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Association between dietary inflammatory index and inflammatory markers in the HELENA study

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Disclosure: Dr. James R. Hébert owns controlling interest in Connecting Health Innovations LLC (CHI), a company planning to license the right to his invention of the dietary inflammatory index (DII) from the University of South Carolina in order to develop computer and smart phone applications for patient counselling and dietary intervention in clinical settings. Dr. Nitin Shivappa is an employee of CHI. None of the other authors declare any conflicts of interest.

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Abstract

Background—Previous research has shown that diet is associated with low-grade systemic inflammation among adults. However, no study has yet been conducted to explore the association between inflammatory potential of diet and low-grade systemic inflammation among adolescents whose dietary behavior may be different from adults.

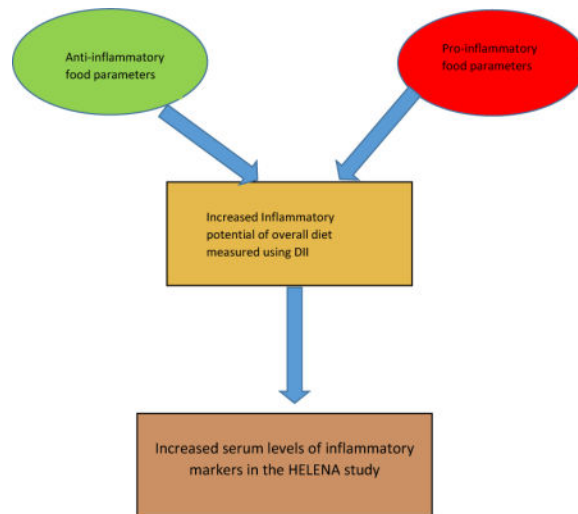
Methods—We examine the predictive ability of 24-hour recall-derived dietary inflammatory index (DII) scores on inflammation among 532 European adolescents in the HELENA cross-sectional study. The DII is a literature-derived dietary index developed to predict inflammation. The DII was calculated per 1000 calories and was tested against C-reactive protein (CRP), interleukins (IL)-1,2,4, 10, TNF- α , ICAM, VCAM and IFN- γ . All inflammatory markers had non-normal distributions and therefore were log transformed. Analyses were performed using multivariable linear regression, adjusting for age, sex, city, BMI, smoking and physical activity.

Results—Pro-inflammatory diet (higher DII scores) was associated with increased levels of various inflammatory markers: TNF- α , IL-1, 2, IFN- γ and VCAM ($b_{\text{DII}t3\text{vs}1} = 0.13$, 95% CI: 0.001, 0.25; 0.13, 95% CI 0.001, 0.25; 0.40, 95% CI: 0.03, 0.77; 0.53, 95% CI: 0.05, 1.01; 0.07, 95% CI: 0.01, 0.13 respectively).

Conclusion—These results reinforce the fact that diet, as a whole, plays an important role in modifying inflammation in adolescents.

Graphical Abstract

Previous research has shown that diet is associated with low-grade systemic inflammation among adults. However, no study has yet been conducted to explore the association between inflammatory potential of diet and low-grade systemic inflammation among adolescents whose dietary behavior may be different from adults. A pro-inflammatory diet as evidenced by higher DII scores is associated with increased levels of various inflammatory markers in the HEalthy Lifestyle in Europe by Nutrition in Adolescents (HELENA) cross-sectional study.



Introduction

Inflammation is body's natural response to dealing with injury or infection in order to heal wounds and promote tissue regeneration (1–4). A chronic, low-grade inflammatory state results when normal negative feedback on acute inflammation does not occur (4). Chronic inflammation has been shown to be associated with cancer (5, 6) and cardiovascular diseases (7–10) such as coronary heart disease (11), myocardial infarction (10) and ischemic stroke (12, 13). Major inflammatory markers that are implicated in these chronic diseases are interleukins (10, 14), tumor necrosis factor (TNF)- α (15), C-reactive protein (11) and vascular cell adhesion molecule (VCAM)-1 (16).

