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ORIGINAL CONTRIBUTION

Diet as a moderator in the association of sedentary behaviors with inflammatory biomarkers among adolescents in the HELENA study

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Abstract

Aim To assess if a healthy diet might attenuate the positive sedentary–inflammation relation, whereas an unhealthy diet may increase the effect of sedentary behaviors 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 assessed by a self-administered computerized 24 h dietary recall for 2 days. A 9-point Mediterranean diet score and an antioxidant-rich diet *z*-score were used as dietary indices and tested as moderators. A set of low-grade inflammatory characteristics was used as outcome: several cytokines in an inflammatory ratio (IL-6, IL-10, TNF-α, TGFβ-1), C-reactive protein, three cell-adhesion molecules (sVCAM-1, sICAM-1, sE-selectin), three cardiovascular risk markers (GGT, ALT, homocysteine) and three immune cell types (white blood cells, lymphocytes, CD3). Sedentary behaviors were self-reported and analyzed as total screen time. Multiple linear regression analyses tested moderation by diet in the sedentary behaviors–inflammation association adjusted for age, sex, country, adiposity (sum of six skinfolds), parental education, and socio-economic status.

Results Both diet scores, Mediterranean and antioxidant-rich diet, were significant protective moderators in the effect of sedentary behaviors on alanine-transaminase enzyme ($P = 0.014$; $P = 0.027$), and on the pro/anti-inflammatory cytokine ratio ($P = 0.001$; $P = 0.004$), but not on other inflammatory parameters.

Conclusion A higher adherence to the Mediterranean diet or an antioxidant-rich diet may attenuate the onset of oxidative stress signs associated by sedentary behaviors, whereas a poor diet seems to increase inflammation.

Keywords Sedentary behavior · Mediterranean diet · Low-grade inflammation · Moderation · Adolescents · HELENA study

Introduction

Sedentary behaviors were identified as the fourth leading risk factor for cardiovascular disease and all-cause mortality in 2010 [1–3], and it has been defined as "activities that do not increase energy expenditure substantially above the resting level". These are activities such as sleeping, sitting, lying down and screen-based time (e.g., computer or watching television), which involve energy expenditure of < 1.5 metabolic equivalents units (MET) [4, 5]; in fact, any waking sitting activity with a low energy expenditure is considered a sedentary behavior [6]. The Pediatric Recommendations and Public Health guidelines in many countries currently recommend limiting screen time for children and adolescents to less than 2 h per day [7–11].

Sedentary behaviors are highly prevalent in current society. During the past 20 years, total screen time (i.e., using computers, watching TV, playing video games) has increased dramatically [2] across developed and developing countries

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[12]. Moreover, there has recently been a shift in screen-viewing behaviours: mobile devices (smartphones and tablets) are becoming more prominent in young children and adolescent's lives [13, 14]: approximately 20% of 24 800 US high school students used these mobile screen devices for ≥ 5 h daily [14]. A 5-year longitudinal study in 2516 adolescents indicated decreases in moderate to vigorous physical activity coupled with increases in leisure-time computer use [15]. The prevalence of these sedentary behaviors increases even through adolescence, and once installed in this phase, there is a strong tendency it remains into adulthood [16]. As the motivation to be sedentary is a behavioral phenotype [17], this behavior should be targeted for prevention during childhood/adolescence.

Sedentary behaviors may lead to the onset of systemic low-grade inflammation [18, 19] as a result of metabolic dysfunction, such as a high triglycerides/low high-density lipoprotein cholesterol concentrations, insulin resistance and impaired glycemic control [20–22]. Although this dysfunction may also contribute to the increase of adipose tissue and therefore, influence certain inflammatory biomarkers associated with adiposity, independently of BMI or central adiposity, sedentary behaviors have a positive correlation with inflammation markers [23, 24].

Screen-based sedentary time have been showing a positive association with inflammation and several health consequences in studies conducted in youth populations [25]. A study in primary school children showed that each additional hour of TV viewing per week was associated with an increase of 4.2% C-reactive protein (CRP) and 0.6% soluble vascular adhesion molecule 1 (sVCAM-1) [8]. One study showed that reallocating 60 min per day of sitting time for standing posture resulted in a 4% reduction in IL-6, while transitioning from sitting to light stepping resulted in improvements in IL-6 (− 28%), CRP (− 41%), and leptin (− 24%), in a population at high risk of type 2 diabetes; these results suggested that decreasing sedentary behaviors provide sufficient stimulus to elicit benefits upon markers of low-grade inflammation [6]. Other studies found relations with IL-6, IL-1ra, TNF-α and soluble intercellular adhesion molecule 1 (sICAM1) [23, 26].

As shown above, previous studies have examined the effects of sedentary behavior on low-grade inflammation. However, there are still no investigations that take into account interactions between lifestyle factors by testing moderation. A moderator is a third variable affecting the direction and/or strength of the relationship between a predictor and outcome variable, i.e., the relation might only be present in case of low values of the moderator [27, 28]. This moderator can thus act as a vulnerability (stimulates sedentary induced inflammation) or protective (prevents sedentary induced inflammation) factor. This allows to target the moderator in prevention and to identify at-risk populations. An interesting lifestyle factor that might act as moderator towards inflammation is diet.

