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Mediterranean diet and telomere length in Nurses' Health Study: population based cohort study

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Abstract

Objective To examine whether adherence to the Mediterranean diet was associated with longer telomere length, a biomarker of aging.

Design Population based cohort study.

Setting Nurses' Health Study, an ongoing prospective cohort study of 121 700 nurses enrolled in 1976; in 1989-90 a subset of 32 825 women provided blood samples.

Participants 4676 disease-free women from nested case-control studies within the Nurses' Health Study with telomere length measured who also completed food frequency questionnaires.

Main outcome measure Association between relative telomere lengths in peripheral blood leukocytes measured by quantitative real time polymerase chain reaction and Alternate Mediterranean Diet score calculated from self reported dietary data.

Results Greater adherence to the Mediterranean diet was associated with longer telomeres after adjustment for potential confounders. Least squares mean telomere length z scores were −0.038 (SE 0.035) for the lowest Mediterranean diet score groups and 0.072 (0.030) for the highest group (P for trend=0.004).

Conclusion In this large study, greater adherence to the Mediterranean diet was associated with longer telomeres. These results further support the benefits of adherence to the Mediterranean diet for promoting health and longevity.

Introduction

The traditional Mediterranean diet is characterized by a high intake of vegetables, fruits, nuts, legumes, and grains (mainly unrefined); a high intake of olive oil but a low intake of saturated lipids; a moderately high intake of fish; a low intake of dairy products, meat, and poultry; and a regular but moderate intake of alcohol (specifically wine with meals).[1] Observational studies and intervention trials have consistently shown the health benefits of a high degree of adherence to the Mediterranean diet, including reduction of overall mortality[2-4]; reduced incidence of chronic diseases, especially major cardiovascular diseases[5]; and increased likelihood of healthy aging.[6]

Telomeres are repetitive DNA sequences at the ends of eukaryotic chromosomes that undergo attrition each time a somatic cell divides.[7] Telomeres prevent the loss of genomic DNA at the ends of linear chromosomes and in turn protect their physical integrity.[8 9] Telomere attrition has been shown to be accelerated by oxidative stress and inflammation.[10 11] Telomere length is considered to be a biomarker of aging; shorter telomeres are associated with a decreased life expectancy and increased rates of developing age related chronic diseases.[12-14] Telomere length decreases with age and varies considerably among individuals.[15] Studies suggest that telomere attrition is modifiable, as substantial variability exists in the rate of telomere shortening that is independent of chronological age.[16-18] Therefore, variability of telomere length may be partially explained by lifestyle practices, including dietary patterns.[19] As accelerated telomere attrition may underlie many chronic diseases, identifying modifiable factors that affect telomere dynamics is important.

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Given that fruits, vegetables, and nuts, key components of the Mediterranean diet, have well known antioxidant and anti-inflammatory effects, and that telomere length is affected by both of these processes, we hypothesized that greater adherence to the Mediterranean diet would be associated with longer telomere length. Therefore, the main objective of this study was to examine the association between greater adherence to the Mediterranean diet and leukocyte telomere length in US women within the Nurses' Health Study cohort. For comparison, we also evaluated the association between other existing dietary patters (prudent pattern, Western pattern, and Alternative Healthy Eating Index) and leukocyte telomere length.

Methods

Study population

The Nurses' Health Study is a prospective cohort study of 121 700 female registered nurses in 11 US states, aged 30-55 years at enrollment. The study was established in 1976; since then, participants have completed biennial questionnaires to update information on demographic characteristics, lifestyle factors, and newly diagnosed diseases.[20 21] Between 1989 and 1990 32 825 cohort participants provided blood samples. Details of the blood collection and archival methods have been described previously.[22]

Multiple nested case-control studies have been conducted within the Nurses' Health Study blood sub-cohort to investigate the association between leukocyte telomere length and cancer, cardiovascular disease, and cognitive function, among others.[23-28] The analysis reported here included data on 4676 women selected as healthy controls (free of major chronic diseases, including cancer and cardiovascular disease) from nested case-control studies, with previously measured leukocyte telomere length and completed food frequency questionnaires at the time of blood draw.

