Diet Quality Indices and Leukocyte Telomere Length among Healthy US Adults: Data from the National Health and Nutrition Examination Surveys (NHANES) 1999-2002

Cindy W. Leung, Teresa T. Fung, Claire T. McEvoy, Jue Lin, and Elissa S. Epel

Correspondence to Dr. Cindy Leung, Department of Nutritional Sciences, University of Michigan School of Public Health, 1415 Washington Heights, SPH I, Ann Arbor, MI 48104 (phone: 734-647-9087; fax: e-mail: cindyleung@post.harvard.edu)

Author Affiliations: Department of Nutritional Sciences, University of Michigan School of Public Health, Ann Arbor, Michigan (Cindy W. Leung); Department of Nutrition, Simmons School of Nursing and Health Sciences and Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, Massachusetts (Teresa T. Fung); Institute of Global Food Security (Center for Public Health), Queen's University Belfast, Northern Ireland (Claire T. McEvoy); Department of Biochemistry and Biophysics (Jue Lin), Department of Psychiatry (Elissa S. Epel) University of California, San Francisco, San Francisco, California

Funding: This work was supported by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (grant 5K99HD084758 to C.W. Leung) and the National Institute on Aging (grant R01AG033592-01A1).

Conflicts of interest: Dr Jue Lin is a cofounder and consultant to Telomere Diagnostics, Inc. The company played no role in the current study. The other authors have no financial relationships with any organizations that might have an interest in the submitted work in the previous three years and no other relationships or activities that could appear to have influenced the submitted work.

Running head: Diet Quality Indices and Leukocyte Telomere Length

Keywords: leukocyte telomere length; cellular aging; diet patterns; Healthy Eating Index; Alternate Healthy Eating Index; Mediterranean Diet Score; Dietary Approaches to Stop Hypertension score

Abbreviations: AHEI-2010, Alternate Healthy Eating Index-2010; BMI, body mass index; DASH, Dietary Approaches to Stopping Hypertension; HEI-2010, Healthy Eating Index-2010; LTL, leukocyte telomere length; MedDiet, Mediterranean Diet

Aging is the biggest risk factor for the development of chronic diseases. Telomere length may represent one important mechanism by which dietary intake influences age-related diseases; however, it is unknown which diet pattern is most strongly related to telomere length. We compared the relations between four evidence-based diet quality indices and leukocyte telomere length in a nationally representative sample of healthy adults, and the extent to which these associations differ between men and women. Data came from 4,758 adults, aged 20-65 years, with no prior diagnosis of major chronic disease, from the 1999-2002 National Health and Nutrition Examination Surveys (NHANES). Diet was assessed using one 24-hour dietary recall.

After adjusting for sociodemographic and health characteristics, comparing the top and bottom quintiles showed that higher Healthy Eating Index-2010 (β=0.065, 95% CI 0.018, 0.112, *P-trend=0.007*), AHEI-2010 scores (β=0.054, 95% CI 0.010, 0.097, *P-trend=0.007*), Mediterranean Diet scores (β=0.058, 95% CI 0.017, 0.098, *P-trend=0.008*), and Dietary Approaches to Stopping Hypertension (DASH) scores (β=0.052, 95% CI 0.014, 0.090, *P-trend=0.007*) were each associated with longer telomere length in women. These results may provide insight into the complex associations between optimal nutrition and longevity. Further investigation is needed to understand why associations were not observed in men.

Telomeres are the DNA-protein caps at the end of eukaryotic chromosomes that promote chromosomal stability and protect the genomic DNA from damage (1). Telomere length naturally shortens with each cell cycle. When telomere length reaches a critical short length, the cell is no longer able to divide and reaches senescence, a mechanism that may underlie age-related disease processes (2, 3). While chronological age is among the strongest predictors of telomere length, studies have recently shown that telomere length is also sensitive to lifestyle behaviors like physical activity (4), smoking (5), and dietary intake (6). Telomere length has also been inversely related to risk of cardiovascular disease, type 2 diabetes, and some cancers (7-11).

Many studies have examined dietary intake in relation to telomere length, but across studies there is little agreement in specific foods, food groups, or nutrients that are beneficial or harmful for telomere length (6). The lack of consistency in findings may be due, in part, to the fact that foods are not consumed in isolation, but with complementary foods and nutrients that can have synergistic and larger effects on health outcomes (12). Diet pattern analysis captures the cumulative effects of foods and nutrients consumed in one's diet, viewing these dietary components as an integrated system rather than as individual factors. To date, there have been seven studies examining the associations between diet quality indices and telomere length. Three of these studies used empirically derived diet patterns, often termed the Prudent and the Western patterns, which organize foods commonly consumed together in the analytic population (13-15). A diet characterized by high intakes of fruits, vegetables, whole grains, fish, poultry, and legumes, and labeled a 'prudent' pattern, was positively associated with telomere length in a study of middle-aged and older Koreans (14), marginally associated with telomere length in a study of healthy US women (13), and not associated with telomere length in a racial/ethnically diverse group of US adults (15). In contrast, the Western pattern, characterized by intakes of refined grains, white potatoes, red and processed meats, and high-fat dairy, was not associated with telomere length in any of these studies. Other studies of leukocyte telomere length have examined diet quality indices using evidence-based dietary guidelines. Two studies have observed positive associations between high adherence to the Mediterranean diet and telomere length, including a small study of elderly adults in Italy (16), and a large study of female nurses in the US (13).

To date, the Mediterranean diet and other diet quality scores have been frequently studied in relation to risk of major chronic disease (17-20). However, only one study has compared multiple diet quality indices to telomere length in the same study (13). Thus, the objective of this study was to examine the associations of diet quality scores and leukocyte telomere length in a large and nationally representative sample of healthy men and women. Four evidence-based diet scores were examined: the Healthy Eating Index (HEI)-2010, the Alternate Healthy Eating Index (AHEI)-2010, the Mediterranean Diet (MedDiet) score, and the Dietary Approaches for Stopping

Hypertension (DASH) index score. We hypothesized that healthful diet quality, as determined by each of these measures, would be positively associated with leukocyte telomere length.

METHODS

Study population

The National Health and Nutrition Examination Surveys (NHANES) is an ongoing, multistage survey representative of the civilian, noninstitutionalized US population, administered by the National Center for Health Statistics. The analytic population was comprised of 4,758 healthy adults, aged 20 to 65 years, who had complete dietary and leukocyte telomere length (LTL) data measured in 1999-2002 NHANES. The NHANES response rates for these years ranged from 69 to 81%. Adults with a prior diagnosis of coronary heart disease, angina, myocardial infarction, stroke, diabetes, congestive heart failure, and cancer were excluded (n=826), as these conditions are known to influence both health behaviors and telomere length.

Dietary assessment

Dietary intake was assessed using a single 24-hour dietary recall in the Mobile Examination Center (21). Individuals without dietary data (n=214) or implausible total energy intakes (<500 or >5000 kcal/day) (n=134) were excluded from analysis. Data from the USDA MyPyramid Equivalents Database Version 1.0 and the NHANES dietary interview files were used to calculate all diet pattern scores. The validity of the 24-hour recall method has been previously discussed (22, 23).