Certain foods may exacerbate or attenuate the transcription process of pro- or anti-inflammatory factors [29]. An overall healthy diet consumption, that is, a diet rich in essential nutrients, antioxidant vitamins, polyunsaturated (omega 3) and monounsaturated fatty acids, can attenuate low-grade inflammation [29–31]. A healthy diet can be found in high-fiber, and plant-based foods such as vegetables and fruits, whole grains, legumes, and nuts (as the Mediterranean diet is characterized); these features can improve post-prandial dysmetabolism, and blunt the post-meal increase in glucose, triglycerides, oxidative stress, and therefore, inflammation. Instead, an energy-dense, and nutrient-depleted diet, are features of an unhealthy diet, which can induce 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 and future cardiovascular event [32].

A better understanding of these interactive physiological phenomena would be useful in forming the policy initiatives on lifestyle with greater precision. Therefore, the current paper intends to analyze the moderating effect of diet on the association between sedentary behaviors and inflammation. The hypothesis is that the sedentary behaviors–inflammation association would become less or not significant in adolescents with a high adherence to a healthy diet.

Methods

Study design and participants

Details on sampling procedures and study design of the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study have been reported elsewhere [33]. Data were 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 standardized procedures. 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.

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 (in two non-consecutive days), anthropometry (measurement of six skinfold thickness) and a particular set of biomarkers in blood (interleukins 1, 2, 4, 5, 6 and 10; transforming growth factor beta 1; tumor necrosis factor alpha; C-reactive protein; white blood cells; lymphocytes; CD3 T-cell; soluble cell adhesion molecules: sVCAM-1, sICAM-1, and sE-selectin; gamma-glutamyl transferase, and alanine transaminase), 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

Food intake was assessed using the HELENA-Dietary Assessment Tool (HELENA-DIAT) as described in detail by Diethelm et al. [34]. HELENA-DIAT, a self-administered computerized 24-h dietary recall (24HDR), was based on the Young Adolescents' Nutrition Assessment on Computer (YANA-C) [35], a tool validated in Flemish adolescents, which showed strong correlations with all investigated nutrients (r 0.86–0.91) between the methods when validated against 24 h recall interviews. 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 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 have potential anti-inflammatory capacity.

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 first constructed [36], and later revised to include fish intake [37]. 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 0–9, with higher scores indicating greater adherence) [38]. 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

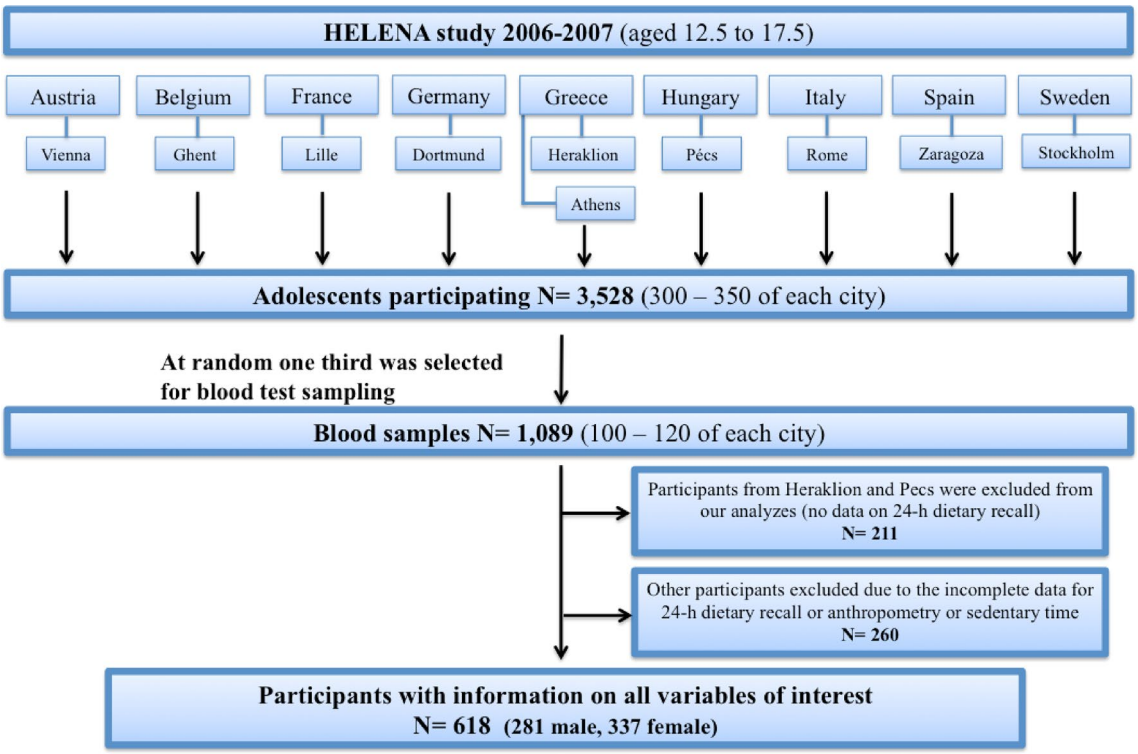

Fig. 1 Sampling procedure schemes

negative in this study because of our focus on an adolescent population. 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.