Dietary patterns and components have been shown to have an effect on inflammation. The Mediterranean dietary pattern, which is high in fruits, vegetables, olive oil, whole grains, and fish, and low in red meat and butter, with moderate alcohol and olive oil intake, has been associated with lower levels of inflammation (17). By contrast, the Western-type diet, which is high in red meat, high-fat dairy products, and refined grains, has been associated with higher levels of CRP, IL-6 and fibrinogen (18, 19). Specific nutrients also have consistently been associated with lower levels of inflammation. These include complex carbohydrates (20), n-3 poly-unsaturated fatty acids (PUFA) (21), fiber (22), vitamin E (23), vitamin C (24), β -carotene (25), and magnesium (26).

The dietary inflammatory index (DII) was developed by researchers at University of South Carolina to estimate the overall inflammatory potential of the diet (27, 28). The DII is based upon an extensive literature search incorporating cell culture, animal, and epidemiologic studies of the effect of diet on inflammation. The DII has been previously shown to be associated with inflammation; specifically CRP, IL-6, TNF- α levels among adults (29–31). To the best of our knowledge no work has yet been conducted on the DII and inflammation among adolescents. Unhealthy eating often starts during childhood or adolescence, and progresses throughout life (32, 33), contributing to the onset of chronic diseases in adulthood. Diet in adolescents is very important and plays a major role in the incidence of

various chronic diseases in later life, such as hypertension and cancer (32, 34). Using the data derived from the HELENA (HEalthy Lifestyle in Europe by Nutrition in Adolescents) study, we aim to test the hypothesis that higher DII scores, indicating a more pro-inflammatory diet, are associated with increased systemic inflammation, as shown by increased levels of plasma inflammatory markers among healthy European adolescents.

Methods

Study design and sample

The HELENA-cross-sectional study was conducted between 2006 and 2007 in ten European Cities [Athens and Heraklion (Greece), Dortmund (Germany), Ghent (Belgium), Lille (France), Pecs (Hungary), Rome (Italy), Stockholm (Sweden), Vienna (Austria) and Zaragoza (Spain)]. Detailed information about the study has been reported elsewhere (35). The main objective of HELENA-CSS was to obtain reliable data using uniform collection procedures on a variety of nutritional and health-related parameters in a representative sample of 3528 European adolescents (boys and girls aged 12.5–17.5 years) (36). The ethical committee of each centre approved the study and signed informed consent was obtained from the adolescents as well as from their parents or guardians (37).

For the present analysis, data on nutritional intake from Heraklion and Pecs (n 678) could not be included because of incomplete data. Furthermore, specific inclusion criteria (complete energy and nutrient intake data on two days obtained by the HELENA-DIAT 24-hour recall (24HR) and data on anthropometry) were defined for the present analysis, which were fulfilled by 1804 adolescents. By design, with the same criteria regarding representativeness, blood was collected in only a randomly selected subset of HELENA participants.

For this study, only data on diet, plasma inflammatory markers and covariates were used to perform cross-sectional analysis. Participants with a recent fever episode or acute disease were excluded for the blood sample collection. Other participants were excluded who did not have complete dietary data, did not have data on any of the inflammatory markers, had undetectable values of inflammatory markers (values <0.12 pg/ml). After exclusion for these covariates, there were 532 subjects with evaluable data. Data on demographic characteristics were obtained through a self-administered questionnaire.

2.5. Inflammatory markers—Blood samples were collected in the early morning after overnight fasting. In all cases, 30 ml of blood was extracted by venipuncture from the antecubital vein by a qualified nurse. The stability of samples during transport and storage in the HELENA study has been reported previously (38). Nine key biomarkers involved in low-grade, systemic inflammation were selected for this study (CRP, TNF- α , IL-6, 1, 2, 4, 10, IFN- γ , sICAM and sVCAM. CRP was measured in serum by immunoturbidimetry (AU2700 biochemistry analyzer; Olympus, Watford, UK). Serum cytokines IL-6, 1, 2, 4, 10 and TNF- α were determined using the High Sensitivity Human Cytokine MILLIPLEX™ MAP kit (Millipore Corp., Billerica, MA, USA) and collected by flow cytometry (Luminex-100 v.2.3, Luminex Corporation, Austin, TX, USA). The intra- and inter-assay

precision CVs were: 3.5% and 4.5%, respectively, for IL-6; and 3.5% and 3.8%, respectively, for TNF- α .