For all diet quality indices, higher scores indicate a more healthful diet pattern. The HEI-2010 is a measure of diet quality that assesses adherence to the 2010 Dietary Guidelines for Americans, developed by the US Department of Agriculture Center for Nutrition Policy and Promotion. It is scored out of 100 points, and assesses the following components: total fruit, whole fruit, total vegetables, greens and beans, whole grains, dairy, total protein foods, seafood and plant proteins, fatty acids, refined grains, sodium, and empty calories (24). Higher scores on components reflect higher intakes, with the exceptions of refined grains, sodium, and empty calories. The AHEI-2010 was developed at the Harvard School of Public Health, as a measure of diet quality inversely related to chronic disease risk (17). It is scored out of 110 points. Maximum scores are awarded for high intakes of vegetables, fruits, whole grains, nuts and legumes, long-chain fats, and polyunsaturated fats; moderate alcohol intake; and minimal intakes of sugar-sweetened beverages and fruit juice, red/processed meats, trans fat, and sodium. In the current analysis, the AHEI-2010 was modified by excluding trans fat, which was unavailable in NHANES, and the overall score was rescaled to the original total. The MedDiet score measures adherence to the traditional Mediterranean diet and was adapted for the US diet (25). Out of 55 points, scores are awarded for high consumption of whole grains, fruits, vegetables, potatoes, legumes, fish, and monounsaturated fats; moderate consumption of alcohol; and low consumption of red meat, poultry, and full-fat dairy products. The MedDiet was originally developed for a Greek population, but has been previously examined in studies of US adults (26-28). The DASH score is a diet pattern associated with improved blood pressure in healthy and hypertensive adults (29). It includes components for fruits, vegetables, nuts and legumes, whole grains, low-fat dairy, sodium, red and processed meats, and sugary beverages, and is scored out of 40 points. Higher intakes on all components, except for sodium, red and processed meats, and sugary beverages, were awarded higher scores. While scoring for the HEI-2010, AHEI-2010, and MedDiet scores are based on concrete guidelines, scoring for the DASH score is based on relative distributions from the analytic sample. In this analysis, sex-specific cutpoints were derived from the study population to estimate each of the DASH components.

Leukocyte telomere length

Leukocyte telomere length (LTL) was assayed from DNA samples purified from whole blood, collected from adult NHANES participants in the 1999 to 2002 waves. LTL was assayed using the quantitative polymerase chain reaction (PCR) method to measure telomere length relative to standard reference DNA (T/S ratio) at the University of California, San Francisco (30). Procedures were taken to exclude outliers (<2% of samples) and ensure validity of the assays. Details of the procedures have been described elsewhere (31). The inter-assay coefficient of variation was 6.5%.

Covariate assessment

Sociodemographic characteristics included in multivariate models included participant's age (in 5-year increments), sex, self-reported race/ethnicity, highest educational attainment, ratio of family income to poverty (FPL), and marital status. Health-related variables included alcohol consumption (non-drinker, moderate drinker: ≤1 drink/day for women or ≤2 drinks/day for men, heavy drinker: >1 drink/day for women or >2 drinks/day for men), smoking status, pack years of smoking, physical activity, self-reported change in physical activity over the past year, and total energy intake (log-transformed). Anthropometric measures of adiposity included body mass index (BMI) and waist circumference. Height, weight and waist circumference were measured by trained personnel in the Mobile Examination Center. BMI categories were defined as underweight (BMI <18.5 kg/m^2), normal weight (BMI = 18.5-24.9 kg/m^2), overweight (BMI = 25.0-29.9 kg/m^2) and obese (BMI ≥30.0 kg/m^2). Elevated waist circumference was defined as ≥102 cm for men ≥88 cm for women.

Missing indicators were used to account for missing educational attainment (n=6), family income (n=373), marital status (n=239), alcohol consumption (n=7), physical activity (n=3), smoking status (n=7), BMI (n=56), and waist circumference (n=82). A complete-case analysis was also conducted as a sensitivity analysis.

Statistical analysis

NHANES complex survey weights were used to account for unequal selection probabilities, patterns of non-response, and to make nationally representative estimates (32). All analyses incorporated the 4-year dietary survey weights for the 1999-2002 period. As the four diet quality indices contained similar components, correlations between the indices were estimated using Pearson correlation coefficients. LTL was skewed and log-transformed prior to analysis. The first set of analyses examined quintiles of diet pattern indices in relation to LTL, in order to allow for non-linear associations. Tests for linear trend were conducted by including the midpoint of each quintile of diet quality index as a trend variable. The second set of analyses examined standardized diet quality indices in relation to LTL, to allow a more appropriate comparison across the four scores. Separate models were examined for men and women in the analyses. Heterogeneity by sex was determined using a Wald test of the cross-product term between sex and diet quality indices. All models adjusted for sociodemographic and health characteristics. Further adjustment for BMI and waist circumference was conducted as a sensitivity analysis, as these anthropometric measures of adiposity could serve as mediators rather than confounders. Supplemental analyses were conducted by examining individual components of diet quality indices in relation to telomere length.

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).

All statistical tests were 2-sided, and statistical significance was considered at $P<0.05$. Statistical analyses were performed using SAS 9.3 (SAS Institute, Cary, NC).

RESULTS

In the analytic sample, the mean HEI-2010 score was 46.3 out of 100; the mean AHEI-2010 score was 37.0 out of 110; the mean MedDiet score was 21.0 out of 55; and the mean DASH score was 23.9 out of 40. Pearson correlation coefficients between the four dietary patterns ranged from 0.56 to 0.73, with the lowest correlation between HEI-2010 and MedDiet, and the highest correlation between AHEI-2010 and DASH. Sociodemographic and health characteristics of the study participants are shown in **Table 1**. In bivariate analyses, being of Hispanic ethnicity, higher educational attainment, being married or living with a partner, higher household income, moderate alcohol intake, and being a former smoker were positively associated with higher mean scores on all four diet patterns.

Associations between quintiles of diet quality scores and log-transformed telomere length are shown in **Table 2**. Among women, a comparison of the top and bottom quintiles showed that higher HEI-2010 scores (β=0.065, 95% CI 0.018, 0.112, *P-trend=0.007*), AHEI-2010 scores (β=0.054, 95% CI 0.010, 0.097, *P-trend=0.007*), MedDiet scores (β=0.058, 95% CI 0.017, 0.098, *P-trend=0.008*), and DASH scores (β=0.052, 95% CI 0.014, 0.090, *P-trend=0.007*) were each significantly associated with longer LTL, after adjustment for sociodemographic and health characteristics (**Figure 1**). Among men, there were no significant associations when comparing the top and bottom quintiles of any diet quality scores to LTL, though there was marginal evidence of a linear trend with DASH scores and longer LTL (*P-trend=0.06*).

We further examined diet quality Z scores in relation to log-transformed telomere length in **Table 3**. Among women, a one-SD increase in HEI-2010 scores (B=0.024, 95% CI 0.008, 0.039), AHEI-2010 scores (B=0.019, 95% CI 0.007, 0.031), MedDiet scores (B=0.020, 95% CI 0.008, 0.033), and DASH scores (B=0.019, 95% CI 0.003, 0.035) were each associated with longer LTL. Further inclusion of BMI and waist circumference in these models did not change these effect estimates and results obtained from the missing indicator method for missing data were identical to those from the complete-case analysis (data not shown). When pairs of HEI-2010, AHEI-2010, MedDiet, and DASH Z scores were included in the models together, estimates were attenuated (data not shown). No diet quality Z scores were associated with LTL among men. Using the age-associated rate of telomere shortening of 14.6 base pairs per year in this sample, a one-standard deviation increase in the HEI-2010 score corresponded to 3.9 additional years of aging; the AHEI-2010 score to 3.2 additional years of aging; the MedDiet score to 3.3 additional years of aging; and the DASH score to 3.2 additional years of aging.

Individual components of the HEI-2010 and MedDiet scores were examined in relation to log-transformed leukocyte telomere length (**Web Tables 1-2**). The HEI-2010 and MedDiet were selected for these analyses because their associations with LTL were largest in magnitude for women. For the HEI-2010 score, a higher empty calories score (reflecting lower empty calories consumption) was associated with longer LTL in women. For the MedDiet score, whole-fat dairy and alcohol scores (reflecting lower whole-fat dairy and moderate alcohol consumption) were associated with longer LTL in women.