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 recalls. Food components were vegetables, fruits, nuts, pulses, fish and a monounsaturated–saturated fat ratio, to represent a high content of essential nutrients, 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

Skinfold thickness was measured to the nearest 0.2 mm in triplicate in the left side at biceps, triceps, subscapular, suprailiac, thigh, and medial calf with a Holtain Caliper (Crymmych, UK). The sum of these six measures was used as marker of overall adiposity. All centers followed the same manual and fieldworkers followed a central training. Intraobserver reliability values were greater than 95% for skinfold thicknesses, while interobserver reliability was greater than 90% [39]. The IOTF cut-offs were used for Body Mass Index (BMI) [40]. The anthropometric methods in the HELENA study has been reported in detail elsewhere [39, 41].

Blood samples and markers associated to inflammation-related biomarkers

Blood samples were collected from fasting state 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 and has been reported elsewhere [42]. 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 centrally 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, 0.009 ng/mL for sICAM-1, and 0.5micromol/L for homocysteine. The intra-assay CVs were 1.9% for CRP, 3.5% for TNF-α, 6.7% for TGFβ-1, <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 was 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, Schawalbach, Germany) at the Central Laboratory of the University of Bonn. Homocysteine was measured by competitive immunoassay (Immulite 2000, DPC Biermann GmbH, Bad Nauheim, Germany) at the IEL laboratory of the University of Bonn. Although there is no real consensus regarding the selection of biomarkers to access inflammation [43], 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) [44], 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) [45], and homocysteine), and alanine transaminase enzyme (ALT) as a marker of non-alcoholic fatty liver disease (NAFLD) [46]. 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 disease. The recruited girls in the study were not in the menstrual period, as well as they did not have Polycystic Ovarian Syndrome. Smoking habits was 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).

2.5 Sedentary behaviors assessment

A validated self-reported questionnaire was used. Adolescents had to report their habitual time devoted to several sedentary behaviors during both week and weekend days: (1) TV viewing, (2) computer games, (3) video games, (4)

Internet for non-study reasons (hobbies), (5) Internet for study reasons and (6) study time (out of scholar schedule); e.g., during weekdays: how many hours do you usually watch TV? Adolescents had to tick one of the following categories: (1) 0 min, (2) > 0–30 min, (3) > 30–60 min, (4) > 60– 120 min, (5) > 120–180 min, (6) > 180–240 min and (7) > 240 min. Sedentary minutes per day were estimated as follows: category 1 = 0 min, 2 = 15 min, 3 = 45 min, 4 = 90 min, 5 = 150 min, 6 = 210 min and 7 = 241 min, respectively. Weekly time was calculated taking the mean time in the selected category and applying this formula: [(week-days × 5) + (weekend × 2)]/7. Moreover, a total sedentary score was obtained by summing up the time reported in each category. The test–retest reliability of the HELENA screen time-based sedentary behaviors questionnaire was assessed using the weighted Cohen's κ-coefficients (quadratic). The strength of the agreement for the κ values was interpreted as follows: 0–0.20, slight; 0.21–0.40, fair; 0.41–0.60, moderate; 0.61–0.80, substantial; and 0.81–1.00, almost perfect. For the majority of sedentary behaviors, κ values showed a moderate, substantial or almost perfect agreement (> 0.7), except for Internet for study reasons (0.46 weekdays, 0.33 weekend). A detailed description of the HELENA screen-based sedentary behaviors questionnaire has been reported elsewhere [47].

Socioeconomic status (SES)

The family affluence scale was based on family car ownership, having an own bedroom, internet availability and computer ownership, which was assessed via questionnaire and used as an indicator of the adolescents' material affluence and a predictor of their health outcomes [41]. A re-categorization into three levels was performed: low (from 0 to 2), medium (from 3 to 5) and high (from 6 to 8). A detailed description of the socioeconomic status has been reported elsewhere [34].

Parental education

The participants reported their parents' educational level as primary education, lower secondary education, higher secondary education or higher education/university degree [34].

Data analyses

Statistics were performed using SPSS (IBM SPSS Statistics, version 23.0), and moderating effects were obtained using interaction [27, 28]. The statistical significance was set at two-sided $p < 0.05$. The regression analyses were adjusted for age, sex, center, adiposity, socio-economic 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: sVCAM-1, sICAM-1, sE-selectin, CRP, GGT, ALT, WBC and homocysteine. Diet and sedentary behaviors parameters were not transformed. The z-scores for inflammation scores are based on the HELENA population data. The effect of sedentary behaviors on inflammatory parameters was first tested with linear regression. The moderating effect (i.e. attenuation) of the diet in the sedentary behaviors–inflammation relation was tested by adding the interaction term "diet*sedentary behavior" as predictor of the markers associated with low-grade inflammation, after centering the diet and sedentary behaviors parameters. In the case of a significant interaction, the sedentary behaviors–inflammation relation was tested for three representative groups: those at the mean, at 1 SD below the mean and 1 SD above the mean of the dietary index.

Results

Characteristics of study participants

The present data analysis included 618 adolescents (281 male, 337 female). Characteristics for age, anthropometry, socio-economic status, diet-related characteristics, sedentary behavior and markers related to inflammation, are presented in Table 1. Based on BMI, 14.1% adolescents (41 male, 46 female) were overweight, while 5% (17 male, 14 female) were obese. The Pediatric Recommendations to limit screen viewing time for less than 120 min [7] were exceeded by approximately 69 min (57.45%) by 198 boys and 174 girls.