Dietary Intake (39) and Dietary Inflammatory Index (DII) (27, 28)

Dietary intake data were obtained using a self-administered, computerized 24HR, named HELENA-DIAT, which was based on the Young Adolescents' Nutrition Assessment on Computer (YANA-C) (40, 41), a tool validated in Flemish adolescents. The basic version was improved by adding dishes representative of cultural/culinary differences observed in European nations participating in HELENA (42). The collection of dietary data is organized in six meal occasions, i.e. breakfast, morning snack, lunch, afternoon snack, evening meal and evening snack. The participants can select from about 400 predefined food items and are free to add non-listed foods manually. Special techniques are used to allow a detailed description and quantification of foods; e.g., pictures of portion sizes and dishes. Amounts eaten could be reported as grams or using common household measures. After a short introduction by a trained researcher, the adolescents completed the HELENA-DIAT 24-HR during school time while a research staff member was present in the classroom to assist the adolescents if necessary. They completed the HELENA-DIAT twice on non-consecutive days within a time span of 2 weeks, to achieve information closer to habitual food intake than assessing food intake on consecutive days. The two 24HR thus comprised weekdays and weekend days, but not necessarily a weekday and weekend day for each individual. To calculate energy and nutrient intake, data from the HELENA-DIAT were linked to the German Food Code and Nutrient Database BLS (Bundeslebensmittelschlüssel) version II. 3.1, 2005) (43). For this purpose, culture-specific composite dishes were disaggregated into their basic food components, all of which were available in the German database (44). Two 24HR were collected in order to allow corrections for within-person variability. The multiple source method (MSM) (45–47), a statistical modelling technique, was used to estimate the usual dietary intake of nutrients and foods.

24HR-derived dietary information was used to calculate DII scores for all subjects, as described in detail elsewhere (27, 28). Briefly, the dietary data for each study participant were first linked to the regionally representative global database that provided a robust estimate of a mean and standard deviation for each of the food parameters (i.e., foods, nutrients, and other food components such as flavonoids) considered (27). A z-score was derived by subtracting the “standard global mean” from the amount reported and then dividing this value by the standard deviation. To minimize the effect of “right skewing” (a common occurrence with dietary data), this value was then converted to a centered percentile score, which was then multiplied by the respective food parameter inflammatory effect score (derived from a literature review and scoring of 1943 “qualified” articles) to obtain the subject's food parameter-specific DII score. All of the food parameter-specific DII scores were then summed to create the overall DII score for every subject in the study. For the current study, data were available for a total of 25 nutrients (carbohydrate, protein, total fat, alcohol, fibre, cholesterol, saturated fat, mono unsaturated fat, poly unsaturated fat, omega-3, omega-6 fatty acid, niacin, thiamin, riboflavin, vitamin B12, vitamin B6, iron, magnesium, zinc, vitamin A, vitamin C, vitamin D, vitamin E, folic acid and betacarotene). A description of validation work of the DII score, based on both dietary recalls and the 7-

day dietary record, a structured questionnaire similar in terms of its layout to an FFQ, is available elsewhere (48). The details of the steps are described in figure 1.

Anthropometry, physical activity and smoking—Anthropometric measurements were performed by trained staff using a standard protocol (49), with the adolescents standing barefoot and in underwear. Weight was measured with an electronic scale to the nearest 0.1 kg, and height was measured with a telescopic stadiometer to the nearest 0.1 cm. BMI was calculated from height and weight (kg/m^2).

