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ORIGINAL CONTRIBUTION

Diet as moderator in the association of adiposity with inflammatory biomarkers among adolescents in the HELENA study

Aline Arouca[1] · Luis A. Moreno[2,3] · Esther M. Gonzalez-Gil[2,3] · Ascensión Marcos[4] · Kurt Widhalm[5] · Dénes Molnár[6] · Yannis Manios[7] · Frederic Gottrand[8] · Anthony Kafatos[9] · Mathilde Kersting[10] · Michael Sjöström[11] · Francisco J. Amaro-Gahete[12] · Marika Ferrari[13] · Inge Huybrechts[1,14] · Marcela Gonzalez-Gross[15] · Stefaan De Henauw[1] · Nathalie Michels[1]

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Abstract

Aim Our aim is to demonstrate that a healthy diet might reduce the relation between adiposity and inflammation, whereas an unhealthy diet may increase the effect of adiposity on inflammatory biomarkers.

Methods In 618 adolescents (13–17 years) of the European HELENA study, data were available on body composition, a set of inflammation markers, and food intake determined by a self-administered computerized 24-h recall. A 9-point Mediterranean diet score and an antioxidant-rich diet score were used as dietary parameters and tested as moderator. Total body fat was represented by the sum of six skinfold thicknesses and central adiposity by waist circumference. A set of inflammation-related biomarkers was used as outcome: a pro/anti-inflammatory interleukins ratio, TGFβ-1, C-reactive protein, TNF-α, 3 cell adhesion molecules, and 3 types of immune cells; gamma-glutamyltransferase (GGT) and homocysteine were used as cardiovascular disease risk biomarkers, and alanine transaminase (ALT) as liver dysfunction biomarker. Multiple linear regression analyses tested moderation by diet in the adiposity-inflammation association and were adjusted for age, sex, country, puberty, socioeconomic status.

Results Both the Mediterranean and antioxidant-rich diet, and overall and central adiposity, were important in the moderation. Diet was a significant protective moderator in the effect of adiposity on the pro/anti-inflammatory interleukins ratio, TGFβ-1, GGT, and ALT.

Conclusion In conclusion, in some cases, a diet rich in antioxidants and essential nutrients may attenuate the concentration of inflammatory biomarkers caused by adiposity, whereas a poor diet appears to contribute to the onset of early oxidative stress signs.

Keywords Low-grade inflammation · Adiposity · Mediterranean diet score · Adolescents

Introduction

The prevalence of overweight and obesity is increasing worldwide at an alarming rate [1]. Obesity is considered to be an important risk factor for the development of metabolic syndrome and chronic diseases, with evidence that it may be established in childhood and adolescence [2]. Obese people have higher concentrations of pro-inflammatory mediators than lean people, since the adipose tissue plays an important immune role and may be a major source of pro-inflammatory mediators, leading to a state of chronic low-grade inflammation [3].

Convincing evidence suggests that low-grade inflammation has a fundamental role in all stages of the atherosclerotic process, and for some individuals it starts in childhood and progresses throughout life [4]. Several studies conducted in adolescent populations have confirmed that a state of low-grade inflammation is detectable early in life, as demonstrated recently by Ferrari et al. [5]. They concluded that the

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✉ Aline Arouca
aline.barbedoarouca@ugent.be

✉ Nathalie Michels
nathalie.michels@ugent.be

Extended author information available on the last page of the article

adiposity found in European adolescents from the HELENA study was sufficient to cause inflammation as reflected by high levels of C-reactive protein (CRP). In addition, abdominal obesity has been associated with low-grade inflammation in healthy pre-pubertal children, and in youth, it has been associated with cardiometabolic alterations such as blood pressure, insulin resistance, total cholesterol, and triglycerides [6, 7].

These cardiometabolic alterations can also mediate a future cardiovascular event when the wrong choices in food are made. A highly processed, calorie-dense, and nutrient-depleted diet induces immediately oxidative stress after a meal. This transient increase in free radicals, acutely triggers atherogenic changes including endothelial dysfunction, hypercoagulability, sympathetic hyperactivity, contributing to the low-grade inflammation [8].

On the other hand, low-grade inflammation can be ameliorated by consumption of certain foods rich in bioactive substances such as polyunsaturated fatty acids and antioxidant vitamins. The antioxidant and anti-inflammatory properties of the nutrients have been shown via decreased inflammation, oxidative stress, insulin resistance, and adipocyte development [9, 10]. Indeed, many epidemiological studies show that maintaining a healthy diet, such as the Mediterranean diet, could counteract the effects of inflammatory responses [11–14]. According to Root et al. [15], there is a negative effect of a healthy diet (rich in fruit and vegetables) on inflammatory biomarkers and a positive effect on antioxidant capacity indicators. A Belgian cross-sectional study reinforced the fact that diet plays an important role in inflammation modification, by a significant positive association between a dietary inflammatory index (a total score from several food groups and dietary constituents) and inflammatory markers [16].

As several studies demonstrate, there is no doubt that the metabolic correlates of excess weight, and the state of chronic low-grade inflammation can be detectable early in life [5–7, 17]. Nevertheless, investigations showing the attenuating effect of a healthy dietary intake on inflammatory biomarkers in overweight/obese adolescents have not been carried out yet. Urgent attention, policy, and action are needed to avoid the enormous future social and health care costs associated with the consequences of obesity in youth [18]. Identifying a change on the inflammatory state in adolescents would provide valuable information for the development of health programs and public opportunities in the prevention of chronic diseases. After all, several nutritional compounds may potentially limit inflammation, helping to maintain a favorable inflammatory status and avoid and/or reduce the progression of obesity-related co-morbidities [9].

The main aim of this study is to examine the relationships between phenotypic factors related to adiposity, nutrition, and low-grade inflammation in adolescents of the European HELENA study. Our hypothesis is that (a) high concentration of inflammatory biomarkers would be associated with adiposity, and (b) that a high adherence to the Mediterranean diet or to an antioxidant-rich diet could attenuate the positive effect of adiposity on inflammation-related biomarkers. Herein, adiposity will be measured by skinfolds and waist circumference, allowing the differentiation between overall and central adiposity. Additionally, with the aim to make the analysis more interesting, several types of markers of the inflammatory status, including some for chronic disease risk, will be checked.

