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Association between the Dietary Inflammatory Index (DII) and telomere length and C-reactive protein from the National Health and Nutrition Examination Survey-1999–2002

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Abstract

Background—Leukocyte telomere length (LTL) is an important biomarker of aging. This study examined whether inflammatory potential of diet, as measured by the Dietary Inflammatory IndexTM (DII) has an impact on telomere shortening in the National Health and Nutrition Examination Survey (NHANES). We also carried out validation of the DII with C-reactive protein (CRP).

Methods—Data came from NHANES 1999–2002. LTL and CRP were assayed from leukocyte DNA and serum specimens, respectively. The DII was calculated from food intakes assessed using 24-hour dietary recalls and expressed per 1,000 calories consumed. Associations were examined using survey-based multivariable linear regression for log-transformed LTL.

Results—After multivariable adjustment, higher DII scores (i.e., relatively more pro inflammatory) were associated with shorter LTL both when used as continuous ($b = -0.003$; 95% confidence interval [CI] = $-0.005, -0.0002$) and as quartiles ($b_{\text{DIIquartile4vs1}} = -0.013$; 95% CI = $-0.025, -0.001$; $P_{\text{trend}} = .03$). In this same sample the DII also was associated with CRP 3mg/l ($OR_{\text{DIIcontinuous}} = 1.10$; 95% CI = $1.06, 1.16$).

Conclusion—In these NHANES data there was an association between DII and LTL. This study also provided a successful construct validation of the DII using CRP in a nationally representative

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Conflict of Interest: None

Disclosures: 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 counseling and dietary intervention in clinical settings. Drs. Nitin Shivappa and Michael Wirth are employees of CHI

sample. These results are consistent with the hypothesis that diet-associated inflammation determines LTL.

INTRODUCTION

Telomeres are important regions of DNA made up of several thousands of repeated 5'-TTAGGG-3' base pairs located at the ends of each chromosome [1]. Telomeres provide an essential protective role for the genetic material, preventing mechanisms of DNA repair from acting on the chromosomal ends, with ensuing genome instability. Because telomere sequences do not fully replicate during DNA replication, they become progressively shorter with each cell division [2,3]. Leukocyte telomere length (LTL) has been proposed to be a marker of biological aging, and shortened LTL has been related with higher all-cause mortality [4]. In this context, several studies in humans have looked into the effects of dietary components on telomere length [5–9]. Higher adherence to a Mediterranean diet was associated with longer LTL in a multi-ethnic elderly population in the US [10] and in Spain [11]. Increased intake of processed meat has been shown to be inversely associated with LTL [8].

Chronic inflammation also has been implicated to play a major role in the shortening of LTL [12,13]. Increasing level of C-reactive protein has been shown to be associated with decreased length of LTL [13]. Therefore, it might be expected that an anti-inflammatory diet would reduce the rate of telomere shortening, which may delay aging. Indeed, a higher intake of specific antioxidants and anti-inflammatory dietary components such as vitamins C and E, polyphenols, curcumin and omega-3 fatty acids have been associated with longer telomeres [14–16]. The Dietary Inflammatory Index (DII) [17], was developed to measure the inflammatory potential of diet and it can be used in diverse populations to predict levels of inflammatory markers including CRP [18] and interleukin-6 [19]. The DII also is associated with the various cancers like colorectal [20–22], pancreatic [23], and prostate cancer [24]. In the PREvención con DIeta MEDiterránea-NAVARRA (PREDIMED-NAVARRA) trial conducted in Spain, results showed both cross-sectional and longitudinal associations between DII scores and telomere shortening [25]. However, no study has yet assessed the inflammatory potential of a dietary pattern and LTL in an American population. Hence, the aim of the present work was to examine the effect of the DII on LTL in the National Health and Nutrition Examination Survey (NHANES) -1999–2002. Our working hypothesis is that increasing inflammatory potential of diet (i.e., higher DII scores) is associated with shorter LTLs. We also sought to validate the DII in the same population by examining associations between DII scores and CRP concentrations.