Patterns of physical activity (PA) also were self-reported using the International Physical Activity Questionnaire for Adolescents (IPAQ-A) (50). The IPAQ-A, covers the following four domains of PA: (i) school-related PA, including activity during physical education and recess; (ii) transportation; (iii) housework; and (iv) extracurricular PA. In each of the domains, the number of days per week and the time periods each day spent walking, in moderate PA and in vigorous PA, were recorded. The data were cleaned and truncated following the guidelines provided by the IPAQ group (www.ipaq.ki.se) (50). Consequently, variables obtained by the IPAQ-A for this study were added to obtain overall PA (moderate to vigorous + walking intensities in minutes/day). BMI was categorised in BMI-categories using the age-sex specific cut-offs from Cole et al (51, 52). Physical activity is measured in activities in minutes per day using the IPAQ questionnaire. Smoking history was assessed with the ever smoked (yes/no).

Statistical analysis

CRP, TNF- α , IL-6, 1, 2,4,10, INF- γ , ICAM and VCAM were log transformed as they were not normally distributed. DII was analysed as both a continuous and categorical (tertiles) variable. All statistical analysis was carried out using SAS[®] statistical software package version 9.3 (SAS Institute Inc., Cary, NC, USA). Comparisons of characteristics and food groups by tertiles of DII were made using χ^2 tests for categorical variables and ANOVA for continuous variables. Multivariable linear analyses were carried out adjusting for age, sex, city, BMI, smoking and physical activity. In the analysis of categorized DII, a test for linear trend was conducted by including the median value for each DII tertile as a continuous term in the regression model, this approach has been used in several previous studies on diet and health outcomes (53, 54). We have also adjusted for multiple testing by dividing the α value (0.05) by the number of inflammatory markers i.e 10, to get the new alpha value of 0.005. Additionally, sensitivity analyses were performed including cytokines with imputed values for interleukins, TNF and INF- γ .

Results

Table 1 shows the characteristics of the participants across tertiles of DII. Adolescents in tertile 3 were older, more likely to be males and to have history of ever smoked cigarettes. Table 2 and Figure 2 shows the results for food groups. Adolescents in the most pro-inflammatory group had higher consumption of bread and rolls, chocolate, margarine, butter and animal fats, and vegetable lipids, carbonated/soft/isotonic drinks, meat and cakes, pies,

biscuits and sugar/honey/jam; and had lower consumption of vegetables (excluding potatoes), fruits, fruits and vegetable juices, and fish.

Analysis of inflammatory markers

Analyses adjusting for covariates showed positive associations between DII and TNF- α , IL-1, IL-2, IFN- γ ($b_{\text{DII}_{3\text{vs}1}} = 0.13$, 95% CI: 0.001, 0.25; 0.13, 95% CI 0.001, 0.25; 0.40, 95% CI: 0.03, 0.77; 0.53, 95% CI: 0.05, 1.01, respectively) and with sVCAM ($b_{\text{DII}_{\text{continuous}}} = 0.03$, 95% CI: 0.01, 0.04) while no significant trend across tertiles of DII were observed for IL-1 and INF- γ with alpha value of 0.005 except for sVCAM ($P_{\text{value}} \leq 0.001$) (Table 3). No significant association was observed with other inflammatory markers (CRP, IL-4, 6, 10 and ICAM). Results for sensitivity analyses with imputed values are shown in supplementary table 1, there were no major changes in the β estimates for any of the cytokines.

Discussion

This is the first study to examine the association between inflammatory potential of diet, as measured by DII scores, and levels of inflammation among adolescents. The results revealed a positive association between DII and TNF- α , IL-1, 2, INF- γ , and sVCAM. Even though the test for linear trend was not significant, these findings reinforce the idea that a diet rich in pro-inflammatory food parameters (sweets, butter and other animal fats, cholesterol, saturated fat), and relatively poor in anti-inflammatory food parameters (vegetables and fruits) may increase inflammation among adolescents. Although we did not observe significant results with CRP, IL-4, 6, 10 and ICAM, the association with these markers was in the expected direction. Also, it should be noted that crp values did not use the high-sensitivity procedure, which we had previously found to be associated with DII scores (48). It is important to note that this is the first study to examine this association among adolescents, the validation potential of DII among adolescents may be limited due to the low inflammation grade in this young population group. Longitudinal studies should investigate the effect of high DII scores on future health outcomes including morbidity and mortality.