Methods

Study design and participants

Data was derived from the HELENA-Cross Sectional Study, which was conducted in 10 European cities from 2006 to 2007. The main objective of the HELENA-CSS study was to obtain reliable and comparable data of a large sample of European adolescents on a variety of nutrition and health related parameters by a standardized procedure. The study was performed following the ethical guidelines of the Declaration of Helsinki 1961 (revision of Edinburgh 2000), the Good Clinical Practice, and the legislation about clinical research in humans in each of the participating countries. All participants and their parents signed an informed consent. Details on sampling procedures and study design of the HELENA study have been reported elsewhere [19]. In the HELENA study, the total sample was 3528 adolescents with a subset of 1089 in which a blood sample was obtained. For the present analysis, data from Heraklion and Pecs ($n=211$ adolescents which had blood test) could not be included due to incomplete dietary intake data. Furthermore, specific inclusion criteria such as data availability from the 24-h dietary recall, anthropometry and a particular set of biomarkers in blood, were defined for the present study. Finally, 618 adolescents (281 male, 337 female), aged 13.0–16.99 years were included (Fig. 1).

Dietary intake assessment

Eating habits were determined from the HELENA-Dietary Assessment Tool (HELENA-DIAT) as described in detail by Diethelm et al. [20]. HELENA-DIAT, a self-administered computerized 24-h recall, was based on the Young Adolescents' Nutrition Assessment on Computer (YANA-C) [21], a tool validated in Flemish adolescents. Participants completed the HELENA-DIAT twice on non-consecutive days within a time span of 2 weeks, to achieve information closer to habitual food intake. Based on these data, two dietary indices were calculated: the Mediterranean diet

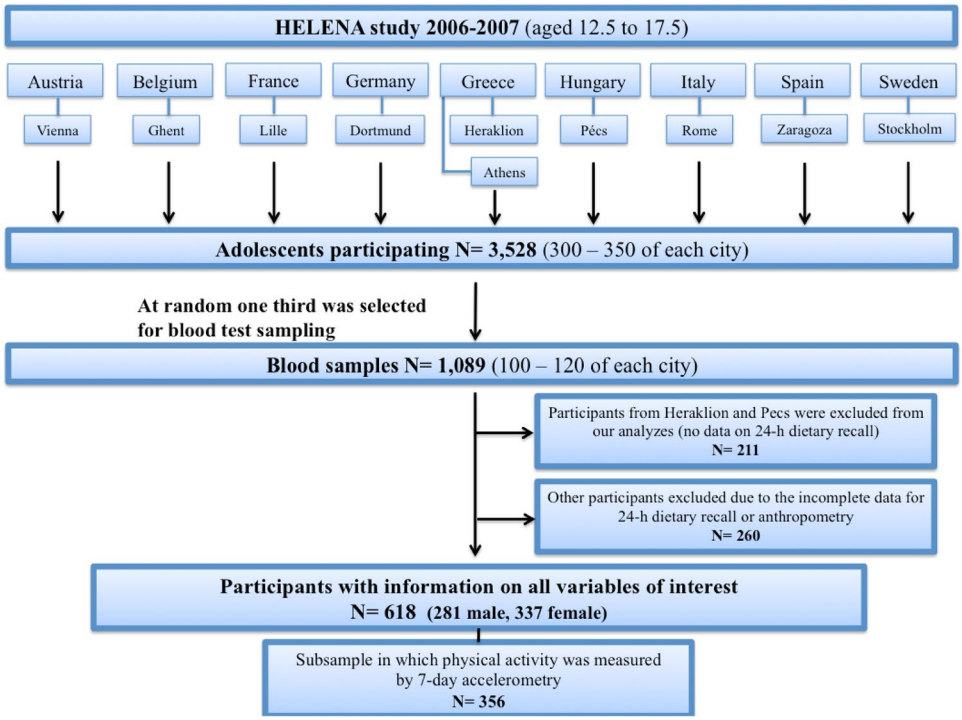

Fig. 1 Sampling procedure schemes

score calculates a balance of generally healthy food versus unhealthy food, while the antioxidant-rich diet score only takes into account food components which are rich in antioxidants—and thus potential anti-inflammatory—capacity. The mean of alcohol consumption in milliliters (from alcoholic beverages) among the adolescents (27 boys and 80 girls), was 1.42 (SD 3.10) and 0.40 (SD 0.99) for boys and girls, respectively.

Mediterranean diet score

The Mediterranean diet score consists of nine single components, namely monounsaturated/ saturated fatty acids, legumes, fruits and nuts, vegetables, meat, cereals, alcohol, dairy, and fish. A scale indicating the degree of adhesion to the traditional Mediterranean diet was firstly constructed [22], and later, revised to include fish intake [23]. The adherence to the traditional Mediterranean diet was assessed by a 9-point Mediterranean diet score that incorporated the salient characteristics of this diet (range of scores 0 to 8, with higher scores indicating greater adherence) [24]. In this study, six component characteristics in the Mediterranean diet score were considered as positive: (1) high ratio of monounsaturated to saturated dietary lipids (mainly olive oil), (2) high consumption of vegetables, (3) high consumption of fruits and nuts, (4) high consumption of fish, (5) high consumption of cereals, and (6) high consumption of pulses; while three components were considered as negative, (7) high consumption of meat and meat products, (8) high consumption of milk and dairy products, and (9) any consumption of alcohol. The consumption of alcohol was considered negative in this study because of our focus on an adolescent population.

Antioxidant-rich diet score

The score was based on the sum of six food components, which reflected the intake (absolute quantity) reported by adolescents through 24-h dietary recall. Food components were vegetables, fruits, nuts, pulses, fish and a monounsaturated–saturated fat ratio, to represent a high content of nutrients such as essential minerals, antioxidant vitamins, monounsaturated and polyunsaturated fatty acids. To have a normal distribution and equal weight, each food component was rank transformed before summing up, and the score ranges from − 7.50 to 10.34.

Adiposity assessment

The sum of six skinfold thicknesses (biceps, triceps, subscapular, suprailiac, thigh and calf) was used as marker of overall adiposity, while waist circumference was used as marker of central adiposity. The IOTF cut-offs were used for Body Mass Index (BMI) [25]. The anthropometric methods in the HELENA study were described in detail by Nagy et al. [26]. All centers followed the same manual and fieldworkers followed a central training. All measures were performed in triplicate. Intraobserver reliability values were greater than

95 and 97% for skinfold thicknesses and circumferences, while interobserver reliability was greater than 90%. Pubertal stage was used as confounder and was evaluated by a medical doctor according to Tanner and Whitehouse [27].