METHODS

NHANES is an ongoing, multistage cross-sectional survey administered by the National Center for Health Statistics. NHANES program is designed to assess the health and nutritional status of children and adults in the United States. In two-year cycles, NHANES employs a complex, multistage, probability sampling design and constructs sample weights to produce nationally representative data of the US population. More detailed descriptions of the NHANES methods and protocols can be found on their website (<http://www.cdc.gov/>)

[nchs/nhanes.htm](https://nchs.nhanes.htm)). The study population was restricted to 7215 adults, aged >19 years, who had complete dietary data and no missing data on any of the covariates. The 1999 to 2002 NHANES was used as it is the only cycle in which LTL was measured.

Leukocyte Telomere Length

DNA samples purified from whole blood were collected from NHANES participants aged 20 years and older in the 1999 to 2002 two-year cycles to establish a national probability sample of genetic material for future research [26]. DNA aliquots were processed by the Division of Laboratory Sciences at the National Center for Environmental Health and provided by the Division of Health and Nutrition Examination Surveys, National Center for Health Statistics, Centers for Disease Control and Prevention. The LTL assay was performed in the laboratory of Elizabeth Blackburn at the University of California, San Francisco, using the quantitative polymerase chain reaction method to measure telomere length relative to standard reference DNA (T/S ratio), as described in detail elsewhere [27]. The polymerase chain reaction method was preferred over the Southern blot method because of the smaller amount of DNA required for the assay [28].

Each LTL sample was assayed 3 times on 3 different days. The samples were assayed on duplicate wells, resulting in 6 data points. Sample plates were assayed in groups of 3 plates, and no 2 plates were grouped together more than once. Each assay plate contained 96 control wells with 8 control DNA samples. Assay runs with 8 or more invalid control wells were excluded from further analysis (<1% of runs). Control DNA values were used to normalize between-run variability. Runs with more than 4 control DNA values falling outside 2.5 SDs from the mean for all assay runs were excluded from further analysis (< 6% of runs). For each sample, any potential outliers were identified and excluded from the calculations (< 2% of samples). The mean and SD of the T/S ratio were then calculated normally. The inter-assay coefficient of variation was 6.5%. Throughout this article, we refer to the T/S ratio and relative telomere length as telomere length for brevity. The conversion from the T/S ratio to base pairs was calculated based on comparison of telomeric restriction fragment length from Southern blot analysis and T/S ratios using DNA samples from the human diploid fibroblast cell line IMR90 at different population doublings. The formula used to convert the T/S ratio to base pairs was $3274 + 2413 * (T/S)$.

High Sensitivity-C-Reactive Protein (hs-CRP)

Serum CRP was assayed at the University of Washington Medical Center Department of Laboratory Medicine using a Dade Behring Nephelometer II Analyzer system (Dade Behring Diagnostics). Hs-CRP was log transformed, as values were not normally distributed. As recommended by the CDC and the American Heart Association, hs-CRP was dichotomized at the level of 3 mg/L [29], considering measurements greater than this place individuals at higher cardiovascular disease (CVD) risk.

Diet and Dietary Inflammatory Index

The development and validation of the DII have been discussed in detail elsewhere [18,17]. In short, nearly 2,000 research articles, published between 1950 and 2010, examining the relationship between 45 different food parameters (mostly micro, macro nutrients and