Overall, our study results are consistent with the hypothesis that diet modulates inflammation. The inference is that through this process of modulating inflammation there is an effect on chronic diseases such as several cancers and cardiovascular diseases. Food items that tended to increase DII scores include butter, other animal fats, and chocolate. Higher scores also could result from low consumption of food items considered to be anti-inflammatory such as fruits, vegetables and juices. There is considerable evidence that adequate and healthy nutrition in children and adolescents is associated with a lower risk of youth (55, 56) and adult obesity (57) as well as of subsequent nutrition-related morbidities in adulthood such as cardiovascular diseases (58), several types of cancers (59, 60) and type 2 diabetes (61). Adherence to an anti-inflammatory dietary pattern such as the Mediterranean diet has been shown to reduce the risk of cardiovascular and cerebrovascular diseases (62, 63). All of these chronic health conditions are related to chronic inflammation (64–66).

Thus far, there has been no report from the HELENA study looking at dietary components and inflammation. In a published report from the National Health and Nutrition Examination Survey (NHANES) – 2003–2006, consuming healthy diet was associated with lower CRP levels; however, no association was observed in children (67). Another report from NHANES-1999–02, showed that children and adolescents with higher CRP levels had significantly lower intakes of grains and vegetables (68).

Some limitations of the study need to be mentioned. First, the 24HR used in the HELENA Study is unusual in that it uses a list of foods. In a previous validation study it has been shown to be prone to under-reporting (42, 69). However, the exclusion of misreporters also might induce selection bias because the misreporters might have differential food choices or eating behaviors as previously reported by Bel-Serrat et al. (69). Second, it also should be noted that the cross-sectional nature of the study precluded inferences related to temporality of effects. Another limitation could be the non-availability of data on other potential confounders such as the use of aspirin and other anti-inflammatory medications, all of which could have played a role in modifying this association.

Another limitation is the fact that all of the 24HR were completed during school days (focusing on the dietary intake of the previous day); thus they did not include information about the adolescents' diet on Saturdays or on some holidays (i.e., those not followed by a school day). Fourth, in this current study the DII was calculated using data on only 25 nutrients and food components derived from 24HR. In the DII validation study (48), sensitivity analysis was conducted comparing DII calculated from multiple (up to 15/person) 24HR (which provided data on 44 nutrients and food components) with DII calculated from 7-day dietary recalls (which provided data on 28 nutrients and food components). We found that the association with CRP was not attenuated when using the more limited list available with the 7-day dietary recalls (48). Still, the attenuation in the food parameter list could explain the absence of an association between DII and inflammatory markers in this study. However, despite the large reduction in the number of parameters, we still were able to successfully observe associations with some of the inflammatory markers.

In addition to its large sample size, one of the major strengths of the present study is the geographical spread over eight European cities. The sample consists of adolescents assessed using highly standardized and validated procedures; for example, all countries used the same 24HR (HELENA-DIAT) and the same food composition database (BLS). Also, the large number of inflammation markers measured in serum and analysed centrally in the same laboratory and the huge battery of lifestyle and anthropometric variables assessed according to standardized procedures is an important advantage of this study as it allows adjustment for several possible confounding factors. Another strength is the use of the dietary data from multiple countries taking into account both between- and within-individual variability of dietary intake data. Moreover, the current study is one of the first to examine overall effect of dietary intake on inflammation and not just specific nutrients among adolescents at a pan-European level.