DISCUSSION

In this national sample of 4,758 healthy, nonelderly adults, we examined four evidence-based diet quality indices in relation to LTL. Our results showed that HEI-2010, AHEI-2010, MedDiet, and DASH scores were each positively associated with LTL in women, roughly equivalent to 3.2 to 3.9 additional years of aging for every one-standard deviation higher score on these diet quality indices. The magnitudes of these associations are comparable to other studies of dietary intake and cellular aging, 4.5 additional years for a 33% increase in the Alternate Mediterranean Diet score (13), 4 fewer years for processed meat intake (33), and 4.6 fewer years of aging for consuming a 20-ounce bottle of sugared soda (34). These magnitudes are also similar to analyses of other lifestyle behaviors and cellular aging, such as smoking (4.6 fewer years) (5). and physical activity (4.4 additional years) (4). When pairs of indices were examined together in the same model, the effect sizes were attenuated, indicating their associations were not different from each other. These results corroborate a prior study that found positive associations between adherence to the Mediterranean diet and to a lesser extent, the AHEI-2010, with longer telomere length in U.S. women (13).

When individual components of the diet quality indices were examined, only one component of the HEI-2010 score, empty calories, was associated with LTL in women. This suggests that there may be synergistic effects of the other dietary components driving the positive association between HEI-2010 scores and LTL. For the MedDiet score, lower whole-fat dairy intake and moderate alcohol consumption were both associated with LTL, which suggests these components may be driving the positive association between MedDiet score and LTL. However, we caution against placing too much focus on one individual dietary component in this analysis, as examining foods in isolation could potentially ignore the synergistic effects of foods commonly consumed together. Overall, our findings suggest that the protective associations with LTL extend to a general healthful diet pattern high in fruits, vegetables, whole grains, dairy products, and plant-based proteins, and low in red and processed meats, sodium, and added sugars, and that consuming these foods in combination is related to healthy cellular aging among women.

High scores on the HEI-2010, AHEI-2010, Mediterranean Diet, and DASH scores have been consistently shown to reduce the risk of major chronic disease, including type 2 diabetes (17, 19, 35), cardiovascular disease (17), cancers (17), and all-cause, cardiovascular disease, and cancer mortality (18). The fact that these four evidence-based diet quality indices are all predictive of lower chronic disease risk may be expected given their shared components; however, the correlations between scores in the present study ranged from 0.56 to 0.73. Other qualitative differences between the four indices include that the HEI-2010 awards points for consuming any types of protein, including red and processed meats, whereas the others prioritize plant-based protein sources (e.g. nuts, legumes) and fish. The HEI-2010 also awards points for consuming any dairy product, but full-fat dairy is penalized in the MedDiet score and only low-fat dairy is awarded points in the DASH score. In contrast, the AHEI-2010 and MedDiet are the only patterns to award points for moderate alcohol consumption. Regardless of these differences, high scores on all diet quality indices were associated with longer LTL in women, suggesting that telomere length maintenance may represent an additional mechanism by which high diet quality is protective for major chronic disease and mortality in women (20).

The relations between multiple healthful diet quality indices and telomere length was significantly different between men and women. Although the mechanisms underlying the associations between diet and LTL are unknown, the differential sex-specific associations we observed agree with findings from other studies. For example, in the PREDIMED-NAVARRA trial, García-Calzón and colleagues observed a positive association between baseline Mediterranean diet adherence and basal telomere length in women, but not in men (36). In a cohort of older Finnish adults, Tiainen and colleagues found that vegetable intake was positively associated with LTL in women, while fruit intake was inversely associated with LTL in men (37). Lastly, in a sample of Chinese women, an empirically derived high vegetable dietary pattern was associated with longer LTL in women but not men, and this relationship as partly mediated by C-reactive protein (inflammation) (38). Telomere length is impacted by inflammation, insulin resistance, and oxidative stress (39-41), all of which can be affected by specific dietary behaviors. In fact, another study using the same data set found that a pro-inflammatory diet was significantly associated with shorter LTL (42). In the present study, men consumed higher levels of red and processed meats and sugar-sweetened beverages across quartiles of LTL (data not shown) – these are all foods known to induce inflammation, insulin resistance, and oxidative stress. The more prevalent consumption of these specific foods may largely negate the beneficial properties of an otherwise healthful diet quality on LTL in men.

Our study was strengthened by the use of a large, nationally representative sample of adults, the availability of information on a large number of sociodemographic and health characteristics that allows for careful adjustments for potential confounding, and high response rates, which improves the generalizability of our findings. However, this study also has limitations. This primary limitation is the cross-sectional nature of the data, which prevents us from making causal inferences about our findings. To date, the majority of epidemiologic studies examining diet and telomere length have relied on cross-sectional data, even those embedded in prospective cohort studies. This highlights the need for longitudinal studies, with repeated concurrent measures of dietary intake and telomere length over time, to better understand how dietary changes influence telomere length. Furthermore, dietary intake was assessed using one 24-hour dietary recall. A single 24-hour dietary recall may not approximate usual dietary intake due to day-to-day variation, and future studies with more robust methods of dietary assessment are needed to confirm these findings.

In conclusion, the results of this large and nationally representative study suggest that a diet high in fruits, vegetables, whole grains, dairy products, and plant-based proteins, and low in red and processed meats, sodium, and added sugars is related to healthy cellular aging, particularly among women. Although further research is still needed to understand how dietary intake and diet patterns relate to cellular aging among men, efforts to promote longevity and reduce chronic disease risk through optimal nutrition should focus on improving overall diet quality rather than emphasizing individual foods or nutrients.

REFERENCES

1. Blackburn EH. Structure and function of telomeres. *Nature* 1991;350(6319):569-573.

2. Greider CW. Telomeres, telomerase and senescence. *BioEssays : news and reviews in molecular, cellular and developmental biology* 1990;12(8):363-369.

3. Blasco MA. Telomeres and human disease: ageing, cancer and beyond. *Nature reviews Genetics* 2005;6(8):611-622.

4. Du M, Prescott J, Kraft P, et al. Physical activity, sedentary behavior, and leukocyte telomere length in women. *American journal of epidemiology* 2012;175(5):414-422.

5. Valdes AM, Andrew T, Gardner JP, et al. Obesity, cigarette smoking, and telomere length in women. *Lancet* 2005;366(9486):662-664.

6. Rafie N, Golpour Hamedani S, Barak F, et al. Dietary patterns, food groups and telomere length: a systematic review of current studies. *European journal of clinical nutrition* 2017; 71(2): 151-158.

7. Willeit P, Raschenberger J, Heydon EE, et al. Leucocyte telomere length and risk of type 2 diabetes mellitus: new prospective cohort study and literature-based meta-analysis. *PloS one* 2014;9(11):e112483.

8. D'Mello MJ, Ross SA, Briel M, et al. Association between shortened leukocyte telomere length and cardiometabolic outcomes: systematic review and meta-analysis. *Circulation Cardiovascular genetics* 2015;8(1):82-90.

9. Haycock PC, Heydon EE, Kaptoge S, et al. Leucocyte telomere length and risk of cardiovascular disease: systematic review and meta-analysis. *BMJ* 2014;349:g4227.

10. Ma H, Zhou Z, Wei S, et al. Shortened telomere length is associated with increased risk of cancer: a meta-analysis. *PloS one* 2011;6(6):e20466.

11. Wentzensen IM, Mirabello L, Pfeiffer RM, et al. The association of telomere length and cancer: a meta-analysis. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 2011;20(6):1238-1250.

12. Jacobs DR, Tapsell LC. Food synergy: the key to a healthy diet. *The Proceedings of the Nutrition Society* 2013;72(2):200-206.

13. Crous-Bou M, Fung TT, Prescott J, et al. Mediterranean diet and telomere length in Nurses' Health Study: population based cohort study. *BMJ* 2014;349:g6674.