flavanoids plus some individual food items) and inflammation were reviewed. Articles showing a positive association between the food parameters and pro-inflammatory cytokines (i.e., IL-1 β , IL-6, tumor necrosis factor [TNF]- α , and CRP) or a negative association with anti-inflammatory cytokines (IL-4 and IL-10) received a value of +1. If the food parameters were associated with reduced pro-inflammatory or increased anti-inflammatory cytokines, the article received a value of -1. Null values were set to 0. These scores were weighted based on study design. For example, randomized control trials received the greatest weight and cell culture the lowest weight. These scores and the weights were used to create pro- and anti-inflammatory fractions for each food parameter. The anti-inflammatory fraction was subtracted from the pro-inflammatory fraction to create the “article effect score” for each of the 45 food parameters. Additionally, DII calculation is linked to a regionally representative world database. The world database contains standard means and deviations for the 45 food parameters from 11 populations around the world (i.e., United States, United Kingdom, Bahrain, Mexico, Australia, South Korea, Taiwan, India, New Zealand, Japan, and Denmark) [17].

Dietary data in NHANES were collected using 24-hour dietary recall interviews (24HR) conducted at the mobile examination center (MEC). The dietary interviews were administered by trained staff and the USDA’s Food Surveys Research Group was responsible for the dietary data collection methodology, maintenance of the databases used to code and process the data, and data review and processing. 24HR-derived dietary information was used to calculate DII scores for all subjects, as described in detail elsewhere [17]. The DII food parameters available through NHANES data included carbohydrates; protein; fat; grams of alcohol; fiber; cholesterol; saturated, monounsaturated, and polyunsaturated fatty acids; omega3 and omega6 polyunsaturated fatty acids; niacin; vitamins A, B1, B2, B6, B12, C, D, E; iron; magnesium; zinc; selenium; folic acid; beta carotene; and caffeine. Higher (i.e., more positive) scores tend to indicate more pro-inflammatory diets and more negative values are more anti-inflammatory [17]. To control for the effect of total energy intake, the DII was calculated per 1,000 calories of food consumed, which requires using the energy-standardized version of the world database.

Study Covariates

Potential confounders included sociodemographic characteristics, such as participant’s age (years), sex, self-reported race/ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic, other race/multiracial), highest educational attainment (< 12 years, high school diploma or equivalent, some college, college graduate), the ratio of family income to poverty using the Department of Health and Human Services annual poverty guidelines (FPL; 0%---100% FPL, 100%---200% FPL, 200%---300% FPL, > 300% FPL). Other health-related variables included smoking status (never, ever) and physical activity (no activity, some activity). Subjects with missing information on any of these covariates were removed from the analyses.

Adiposity measures included calculated body mass index (BMI) from self-reported height (in meters) and weight (in kilograms) squared, measured by trained personnel using a stadiometer and Toledo® weight scale (Toledo Scale, Honolulu, HI) [30].

Statistical Analyses

Survey design procedures in SAS® (version 9.4, Cary, NC) were used for all analyses, the associations between LTL or CRP and DII were investigated using linear regression (SURVEYREG procedure) and logistic regression (SURVEYLOGISTIC) models, respectively, adjusted for potential confounders. ANOVA and chi-square tests compared population characteristics according to quartiles of DII. The DII was analyzed as both continuous and has quartiles with telomere length and CRP as the outcomes. These procedures allow for control of the stratification and clustering employed by NHANES for sampling. The NHANES-provided sample weights accounted for different sampling probabilities and the potential non-response bias of the participants in the NHANES subsample who consented to the use of DNA specimens for future genetic research.