Conclusion

Results from this study suggest that eating a diet high in food components such as sugar and saturated fat leads to a pro-inflammatory state, which may increase the risk of a range of chronic diseases throughout life. These results will have to be replicated in other studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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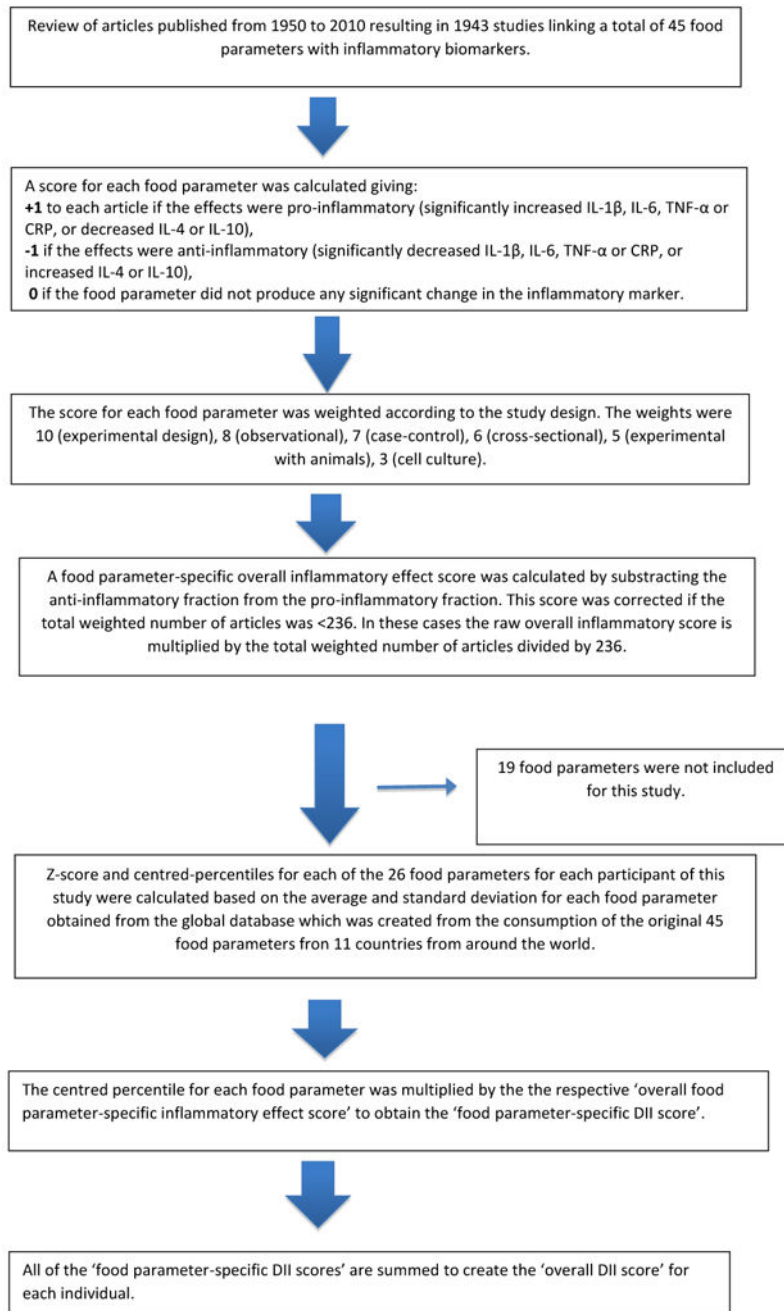


Figure 1. Sequence of steps in creating the dietary inflammatory index in the HELENA study