The association between sedentary behavior and markers related to low-grade inflammation

Table 2 shows the association between sedentary behavior (total screen time i.e., using computers, watching TV, playing video games) and markers related to inflammation for all subjects. A lower concentration of ALT was found when more time was spent on sedentary behavior ($P = 0.027$).

Moderating effect of diet (higher adherence to the Mediterranean diet Score and the antioxidant-rich diet) on the relation between sedentary behavior and low-grade inflammation markers

The association of sedentary–diet interaction terms with inflammation markers can be found in Table 3. The dietary indices were significantly related to inflammation [48] but not with sedentary behavior (correlation − 0.059 for Mediterranean and − 0.087 for antioxidant-rich diet). Figures 2a, b and 3a, b show the moderating effect of the higher

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

Variables	Boys (*n* 281)		Girls (*n* 337)		*P* for sex difference
	Mean	SD	Mean	SD	
Age (years)	14.79	1.25	14.74	1.19	0.085
Anthropometric characteristics					
Skinfold thickness sum (mm)	74.69	38.72	102.14	37.97	**< 0.001**
Waist circumference (cm)	73.70	8.43	70.74	7.79	**< 0.001**
Body Mass Index (*z*-score)	0.45	1.12	0.35	1.06	0.224
Socio-economic characteristics					
High maternal education (%)[a]	72.2		68.0		0.182
High paternal education (%)[a]	69.3		63.8		0.133
High familial affluence (%)[b]	47.7		52.2		**0.021**
Diet-related characteristics					
Mediterranean diet Score (score 0–9)	4.14	1.45	4.21	1.42	0.071
Antioxidant-rich diet (*z*-score)	− 0.92	2.57	0.67	2.52	**0.001**
Sedentary behavior[c] (min/day)	226.60	151.39	157.98	111.76	**< 0.001**
Markers related to inflammation	Median [P25; P75]		Median [P25; P75]		*P* for sex difference
Pro/anti ratio (*z*-score)	0.77 [0.16; 1.32]		0.77 [− 0.02; 1.39]		0.345
CRP (mg/L)	0.44 [0.18; 0.92]		0.31 [0.15; 0.83]		**0.043**
WBC (10^/µL)	5.95 [5.29; 6.81]		6.34 [5.35; 7.37]		**< 0.001**
Lymphocytes (10^3/µL)	2.25 [1.90; 2.50]		2.04 [1.76; 2.42]		0.077
CD3 (T-cells) (%)	67.60 [63.85; 72.80]		70.85 [66.02; 73.90]		**< 0.001**
sVCAM-1 (ng/mL)	1310.75 [1093.62; 1594.12]		1149.25 [935.12; 1422.00]		**< 0.001**
sICAM-1 (ng/mL)	153.75 [116.12; 198.00]		127.50 [99.25; 167.75]		**< 0.001**
sE-selectin (ng/mL)	35.75 [26.50; 51.37]		32.50 [24.00; 42.75]		**< 0.001**
GGT (U/L)	17.00 [15.00; 20.00]		14.00 [12.00; 16.00]		**< 0.001**
ALT (U/L)	21.00 [18.00; 26.00]		19.00 [16.00; 22.00]		**< 0.001**
Homocysteine (µmol/L)	6.96 [5.51; 8.93]		6.39 [5.10; 7.99]		0.052

Significant differences between groups tested using *T* test (parametric) or Mann–Whitney (non-parametric) for a continuous variable; Chi square for categorical variables

Bold: statistical significance when $P < 0.05$

P25 25th percentile, *P75* 75th percentile, *SD* standard deviation, *P p* value, *CRP* C-reactive protein, *WBC* white blood cells, *CD* cluster of differentiation expressed as percentage of total lymphocytes, *sVCAM-1* sICAM-1, *sE-selectin* soluble cell adhesion molecules, *GGT* gamma-glutamyl transferase, *ALT* alanine transaminase

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

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

[c]Total screen time (i.e., using computers, watching TV, playing video games) in minutes a day. Pro-anti inflammatory cytokines ratio ((TNF-α + IL-6)/(TGFβ-1 + IL-10))

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

Inflammation markers (as outcome of sedentary behavior)	B	95% CI	As % change[a]	β	*P*
Pro/anti ratio (*z*-score)	6.00×10^{-3}	[-1.0×10^{-3} to 0.01]	1.391	0.08	0.122
CRP (log mg/L)	1.14×10^{-4}	[-5.0×10^{-4} to 2.8×10^{-4}]	− 0.026	− 0.02	0.570
WBC (log 10^3/μL)	2.04×10^{-5}	[-4.6×10^{-5} to 8.7×10^{-5}]	0.004	0.02	0.548
Lymphocytes (10^3/μL)	-3.00×10^{-3}	[-8.0×10^{-3} to 2.0×10^{-3}]		− 0.05	0.229
CD3 (T-cells) (%)	3.96×10^{-6}	[-2.5×10^{-5} to 3.3×10^{-5}]		0.01	0.793
sVCAM-1 (log ng/mL)	-2.12×10^{-5}	[-1.1×10^{-4} to 6.7×10^{-4}]	− 0.004	− 0.02	0.637
sICAM-1 (log ng/mL)	1.61×10^{-5}	[-1.3×10^{-4} to 1.6×10^{-4}]	− 0.003	0.01	0.829
sE-selectin (log ng/mL)	1.06×10^{-4}	[-2.5×-10^{-4} to 3.5×10^{-4}]	− 0.024	− 0.07	0.141
GGT (log U/L)	-1.42×10^{-5}	[-8.9×10^{-5} to 6.0×10^{-5}]	− 0.003	− 0.01	0.706
ALT (log U/L)	-9.97×10^{-5}	[-1.9×10^{-4} to -1.1×10^{-5}]	− 0.022	− 0.10	**0.027**
Homocysteine (log μmol/L)	2.00×10^{-5}	[-8.8×10^{-5} to 1.3×10^{-4}]	0.004	0.01	0.716