RESULTS

The mean DII in this study was 0.01 (SE: 0.02) and the scores ranged from +3.98 (most pro-inflammatory) to -4.79 (most anti-inflammatory). Table 1 shows distribution of characteristics across quartiles of the DII. As compared to subjects in the most anti-inflammatory DII category, those in the most pro-inflammatory were significantly more likely to be males, non-Hispanic Blacks, obese, smokers and of lower socioeconomic status (Poverty Index < 100). Table 2, shows the results with log transformed LTL as the outcome. In the multivariable model, when used as quartiles, subjects in the most pro-inflammatory DII group (quartile 4) had significantly lower LTL compared to subjects in quartile 1 ($b_{\text{DIIquartile4vs1}} = -0.013$; 95% CI = -0.024, -0.001; $P_{\text{trend}} = 0.03$) and similar result was observed when the DII was used as a continuous variable, ($b = -0.003$; 95% CI = -0.005, -0.0002). Table 3, shows the results with CRP > 3mg/l. In the multivariable model, subjects in quartile 4 had 53% higher odds of having CRP > 3mg/l compared to subjects in quartile 1 ($OR_{\text{DIIquartile4vs1}} = 1.53$; 95% CI = 1.20, 1.95; $P_{\text{trend}} = < 0.0001$). Additionally, higher odds were observed when the DII was used as continuous ($OR_{\text{DIIcontinuous}} = 1.11$; 95% CI = 1.06, 1.17).

DISCUSSION

In the present study, we found a direct association between the pro-inflammatory capacity of the diet (as measured by the DII) and telomere length when the DII was used as both continuous and categorical. Indeed, the DII was inversely associated with LTL, indicating that the higher the DII (more pro-inflammatory values) the shorter the telomeres. We also found a positive association between the DII and CRP in this population.

To our knowledge, this is the first study specifically looking at the association between the inflammatory potential of the overall dietary pattern and LTL in a US population. Previously, in the same NHANES sample, sugar-sweetened soda consumption was associated with shorter telomeres ($b = -0.010$; 95% confidence interval [CI] = -0.020, -0.001; $P = .04$). Consumption of 100% fruit juice was marginally associated with longer telomeres ($b = 0.016$; 95% CI = -0.000, 0.033; $P = .05$). No significant association was observed between consumption of diet sodas or noncarbonated sugar sweetened beverages and telomere length [31]. However, there are other studies that have investigated the

association between specific dietary components and TL. In this context, omega-3 fatty acids, which are known for their anti-inflammatory potential [32] and have an anti-inflammatory score on DII [17], were shown to prevent telomere erosion after 4 and 6 months in a randomized intervention [14,33]. In contrast, total and saturated fat intake, both which have pro-inflammatory DII scores, and consumption of refined flour cereals, meat and meat products, and sugar-sweetened beverages are associated with shorter telomeres [34]. Our findings are consistent in showing the effect of the DII, which includes omega-3, saturated fat and several other dietary components which are known to have an impact on inflammation. The DII has been shown to be associated with lower LTL in only one study previously; i.e., the PREDIMED-NAVARRA study, in which increasing DII scores were associated with LTL shortening both cross-sectionally and longitudinally [25].

The possible mechanism for this association may be through the effect of diet on inflammatory markers such as CRP, which increases generation of oxygen free radicals by neutrophils [35]. It is well known that telomeres are highly sensitive to damage by oxidative stress [36]; therefore, it is biologically plausible that CRP, through oxygen free radicals, could damage telomeric DNA and thereby contribute to telomere shortening. Because inflammation negatively influences telomere length [13], it is reasonable to expect that an anti-inflammatory treatment may prevent telomere shortening. Therefore, a more anti-inflammatory diet may decrease the cumulative inflammatory burden thus leading to a decrease in the rate of telomere shortening. On the other hand, a more pro-inflammatory diet could result in an increase in inflammatory molecules causing accelerated telomere erosion. However, it also is possible that an increase in telomerase activity occurs as a consequence of dietary intervention. Indeed, a few studies have observed that intensive lifestyle changes are associated with increases (of about 30% to 40%) in telomerase activity of peripheral blood mononuclear cells [37]. In addition, exposure to TNF- α shortens TL through negative regulation of telomerase activity [38].