Dietary assessment

In 1980 participants completed a 61 item semi-quantitative food frequency questionnaire to assess their diet in the previous year. In 1984, 1986, and every four years thereafter, an expanded food frequency questionnaires containing 116-130 food items was sent to the nurses to update their dietary information. Participants were asked about the frequency of consumption of each food item of a pre-specified standard portion size in the previous year. The validity of these questionnaires has been previously described.[29] We calculated all dietary patterns and diet quality scores from self reported dietary data on the 1990 food frequency questionnaire, the closest to the blood draw (1989-90).

Trichopoulou adapted the Alternate Mediterranean Diet score from the Mediterranean Diet Score to assess adherence to the traditional Mediterranean diet.[2] The Alternate Mediterranean Diet score includes the following nine components: vegetables (excluding potatoes), fruits, nuts, whole grains, legumes, fish, monounsaturated:saturated fatty acid ratio, red and processed meats, and moderate alcohol intake. The possible score range is 0-9, with a higher score representing a closer resemblance to the Mediterranean diet. We dichotomized each of the nine dietary components at the median. We gave participants with intake above the median 1 point each for vegetables, fruits, nuts, whole grains, legumes, fish, and monounsaturated:saturated fatty acid ratio; we scored intake equal to or below the median as 0 points for each component. We scored red and processed meat consumption below the median as 1 point, with 0 points for intake equal to or above the median. Participants received

1 point for alcohol intake between 5 and 15 g/day; otherwise, they got 0 points. Details of the Alternate Mediterranean Diet scoring system are described elsewhere.[3]

We used principal component analysis of the food frequency questionnaires to identify prudent and Western dietary patterns.[30 31] The prudent dietary pattern is characterized by high intakes of fruits, vegetables, legumes, fish and other seafood, poultry, and whole grains; the Western dietary pattern includes high intakes of red and processed meats, butter, high fat dairy products, eggs, sweets and desserts, French fries, and refined grains. Details of the reproducibility and validity of dietary patterns assessed with a food frequency questionnaire have been previously described.[30 31]

The Alternative Healthy Eating Index measures adherence to a dietary pattern based on foods and nutrients most predictive of risk for chronic disease in the literature.[32] The Alternative Healthy Eating Index ranges from 0 to 110 points. Each of the 11 components of the score (vegetables, fruits, whole grains, sugar sweetened drinks, nuts and legumes, red and processed meat, trans fat, long chain (n-3) fats, polyunsaturated fatty acids, sodium, and alcohol) has a minimum score of 0 (worst) and a maximum score of 10 (best), according to component specific criteria reflecting either the current dietary guidelines or associations reported in the literature. Details of component selection and Alternative Healthy Eating Index calculation have been previously described.[32]

Covariate assessment

In addition to age at blood draw, we collected information on factors potentially associated with Mediterranean diet and telomere length. Using the biennial questionnaires completed proximal to blood collection and a supplemental questionnaire administered at blood collection, we assessed various anthropometric, reproductive, and lifestyle factors, as well as factors that have been related to telomere length in the Nurses' Health Study elsewhere: body mass index (kg/m²), cigarette smoking (pack years),[33-35] physical activity (total metabolic equivalent hours of activity per week).[16] We calculated dietary factors, including energy intake (total calories), from the 1990 food frequency questionnaire.

Telomere length measurement

Genomic DNA was extracted from peripheral blood leukocytes using the QIAmp 96-spin blood protocol (Qiagen, Chatsworth, CA, USA). Pico-Green quantification of genomic DNA was performed using a Molecular Devices 96 well spectrophotometer (Sunnyvale, CA, USA).

Relative telomere length was determined using a modified, high throughput version of the quantitative real time polymerase chain reaction based telomere assay.[36 37] The quantitative real time polymerase chain reaction telomere assay was run on the Applied Biosystems 7900HT Sequence Detection System (Foster City, CA, USA). Laboratory personnel were blinded to participants' characteristics, and all assays were processed in triplicate by the same technician and under identical conditions.