Linear regression analyses for sedentary behavior as predictor of inflammation markers adjusted for age, sex, center, adiposity, socioeconomic status, maternal and paternal educational status. Pro/anti-inflammatory cytokines ratio [(TNF-α + IL-6)/(TGFβ-1 + IL-10)]

Bold: statistical significance when $P<0.05$

CRP C-reactive protein, *WBC* white blood cells, *CD* cluster of differentiation expressed as percentage of total lymphocytes, *sVCAM-1* sICAM-1, *sE-selectin* soluble cell adhesion molecules, *GGT* gamma-glutamyl transferase, *ALT* alanine transaminase, *B* unstandardized coefficient, *β* 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 "$[(10^{\text{coefficient}})-1]\times100$"

Table 3 Moderating effect of Mediterranean and antioxidant-rich diet adherence on the relation between sedentary behavior and low-grade inflammation markers in 618 adolescents from the HELENA study

Inflammation markers (outcome)	Interaction term with Mediterranean diet			Interaction term with Antioxidant-rich diet		
	B	95% CI	*P*	B	95% CI	*P*
Pro/anti ratio (*z*-score)	-8.00×10^{-3}	[-0.01×10^{-3} to -3.0×10^{-3}]	**0.001**	-4.00×10^{-3}	[-7.0×10^{-3} to -1.0×10^{-3}]	**0.004**
CRP (log mg/L)	-3.15×10^{-5}	[-2.8×10^{-4} to 2.1×10^{-4}]	0.803	-4.93×10^{-6}	[-1.5×10^{-4} to 1.4×10^{-4}]	0.945
WBC (log 10^3/μL)	1.04×10^{-5}	[-3.2×10^{-5} to 5.3×10^{-5}]	0.632	7.86×10^{-6}	[-1.6×10^{-5} to 3.2×10^{-5}]	0.527
Lymphocytes (10^3/μL)	-2.00×10^{-3}	[-5.0×10^{-3} to 9.0×10^{-4}]	0.158	-1.00×10^{-3}	[-3.0×10^{-3} to 5.0×10^{-4}]	0.162
CD3 (T-cells) (%)	-1.34×10^{-5}	[-3.2×10^{-5} to 5.5×10^{-6}]	0.164	-2.40×10^{-6}	[-1.3×10^{-5} to 8.4×10^{-6}]	0.664
sVCAM-1 (log ng/mL)	1.46×10^{-6}	[-5.2×10^{-5} to 5.5×10^{-5}]	0.957	9.30×10^{-6}	[-2.1×10^{-5} to 4.0×10^{-5}]	0.552
sICAM-1 (log ng/mL)	7.09×10^{-6}	[-8.3×10^{-5} to 9.7×10^{-5}]	0.877	1.39×10^{-5}	[-3.7×10^{-5} to 6.5×10^{-5}]	0.530
sE-selectin (log ng/mL)	-4.93×10^{-5}	[-1.3×10^{-4} to 3.5×10^{-5}]	0.254	5.43×10^{-6}	[-4.3×10^{-5} to 5.4×10^{-5}]	0.826
GGT (log U/L)	-3.53×10^{-5}	[-8.3×10^{-5} to 1.2×10^{-5}]	0.145	-9.65×10^{-6}	[-3.6×10^{-5} to 1.7×10^{-5}]	0.482
ALT (log U/L)	-7.00×10^{-5}	[-1.2×10^{-4} to -1.4×10^{-5}]	**0.014**	-3.56×10^{-5}	[-6.7×10^{-5} to -3.9×10^{-6}]	**0.027**
Homocysteine (log μmol/L)	8.50×10^{-7}	[-6.7×10^{-5} to 6.9×10^{-5}]	0.980	1.57×10^{-5}	[-2.2×10^{-5} to 5.4×10^{-5}]	0.420

Moderation was tested using interaction (by Process macro for SPSS) based on including the diet × sedentary behavior predictor, next to diet, overall adiposity (sum of six skinfold thickness), age, center, socioeconomic status, maternal and paternal educational status in the prediction of inflammatory parameters. Pro/anti-inflammatory cytokines ratio [(TNF-α + IL-6)/(TGFβ-1 + IL-10)]