The DII provides a summary measure for assessing the inflammatory potential of the diet [39]. Results based on this index indicate that it reliably predicts levels of inflammatory markers, such as CRP, IL-6 or homocysteine [18,19]. These findings reinforce the idea that diet, as a whole, plays an essential role in modifying inflammation. Interestingly, a previous study also carried out in the PREDIMED trial showed that the DII was inversely associated with the intake of healthy foods, nutrients and adherence to the Mediterranean Diet [40]. That study also reported a positive relationship between a pro-inflammatory diet and elevated indices of central and abdominal obesity [40]. In addition, higher DII values have been associated with the glucose component of the metabolic syndrome [41] and with higher risks of cancer [42,43] and asthma [44]. Regarding survival after cancer, there is one study, conducted in Italy, showing an association between DII, scores and a shorter survival time among survivors of prostate cancer [45].

The present study has several strengths. To the best of our knowledge, this is the first study to assess the inflammatory potential of diet in relation to LTL in a nationally representative US population. Second, the present study also included CRP as well as the DII in order to provide a well-recognized inflammatory biomarker and a method to gauge the inflammatory potential of the diet in the same study population. This has not been possible in many of the

previous DII-related studies. Third, numerous confounding factors, including sociodemographic factors and smoking status could be controlled in this study. Finally, this study provides a unique opportunity to validate the DII with CRP, while also allowing for examining its association with LTL in the same population.

Despite its strengths, the present study has potential limitations. TL technique could lead to errors in measurements due to the high variability of qPCR analysis. For this reason, experimental conditions were carefully controlled to avoid potential errors [46], which is reflected in the low CVs obtained. Another limitation is the lack of telomerase activity and inflammation or oxidative stress measurements. Without these measurements it is not possible to confirm the exact mechanisms involved in the observed associations between the DII and TL.

Additionally, the cross-sectional nature of the data made it difficult to infer causation. LTL was measured from a single DNA specimen, which did not provide information on rates of telomere shortening. Similarly, dietary intakes were estimated from a 24HR conducted at the time of the survey, which might not reflect food or beverage patterns over the life course. Another limitation could be the non-availability of the remaining 18 food parameters that could be used to perform the DII calculation. The food parameters that are missing include turmeric, thyme, saffron and others. Out of these, food parameters such as turmeric and saffron are probably consumed in small amounts, infrequently or not consumed at all in this population; hence, they may not have had a major impact on the scoring. However, anti-inflammatory food parameters such as garlic and onion are more likely to be consumed in this population; hence, the resultant additional noise in calculating the DII due to missing information on those foods may have caused an underestimate of the association. Finally, only one 24HR was used to assess dietary information in the current analysis. Using just one day of dietary information may not account for day-to-day variability in diet leading to imprecise estimates [47].

In conclusion, DII scores were inversely associated with LTL, revealing that anti-inflammatory values of the DII were related to longer telomeres in a representative population of US adults. These findings suggest the beneficial effects of adherence to an anti-inflammatory diet on aging and health, by preventing telomere shortening.

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Distribution of characteristics across quartiles of DII National Health and Nutrition Examination Surveys, United States, 1999–2002^{a,b}**Table 1**