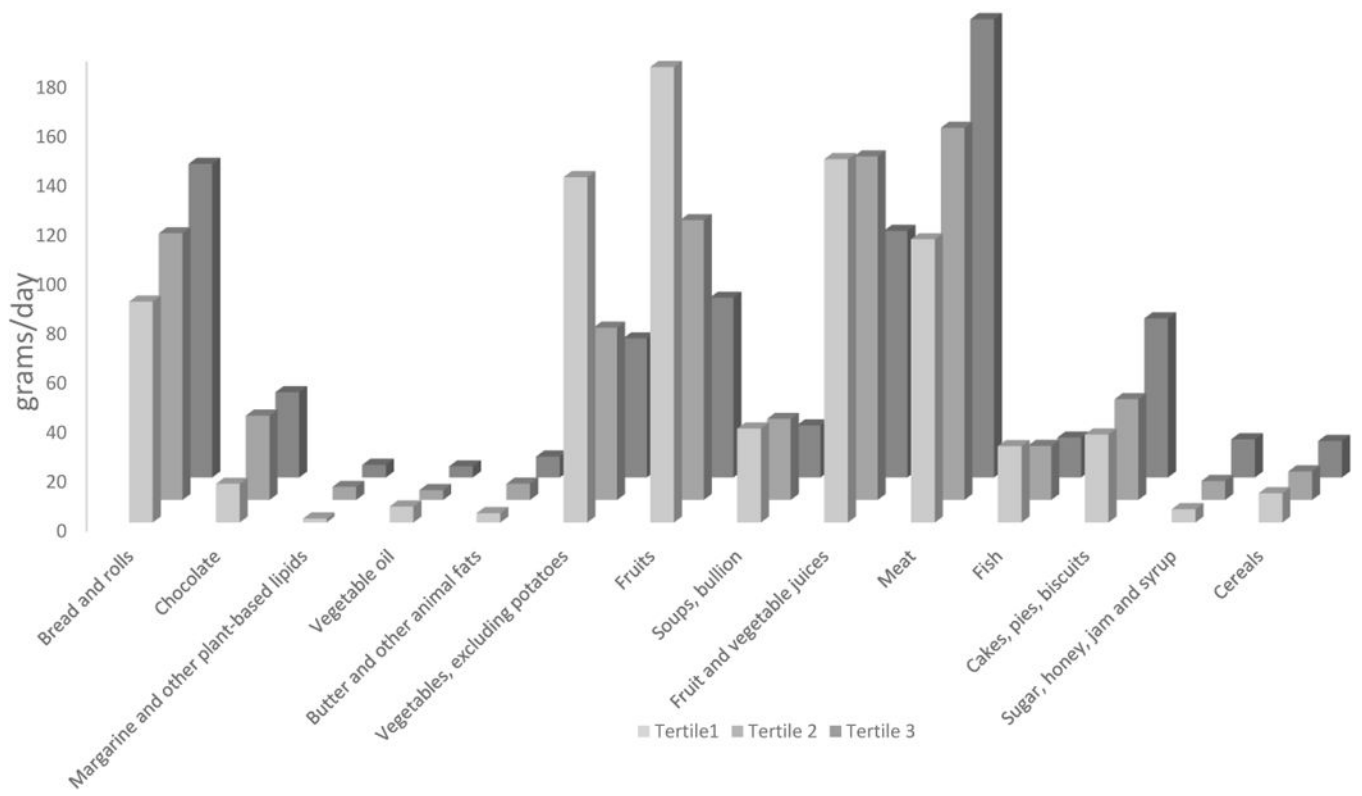


Figure 2. Distribution of food groups across tertiles of DII.

Table 1

Distribution of selected characteristics across tertiles of Dietary Inflammatory Index (DII)^{a,b,c}, HELENA Study, 2006–7.

Characteristics	Tertile 1	Tertile 2	Tertile 3	P-value
Age (years) (mean±sd)	14.7±1.12	14.7±1.2	15.0±1.2	0.005
BMI (kg/m ²) (mean±sd)	21.1±3.8	20.9±3.1	20.8±3.3	0.44
Total physical activity (minutes/week) (mean±sd)	1379.3±1274.1	1215.4±1055.9	1277.1±1204.4	0.41
Sex, n (%)				<0.0001
Smoking				0.006
No	122 (69.7)	105 (59.0)	91 (50.8)	
Yes	51 (29.1)	69 (38.8)	86 (48.0)	

^{a,b} ANOVA and Chi-square statistics were used to test the difference across the tertiles.

^c Higher DII scores indicate a pro-inflammatory diet.