Bold: statistical significance when $P<0.05$

CRP C-reactive protein, *WBC* white blood cells, *CD* cluster of differentiation expressed as percentage of total lymphocytes, *sVCAM-1* sICAM-1, *sE-selectin* soluble cell adhesion molecules, *GGT* gamma-glutamyl transferase, *ALT* alanine transaminase, *B* unstandardized coefficient, *β* standardized coefficient

adherence to the Mediterranean and antioxidant rich-diet, respectively, on the relation between sedentary behavior and inflammation for ALT ($B=-7.003\times10^{-5}$, $P=0.014$; $B=-3.561\times10^{-5}$, $P=0.027$; Mediterranean and antioxidant-rich diet, respectively); and the pro/anti-inflammatory cytokines ratio ($B=-0.0082$, $P=0.001$; $B=-0.0043$, $P=0.004$; Mediterranean and antioxidant-rich diet, respectively). The same significant results remained without adjusting for adiposity, and also after adjusting for waist circumference instead of using the sum of skinfold thickness. The

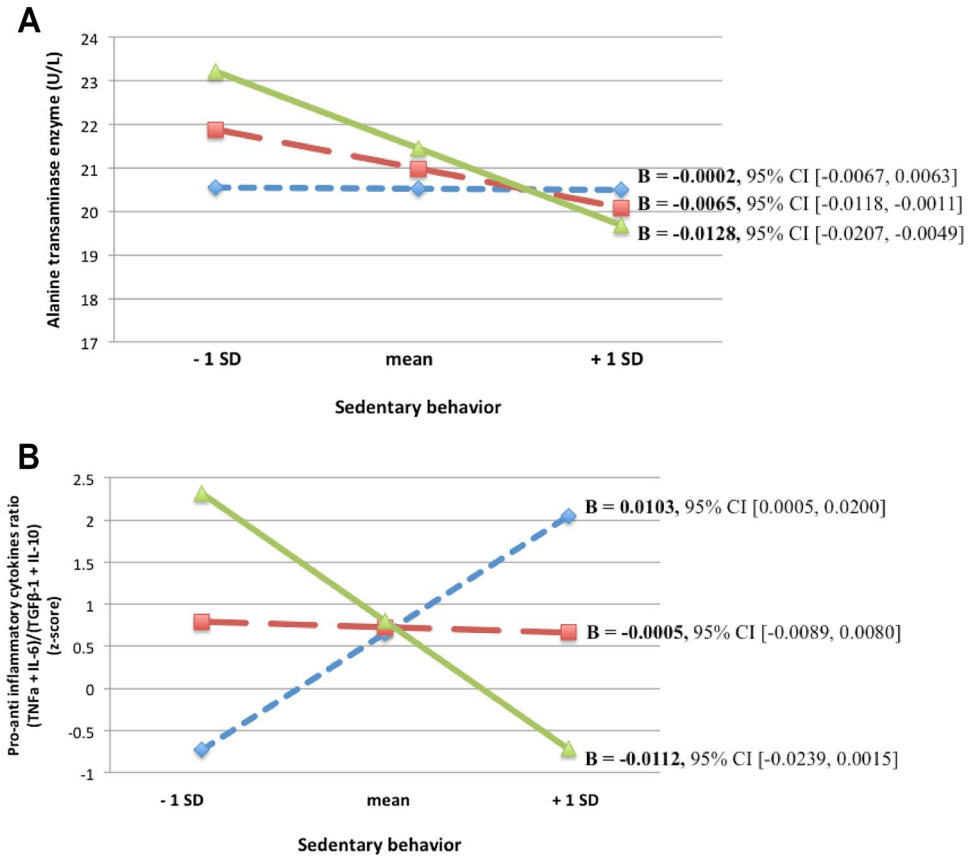

Fig. 2 Moderating effect of the Mediterranean diet on the relation between inflammation-related biomarkers and sedentary behavior (**a, b**). **a** Moderating effect of the adherence to the Mediterranean diet on alanine transaminase enzyme in sedentary behavior in all subjects. Dotted lines low adherence, dashed lines average adherence, straight line high adherence. Sedentary behavior is based on total screen time (i.e., using computers, watching TV, playing video games). In the case of a significant moderation, the sedentary behavior-inflammation relation was tested for 3 representative groups: those at the mean (average adherence), at 1 SD below the mean (lower adherence), and 1 SD above the mean (higher adherence) of the dietary index. **b** Moderating effect of the adherence to the Mediterranean diet on pro/anti-inflammatory cytokines ratio in sedentary behavior in all subjects. Dotted lines low adherence, dashed lines average adherence, straight line high adherence. Sedentary behavior is based on total screen time (i.e., using computers, watching TV, playing video games). In the case of a significant moderation, the sedentary behavior-inflammation relation was tested for 3 representative groups: those at the mean (average adherence), at 1 SD below the mean (lower adherence), and 1 SD above the mean (higher adherence) of the dietary index

R^2 without interaction term was around 5% and increased by 2% when the diet × sedentary behavior parameter was included.