Blood samples and markers associated to inflammation-related biomarkers

Blood samples were collected from fasting in a randomly selected one-third subset of the total HELENA study population. The methodology for blood collection, transport and analysis was standardized among all participating centers. A description of the blood analysis has been reported elsewhere [28]. The quality control for all parameters was in the range of the recommended levels reported in the literature and transport had no influence. As all analyses were executed by certified laboratories, blinded quality controls were implemented, e.g., for homocysteine, a high and low control concentration was tested following the test instructions. Detection limits (sensitivity) were 0.007 mg/L for CRP, 0.05 pg/mL for TNF-α, 2.5U/l for GGT, 0.079 ng/mL for sE-Selectin, 0.016 ng/mL for sVCAM-1 and 0.009 ng/mL for sICAM-1 and 0.5 micromol/L for homocysteine. The intra-assay CVs were 1.9% for CRP, 3.5% for TNF-a α, 6.7% for TGFb, < 2.5% for blood cell counts, 1.3% for GGT, 11.2% for sE-Selectin, 4.5% for sVCAM-1, 7.9% for sICAM-1 and 3.4% for homocysteine. CRP was measured in serum by immunoturbidimetry (AU2700 biochemistry analyzer, Olympus, Watford, UK). Serum cytokines were determined using the High Sensitivity Human Cytokine MILLIPLEXTM MAP kit (Millipore Corp., Billerica, MA, USA) and collected by flow cytometry (Luminex-100 v.2.3, Luminex Corporation, Austin, TX, USA). WBC counts were determined with automated blood cell counters. Lymphocytes were measured in the Immunonutrition laboratory at the Spanish National Research Council after incubated with monoclonal antibodies (BD Biosciences, San José, CA, USA). The serum adhesion molecules were analyzed through commercial ELISA kit (Diaclone, France). ALT and GGT levels were measured in serum using standard protocols with the clinical chemistry system RxL (Dade Behring, Schwalbach, Germany). Homocysteine was measured by competitive immunoassay (Immulite 2000, DPC Biermann GmbH, Bad Nauheim, Germany). Although there is no real consensus regarding the selection of biomarkers to access inflammation [29], there are some cytokines, adhesion molecules, immune cells and acute-phase proteins that have been previously used. These include: cytokines (IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, TGFβ-1 and TNF-α) [30], adhesion molecules which are stimulated by cytokines (soluble vascular adhesion molecule 1 (sVCAM1), soluble intercellular adhesion molecule 1 (sICAM1), soluble E-selectin), immune cells (white blood cell count, lymphocyte count and T-cell count by CD3-recognition), the acute-phase protein CRP, cardiovascular risk factors (gamma-glutamyltransferase enzyme (GGT) [31], and homocysteine), and alanine transaminase enzyme (ALT) as a marker of Non-Alcoholic Fatty Liver Disease (NAFLD) [32]. To represent the interleukins, a ratio of pro versus anti-inflammatory interleukins was calculated after z-score transformation of the individual interleukins, to give them equal weight in the equation: pro/anti-inflammatory ratio = (IL-1 + IL-2 + IL-6)/(IL-4 + IL-5 + IL-10). None of the adolescents took nonsteroidal anti-inflammatory drugs, and or had an active inflammatory diseases. The recruited girls in the study were not in the menstrual period, as well as they did not present polycystic ovarian syndrome. Smoking habits were reported as follows: 8.6% smoked every day; 4.9% at least once a week; 4.9% less than once a week; and 81.6% were not smokers (0.4% missing data).

Physical activity

Moderate-to-vigorous physical activity was measured by 7-day accelerometry in only a subsample ($n = 356$) of the participants in our study. A detailed description of the physical activity assessment has been reported elsewhere [33]. Physical activity was assessed using uni-axial accelerometer (Actigraph GT1M, FL, USA) during 7 days at the lower back. Data with periods of continuous zero values for more than 20 min were considered 'accelerometer non-wear' periods and were, therefore, excluded from the analyses. At least 3 days of recording with a minimum of 8 or more hours of registration per day were necessary for the adolescent to be included in the study. In addition, a diary had to be completed. The amount of time engaged in moderate-to-vigorous activity was based on the standardized cut-off point of ≥ 2000 counts per min [34].

Socioeconomic status (SES)

The family affluence scale was assessed via questionnaire and used as an indicator of the adolescents' material affluence and a predictor of their health outcomes [35]. A recategorization into three levels was performed: low (from 0 to 2), medium (from 3 to 5) and high (from 6 to 8). The adolescents reported their parents' educational level as primary education, lower secondary education, higher secondary education or higher education/university degree. A detailed description of the socioeconomic status has been reported elsewhere [20].

Data analyses

Statistics were performed using SPSS (IBM SPSS Statistics, version 23.0), and moderation effects were obtained using interaction [36, 37]. The statistical significance was set at

two-sided $P < 0.05$. The regression analyses were adjusted for age, sex, center, socioeconomic status, pubertal status, maternal, and paternal educational status. To get a normal distribution of the variables, some biological parameters had to be log-transformed when used as outcome variable: IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, TNF-α, sVCAM-1, sICAM-1, sE-selectin, CRP, GGT, ALT, WBC and homocysteine. Diet and adiposity parameters were not transformed. The z-scores for inflammation scores are based on HELENA population data. The effect of adiposity on inflammatory parameters was first tested with linear regression. The moderation effect (i.e., attenuation) of the diet in the adiposity–inflammation relation was tested by adding the interaction term "diet × adiposity" as predictor of the markers associated with low-grade inflammation, after centering the diet and adiposity parameters. In the case of a significant interaction, the adiposity–inflammation relation was tested for 3 representative groups: those at the mean, at 1 SD below the mean and 1 SD above the mean of the dietary index. As a sensitivity analysis, an additional adjustment was done for moderate-to-vigorous physical activity measured by 7-day accelerometry in only a subsample ($n = 356$).

Results

Characteristics of study participants

The present data analysis included 618 adolescents (281 male, 337 female). Characteristics for age, anthropometry, socio-economic status, maternal and paternal education, diet-related characteristics and markers related to inflammation, are presented in Table 1. Significant differences between boys and girls were found regarding anthropometric characteristics, paternal education, high familial affluence, antioxidant-rich diet score, and 10 of the selected biomarkers in this study. Based on BMI, 14.1% adolescents (41 male, 46 female) were overweight, while 5% (17 male, 14 female) were obese. The higher adherence to the Mediterranean or antioxidant-rich diet was associated with healthier intakes of several macro- and micronutrients (see Supplemental Tables 1 and 2), but not with energy intake differences between low, average, and high adherence to the diets.

The effect of adiposity on markers related to low-grade inflammation

Table 2 shows the effect of adiposity (skinfold thickness and waist circumference, representing overall and central adiposity, respectively) on markers related to inflammation for all subjects. In the presence of adiposity, was found a higher concentration of TGFβ-1 ($P = 0.011$, $P = 0.013$ for overall and central adiposity, respectively), CRP ($P < 0.001$ for overall and central adiposity), WBC ($P < 0.001$ for overall and central adiposity), GGT ($P < 0.001$ for overall and central adiposity), and ALT ($P < 0.001$, for overall and central adiposity); while a lower concentration of lymphocytes ($P = 0.030$ for overall adiposity), sVCAM-1 ($P = 0.008$, $P = 0.001$ for overall and central adiposity, respectively) and sICAM-1 ($P = 0.016$, $P = 0.045$ for overall and central adiposity, respectively).

