) categories, wherein for each category was assigned one point if the biomarker was above the median adjusted by age and sex or zero if below the median

The strengths of this study include the novelty of its aim and the use of objectively measured physical activity and sedentary time. We also included sedentary time as a covariate in our models once it was considered a risk factor for cardiovascular health independently of physical activity levels [50]. In addition, we used only accurate food-frequency questionnaires, according to Goldberg's method [41]. This method is useful to evaluate the mean population bias in reporting energy intake and recommends the use of information about physical activity, as we did. Moreover, our models considered other important potential confounders such as age, body mass index, sex and smoking, considering them as modifying factors that affect the inflammatory biomarker concentration [1, 2].

This study is not without limitations. First, due to lack of cut-offs established for inflammatory biomarkers, we used median values age- and sex-adjusted. For IL-6, we considered 1.9ng/L (1.9–6.95ng/L) for most age/sex group. Nevertheless, our cut-offs are very close to those reported by the Asklepios Study (1.6ng/L for IL-6) [51], where authors also found an association between DII score and IL-6. For CRP, our cut-offs (0.11–0.79 mg/dL) are close to that reported by Visser (0.22 mg/dL) [11], reporting a positive association with overweight in children and adolescents. Second, we used inflammatory biomarkers considered non-specificity in order to measure low-grade inflammation in healthy subjects [1, 2]; however, we attempted to overcome this with adjusted models and the overall inflammatory biomarker score. Third, we calculated the DII score using only 31 out of a possible 45 food parameters because only these components are present in our database. Thus, DII score in our sample has a lower range (−5.36 to 4.02) than the original possible ranges (−8.87 to 7.98) [23]. However, it represents 56% of the score range and is similar to the SEASONS cohort (57%) [26].

In summary, DII score was associated with IL-6, C4 and the overall inflammatory biomarker score after adjustments for biological and lifestyle characteristics. DII score was not associated with CRP and C3 in Portuguese adolescents.

DII score can be useful to assess the diet's inflammatory properties and its association with low-grade inflammation in adolescents.

Acknowledgements The authors gratefully acknowledged the participation of all adolescents and their parents, teachers and schools of the LabMed and Physical Activity Study, the cooperation of volunteer's, the Department of Hygiene and Epidemiology (University of Porto) for the conversion food frequency questionnaire data into nutrients, and the Research Centre in Physical Activity, Health and Leisure (University of Porto) for the sponsoring the LabMed and Physical Activity Study.

Competing interests The authors declare that they have no competing interests.

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