Discussion

Previous studies found a relationship between diet and inflammatory markers [48–52]. Our results add evidence to the capacity of the diet in attenuating or increasing inflammation in adolescents' sedentary behaviors: in the presence of higher sedentary behaviors, the highest adherence to the Mediterranean or antioxidant-rich diet was able to decrease the pro/anti-inflammatory cytokines ratio and ALT enzyme. Indeed, an R^2 change of 2% is a rather small effect, but the moderation on the pro-anti inflammatory cytokines ratio (Fig. 3b) seems substantial as the outcome points between low and high adherence to diet are an interquartile range different, depending on one standard deviation above (+ 1 SD) and one below (− 1 SD) of the mean. There is a high probability that this attenuating effect of the diet was mostly due to specific food groups present in both dietary indices such as vegetables, fruits, nuts, pulses, fish and a high monounsaturated–saturated fat ratio, which represent a high content of essential nutrients, monounsaturated or polyunsaturated fatty acids, and antioxidant vitamins. Other studies also assessed the moderating effect of the diet on inflammatory response through pro- and anti-inflammatory pathways [53]. As an example, a study examined the interaction of polymorphism in the heat shock protein gene (*HSP70*) with energy intake on the risk of high serum concentrations of CRP in coronary artery disease (CAD) patients: compared to control subjects,

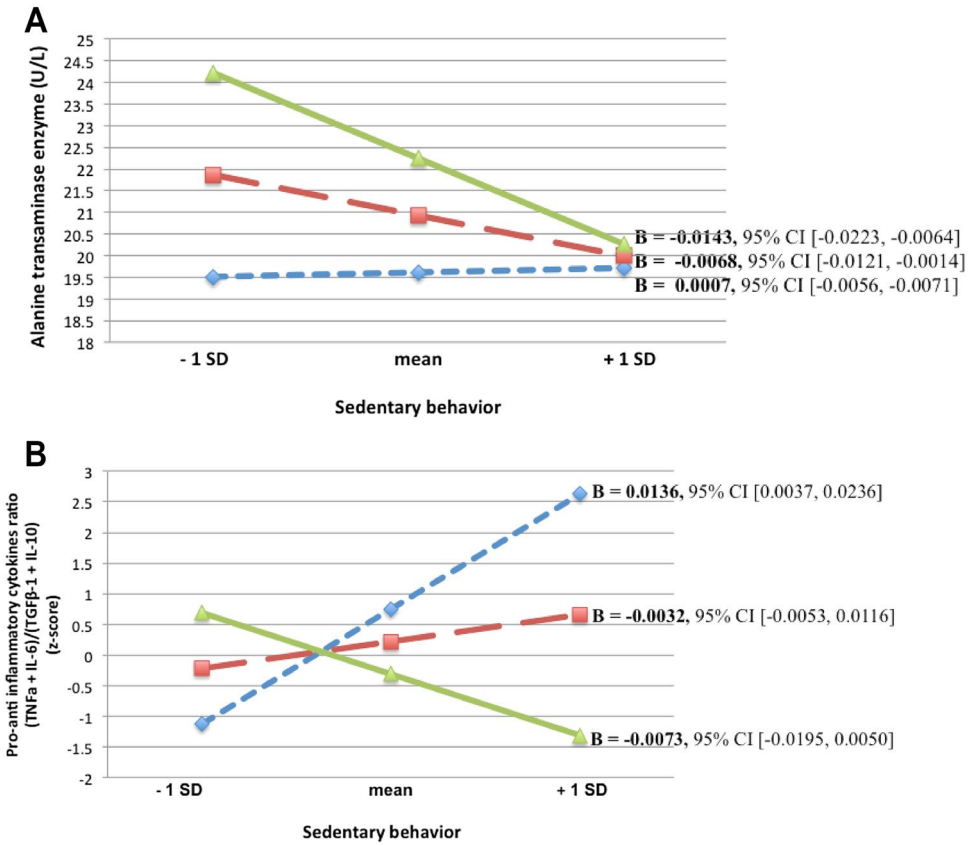

Fig. 3 Moderating effect of the Antioxidant-rich diet on the relation between inflammation-related biomarkers and sedentary behavior (**a, b**). **a** Moderating effect of the adherence to the Antioxidant-rich diet on alanine transaminase enzyme in sedentary behavior in all subjects. Dotted lines low adherence, dashed lines average adherence, straight line high adherence. Sedentary behavior is based on total screen time (i.e., using computers, watching TV, playing video games). In the case of a significant moderation, the sedentary behavior-inflammation relation was tested for 3 representative groups: those at the mean (average adherence), at 1 SD below the mean (lower adherence), and 1 SD above the mean (higher adherence) of the dietary index. **b** Moderating effect of the adherence to the Antioxidant-rich diet on pro/anti-inflammatory cytokines ratio in sedentary behavior in all subjects. Dotted lines low adherence, dashed lines average adherence, straight line high adherence. Sedentary behavior is based on total screen time (i.e., using computers, watching TV, playing video games). In the case of a significant moderation, the sedentary behavior-inflammation relation was tested for 3 representative groups: those at the mean (average adherence), at 1 SD below the mean (lower adherence), and 1 SD above the mean (higher adherence) of the dietary index

the high energy consumption was associated with a higher concentration of CRP, suggesting that the energy-dense diet may exacerbate the inflammation state in CAD patients who carry the genotype of risk [54]. In another study, the moderating effect of a monounsaturated fatty acids (MUFA)-rich diet on polymorphisms of the adiponectin gene (*adipoQ*), which is associated with type 2 diabetes mellitus and insulin resistance, was shown: there was less insulin resistance in men carrying genetic variants who followed a MUFA-rich diet, compared to a saturated fatty acids (SFA)-rich diet [55]. Regarding the Mediterranean diet, studies show that a greater adherence has significant improvements in lipid profile [56], and a reduced risk of developing type 2 diabetes [57]; whereas at lower adherence, it leads to a worse profile of plasmatic inflammation markers in adults and adolescents [58].