Moderation effect between adiposity and diet in predicting inflammatory status

Table 3 shows that there was an overall moderation by diet in the obesity-inflammation relation. Both the effect of overall and central adiposity was moderated by the Mediterranean diet and by the antioxidant-rich diet. After additional adjustment for moderate-to-vigorous physical activity measured by 7-day accelerometry in a subsample ($n = 356$), only the significances for Mediterranean diet remained.

Moderator effect of diet (high adherence to the Mediterranean diet score and the antioxidant–rich diet) on the relation between adiposity and low-grade inflammation markers

Significances for separated inflammation markers can be found in Table 4. A moderator effect by a higher adherence to the diets (Mediterranean or antioxidant-rich) was found on the relation between adiposity and inflammation for the pro/anti-inflammatory interleukins ratio, TGFβ-1, GGT, and ALT (Figs. 2a–c, 3a, b). A positive adiposity–inflammation relation was seen only in those with a lower or average adherence. Only for TGFβ-1, the lower adherence to the Mediterranean diet showed just a borderline trend to increase TGFβ-1 at the highest adiposity ($P = 0.051$; Fig. 2a). When using pro/anti-inflammatory interleukins ratio, even a negative adiposity–inflammation relation was seen in high-quality diet adherence. In the sensitivity analysis adjusting for physical activity in a subsample ($n = 356$), all significant findings for the Mediterranean diet remained (data not shown). Maximum R^2 change by including the diet × adiposity parameter was 2%.

Discussion

Previous studies have found a relationship between diet and inflammatory markers [11, 16, 38, 39]. Now our results add evidence to this anti-inflammatory power of the diet by indicating the attenuating or amplifying effect of nutrition in adiposity (both overall and central) leading to low-grade inflammation in an adolescent population. In the presence of

Table 1 Characteristics of the study sample: 618 included European adolescents from the Healthy Lifestyle in Europe by Nutrition in Adolescents (HELENA) study

Variables	(*n* = 618)	
	Mean	SD
Age (years)	14.76	1.22
Anthropometric characteristics		
Weight (kg)	58.39	12.52
Body mass index [kg/stature (m^2)]	21.09	3.51
Body mass index (*z*-scores)	0.39	1.09
Skinfold thickness sum (mm)	89.66	40.65
Waist circumference (cm)	72.09	8.22
Socio-economic characteristics		
High maternal education (%)[a]	70.0	
High paternal education (%)[a]	66.3	
High familial affluence (%)[b]	78.3	
Diet-related characteristics		
Mediterranean diet score (score 0–8)	4.18	1.43
Antioxidant-rich diet score (*z*-score)	0.31	2.57
Markers related to inflammation	Median [P25; P75]	
Pro/anti infl. interleukins ratio (*z*-score)	0.77 [−0.07; 1.37]	
TGFβ-1 (ng/mL)	97.75 [59.22; 146.15]	
TNF-α (pg/mL)	5.50 [4.07; 7.66]	
CRP (mg/L)	0.36 [0.16; 0.88]	
WBC (10/μL)	6.15 [5.34; 7.09]	
Lymphocytes (10^3/μL)	2.11 [1.81; 2.45]	
T-cell count (CD3) (%)	79.60 [64.55; 73.40]	
sVCAM-1 (ng/mL)	1218.25 [1009.50; 1490.00]	
sICAM-1 (ng/mL)	138.00 [106.50; 185.75]	
sE-selectin (ng/mL)	34.00 [25.00; 47.50]	
GGT (U/L)	15.00 [13.00; 18.00]	
ALT (U/L)	20.00 [16.00; 24.00]	
Homocysteine (μmol/L)	6.61 [5.21; 8.34]	

Confidence interval for the mean sex difference using *t* test; Chi-square for categorical variables

P25 25th percentile, *P75* 75th percentile, *SD* standard deviation, *LLCI and ULCI* lower and upper confidence interval respectively, *TGFβ-1* transforming growth factor beta 1, *TNF-α* tumor necrosis factor alpha, Pro/anti-inflammatory interleukins ratio [(IL-1 + IL-2 + IL-6)/(IL-4 + IL-5 + IL-10)], *CRP* C-reactive protein, *WBC* white blood cells, *sVCAM-1, sICAM-1, sE-selectin*, soluble cell adhesion molecules, *GGT* gamma-glutamyltransferase, *ALT* alanine transaminase

[a]High education, higher secondary education and higher education or university degree

[b]Score 4 to 8 of socio-economic characteristics based on family affluence scale (family car ownership, having an own bedroom, internet availability, and computer ownership)

higher adiposity, the highest adherence to the Mediterranean or antioxidant-rich diet was able to decrease the concentration of some markers related to low-grade inflammation, such as a pro/anti- inflammatory interleukins ratio, TGFβ-1, GGT, and ALT.

Moderation effects on the adiposity-cytokine relation were mainly present when considering the whole picture, i.e., using the pro/anti-inflammatory interleukins ratio, with the antioxidant-rich diet as main moderator. Moderation effects on cardiovascular risk factors were seen for both the antioxidant-rich and Mediterranean diet, although more frequently for the Mediterranean diet. These different roles of the 2 dietary indices might be due to the fact that the antioxidant-rich diet does not consider food groups with potential pro-inflammatory capacity like red meat, while the Mediterranean diet considers this using score penalization. For a better understanding, findings will be properly discussed now for those that were moderated by the diets.