The average relative leukocyte telomere length was calculated as the ratio of telomere repeat copy number to a single gene (36B4) copy number. Leukocyte telomere length is reported as the exponentiated ratio of telomere repeat copy number to a single gene copy number corrected for a reference sample. In all nested case-control studies, the telomere and single gene assay coefficients of variation for triplicates were less than 4%. Coefficients of variation for the exponential ratio of telomere repeat copy number to a single gene copy number were under

18%. In the Nurses' Health Study, the three year and 10 year interclass correlations for reliability were determined to be 0.80 and 0.60. Although this assay provides a relative measurement of telomere length, ratios of telomere repeat copy number to a single gene copy number highly correlate with absolute telomere lengths determined by Southern blot (r=0.677; P<0.001).[36]

Telomere length was assayed in various batches corresponding to each study. To minimize the impact of potential batch effect on leukocyte telomere length measurements across different studies, we calculated z scores of log transformed leukocyte telomere length by standardizing the leukocyte telomere length in comparison with the mean within each individual study.[18 19 38]

Statistical analysis

We used Spearman's partial rank correlation coefficients to examine age adjusted associations between characteristics of the study population, quarters of leukocyte telomere length, and Alternate Mediterranean Diet score groups.

We used multivariable linear regression models to determine the cross sectional association between leukocyte telomere length and adherence to the Mediterranean diet. We calculated multivariable adjusted least squares mean leukocyte telomere length z scores (and their corresponding 95% confidence intervals) across all dietary patterns score groups by using generalized linear models. Firstly, only age at blood draw was included in the models. Models were then additionally adjusted for other potential confounders (body mass index, smoking, physical activity, energy intake, and batch). Finally, other variables were included as potential covariates (for example, postmenopausal hormone therapy, history of hypertension, and socioeconomic status), but these were removed from the final models as estimates remained the same. All P values are two sided, and an α level of 0.05 was used. We used SAS version 9.2 for all statistical analyses. Reported STROBE guidelines have been the basis for reporting our results.[39]

Results

Table 1⇓ shows the main age standardized characteristics of the study population by quarters of telomere length. The mean age of the participants was 59 (SD 6.6) years, and the exact age range of the participants included in our study was 42-70 years. As expected, a statistically significant inverse correlation existed between age at blood draw and telomere length: younger women had longer telomeres (P<0.001). Moreover, women with shorter telomere length smoked slightly more (P=0.02). Distributions of main lifestyle factors between the study participants (n=4676), in comparison with the rest of the Nurses' Health Study blood sub-cohort (n=32 825) after age standardization, were largely similar (results not shown).

Table 2⇓ shows the baseline characteristics of the study participants across Alternate Mediterranean Diet score groups. The highest score group (score ≥6) represents the closest resemblance to the Mediterranean diet. Compared with women in the lowest score group (score ≤2), those in the highest score group were older at blood collection (P<0.001), had slightly lower body mass index (P=0.01), smoked less (P<0.001), had higher intake of total energy (P<0.001), and were more physically active (P<0.001). As expected, women with the highest Alternate Mediterranean Diet score also had higher intakes of vegetables, fruits, grains, fish, legumes, nuts, and total fat, as well as lower meat intake.

Table 3⇓ shows the association between adherence to a Mediterranean diet and telomere length. Higher Alternate

Mediterranean Diet scores were associated with higher age adjusted mean leukocyte telomere length z scores (P for trend=0.02). The association remained statistically significant after inclusion of body mass index, pack years of smoking, physical activity, and total caloric intake in the models; the multivariable adjusted least squares mean leukocyte telomere length z score across Alternate Mediterranean Diet groups (≤2, 3, 4, 5, ≥6) was −0.038, −0.049, −0.010, 0.039, and 0.072 (P for trend=0.004) (table 3⇓). Additional adjustment for postmenopausal hormone therapy, age at menopause, history of hypertension, socioeconomic status, and case-control study set (batch), did not change the estimates (data not shown).

We also evaluated the association between leukocyte telomere length z score and each component in the Alternate Mediterranean Diet score (table 4⇓). None of the individual components was significantly associated with telomere length, even after adjustment for multiple confounders (age, body mass index, pack years of smoking, physical activity, total caloric intake, and mutual adjustment for other components of the Alternate Mediterranean Diet score) (table 4⇓).