14. Lee JY, Jun NR, Yoon D, et al. Association between dietary patterns in the remote past and telomere length. *European journal of clinical nutrition* 2015; 69(9): 1048-1052.

15. Nettleton JA, Diez-Roux A, Jenny NS, et al. Dietary patterns, food groups, and telomere length in the Multi-Ethnic Study of Atherosclerosis (MESA). *The American journal of clinical nutrition* 2008;88(5):1405-1412.

16. Boccardi V, Esposito A, Rizzo MR, et al. Mediterranean diet, telomere maintenance and health status among elderly. *PloS one* 2013;8(4):e62781.

17. Chiuve SE, Fung TT, Rimm EB, et al. Alternative dietary indices both strongly predict risk of chronic disease. *The Journal of nutrition* 2012;142(6):1009-1018.

18. Reedy J, Krebs-Smith SM, Miller PE, et al. Higher diet quality is associated with decreased risk of all-cause, cardiovascular disease, and cancer mortality among older adults. *The Journal of nutrition* 2014;144(6):881-889.

19. Cespedes EM, Hu FB, Tinker L, et al. Multiple Healthful Dietary Patterns and Type 2 Diabetes in the Women's Health Initiative. *American journal of epidemiology* 2016;183(7):622-633.

20. Schwingshackl L, Hoffmann G. Diet quality as assessed by the Healthy Eating Index, the Alternate Healthy Eating Index, the Dietary Approaches to Stop Hypertension score, and health outcomes: a systematic review and meta-analysis of cohort studies. *Journal of the Academy of Nutrition and Dietetics* 2015;115(5):780-800 e5.

21. Dietary Interviewers Procedures Manual. *National Health and Nutrition Examination Surveys*. Atlanta, Georgia: Centers for Disease Control, 2000. (https://wwwn.cdc.gov/nchs/data/nhanes/1999-2000/manuals/dr-1-5.pdf) (Accessed October 25, 2016).

22. Ahluwalia N, Dwyer J, Terry A, et al. Update on NHANES Dietary Data: Focus on Collection, Release, Analytical Considerations, and Uses to Inform Public Policy. *Adv Nutr* 2016;7(1):121-134.

23. Baranowski T. 24-Hour Recall and Diet Record Methods. In: Willett WC, ed. *Nutritional Epidemiology*. New York, NY: Oxford University Press, 2012:49-69.

24. Guenther PM, Casavale KO, Reedy J, et al. Update of the Healthy Eating Index: HEI-2010. *Journal of the Academy of Nutrition and Dietetics* 2013;113(4):569-580.

25. Panagiotakos DB, Pitsavos C, Arvaniti F, et al. Adherence to the Mediterranean food pattern predicts the prevalence of hypertension, hypercholesterolemia, diabetes and obesity, among healthy adults; the accuracy of the MedDietScore. *Prev Med* 2007;44(4):335-340.

26. McEvoy CT, Guyer H, Langa KM, et al. Neuroprotective Diets Are Associated with Better Cognitive Function: The Health and Retirement Study. *Journal of the American Geriatrics Society* 2017;65(8):1857-1862.

27. Tangney CC, Kwasny MJ, Li H, et al. Adherence to a Mediterranean-type dietary pattern and cognitive decline in a community population. *The American journal of clinical nutrition* 2011;93(3):601-607.

28. Koyama A, Houston DK, Simonsick EM, et al. Association between the Mediterranean diet and cognitive decline in a biracial population. *J Gerontol A Biol Sci Med Sci* 2015;70(3):354-359.

29. Fung TT, Chiuve SE, McCullough ML, et al. Adherence to a DASH-style diet and risk of coronary heart disease and stroke in women. *Archives of internal medicine* 2008;168(7):713-720.

30. Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic acids research* 2002;30(10):e47.

31. NHANES 1999-2000: Telomere Mean and Standard Deviation (Surplus) Data Documentation, Codebook, and Frequencies. National Center for Health Statistics, Centers for Disease Control and Prevention; 2015. (https://wwwn.cdc.gov/Nchs/Nhanes/1999-2000/TELO_A.htm). (Accessed October 25 2016).

32. Key Concepts about Weighting in NHANES I. Atlanta, GA: National Center for Health Statistics, Centers for Disease Control; 2013. (https://www.cdc.gov/nchs/tutorials/NHANES/SurveyDesign/Weighting/OverviewKey.htm). (Accessed May 12 2017).

33. Fretts AM, Howard BV, Siscovick DS, et al. Processed Meat, but Not Unprocessed Red Meat, Is Inversely Associated with Leukocyte Telomere Length in the Strong Heart Family Study. *The Journal of nutrition* 2016;146(10):2013-2018.

34. Leung CW, Laraia BA, Needham BL, et al. Soda and cell aging: associations between sugar-sweetened beverage consumption and leukocyte telomere length in healthy adults from the National Health and Nutrition Examination Surveys. *American journal of public health* 2014;104(12):2425-2431.

35. de Koning L, Chiuve SE, Fung TT, et al. Diet-quality scores and the risk of type 2 diabetes in men. *Diabetes care* 2011;34(5):1150-1156.

36. Garcia-Calzon S, Martinez-Gonzalez MA, Razquin C, et al. Mediterranean diet and telomere length in high cardiovascular risk subjects from the PREDIMED-NAVARRA study. *Clin Nutr* 2016;35(6):1399-1405.

37. Tiainen AM, Mannisto S, Blomstedt PA, et al. Leukocyte telomere length and its relation to food and nutrient intake in an elderly population. *European journal of clinical nutrition* 2012;66(12):1290-1294.

38. Gong Y, Tian G, Xue H, et al. Higher adherence to the 'vegetable-rich' dietary pattern is related to longer telomere length in women. *Clin Nutr* 2017. S0261-5614(17): 30166-30168.

39. Demissie S, Levy D, Benjamin EJ, et al. Insulin resistance, oxidative stress, hypertension, and leukocyte telomere length in men from the Framingham Heart Study. *Aging cell* 2006;5(4):325-330.

40. Shiels PG, McGlynn LM, MacIntyre A, et al. Accelerated telomere attrition is associated with relative household income, diet and inflammation in the pSoBid cohort. *PloS one* 2011;6(7):e22521.

41. von Zglinicki T. Oxidative stress shortens telomeres. *Trends in biochemical sciences* 2002;27(7):339-344.

42. Shivappa N, Wirth MD, Hurley TG, et al. Association between the dietary inflammatory index (DII) and telomere length and C-reactive protein from the National Health and Nutrition Examination Survey-1999-2002. *Mol Nutr Food Res* 2017;61(4).

Table 1: Mean leukocyte telomere length (LTL) and diet quality scores by characteristics of study participants: National Health and Nutrition Examination Surveys 1999-2002