Pro/anti-inflammatory interleukins ratio

A pro/anti-inflammatory interleukins ratio [(IL-1 + IL-2 + IL-6)/(IL-4 + IL-5 + IL-10)] was created to consider the net result from pro-inflammatory and

Table 2 The association between adiposity and markers related to low-grade inflammation in 618 adolescents from the HELENA study

Inflammation markers (outcome)	Adiposity marker (predictor)	*B*	LLCI	ULCI	As % change[a]	*β*	*P*
Pro/anti ratio (*z*-score)	Skinfold thickness	0.005	−0.173	0.183	1.157	0.002	0.960
	Waist circumference	−0.029	−0.111	0.053	−6.459	−0.027	0.495
TGFβ-1 (ng/mL)	Skinfold thickness	0.135	0.032	0.238		0.090	**0.011**
	Waist circumference	0.668	0.143	1.193		0.094	**0.013**
TNF-α (log pg/mL)	Skinfold thickness	1.66×10^{-4}	-3.31×10^{-4}	6.63×10^{-4}	0.038	0.024	0.514
	Waist circumference	-4.19×10^{-4}	−0.002	0.001	−0.096	−0.013	0.738
CRP (log mg/L)	Skinfold thickness	0.004	0.003	0.005	0.925	0.273	**< 0.001**
	Waist circumference	0.015	0.012	0.018	3.514	0.220	**< 0.001**
WBC (log 10/μL)	Skinfold thickness	4.77×10^{-4}	3.01×10^{-4}	6.53×10^{-4}	0.109	0.178	**< 0.001**
	Waist circumference	0.002	0.001	0.002	0.461	0.125	**< 0.001**
Lymphocytes (10^3/μL)	Skinfold thickness	−0.015	−0.028	−0.002		−0.073	**0.030**
	Waist circumference	−0.012	−0.080	0.056		−0.012	0.733
T-cell count (CD3) (%)	Skinfold thickness	1.53×10^{-5}	-6.11×10^{-5}	9.18×10^{-5}		0.015	0.696
	Waist circumference	-1.11×10^{-5}	-4.09×10^{-4}	4.09×10^{-4}		−0.002	0.956
sVCAM-1 (log ng/mL)	Skinfold thickness	-3.17×10^{-4}	-5.50×10^{-4}	-8.38×10^{-5}	−0.072	−0.090	**0.008**
	Waist circumference	−0.002	−0.003	−0.001	−0.459	−0.115	**0.001**
sICAM-1 (log ng/mL)	Skinfold thickness	-4.55×10^{-4}	-8.25×10^{-4}	-8.46×10^{-5}	−0.104	−0.084	**0.016**
	Waist circumference	−0.002	−0.003	−0.001	−0.459	−0.073	**0.045**
sE-selectin (log ng/mL)	Skinfold thickness	2.31×10^{-4}	-1.33×10^{-4}	5.95×10^{-4}	0.053	0.043	0.215
	Waist circumference	0.001	-2.91×10^{-4}	0.003	0.230	0.060	0.101
GGT (log U/L)	Skinfold thickness	4.23×10^{-4}	2.23×10^{-4}	6.23×10^{-4}	0.097	0.132	**< 0.001**
	Waist circumference	0.002	0.001	0.002	0.461	0.162	**< 0.001**
ALT (log U/L)	Skinfold thickness	0.001	0.001	0.001	0.230	0.178	**< 0.001**
	Waist circumference	0.004	0.003	0.005	0.925	0.216	**< 0.001**
Homocysteine (log μmol/L)	Skinfold thickness	-2.54×10^{-7}	-2.65×10^{-4}	2.64×10^{-4}	-5.84×10^{-5}	6.20×10^{-5}	0.998
	Waist circumference	0.001	-2.48×10^{-4}	0.002	0.230	0.055	0.112

Linear regression analyses adjusted for age, sex, center, socioeconomic status, maternal, and paternal educational status

TGFβ-1 transforming growth factor beta 1, *TNF-α* tumor necrosis factor alpha, pro/anti-inflammatory interleukins ratio [(IL-1 + IL-2 + IL-6)/(IL-4 + IL-5 + IL-10)], *CRP* C-reactive protein, *WBC* white blood cells, *sVCAM-1, sICAM-1, sE-selectin* soluble cell adhesion molecules, *GGT* gamma-glutamyl transferase, *ALT* alanine transaminase, *B* unstandardized coefficient, LLCI and ULCI, lower and upper confidence interval, respectively, *beta* standardized coefficient

[a]As the outcome value was log-transformed, the coefficient should be interpreted as 1 point change in the predictor results in % change in the outcome, based on the formula "$((10^{\text{coefficient}})-1)\times100$". Bold: statistical significance when $P<0.05$

Table 3 Interaction effect between adiposity and diet in predicting overall inflammatory status in 618 adolescents from the HELENA study

Interaction	*P*	E^2
Skinfold thickness × Mediterranean diet score	**0.026**	0.089
Waist circumference × Mediterranean diet score	**0.017**	0.095
Skinfold thickness × antioxidant-rich diet score	0.127	0.086
Waist circumference × antioxidant-rich diet score	**0.042**	0.108

E^2 = partial eta-squared as effect size. Multivariate regression analyses with as outcome the inflammatory parameters IL-1, IL-2, IL-4, IL-5, IL-6, IL-10, TGFβ-1, TNF-α, soluble VCAM-1, soluble ICAM-1, soluble E-selectin, white blood cell count, T-cell count by CD3-recognition, CRP, GGT, ALT and homocysteine. Central adiposity (waist circumference) adjusted for pubertal status. Bold: statistical significance when $P<0.05$

anti-inflammatory interleukins. Herein, the interleukins 1, 2 and 6 (IL-1, IL-2, IL-6) are correlated with inflammatory, autoimmune, or infectious disease; on the other hand, IL-4, IL-5 and IL-10 can be considered anti-inflammatory interleukins by exerting important roles such as inhibition of macrophage activation and T-cell proliferation, and inhibition of pro-inflammatory cytokine production [30, 40].

The higher adherence to the antioxidant-rich diet exerted a significant enhancing moderator effect on the pro/anti-inflammatory interleukins ratio related to the higher adiposity (Fig. 3a). This moderation can be explained through the presence of omega-3 polyunsaturated fatty acids, which are found in fish oils. They can decrease cytokine production by suppressing the production of arachidonic-acid-derived eicosanoids [4, 41]. During the metabolism of food,

Table 4 Moderator effect of diet (high adherence to the Mediterranean diet score and the antioxidant–rich diet) on the relation between adiposity and low-grade inflammation markers in 618 adolescents from the HELENA study