The figure⇓ shows the association between other dietary patterns and leukocyte telomere length. Similar to the Alternate Mediterranean Diet score, a higher Alternative Healthy Eating Index (that is, healthy eating) showed a weak positive association with longer telomere length (P=0.02), but we found no statistically significant associations for the prudent (P=0.09) or Western dietary patterns (P=0.32) (fig⇓). Additional adjustment for body mass index, pack years of smoking, physical activity, and total caloric intake did not alter the estimates.

Discussion

In this large cross sectional study of 4676 healthy middle aged and older women from the well characterized Nurses' Health Study, greater adherence to the Mediterranean diet was significantly associated with longer leukocyte telomere length. Notably, whereas higher Alternate Mediterranean Diet score was significantly associated with longer leukocyte telomere length, none of the individual components showed an association with leukocyte telomere length, emphasizing the importance of examining the relation between dietary patterns, in addition to separate dietary factors, and health. This suggests that the association may be a consequence of the global effect of the overall Mediterranean diet. The Alternative Healthy Eating Index was also associated with longer telomere length; however, the strongest association was observed among women with greater adherence to the Mediterranean diet.

The difference in telomere length for each one point change in the Alternate Mediterranean Diet score corresponded on average to 1.5 years of aging. A three point change in the Alternate Mediterranean Diet score would correspond to on average 4.5 years of aging, which is comparable to the difference observed when comparing smokers with non-smokers (4.6 years),[35] highly active women with less active women (4.4 years),[16] and women with high phobic anxiety scores with women with low phobic anxiety (6 years).[17]

Comparison with previous literature

To our knowledge, this is the largest population based study specifically looking at the association between adherence to the Mediterranean diet and telomere length in healthy, middle aged women. Our results are consistent with previously published literature on the benefits of a healthy lifestyle, particularly diet, and telomere length.

To date, only one study has assessed the association between telomere length, telomerase activity, and different adherence to a Mediterranean diet.[40] The 217 older people in this study had a mean age of 77, and the upper age limit for reliable assessment of telomere length is 75.[41] Using an older age group is considered inappropriate because of "survivor" bias[42 43]: people who live longer tend to be more resilient to chronic disease and possess longer telomeres. The authors may be capturing survivor bias rather than an association between telomere length and adherence to a Mediterranean diet.

García-Calzón et al studied the association between telomere length and changes in adiposity indices after a five year intervention study with a Mediterranean diet,[44] in the context of the PREDIMED-NAVARRA trial.[5] In this particular study, the outcome was obesity, and the association between telomere length and the whole Mediterranean diet was not specifically examined.

Only two studies have examined the relation between a healthy lifestyle pattern and leukocyte telomere length.[19 34] Mirabello et al observed a positive association between a healthy lifestyle (defined by low risk factors including low or no smoking, higher intakes of fruits and vegetables, lower body mass index, and greater physical activity) and leukocyte telomere length in men.[34] Similarly, Sun et al reported that a healthy lifestyle pattern (defined by five components: smoking, physical activity, adiposity, alcohol use, and Alternative Healthy Eating Index) was associated with longer leukocyte telomere length in women.[19]

One of the first studies to show a strong relation between physical fitness and telomere length in a large sample of patients with existing coronary heart disease was conducted by Krauss et al.[45] They found that poor physical fitness was associated with shorter leukocyte telomere length in patients with coronary heart disease. Du et al reported, in the Nurses' Health Study, that physical activity (even moderate amounts of activity) was associated with longer telomeres.[16] Moreover, Ornish et al recently published an intervention trial on the effect of comprehensive lifestyle changes (diet, activity, stress management, and social support) on telomerase activity and telomere length in men with low risk prostate cancer.[46] Results from this pilot study showed that a comprehensive lifestyle intervention was associated with increases in relative telomere length after five years of follow-up, compared with controls.