	Mean (SE) or n (weighted %)	LTL	HEI-2010 score	AHEI-2010 score	MedDiet score	DASH score
		Mean (SE)				
Age,	39.5 (0.3)	1.10 (0.02)	46.3 (0.5)	37.0 (0.3)	21.0 (0.1)	23.9 (0.2)
Sex						
Men	2208 (49.0)	1.09 (0.02)	46.0 (0.5)	35.1 (0.4)[a]	20.4 (0.1)[a]	24.4 (0.2)[a]
Women	2550 (51.0)	1.10 (0.02)	46.6 (0.7)	38.8 (0.4)	21.5 (0.2)	23.4 (0.2)
Race/ethnicity						
Non-Hispanic White	2239 (71.2)	1.09 (0.02)	46.3 (0.7)[a]	37.1 (0.4)[a]	21.0 (0.2)[a]	24.1 (0.2)[a]
Non-Hispanic Black	814 (9.6)	1.15 (0.02)	43.9 (0.8)	34.8 (0.5)	19.9 (0.2)	22.4 (0.2)
Hispanic	1544 (15.1)	1.09 (0.02)	48.1 (0.7)	37.7 (0.6)	21.5 (0.2)	24.0 (0.2)
Other	161 (4.1)	1.07 (0.03)	46.2 (1.5)	36.2 (1.6)	21.0 (0.5)	23.5 (0.4)
Education						
Less than 12 years	1378 (17.5)	1.07 (0.02)	43.0 (0.6)[a]	35.2 (0.6)[a]	20.2 (0.2)[a]	22.8 (0.2)[a]
High school graduate or equivalent	1088 (24.8)	1.09 (0.02)	43.5 (0.6)	34.3 (0.7)	20.0 (0.2)	22.7 (0.2)
Some college	1293 (30.5)	1.11 (0.02)	45.7 (0.6)	36.1 (0.4)	20.8 (0.2)	23.6 (0.2)
College graduate	993 (27.0)	1.12 (0.02)	51.7 (0.7)	41.6 (0.7)	22.6 (0.3)	26.0 (0.2)
Marital status						
Single	911 (19.6)	1.18 (0.02)	43.5 (0.8)[a]	33.9 (0.7)[a]	19.9 (0.3)[a]	23.1 (0.2)[a]
Married or living with partner	2979 (61.2)	1.08 (0.02)	47.4 (0.6)	38.0 (0.4)	21.4 (0.2)	24.3 (0.2)
Separated, widowed, or divorced	629 (13.1)	1.04 (0.02)	45.3 (1.0)	37.0 (0.7)	20.7 (0.3)	23.4 (0.3)
Household income						
0-100% FPL	777 (12.6)	1.14 (0.02)	43.2 (1.0)[a]	35.2 (1.0)[a]	20.4 (0.3)[a]	22.9 (0.3)[a]
100.1-200% FPL	994 (16.7)	1.08 (0.02)	42.4 (0.8)	33.8 (0.7)	19.6 (0.2)	22.4 (0.3)
200.1-300% FPL	694 (14.0)	1.10 (0.02)	46.0 (0.9)	35.2 (0.8)	20.6 (0.3)	23.3 (0.2)

300.1-400% FPL	556 (13.2)	1.11 (0.03)	46.4 (0.7)	36.7 (0.6)	20.9 (0.3)	23.9 (0.3)
>400% FPL	1364 (36.7)	1.08 (0.02)	49.0 (0.7)	39.9 (0.5)	22.0 (0.2)	25.2 (0.2)
Alcohol intake						
Never drinker	277 (4.8)	1.11 (0.03)	44.5 (1.4)[a]	35.3 (1.2)[a]	20.8 (0.6)[a]	23.0 (0.3)[a]
Moderate drinker	2656 (55.5)	1.09 (0.02)	47.8 (0.6)	38.8 (0.4)	21.5 (0.2)	24.6 (0.2)
Heavy drinker	1818 (39.6)	1.11 (0.02)	44.5 (0.7)	34.7 (0.5)	20.3 (0.2)	23.0 (0.2)
Smoking status						
Never smoker	2582 (52.4)	1.11 (0.02)	47.7 (0.6[a]	38.4 (0.4)[a]	21.6 (0.2)[a]	24.3 (0.2)[a]
Former smoker	987 (21.3)	1.06 (0.02)	49.4 (0.7	39.5 (0.6)	21.7 (0.3)	25.0 (0.2)
Current smoker	1182 (26.2)	1.10 (0.02)	41.0 (0.6	32.2 (0.4)	19.1 (0.2)	22.1 (0.2)
Physical activity						
No activity	1730 (27.8)	1.11 (0.02)	43.6 (0.6)[a]	35.6 (0.6)[a]	20.2 (0.2)[a]	22.9 (0.2)[a]
Any activity	3025 (72.1)	1.07 (0.01)	47.3 (0.6)	37.5 (0.3)	21.3 (0.2)	24.2 (0.2)
Body mass index categories						
Underweight	70 (1.9)	1.14 (0.04)	44.1 (2.1)[a]	33.1 (1.7)	20.1 (0.8)	23.2 (0.7)[a]
Normal	1487 (34.6)	1.13 (0.02)	48.0 (0.8)	37.7 (0.6)	21.2 (0.3)	24.0 (0.3)
Overweight	1685 (34.0)	1.08 (0.02)	46.7 (0.6)	36.9 (0.4)	20.9 (0.2)	24.2 (0.2)
Obese	1460 (28.3)	1.07 (0.02)	44.1 (0.6)	36.5 (0.5)	20.8 (0.2)	23.3 (0.2)
Waist circumference						
Normal	2414 (55.0)	1.12 (0.02)	47.2 (0.6)[a]	36.9 (0.4)	21.0 (0.2)[a]	24.1 (0.2)
Elevated	2262 (43.5)	1.06 (0.02)	45.3 (0.7)	37.1 (0.5)	20.9 (0.2)	23.5 (0.2)

LTL, leukocyte telomere length; HEI, Healthy Eating Index; AHEI, Alternate Healthy Eating Index; MedDiet, Mediterranean Diet score; DASH, Dietary Approaches to Stopping Hypertension; FPL, federal poverty level

[a] Significant differences in diet quality within category levels

Table 2: Diet quality quintiles and log-transformed leukocyte telomere length: National Health and Nutrition Examination Surveys 1999-2002

Diet quality	All adults			Men			Women		
index	Mean (SE)	β	95% CI	Mean (SE)	β[a]	95% CI	Mean (SE)	β[a]	95% CI
HEI-2010 score									
Age-adjusted									
Quintile 1	0.027 (0.022)	0	Referent	0.026 (0.018)	0	Referent	0.027 (0.029)	0	Referent
Quintile 2	0.032 (0.019)	0.006	–0.024, 0.036	0.033 (0.023)	0.007	–0.033, 0.046	0.032 (0.022)	0.006	–0.041, 0.052
Quintile 3	0.042 (0.018)	0.015	–0.022, 0.053	0.028 (0.021)	0.003	–0.042, 0.047	0.053 (0.019)	0.026	–0.026, 0.079
Quintile 4	0.050 (0.016)	0.023	–0.010, 0.057	0.046 (0.018)	0.020	–0.015, 0.055	0.052 (0.017)	0.025	–0.022, 0.073
Quintile 5	0.076 (0.018)	0.050	0.006, 0.095	0.059 (0.021)	0.033	–0.016, 0.082	0.094 (0.018)	0.067	0.015, 0.120
P-trend			*0.02*			*0.12*			*0.01*
Multivariate-adjusted [b]									
Quintile 1	0.113 (0.031)	0	Referent	0.134 (0.039)	0	Referent	0.004 (0.063)	0	Referent
Quintile 2	0.119 (0.030)	0.006	–0.024, 0.036	0.137 (0.043)	0.003	–0.039, 0.046	0.010 (0.064)	0.007	–0.037, 0.051
Quintile 3	0.126 (0.030)	0.014	–0.021, 0.049	0.139 (0.042)	0.005	–0.039, 0.049	0.024 (0.064)	0.020	–0.026, 0.067
Quintile 4	0.128 (0.031)	0.015	–0.016, 0.047	0.139 (0.046)	0.006	–0.031, 0.042	0.027 (0.061)	0.024	–0.020, 0.067
Quintile 5	0.152 (0.031)	0.039	–0.001, 0.079	0.149 (0.045)	0.015	–0.033, 0.064	0.069 (0.064)	0.065	0.018, 0.112
P-trend			*0.04*			*0.50*			*0.007*
AHEI-2010 score									
Age-adjusted									
Quintile 1	0.048 (0.019)	0	Referent	0.052 (0.020)	0	Referent	0.044 (0.021)	0	Referent
Quintile 2	0.029 (0.017)	–0.019	–0.050, 0.011	0.014 (0.019)	–0.038	–0.077, 0.000	0.045 (0.021)	0.001	–0.038, 0.040
Quintile 3	0.030 (0.020)	–0.018	–0.041, 0.006	0.039 (0.020)	–0.013	–0.051, 0.024	0.023 (0.023)	–0.021	-0.058, 0.016
Quintile 4	0.049 (0.017)	0.001	–0.025, 0.027	0.038 (0.021)	–0.015	–0.045, 0.016	0.058 (0.019)	0.014	–0.030, 0.058