Inflammation markers (outcome)	Adiposity marker (predictor)	Interaction term with Mediterranean diet				Interaction term with antioxidant-rich diet			
		B	LLCI	ULCI	*P*	*B*	LLCI	ULCI	*P*
Pro/anti ratio (*z*-score)	Skinfold thickness	−0.0059	−0.0130	0.0013	0.1095	−0.0980	−0.1628	−0.0331	**0.0031**
	Waist circumference	−0.0385	−0.0773	0.0002	0.0513	−0.4705	−0.8203	−0.1207	**0.0085**
TGFβ-1 (ng/mL)	Skinfold thickness	−0.0866	−0.1672	−0.0059	**0.0354**	−0.0229	−0.0813	0.0355	0.4411
	Waist circumference	−0.2570	−0.6862	0.1723	0.2402	−0.2293	−0.5386	0.0801	0.1459
TNF-α (log pg/mL)	Skinfold thickness	0.0002	−0.0002	0.0006	0.4188	0.0000	−0.0003	0.0003	0.8128
	Waist circumference	0.0013	−0.0008	0.0034	0.2257	0.0000	−0.0016	0.0016	0.9918
CRP (log mg/L)	Skinfold thickness	0.0004	−0.0003	0.0012	0.2722	0.000	−0.0005	0.0006	0.8641
	Waist circumference	0.0030	−0.0011	0.0071	0.1497	−0.0004	−0.0035	0.0027	0.8007
WBC (log 10/μL)	Skinfold thickness	0.0000	−0.0001	0.0002	0.6118	0.0000	−0.0001	0.0001	0.7436
	Waist circumference	0.0004	−0.0003	0.0011	0.2960	0.0000	−0.0005	0.0005	0.8926
Lymphocytes (10^3/μL)	Skinfold thickness	0.0000	−0.0007	0.0008	0.9055	−0.0005	−0.0010	0.0000	0.0629
	Waist circumference	0.0008	−0.0031	0.0047	0.6872	0.0000	−0.0027	0.0027	0.9784
T-cell count (CD3) (%)	Skinfold thickness	0.0000	−0.0001	0.0000	0.2816	0.0000	0.0000	0.0001	0.2207
	Waist circumference	−0.0003	−0.0005	0.0002	0.4483	−0.0001	−0.0003	0.0001	0.5038
sVCAM-1 (log ng/mL)	Skinfold thickness	−0.0001	−0.0003	0.0001	0.1928	−0.0001	−0.0002	0.0001	0.2896
	Waist circumference	−0.0005	−0.0014	0.0004	0.2488	−0.0002	−0.0009	0.0004	0.5244
sICAM-1 (log ng/mL)	Skinfold thickness	0.0002	−0.0001	0.0005	0.1333	0.0002	0.0000	0.0004	0.1176
	Waist circumference	0.0012	−0.0003	0.0027	0.1181	0.0006	−0.0005	0.0017	0.2884
sE-selectin (log ng/mL)	Skinfold thickness	−0.0001	−0.0004	0.0002	0.5139	0.0000	−0.0002	0.0002	0.7218
	Waist circumference	−0.0006	−0.0020	0.0009	0.4510	0.0000	−0.0010	0.0010	0.9686
GGT (log U/L)	Skinfold thickness	−0.0003	−0.0004	−0.0001	**0.0017**	−0.0001	−0.0002	0.0000	**0.0132**
	Waist circumference	−0.0007	−0.0016	0.0002	0.1137	−0.0008	−0.0014	−0.0002	**0.0077**
ALT (log U/L)	Skinfold thickness	−0.0003	−0.0004	−0.0001	**0.0072**	−0.0001	−0.0002	0.0000	0.0949
	Waist circumference	−0.0011	−0.0021	−0.0001	**0.0354**	0.0001	−0.0006	0.0008	0.8391
Homocysteine (log μmol/L)	Skinfold thickness	−0.0001	−0.0012	0.0010	0.8519	0.0000	−0.0002	0.0001	0.5943
	Waist circumference	−0.0001	−0.0012	0.0010	0.8519	0.0003	−0.0005	0.0011	0.4399

produced oxidants such as superoxide radicals or hydrogen peroxide also may activate the NF-kB pathway, contributing to inflammation; however, the presence of antioxidant properties from vegetables and fruits may limit the pro-inflammatory responses [42, 43].

Transforming growth factor beta 1 (TGFβ-1)

A lower adherence to the Mediterranean diet enhanced a positive association between adiposity and TGFβ-1 (Fig. 2a). TGFβ-1 is considered a multifunctional cytokine and plays an important role in regulating repair and tissue regeneration [44]. A high concentration can be present in obesity development and fibrosis of the liver or white adipose tissue [45]. Fibrosis is generated by persistent inflammatory stimuli, and it is attributed to an excessive deposition of extracellular matrix proteins, as an unresolved chronic inflammation [46, 47]. This is the first study that found a positive correlation between a lower adherence to the Mediterranean diet and TGFβ-1 concentrations in adolescents with high adiposity. The trend to decrease the TGFβ-1 concentration in those with higher adherence to the Mediterranean diet may be explained by the presence of vitamin E, an antioxidant vitamin, since it inhibits TGFβ-1 gene expression in the liver, and also contributes to decrease lipid peroxidation and oxidative stress, and therefore improving inflammation and fibrosis [45, 48].

Gamma-glutamyltransferase (GGT)

A higher adherence to the Mediterranean and antioxidant-rich diet showed a moderator effect on GGT in subjects who presented high central or overall adiposity (Figs. 2b, 3b). Serum GGT enzyme activity has long been used as a reliable marker of liver dysfunction and excessive alcohol intake [31]. However, in recent years the knowledge of the physiological functions of this enzyme has expanded and several important epidemiological associations have been reported,