Studies on the association between specific dietary factors and telomere length have yielded inconsistent results.[34 47-50] Cassidy et al, for instance, reported a positive association between leukocyte telomere length and dietary fiber intake but an inverse association between total fat intake (particularly, polyunsaturated fatty acids) and leukocyte telomere length. No significant associations were observed for intakes of vitamin D or fruits and vegetables.[50] These findings support the importance of examining an overall lifestyle dietary pattern, rather than individual food components.

The Mediterranean diet score has been used to evaluate the association between the Mediterranean diet and overall mortality, as well as Alzheimer's disease, diabetes mellitus, coronary heart disease, and cancer overall.[2 51-55] Trichopoulou A et al evaluated the contribution of the nine widely accepted components, which are used to build the Mediterranean diet score, and reported an inverse association of this diet with all cause mortality in a population based cohort in Greece.[56] More interestingly, they found additive associations among components. The authors report that one of the advantages of using a Mediterranean diet score rather than focusing on the food components is the generation of fairly consistent results with respect to health benefits, whereas studies focusing on the components or food groups are often contradictory. As summarized by Trichopoulou et al,[56] chance, non-differential misclassification, and residual confounding may have more important consequences when individual food items are evaluated rather than a multi-component, unidimensional score.

The benefits on health and aging from a greater adherence to the Mediterranean diet have been largely described. Adherence to the Mediterranean diet has been linked to lower all cause mortality, reduced incidence of major cardiovascular diseases and other chronic diseases, and greater health and wellbeing in people surviving to older ages.[2-6]

Possible biologic mechanisms

Oxidative stress and inflammation have been reported to accelerate telomere attrition.[10 11 57] Moreover, given human telomere composition, these DNA regions are more sensitive to damage by oxidation.[58 59] In contrast, high antioxidative capacity slows telomere shortening.[58 60-62] The established protective effects of the Mediterranean diet on oxidative stress and chronic inflammation may explain the favorable influence of the Mediterranean diet on telomere length, pointing to a potential biologic mechanism behind the well known anti-aging effects of the Mediterranean diet.

The lack of an association with individual components is not surprising. Studies have proposed a possible synergy among the nutrient rich foods included in the Mediterranean diet that fosters favorable changes in intermediate pathways of cardiometabolic risk, such as insulin sensitivity and resistance to oxidation and inflammation,[5 63] supporting the role of the Mediterranean diet as a whole.

Strengths and limitations of study

The strengths of our study include the large and well characterized study population, detailed assessment of dietary habits as well as other sociodemographic and lifestyle characteristics, and validated food frequency questionnaires and dietary scores. However, potential limitations should be considered. The cross sectional design precludes us from establishing a temporal association between dietary habits and telomere length. Leukocyte telomere length was assessed using a single measure, preventing the estimation of associations between Mediterranean diet and telomere attrition rate; assessing this requires a prospective study with repeated assessments of leukocyte telomere length. Although all analyses were adjusted for multiple covariates, unmeasured or residual confounding could still be present. Validation of self reported variables potentially associated with telomere length, such as self reported diabetes, hypertension, and obesity, has been done previously.[64-66] Finally, the Nurses' Health Study population predominantly includes women of European ancestry, and telomere dynamics may differ among other ethnicities[67-69]; therefore, results may not be generalized. However, the homogeneity among Nurses' Health Study participants strengthens the internal validity of these findings by maximizing the quality of reported data.

Conclusion

In summary, greater adherence to the Mediterranean diet was significantly associated with longer leukocyte telomere length, a marker of biological aging. Our results further support the health benefits of adherence to the Mediterranean diet.

What is already known on this topic

The Mediterranean diet has been consistently linked with health benefits, including reduced mortality and reduced risk of chronic diseases

No study has examined whether adherence to the Mediterranean diet is associated with longer telomere length, a biomarker of aging

What this study adds

Greater adherence to the Mediterranean diet was associated with longer telomeres

Telomere length variability may be partially explained by lifestyle practices; as accelerated telomere attrition may underlie many chronic diseases, identifying modifiable factors that affect telomere dynamics is important

The results further support the benefits of adherence to the Mediterranean diet for promoting health and longevity

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Tables

**Table 1| Age standardized characteristics of study population at blood draw, by quarters of leukocyte telomere length z score (n=4676).
Values are mean (SD)**