Concerning the relationship between sedentary behaviors and inflammatory markers, several studies have shown positive associations, with CRP as the most investigated inflammatory biomarker [18, 19, 25, 59]. In our study, this was not confirmed; even a lower concentration of ALT was found in the highest sedentary behaviors. Depending on adherence to diets, the relation between sedentary behaviors and inflammation parameters was positive, non-significant or negative. For a better understanding, inflammatory parameter specific findings will be properly discussed.

Alanine transaminase (ALT)

A higher adherence to the Mediterranean or Antioxidant-rich diet was able to attenuate significantly the concentrations of ALT in highly sedentary adolescents (Figs. 2a, 3a). ALT is

associated to metabolic syndrome, overweight, total body fat, and also considered a marker of oxidative stress [60]. ALT when used along with other techniques, can predict NAFLD liver dysfunction [46, 61]. 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. While the prevalence of NAFLD in the pediatric population can range from 3 to 11%, it may reach 20–53% among those who are overweight or have high adiposity [62, 63]. 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 [60, 64].

Some studies conducted in adults have found a positive association between longer sitting time and prevalence of NAFLD by showing increased ALT [65, 66], although also non-significant findings exist [67]. However, in the current adolescent sample, ALT was negatively associated with sedentary behaviors, especially in the case of high Mediterranean or antioxidant-rich diet adherence. It is likely that the low prevalence of obesity in our study (5%, $n = 31$) and therefore, the limited available cases of obesity-damaging effects in the liver enzyme levels of the study may explain this association. Most important is, however, the observed attenuating effect of the 'healthy' diet. An increased fat intake rich in saturated fat, omega-6 fatty acids and cholesterol, as well as simple carbohydrates as fructose, is strongly associated with NAFLD [63, 68]. Presumably, the moderating effect of the Mediterranean and antioxidant-rich diets 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 on blood ALT concentrations, systolic blood pressure, fasting insulin, triglyceride levels and also in normalizing the ultrasonographic findings [68–70].

Pro/anti-inflammatory cytokines ratio

A pro/anti-inflammatory cytokines ratio [(TNF-α + IL-6)/(TGFβ-1 + IL-10)] was created to consider the net result from pro-inflammatory and anti-inflammatory cytokines. Herein, IL- 6 and TNF-α are associated with inflammatory, autoimmune, or infectious disease; on the other hand, TGFβ-1 correlates with protection and/or recovery from autoimmune diseases [71], while IL-10 can be considered an anti-inflammatory interleukin by exerting important roles such as inhibition of macrophage activation and T-cell proliferation, and inhibition of pro-inflammatory cytokine production [44, 72].

Using this pro/anti-inflammatory cytokines ratio as outcome, a clear inflammation-attenuating effect by diet was seen. A positive relation between sedentary behaviors and the pro/anti-inflammatory cytokines ratio was found in case of low Mediterranean or antioxidant-rich diet adherence, while this relation was attenuated (thus became non-significant) in high Mediterranean or antioxidant-rich diet adherence (Figs. 2b, 3b). During the metabolism of food, produced oxidants such as superoxide radicals or hydrogen peroxide may activate the NF-kB pathway, contributing to cytokine production. However, antioxidants from vegetables and fruits may limit these pro-inflammatory responses [73, 74], and omega-3 polyunsaturated fatty acids can suppress the production of arachidonic-acid-derived eicosanoids thus decreasing the cytokine production [75, 76].

Strengths and limitations

A first strength is the multi-country design, necessary to capture the diversity in dietary patterns. A second asset 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. Another strength is that the sum of six skinfold thickness was used to reflect overall adiposity and used as confounder. 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 [77]. 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 [78]. All countries used the same self-administered computerized 24-h dietary recall (HELENA-DIAT). Self-administered computer tools have many advantages: standardization of the questions and questioning sequence, fast and easy data processing, immediate results, and it is considered a valid instrument for the assessment of energy and nutrients. The use of two dietary indices allowed us to test the replicability of the moderating effect: the Mediterranean diet and Antioxidant-rich diet showed the same findings.

The present study has some limitations. The HELENA-DIAT 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 2 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 include: two cities did not assess data on dietary intake, and therefore, could not be added in

the analyses; and adiponectin, an important anti-inflammatory adipokine, was not assessed in the study.

Conclusion

The results of the present study are consistent with the hypothesis of diet as an attenuating moderator in the association between sedentary behaviors (which is reflected by the total screen time in this study) and inflammation: sedentary behaviors together with a nutrient-poor diet increased the inflammatory cytokines ratio, while a healthy diet decreased the concentrations of ALT enzyme. Nevertheless, other inflammatory parameters did not undergo this moderation. This study brings light to the research community, healthcare, professionals, and public policy makers, on how diet can attenuate the negative health effects of current society exposure such as sedentary behaviors. Consequently, a diet rich in anti-oxidants and polyunsaturated fatty acids (omega 3) should be adopted to help in the prevention of the development and progression of inflammatory diseases. Undoubtedly, other lifestyle factors should be considered.

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Author contributions ABA 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 ABA; 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. MG was responsible for the complete blood sampling and collection. The authors would like to thank Anke Carstensen and Petra Pickert for their contribution to laboratory work. All authors have read the draft and agreed on the final version.

Compliance with ethical standards

Conflict of interest The author declares that there is no competing interest.

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