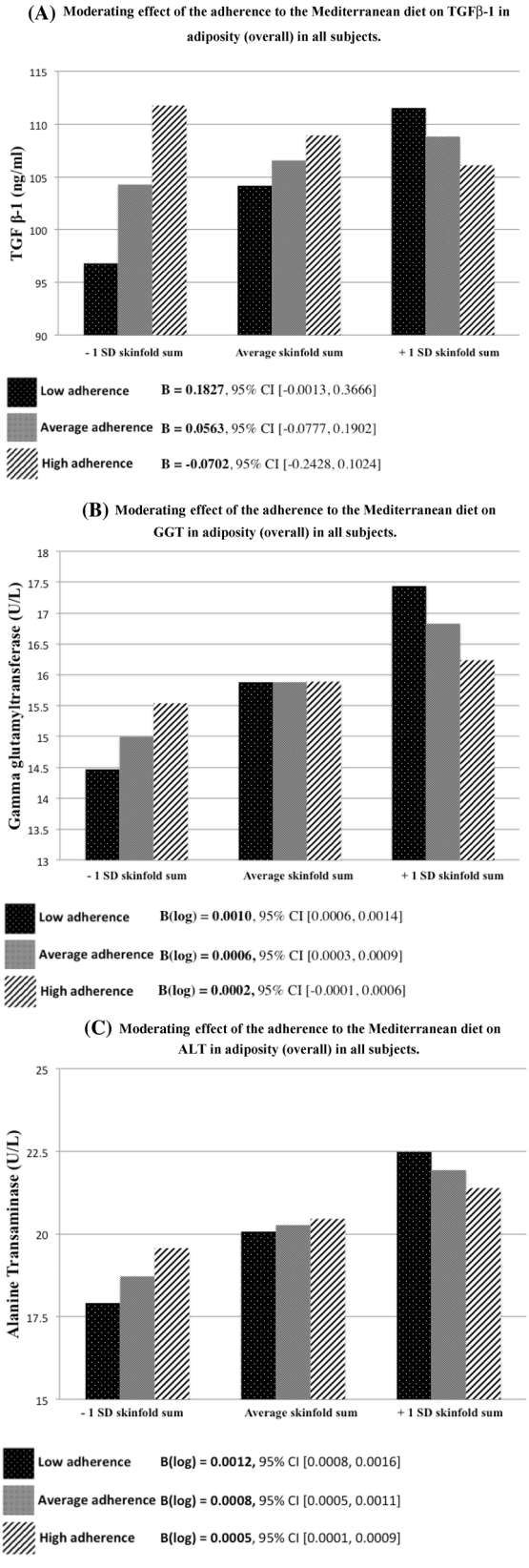

Fig. 2 Moderation effect of the Mediterranean diet on the relation ▶ between inflammation-related biomarkers and adiposity (**a**–**c**). TGFβ-1, Transforming growth factor beta 1; overall adiposity is based on skinfold thickness; central adiposity is based on waist circumference. In the case of a significant moderation, the adiposity–inflammation relation was tested for three representative groups: those at the mean (average adherence), at 1 SD below the mean (low adherence), and 1 SD above the mean (high adherence) of the dietary index

showing that GGT is a superior marker for future disease risk, and early oxidative stress signal, when compared against multiple other known mortality risk factors [49, 50]. At physiological serum values, GGT acts as a protein catalyst in the degradation of glutathione, the major thiol intracellular antioxidant in the body [51]. Pro-inflammatory and pro-oxidant activities have been proposed to explain the reason why GGT correlates with cardiovascular disease risk [50–53]. Glutathione is hydrolyzed into glutamate and a cysteinyl-glycine dipeptide, the latter acting as a strong reducing agent of iron leading to the formation of reactive oxygen species. As result, the super-oxide ion and hydrogen peroxide may cause oxidization of low- density lipoprotein cholesterol particles, contributing for the formation of inflammatory atheroma within the vascular endothelial wall [51, 53]. Several dietary or lifestyle measures, including fruits and meat intake, vitamin status and markers of oxidative stress are associated with GGT concentration [50, 54, 55]. In addition, studies have shown that GGT has the strongest association with BMI compared to other risk markers, and it increases progressively in all classes of BMI [51]. In conclusion, the attenuating power from both diet types on GGT may be due to their antioxidant and anti-inflammatory properties by the presence of essential nutrients [9].

Alanine transaminase (ALT)

At the higher adiposity, the Mediterranean diet attenuated significantly the concentrations of ALT in those adolescents who had a higher adherence, whereas those with lower adherence had high concentrations of this biomarker (Fig. 2c). High ALT concentration is correlated to metabolic syndrome, overweight, and total body fat, and is also considered a marker of early oxidative stress [54]. ALT when used along with other techniques, can predict NAFLD liver dysfunction [32, 56]. The NAFLD is defined by hepatic fat infiltration involving > 5% hepatocytes in the absence of excessive alcohol intake or other liver diseases, and it has become the most common form of liver disease in childhood. Hyperinsulinemia and insulin resistance, which are often accompanied by the adiposity, lead to the hepatic steatosis by increasing the absolute absorption of non-esterified fatty acids in the liver, and by esterification to form triacylglycerol [57]. While the prevalence of NAFLD in the pediatric population can range from 3 to 11%, it may reach

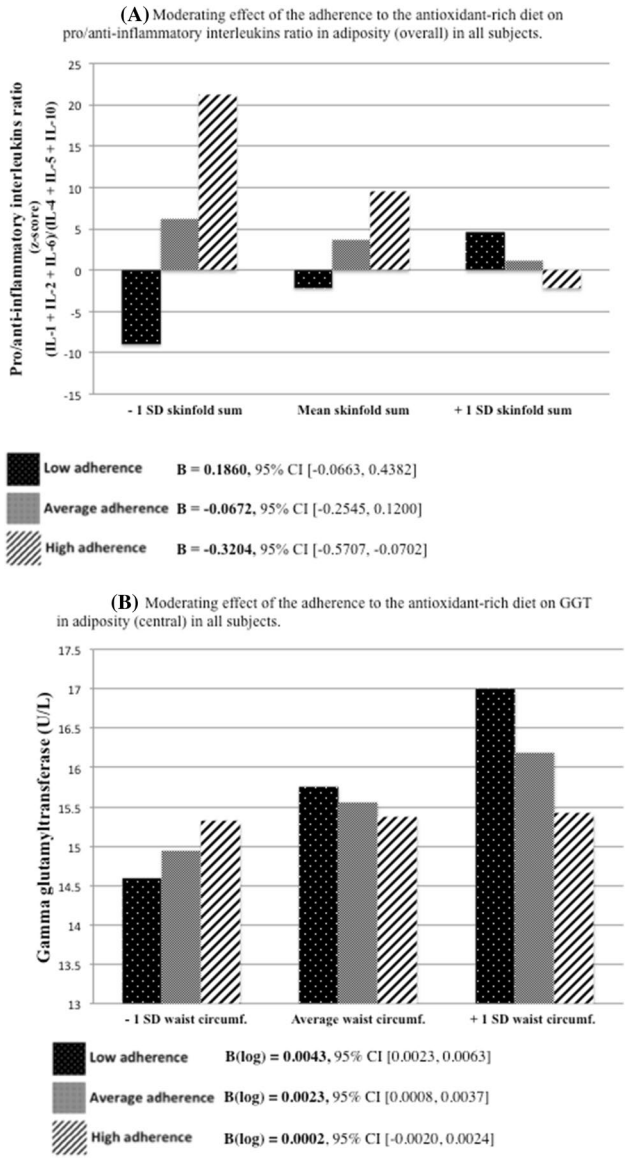

Fig. 3 Moderation effect of the antioxidant-rich diet on the relation between inflammation-related biomarkers and adiposity (**a, b**). Pro/anti-inflammatory interleukins ratio [(IL-1 + IL-2 + IL-6)/(IL-4 + IL-5 + IL-10)]; overall adiposity is based on skinfold thickness; central adiposity is based on waist circumference. In the case of a significant moderation, the adiposity–inflammation relation was tested for three representative groups: those at the mean (average adherence), at 1 SD below the mean (lower adherence), and 1 SD above the mean (high adherence) of the dietary index

20–53% among those who are overweight or have high adiposity [57, 58]. It is proposed that elevated serum ALT is an independent marker of the activation of systemic inflammation (positive association with CRP) and increased oxidative stress, independent of its relationship to metabolic syndrome and NAFLD. As a result, ALT is a predictor of mortality/morbidity more specific cardiovascular health, independent of liver disease [54, 59].