Quintile 5	0.074 (0.017)	0.026	–0.008, 0.060	0.050 (0.020)	–0.002	–0.047, 0.044	0.095 (0.019)	0.051	0.007, 0.094
P-trend			*0.04*			*0.73*			*0.01*
Multivariate-adjusted [b]									
Quintile 1	0.136 (0.032)	0	Referent	0.159 (0.036)	0	Referent	0.021 (0.057)	0	Referent
Quintile 2	0.113 (0.032)	–0.023	–0.053, 0.007	0.113 (0.040)	–0.045	–0.081, –0.010	0.016 (0.060)	–0.005	–0.045, 0.036
Quintile 3	0.118 (0.033)	–0.018	–0.041, 0.005	0.142 (0.043)	–0.017	–0.049, 0.016	0.005 (0.067)	–0.016	–0.057, 0.025
Quintile 4	0.132 (0.032)	–0.004	–0.030, 0.022	0.131 (0.040)	–0.028	–0.057, 0.002	0.039 (0.063)	0.018	–0.029, 0.064
Quintile 5	0.155 (0.030)	0.019	–0.010, 0.049	0.140 (0.044)	–0.019	–0.064, 0.026	0.075 (0.058)	0.054	0.010, 0.097
P-trend			*0.08*			*0.63*			*0.007*
MedDiet score									
Age-adjusted									
Quintile 1	0.027 (0.022)	0	Referent	0.027 (0.022)	0	Referent	0.027 (0.024)	0	Referent
Quintile 2	0.039 (0.018)	0.012	–0.021, 0.046	0.025 (0.020)	–0.003	–0.048, 0.043	0.051 (0.021)	0.024	–0.014, 0.061
Quintile 3	0.055 (0.017)	0.028	–0.007, 0.063	0.046 (0.020)	0.019	–0.028, 0.065	0.065 (0.017)	0.038	0.002, 0.073
Quintile 4	0.045 (0.017)	0.018	–0.010, 0.046	0.047 (0.020)	0.019	–0.017, 0.055	0.043 (0.021)	0.016	–0.026, 0.058
Quintile 5	0.066 (0.019)	0.039	0.004, 0.073	0.045 (0.020)	0.018	–0.029, 0.065	0.087 (0.023)	0.059	0.013, 0.105
P-trend			*0.02*			*0.31*			*0.02*
Multivariate-adjusted [b]									
Quintile 1	0.117 (0.032)	0	Referent	0.140 (0.039)	0	Referent	0.006 (0.063)	0	Referent
Quintile 2	0.126 (0.035)	0.009	–0.025, 0.043	0.129 (0.047)	–0.010	–0.059, 0.038	0.028 (0.061)	0.021	–0.014, 0.057
Quintile 3	0.140 (0.031)	0.023	–0.008, 0.053	0.152 (0.041)	0.012	–0.030, 0.054	0.040 (0.058)	0.034	0.001, 0.067
Quintile 4	0.130 (0.034)	0.013	–0.011, 0.037	0.152 (0.044)	0.012	–0.022, 0.046	0.020 (0.064)	0.013	–0.025, 0.052
Quintile 5	0.149 (0.032)	0.032	0.003, 0.061	0.146 (0.049)	0.007	–0.038, 0.051	0.064 (0.061)	0.058	0.017, 0.098
P-trend			*0.03*			*0.56*			*0.008*
DASH score									
Age-adjusted									

Quintile 1	0.032 (0.023)	0	Referent	0.024 (0.021)	0	Referent	0.040 (0.027)	0	Referent
Quintile 2	0.035 (0.017)	0.003	–0.026, 0.032	0.026 (0.016)	0.002	–0.035, 0.039	0.044 (0.021)	0.004	–0.035, 0.044
Quintile 3	0.026 (0.021)	–0.006	–0.044, 0.032	0.020 (0.025)	–0.005	–0.050, 0.040	0.030 (0.023)	–0.009	–0.053, 0.035
Quintile 4	0.044 (0.019)	0.012	–0.032, 0.056	0.031 (0.020)	0.006	–0.035, 0.047	0.057 (0.024)	0.018	–0.048, 0.083
Quintile 5	0.088 (0.017)	0.056	0.012, 0.100	0.089 (0.021)	0.064	0.014, 0.115	0.087 (0.016)	0.047	–0.003, 0.097
P-trend			*0.002*			*0.01*			*0.006*
Multivariate-adjusted [b]									
Quintile 1	0.116 (0.033)	0	Referent	0.134 (0.041)	0	Referent	0.008 (0.067)	0	Referent
Quintile 2	0.119 (0.033)	0.004	–0.024, 0.032	0.135 (0.039)	0.001	–0.039, 0.041	0.016 (0.067)	0.007	–0.025, 0.040
Quintile 3	0.112 (0.038)	–0.003	–0.040, 0.033	0.124 (0.050)	–0.009	–0.057, 0.038	0.009 (0.067)	0.001	–0.041, 0.043
Quintile 4	0.130 (0.038)	0.014	–0.029, 0.057	0.130 (0.048)	–0.004	–0.049, 0.042	0.042 (0.064)	0.034	–0.028, 0.096
Quintile 5	0.166 (0.033)	0.05	0.015, 0.085	0.177 (0.045)	0.043	–0.004, 0.091	0.060 (0.063)	0.052	0.014, 0.090
P-trend			*0.002*			*0.06*			*0.007*

HEI, Healthy Eating Index; AHEI, Alternate Healthy Eating Index; MedDiet, Mediterranean Diet score; DASH, Dietary Approaches to Stopping Hypertension

[a] *P* values from Wald tests for heterogeneity of OR by sex were: *0.22* for HEI-2010 score, *0.01* for AHEI-2010 score; *0.50* for MedDiet score, and *0.94* for DASH score

[b] Model includes age, sex (except for sex-specific models), race/ethnicity, educational attainment, marital status, poverty income ratio, alcohol consumption, smoking status, pack years of smoking, physical activity, change in activity over the past year, and total energy intake (log)

Table 3: Standardized diet quality scores and log-transformed leukocyte telomere length: National Health and Nutrition Examination Surveys 1999-2002

Diet quality index	All adults		Men only[a]		Women only[a]	
	β	95% CI	β	95% CI	β	95% CI
HEI-2010 score						
Age-adjusted	0.020	0.006, 0.034	0.015	0.000, 0.030	0.025	0.008, 0.041
Multivariate-adjusted[b]	0.015	0.003, 0.028	0.007	-0.007, 0.021	0.024	0.008, 0.039
AHEI-2010 score						
Age-adjusted	0.011	0.001, 0.022	0.004	-0.009, 0.018	0.017	0.004, 0.030
Multivariate-adjusted[b]	0.009	–0.000, 0.018	–0.002	-0.015, 0.012	0.019	0.007, 0.031
MedDiet score						
Age-adjusted	0.014	0.003, 0.025	0.006	–0.008, 0.021	0.021	0.007, 0.035
Multivariate-adjusted[b]	0.011	0.002, 0.020	0.003	–0.010, 0.016	0.020	0.008, 0.033
DASH score						
Age-adjusted	0.018	0.003, 0.033	0.020	0.005, 0.034	0.015	–0.003, 0.034
Multivariate-adjusted[b]	0.016	0.003, 0.029	0.012	–0.002, 0.027	0.019	0.003, 0.035