Baseline characteristics	Quarters of leukocyte telomere length z score				P for trend
Age at blood draw*, years	59.6 (6.3)	59.2 (6.6)	58.6 (6.6)	57.7 (6.8)	<0.001
Body mass index at blood draw, kg/m²	25.5 (4.7)	25.2 (4.4)	25.0 (4.5)	25.3 (4.3)	0.13
Pack years†	23.9 (20.8)	22.8 (18.8)	23.5 (20.4)	22.9 (18.9)	0.024
Physical activity, metabolic equivalent hours of activity/week	16.4 (24.4)	16.7 (21.5)	16.4 (19.5)	16.6 (18.8)	0.87
Total energy, kcal	1790 (508)	1757 (485)	1743 (487)	1768 (494)	0.72
Vegetables, servings/day	3.1 (1.8)	3.1 (1.6)	3.1 (1.7)	3.2 (1.7)	0.51
Fruits, servings/day	2.4 (1.3)	2.4 (1.3)	2.4 (1.5)	2.5 (1.4)	0.81
Whole grains, servings/day	1.5 (1.3)	1.5 (1.2)	1.5 (1.2)	1.5 (1.3)	0.77
Fish, servings/day	0.4 (0.4)	0.3 (0.3)	0.3 (0.3)	0.4 (0.3)	0.68
Meats, servings/day	0.8 (0.5)	0.7 (0.5)	0.7 (0.5)	0.8 (0.5)	0.54
Legumes, servings/day	0.4 (0.3)	0.4 (0.3)	0.4 (0.3)	0.4 (0.4)	0.72
Nuts, servings/day	0.4 (0.6)	0.4 (0.5)	0.3 (0.5)	0.4 (0.6)	0.91
Alcohol, g/day	5.5 (9.8)	5.7 (9.9)	5.3 (10.1)	5.5 (9.2)	0.90
Total fat, g/day	62.5 (22.5)	61.4 (23.2)	60.3 (21.4)	61.4 (22.7)	0.76
Total saturated fat, g/day	21.0 (8.0)	20.7 (8.6)	20.3 (8.0)	20.5 (8.1)	0.95
Total monounsaturated fat, g/day	24.0 (9.2)	23.6 (9.4)	23.2 (8.7)	23.6 (9.3)	0.78

*Values for age at blood draw are not age adjusted.

†Among ever smokers.

Table 2| Age standardized characteristics of study population at blood draw, by groups of Alternate Mediterranean Diet score (n=4676). Values are mean (SD)

Baseline characteristics	Alternate Mediterranean Diet score groups					P for trend
Age at blood draw*, years	57.5 (7.0)	58.4 (6.8)	58.7 (6.5)	59.2 (6.5)	59.6 (6.2)	<0.001
Body mass index at blood draw, kg/m^2	25.6 (4.5)	25.2 (4.2)	25.3 (4.7)	25.2 (4.3)	25.0 (4.5)	0.010
Pack years†	27.6 (21.6)	24.1 (20.0)	23.8 (19.3)	22.2 (19.1)	20.2 (18.5)	<0.001
Physical activity, metabolic equivalent hours of activity/week	13.2 (18.6)	14.0 (17.9)	16.2 (27.0)	17.9 (20.0)	20.1 (21.2)	<0.001
Total energy, kcal	1534 (432)	1630 (459)	1736 (468)	1847 (480)	1984 (494)	<0.001
Vegetables, servings/day	1.9 (0.9)	2.4 (1.3)	3.0 (1.4)	3.5 (1.5)	4.3 (1.8)	<0.001
Fruits, servings/day	1.5 (0.9)	1.9 (1.1)	2.4 (1.4)	2.7 (1.3)	3.2 (1.4)	<0.001
Whole grains, servings/day	0.8 (0.9)	1.2 (1.1)	1.4 (1.2)	1.7 (1.2)	2.1 (1.3)	<0.001
Fish, servings/day	0.2 (0.1)	0.2 (0.2)	0.3 (0.2)	0.4 (0.4)	0.5 (0.3)	<0.001
Meats, servings/day	0.9 (0.5)	0.8 (0.5)	0.8 (0.5)	0.7 (0.5)	0.6 (0.5)	<0.001
Legumes, servings/day	0.2 (0.2)	0.3 (0.2)	0.4 (0.3)	0.4 (0.3)	0.6 (0.3)	<0.001
Nuts, servings/day	0.2 (0.5)	0.3 (0.5)	0.3 (0.5)	0.4 (0.5)	0.5 (0.6)	<0.001
Alcohol, g/day	5.1 (11.0)	5.5 (9.9)	5.5 (10.4)	5.5 (10.1)	5.9 (8.2)	0.070
Total fat, g/day	57.8 (22.1)	59.0 (22.4)	60.7 (21.7)	63.1 (23.2)	64.8 (22.6)	<0.001
Total saturated fat, g/day	20.9 (8.7)	20.5 (8.4)	20.7 (8.2)	20.7 (8.2)	20.4 (7.9)	0.084
Total monounsaturated fat, g/day	22.1 (8.8)	22.7 (9.2)	23.2 (8.7)	24.2 (9.5)	25.1 (9.3)	<0.001