An increased fat intake rich in saturated fat, omega-6 fatty acids and cholesterol, as well as sweet carbohydrates as fructose, is strongly associated with NAFLD [58, 60]. Presumably, the moderator effect of the Mediterranean diet on ALT may be due to the presence of omega-3 polyunsaturated fatty acids, especially eicosapentaenoic acid and docosahexaenoic acid, since they regulate gene transcription factors that control key pathways involved in hepatic lipid metabolism, resulting in increased fat oxidation and improvement of insulin sensitivity. Some studies have already shown the benefits of omega-3 polyunsaturated fatty acids in liver ALT levels, systolic blood pressure, fasting insulin, triglycerides levels and also in normalizing the ultrasonographic findings [60–62].

Strengths and limitations

A first strength is the multi-country design, necessary to capture the diversity in diet patterns. A second asset is the use of both skinfolds and waist as marker of adiposity. The waist circumference was used to reflect central adiposity. Waist circumference is part of the diagnosis of metabolic syndrome and is also almost as useful as ultrasonography although with much more simplicity to implement in epidemiological studies [58]. For the overall adiposity, we applied the sum of six skinfolds. The sum has been shown as a more reliable marker than body fat percentage equations based on the sum, when comparing to the reference method dual-energy X-ray absorptiometry [63]. The skinfold measurement is considered a better predictor of body fat than other simple anthropometric variables or ratios as BMI, since subcutaneous fat (40–60% of total body fat) can be directly measured with a caliper [64]. Another strength is the wide spectrum of inflammatory markers to detect specific inflammatory effects with a better interpretation on health risk, including some markers for chronic disease risk. Interestingly, a multivariate regression (including all inflammatory outcomes at once) showed a consistent significant moderation. Finally, two theory-based dietary indices were applied.

The present study has some limitations. The HELENA-DIAT, a self-administered computerized 24-h recall relies on the respondents' memory and their capabilities to interpret those questions on frequency and quantity of consumption in the last 24 h. Despite of two days of assessment in non-consecutive days, this may not reflect their eating habits on weekends or holidays. In addition, the cross-sectional nature of our study does not permit causality statements. Other limitations of the study include: the lack of testing the validity of the antioxidant diet score and the pro/anti-inflammatory interleukins ratio, a smaller sample size as the result of blood sampling in only one-third of the population, as well as the lack of a complete set of data regarding physical activity. By excluding the

under-reporters from the analyses (19.4%), all significant results remained, except for the interaction between ALT and Mediterranean diet, which did not show any attenuating effect of the diet on the concentration of this biomarker. Finally, adiponectin, which is considered an important anti-inflammatory adipokine, was not assessed in the study.

Conclusion

The results of the present study are mostly consistent with the hypothesis that diet may modulate inflammation. This is the first study that shows the moderator power of the Mediterranean or an antioxidant-rich diet on markers related to low-grade inflammation caused by adiposity (overall and central), in European adolescents. The benefits generated by the high adherence to the diets can be due to the presence of a higher amount of antioxidant vitamins (vitamin C and E), vitamin D and K, folic acid, essential oils omega 3 (represented by EPA and DHA), fiber, magnesium, and the minerals copper and manganese, necessary for the function of the superoxide dismutase enzyme which works as free radical scavenger. This study brings light to the research community, healthcare, professionals, and public policy makers, on how diet can be a modifier in the negative health effects of current society exposure such as adiposity. Consequently, a high adherence to a diet rich in anti-oxidants and polyunsaturated fatty acids (omega 3) can be adopted to help in the prevention of the development of early oxidative stress signs and inflammatory diseases.

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Author contributions AA formulated the research question, has analyzed the data and wrote a draft of the paper. NM helped in formulating the research question, analyzing the data and did editing of the first draft. NM and SDH are co-supervisor and supervisor of Aline Arouca; LAM was coordinator of the HELENA project. All other authors were involved in the HELENA project (coordinator or data collection). AM was responsible for the inflammatory parameter analyses. IH developed the Mediterranean diet score. MGG was responsible for the complete blood sampling and collection. All authors have read the draft and agreed on the final version. The authors would like to thank Anke Carstensen and Petra Pickert for their contribution to the laboratory work.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Affiliations

Aline Arouca[1] · Luis A. Moreno[2,3] · Esther M. Gonzalez-Gil[2,3] · Ascensión Marcos[4] · Kurt Widhalm[5] · Dénes Molnár[6] · Yannis Manios[7] · Frederic Gottrand[8] · Anthony Kafatos[9] · Mathilde Kersting[10] · Michael Sjöström[11] · Francisco J. Amaro-Gahete[12] · Marika Ferrari[13] · Inge Huybrechts[1,14] · Marcela Gonzalez-Gross[15] · Stefaan De Henauw[1] · Nathalie Michels[1]

1 Department of Public Health, Faculty of Medicine and Health Sciences, Ghent University, De Pintelaan 185, Block K3, 4th Floor, 9000 Ghent, Belgium

2 GENUD: "Growth, Exercise, Nutrition and Development" Research Group, Facultad de Ciencias de la Salud, University of Zaragoza, Instituto Agroalimentario de Aragón (IA2), Instituto de Investigación Sanitaria Aragón (IIS Aragón), Spain, Universidad de Zaragoza, Zaragoza, Spain

3 Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición (CIBERObn), Santiago de Compostela, Spain

4 Department of Metabolism and Nutrition, Institute of Food Science and Technology and Nutrition, Madrid, Spain

5 Department of Pediatrics, Division of Nutrition and Metabolism, Medical University of Vienna, Vienna, Austria

[6] Department of Pediatrics, Medical School, University of Pécs, Pecs, Hungary

[7] Department of Nutrition and Dietetics, School of Health Science & Education, Harokopio University, Athens, Greece

[8] Faculty of Medicine, University Lille, Lille, France

[9] Faculty of Medicine, University of Crete, Crete, Greece

[10] Research Department Institute of Child Nutrition Dortmund, Pediatric University Clinic, Ruhr-University Bochum, Bochum, Germany

[11] Department of Biosciences, Unit for Preventive Nutrition, Karolinska Institutet, Huddinge, Sweden

[12] Department of Medical Physiology, School of Medicine, University of Granada, Granada, Spain

[13] Council for Agricultural Research and Economics, Research Center for Food and Nutrition, Rome, Italy

[14] International Agency for Research on Cancer, Lyon, France

[15] ImFINE Research Group, Department of Health and Human Performance, Facultad de Ciencias de la Actividad Física y del Deporte-INEF, Universidad Politécnica de Madrid, Madrid, Spain