Table 2Distribution of consumption of food groups across tertiles of DII^a, HELENA Study, 2006–7.

Food groups (g/day)	Tertile 1	Tertile 2	Tertile 3	P-Value
Bread and rolls	89.6±83.0	108.2±90.4	127.2±124.8	0.0005
Chocolate	15.7±30.6	34.2±70.4	34.6±75.6	0.004
Margarine and other plant-based lipids	1.6±6.3	5.3±18.3	5.1±15.8	0.02
Vegetable oil	6.5±18.3	4.0±10.2	4.5±14.1	0.19
Butter and other animal fats	3.8±8.5	6.6±15.2	8.5±18.8	0.003
Vegetables, excluding potatoes	140.1±151.2	69.9±113.7	56.4±80.1	<0.0001
Fruits	184.8±187.6	113.5±169.3	72.9±140.8	<0.0001
Soups, bullion	38.1±111.2	32.9±125.0	21.1±84.7	0.14
Fruit and vegetable juices	147.4±240.6	139.4±272.4	100.0±164.8	0.05
Carbonated/soft/isotonic drinks	149.3±317.1	261.6±382.2	486.4±637.7	<0.0001
Meat	114.9±131.3	151.0±154.1	185.9±229.5	0.0002
Fish	31.0±97.1	21.9±65.7	16.2±46.7	0.05
Cakes, pies, biscuits	35.7±61.5	40.9±67.8	64.5±114.0	0.001
Sugar, honey, jam and syrup	5.4±16.7	7.7±22.9	15.5±44.3	0.002
Cereals	11.9±23.4	11.6±26.6	14.8±41.3	0.37

^aANOVA was used to test the difference across the tertiles.

Table 3

Beta estimates and confidence interval for the association between DII and inflammation in the HELENA Study, 2006–7.

DII	Beta Estimates and confidence interval for inflammatory markers ^d						P-trend ^{b,c}
	DII (continuous)	P-value ^c	Tertile 1	Tertile 2	Tertile 3		
CRP mg/l	0.04 (-0.04, 0.11)	0.32	Ref	0.18 (-0.08, 0.44)	0.09 (-0.18, 0.36)		0.41
TNF- α pg/ml	0.04 (0.002, 0.07)	0.03	Ref	-0.01 (-0.13, 0.11)	0.13 (0.007, 0.26)		0.06
IL-6 pg/ml	0.04 (-0.04, 0.13)	0.31	Ref	-0.05 (-0.35, 0.25)	0.09 (-0.22, 0.40)		0.66
IL-1 pg/ml	0.07 (-0.005, 0.15)	0.06	Ref	0.11 (-0.16, 0.38)	0.30 (0.02, 0.58)		0.04
IL-2 pg/ml	0.10 (-0.01, 0.20)	0.05	Ref	-0.14 (-0.50, 0.22)	0.42 (0.04, 0.79)		0.08
IL-4 pg/ml	0.08 (-0.04, 0.20)	0.20	Ref	-0.05 (-0.45, 0.35)	0.17 (-0.25, 0.59)		0.19
IL-10 pg/ml	0.01 (-0.06, 0.08)	0.69	Ref	-0.12 (-0.38, 0.13)	0.09 (-0.17, 0.35)		0.75
INF- γ pg/ml	0.13 (-0.007, 0.27)	0.04	Ref	0.25 (-0.22, 0.72)	0.58 (0.09, 1.06)		0.03
ICAM ng/ml	0.01 (-0.01, 0.04)	0.23	Ref	-0.04 (-0.13, 0.05)	0.02 (-0.08, 0.11)		0.27
VCAM ng/ml	0.03 (0.01, 0.04)	<0.001	Ref	0.03 (-0.03, 0.08)	0.07 (0.01, 0.13)		0.07

^a Adjusted for age, sex, body mass index (BMI)-categories, city, smoking and physical activity.

^b P-trend determined through the median approach

^c P-value <0.005 were deemed significant after Bonferroni approach.