*Values for age at blood draw are not age adjusted.

†Among ever smokers.

Table 3| Estimated least squares mean telomere length z scores (and standard errors) by Mediterranean diet score categories (Alternate Mediterranean Diet score) in Nurses' Health Study (n=4676)

| Analysis	Alternate Mediterranean Diet score					P for trend
	≤2 (n=891)	3 (n=795)	4 (n=909)	5 (n=880)	≥6 (n=1201)	
Age adjusted	−0.028 (0.033)	−0.039 (0.035)	−0.011 (0.033)	0.029 (0.033)	0.054 (0.029)	0.016
Multivariable adjusted*	−0.038 (0.035)	−0.049 (0.036)	−0.010 (0.033)	0.039 (0.034)	0.072 (0.030)	0.004

*Adjusted for age, body mass index, pack years of smoking, physical activity, and total caloric intake.

Table 4| Estimated least squares mean of telomere length z scores by quarters of individual Alternate Mediterranean Diet components in Nurses' Health Study (n=4676)

Component	No	Least squares means (SE)*	95% CI	P for trend
Vegetables (servings/day):				0.37
Q1	1165	−0.005 (0.032)	−0.067 to 0.057	
Q2	1167	−0.039 (0.030)	−0.097 to 0.018	
Q3	1168	0.032 (0.029)	−0.025 to 0.090	
Q4	1176	0.045 (0.032)	−0.017 to 0.108	
Fruit (servings/day):				0.41
Q1	1180	−0.004 (0.031)	−0.066 to 0.057	
Q2	1167	0.0007 (0.030)	−0.057 to 0.059	
Q3	1168	0.021 (0.030)	−0.036 to 0.079	
Q4	1161	0.015 (0.032)	−0.047 to 0.078	
Whole grains (servings/day):				0.35
Q1	1160	−0.047 (0.031)	−0.107 to 0.014	
Q2	1204	0.012 (0.029)	−0.045 to 0.069	
Q3	1177	0.058 (0.029)	0.0003 to 0.115	
Q4	1135	0.008 (0.031)	−0.053 to 0.070	
Fish (servings/day):				0.52
Q1	1257	−0.013 (0.029)	−0.070 to 0.044	
Q2	772	0.004 (0.036)	−0.067 to 0.075	
Q3	1470	0.012 (0.026)	−0.040 to 0.064	
Q4	1177	0.029 (0.031)	−0.031 to 0.089	
Meat (servings/day):				0.15
Q1	1173	0.018 (0.032)	−0.045 to 0.082	
Q2	1170	−0.005 (0.030)	−0.063 to 0.053	
Q3	1179	−0.028 (0.029)	−0.086 to 0.030	
Q4	1154	0.049 (0.033)	−0.016 to 0.113	
Legumes (servings/day):				0.33
Q1	821	0.002 (0.036)	−0.068 to 0.073	
Q2	1546	0.009 (0.026)	−0.041 to 0.060	
Q3	1106	−0.011 (0.030)	−0.071 to 0.048	
Q4	1203	0.029 (0.031)	−0.031 to 0.089	
Nuts (servings/day):				0.24
Q1	1561	−0.034 (0.026)	−0.086 to 0.018	
Q2	797	−0.006 (0.036)	−0.076 to 0.063	
Q3	1183	0.014 (0.029)	−0.043 to 0.071	
Q4	1135	0.070 (0.032)	0.008 to 0.133	
Alcohol (g/day):				0.53
Q1	1804	0.008 (0.024)	−0.039 to 0.055	
Q2	497	−0.012 (0.045)	−0.100 to 0.076	
Q3	1206	−0.018 (0.029)	−0.075 to 0.039	
Q4	1169	0.044 (0.030)	−0.015 to 0.104	
Monounsaturated:saturated fatty acid ratio:				0.81
Q1	1141	0.007 (0.030)	−0.052 to 0.066	
Q2	1139	0.013 (0.030)	−0.045 to 0.072	
Q3	1241	0.032 (0.029)	−0.024 to 0.088	
Q4	1155	−0.021 (0.031)	−0.082 to 0.039	