	Quartile 1 −4.79 to −1.18 N=1803	Quartile 2 −1.17 to 0.15 N=1804	Quartile 3 0.16 to 1.34 N=1805	Quartile 4 1.35 to 3.98 N=1803	P-Values
Age	56.3±18.2	50.3±18.4	46.9±17.8	43.2 ±17.1	<0.0001
Sex					<0.0001
Male	770 (42.7)	856 (47.4)	910 (50.4)	889 (49.3)	
Female	1033 (57.3)	949 (52.6)	894 (49.6)	914 (50.7)	
Ethnicity/Race					<0.0001
Non-Hispanic White	970 (53.8)	880 (48.7)	881 (48.8)	967 (53.6)	
Non-Hispanic Black	203 (11.3)	254 (14.1)	360 (20.0)	395 (21.9)	
Mexican American	493 (27.3)	503 (27.9)	432 (23.9)	316 (17.5)	
Other Race-Including Multi-Racial	49 (2.7)	47 (2.6)	46 (2.6)	33 (1.9)	
Other Hispanic	88 (4.9)	121 (6.7)	85 (4.7)	92 (5.1)	
BMI (kg/m ²)					0.0002
<25	594 (32.9)	530 (29.4)	542 (30.0)	538 (29.8)	
25–30	677 (37.6)	678 (37.6)	604 (33.5)	625 (34.7)	
>30	532 (29.5)	597 (33.1)	658 (36.5)	640 (35.5)	
Education					<0.0001
Less than high school	601 (33.4)	609 (33.7)	593 (33.0)	586 (32.5)	
High School Diploma	349 (19.4)	384 (21.3)	446 (24.8)	489 (27.1)	
More than high school	850 (47.2)	812 (45.0)	760 (42.2)	727 (40.3)	
Smoking					<0.0001
Yes	752 (41.9)	817 (45.3)	929 (51.7)	978 (54.3)	
No	1044 (58.1)	987 (54.7)	867 (48.3)	824 (45.7)	
Poverty Index ^c					0.001
0–100	420 (23.4)	463 (25.7)	458 (25.5)	449 (24.9)	
101–200	392 (21.8)	404 (22.4)	421 (23.4)	453 (24.1)	
201–300	249 (13.9)	244 (13.5)	289 (16.1)	281 (15.6)	

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	Quartile 1 -4.79 to -1.18 N=1803	Quartile 2 -1.17 to 0.15 N=1804	Quartile 3 0.16 to 1.34 N=1805	Quartile 4 1.35 to 3.98 N=1803	P-Values
>300	735 (40.9)	693 (38.4)	628 (35.0)	619 (34.4)	
Physical activity					0.55
None	452 (25.1)	414 (23.1)	428 (23.7)	428 (23.9)	
Some	1349 (74.9)	1380 (76.9)	1375 (76.3)	1365 (76.1)	

^{a,b} ANOVA was used for continuous variable and Chi-square was used for categorical variables.

^c Ratio of family income to poverty, using the Department of Health and Human Services annual poverty guidelines

Table 2

Beta estimates for Associations Between DII and Log-Transformed Leukocyte Telomere Length (T/S Ratio): National Health and Nutrition Examination Surveys, United States, 1999–2002

	Quartile 1 −4.79 to −1.18 N=1803	Quartile 2 −1.17 to 0.15 N=1804	Quartile 3 0.16 to 1.34 N=1805	Quartile 4 1.35 to 3.98 N=1803	P-trend	DII continuous
Age adjusted	Ref	−0.009 (−0.018, −0.0006)	−0.009 (−0.020, 0.001)	−0.016 (−0.030, −0.002)	0.02	−0.004 (−0.007, −0.0005)
Multivariate ^a	Ref	−0.008 (−0.016, 0.0003)	−0.007 (−0.017, 0.003)	−0.013 (−0.024, −0.001)	0.03	−0.003 (−0.005, −0.0002)

^a Adjusted for age, sex, ethnicity, BMI, education, smoking, poverty index and physical activity.

Table 3

Odds Ratio for Associations Between DII and C-reactive protein (>3mg/l): National Health and Nutrition Examination Surveys, United States, 1999–2002

	Quartile 1 −4.79 to −1.18 N=1803	Quartile 2 −1.17 to 0.15 N=1804	Quartile 3 0.16 to 1.34 N=1805	Quartile 4 1.35 to 3.98 N=1803	P-trend	DII continuous
Age adjusted	1	1.22 (0.94, 1.60)	1.50 (1.19, 1.89)	1.55 (1.24, 1.93)	<0.0001	1.12 (1.07, 1.17)
Multivariate ^a	1	1.17 (0.88, 1.56)	1.52 (1.19, 1.95)	1.53 (1.20, 1.95)	<0.0001	1.11 (1.06, 1.17)

^a Adjusted for age, sex, ethnicity, BMI, education, smoking, poverty index and physical activity.