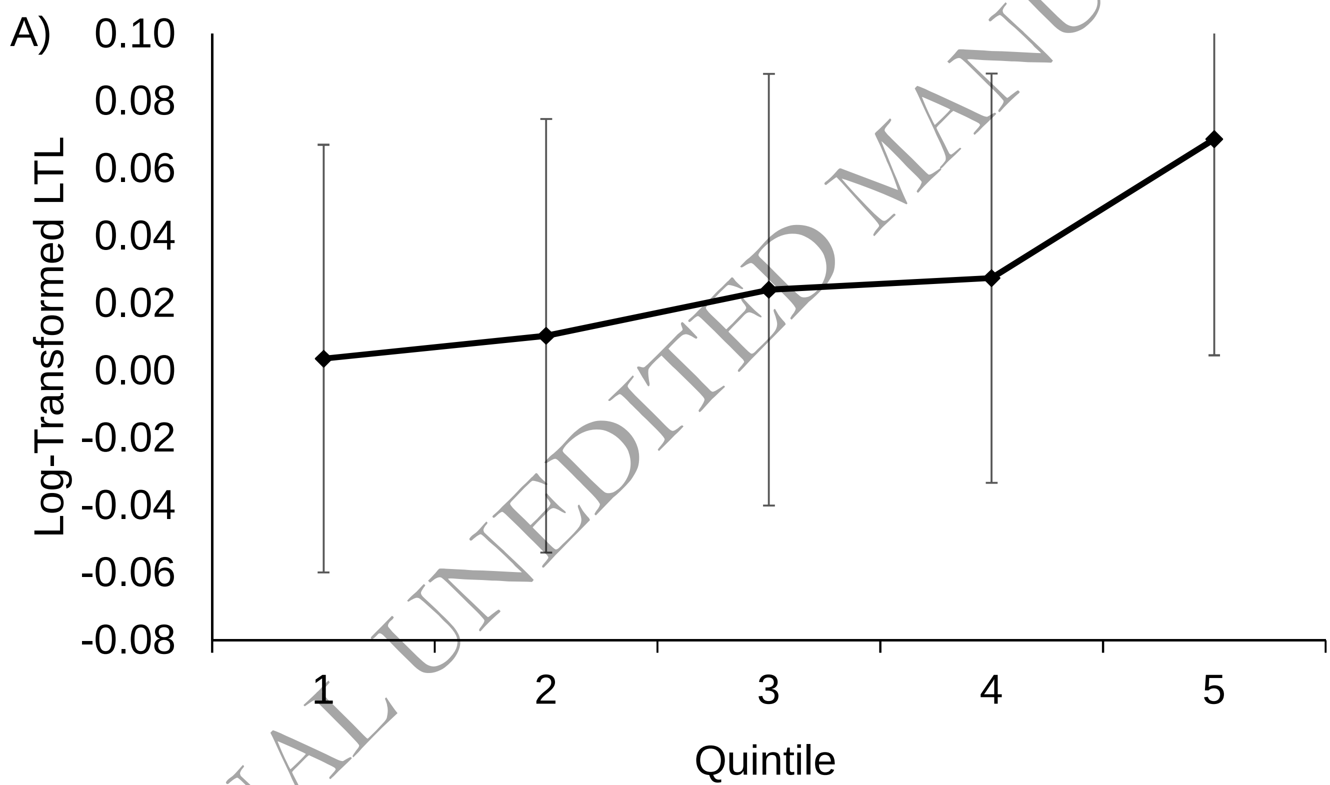
HEI, Healthy Eating Index; AHEI, Alternate Healthy Eating Index; MedDiet, Mediterranean Diet score; DASH, Dietary Approaches to Stopping Hypertension

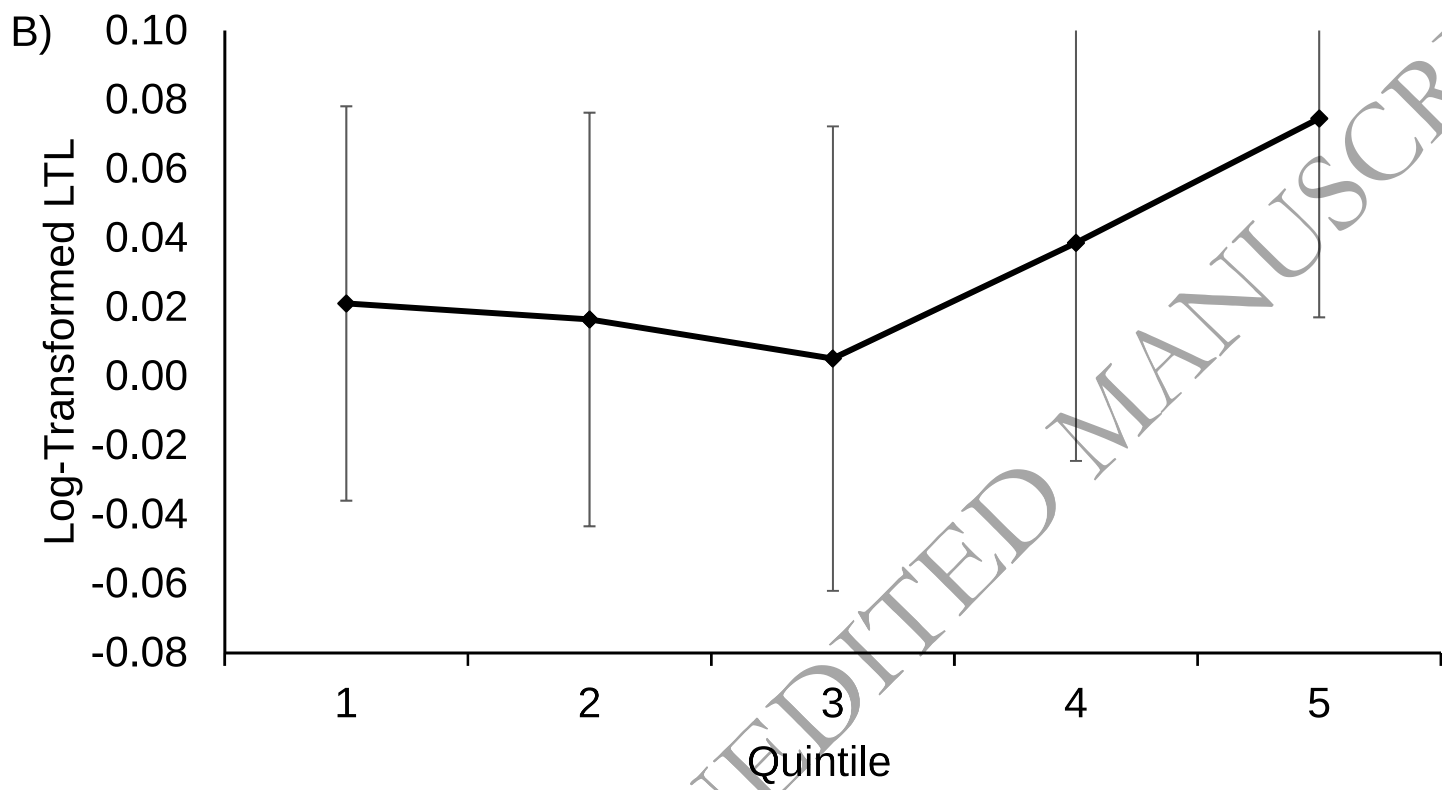
[a] *P* values from Wald tests for heterogeneity of OR by sex were: *0.06* for HEI-2010 score, *0.02* for AHEI-2010 score; *0.04* for MedDiet score; and *0.89* for DASH score