*Adjusted for age, body mass index, pack years of smoking, physical activity, total caloric intake, and intakes of other components of Alternate Mediterranean Diet score.

Figure
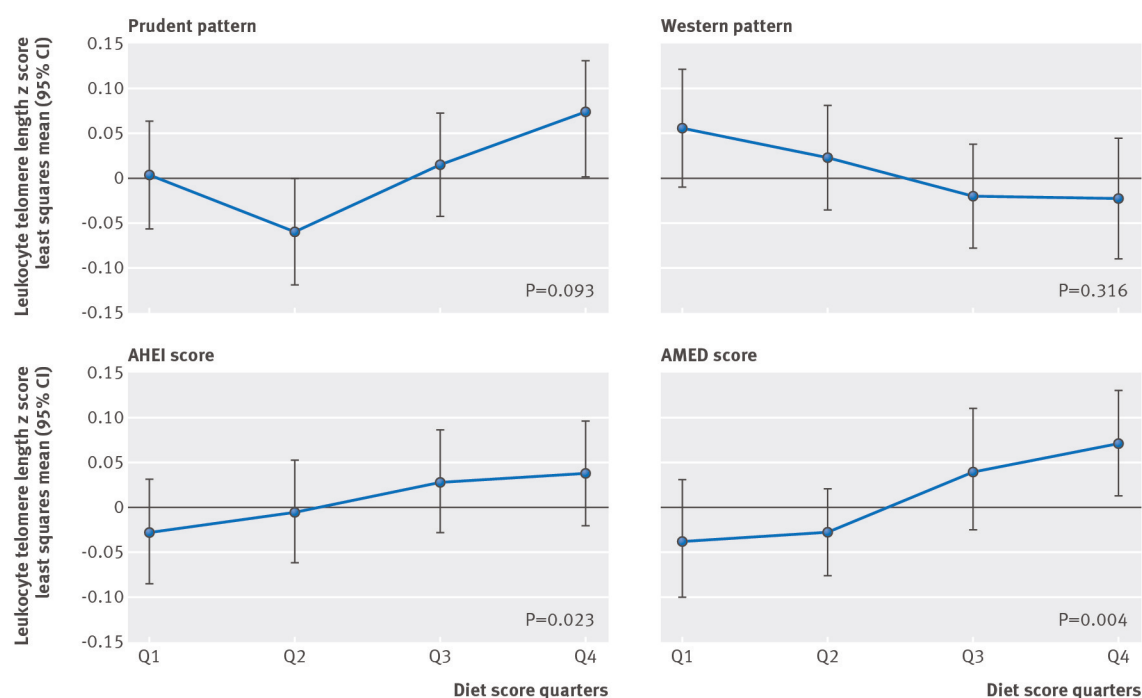
Multivariable adjusted least square means of leukocyte telomere length z scores (and their corresponding confidence intervals) by diet score quarters. All dietary patters are represented: prudent pattern, Western pattern, Alternative Healthy Eating Index (AHEI) score, and Alternate Mediterranean Diet (AMED) score