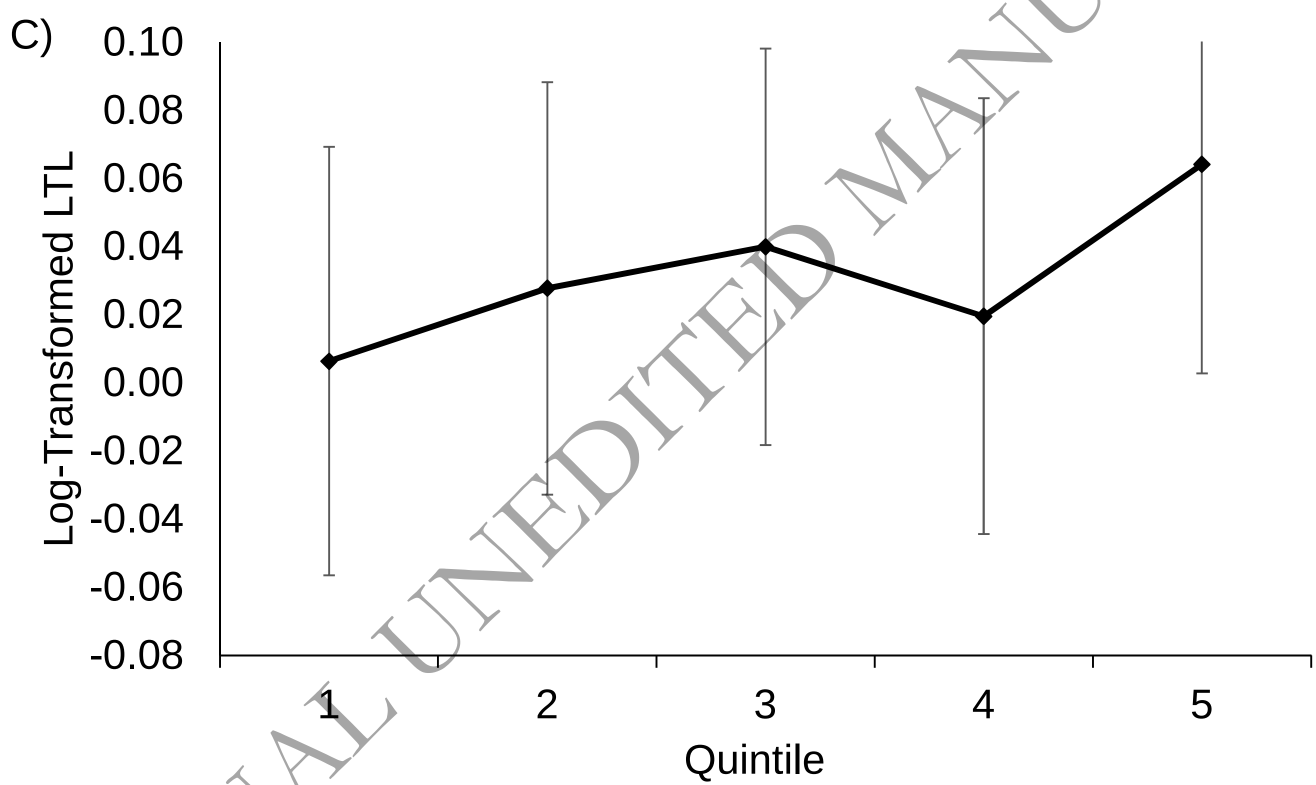
[b] Estimates adjusted for age, sex (except for sex-specific models), race/ethnicity, educational attainment, marital status, poverty income ratio, alcohol consumption, smoking status, pack years of smoking, physical activity, change in activity over the past year, and total energy intake (log)

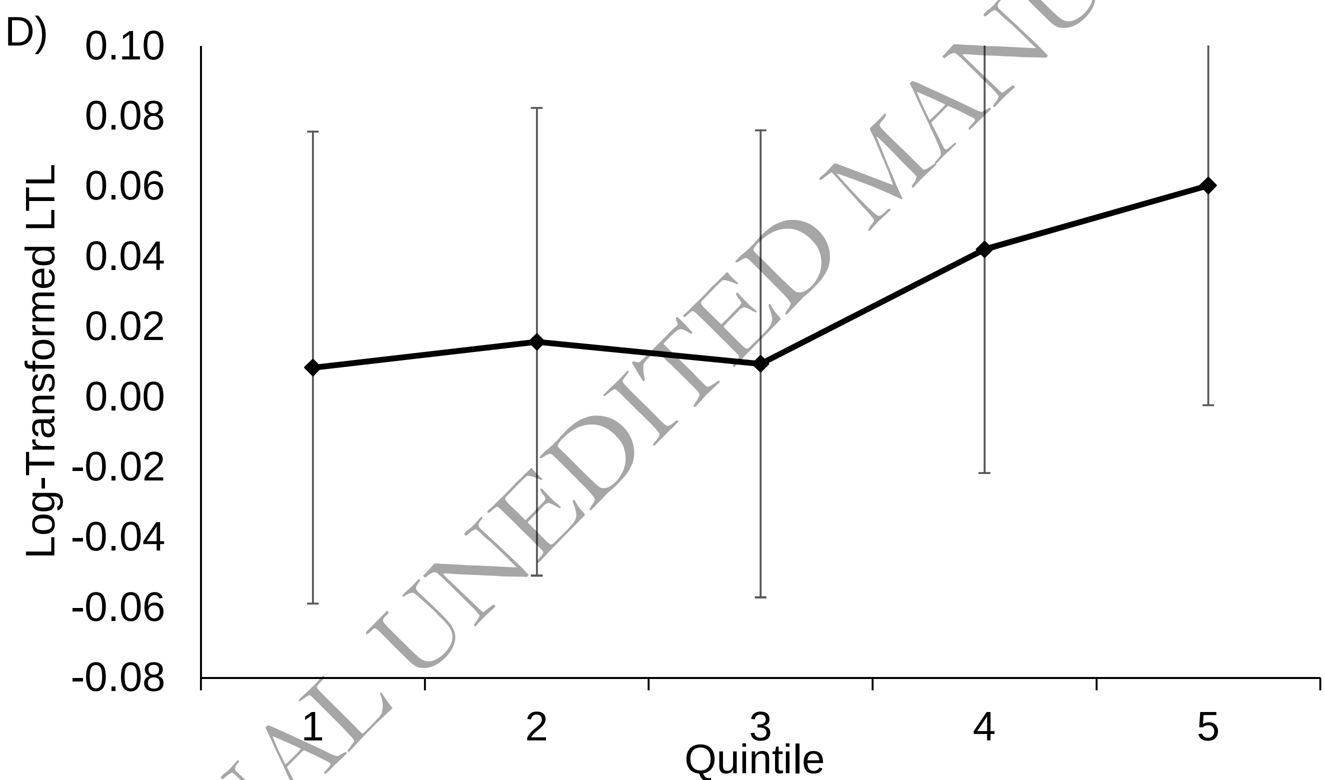
Figure 1:

Multivariate-adjusted least square means (and standard errors) of log-transformed leukocyte telomere length by quintiles of diet quality indices (Healthy Eating Index (HEI)-2010 (Panel A); Alternate Healthy Eating Index (AHEI)-2010 (Panel B); Mediterranean Diet (MedDiet) score (Panel C); Dietary Approaches to Stopping Hypertension (DASH) score) (Panel D) among women. P-trend for diet quality indices and log-transformed LTL is as follows: 0.007 for HEI-2010, 0.007 for AHEI-2010, 0.008 for MedDiet, and 0.007 for DASH.


A)
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
Log-Transformed LTL
1
2
3
4
5
Quintile


B)
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
Log-Transformed LTL
1
2
3
4
5
Quintile


C)
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
Log-Transformed LTL
1
2
3
4
5
Quintile


D)
Log-Transformed LTL
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
1
2
3
4
5
Quintile