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CONSUMPTION OF NUTS AND SEEDS AND TELOMERE LENGTH IN 5,582 MEN AND WOMEN OF THE NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY (NHANES)

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Abstract: *Objectives:* Consumption of nuts and seeds is associated favorably with all-cause mortality. Nuts and seeds could reduce disease and prolong life by influencing telomeres. Telomere length is a good indicator of the senescence of cells. The purpose of the present study was to determine the relationship between nuts and seeds intake and leukocyte telomere length, a biomarker of biologic aging. *Design:* Cross-sectional. *Setting and Participants:* A total of 5,582 randomly selected men and women from the National Health and Nutrition Examination Survey (NHANES), 1999-2002, were studied. *Measurements:* DNA was obtained via blood samples. Telomere length was assessed using the quantitative polymerase chain reaction method. A validated, multi-pass, 24-h recall dietary assessment, administered by NHANES, was employed to quantify consumption of nuts and seeds. *Results:* Nuts and seeds intake was positively and linearly associated with telomere length. For each 1-percent of total energy derived from nuts and seeds, telomere length was 5 base pairs longer ($F=8.6$, $P=0.0065$). Given the age-related rate of telomere shortening was 15.4 base pairs per year ($F=581.1$, $P<0.0001$), adults of the same age had more than 1.5 years of reduced cell aging if they consumed 5% of their total energy from nuts and seeds. *Conclusions:* Consumption of nuts and seeds accounts for meaningful decreases in biologic aging and cell senescence. The findings reinforce the recommendations of the 2015-2020 Dietary Guidelines for Americans, which encourage the consumption of nuts and seeds as part of a healthy diet.

Key words: Cell aging, nuts, DNA, dietary fat, NHANES.

Introduction

The recently published Dietary Guidelines for Americans (2015-2020) provide recommendations for choosing a healthy diet that are designed to reduce the risk of chronic disease. Among the recommendations, Americans are encouraged to follow a healthy eating pattern comprising a variety of foods, including nuts and seeds (1). The latest guidelines also encourage Americans to consume nutrient dense foods, those containing beneficial substances which have not been "diluted" by adding solid fats, sugars, or refined carbohydrates. Again, nuts and seeds are highlighted as healthy options (1).

Numerous studies support the recommendation to include nuts and seeds in the diet. In a randomized trial by Guasch-Ferré et al, adults who ate 3 or more servings of nuts per week had 39% lower all-cause mortality than non-consumers over 5 years (2). Similar outcomes were revealed for cardiovascular and cancer mortality (2). Focusing on postmenopausal women, research by Ellsworth et al also showed that frequent nut consumption was associated with reduced risk of all-cause mortality (3). Moreover, after approximately 10 years of follow-up in the Physician's Health Study, there was a strong linear relationship showing that mortality decreased as nut consumption increased (4). Specifically, physicians eating more than 5 servings of nuts per week had 26% lower risk of mortality compared to those consuming less than 1 serving per month (4).

In an investigation by Sabaté et al, data from 25 intervention studies focusing on nut intake and blood lipid levels were pooled (5). The studies indicated that participants with the highest intake of nuts had the healthiest LDL, HDL, and triglyceride levels. The nut consumption and lipid associations were dose-related (5). Furthermore, according to research by Albert et al, nut consumption is also linked to lower rates of sudden cardiac death in men (6).

According to Ros, nut consumption improves several biomarkers of cardiovascular disease, including lower oxidized LDL concentrations and circulating inflammatory molecules, and increased plasma adiponectin (7, 8). Hence, it is not surprising to find that all epidemiological investigations conducted in the United States have shown favorable associations between nut intake and coronary heart disease incidence. After pooling 4 large observational studies conducted in the U.S., meta-analysis results showed that participants in the group with the highest nut consumption had approximately 35% lower risk of coronary heart disease incidence (9).

Clearly, consumption of nuts and seeds is associated favorably with a number of biomarkers and chronic disease risks, as well as all-cause mortality. The beneficial effects of nuts and seeds are likely a function of healthy concentrations of unsaturated fats, plant-based amino acids, vitamins, minerals, fiber, tocopherols, phytosterols, and polyphenols (7, 8, 10). Given their many nutritional advantages and disease prevention properties, nuts and seeds likely enhance health through a variety of pathways.

Nuts and seeds could reduce the risk of disease and prolong life by influencing telomere length. Telomeres are

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nucleoprotein caps that protect the ends of chromosomes. As a result of mitosis, telomeres shorten. When cells divide, part of the telomeric DNA fails to replicate. Over time, mitotic cells experience a finite number of cell divisions. Therefore, the shortening of telomeres is a mechanism of biologic aging and an index of the senescence of cells.

The primary purpose of the present investigation was to determine the extent to which the consumption of nuts and seeds accounts for differences in telomere length in a large NHANES sample representing non-institutionalized adults in the United States. A secondary objective was to ascertain the extent to which sociodemographic and lifestyle factors affect the relationship between nuts and seeds consumption and telomere length in U.S. adults.

Methods

Sample

To provide estimates of the health and nutritional status of civilians living in the United States, for many decades the Centers for Disease Control and Prevention has conducted the National Health and Nutrition Examination Survey (NHANES). To enable broad generalization of the results, NHANES employs a multistage, probability sampling design (11). In general, counties are randomly selected, followed by city blocks, followed by households, and finally individuals (11).

NHANES included assessment of leukocyte telomeres during a 4-year period only, 1999-2002. The telomere data became available to the public in November, 2014. To afford more accurate estimates, NHANES 1999-2002 oversampled several subgroups, including African Americans, Mexican Americans, low-income individuals, persons 12-19 years old, and individuals age 60+. NHANES data are all cross-sectional (11).

Each respondent of the NHANES 1999-2000 and 2001-2002 cycles who were at least 20 years old were asked to give a DNA sample. A total of 10,291 participants were eligible and 3567 adults from NHANES 1999-2000 and 4260 from NHANES 2001-2002 provided a useable DNA sample (n=7827, 76%). From this sample, participants 85 years and older were excluded because they were all given the age of 85 by NHANES. Only participants with complete data, including values for nuts and seeds consumption, telomere length, and the potential mediating variables, were included in the analyses. The final sample included a total of 5582 participants, 2647 men and 2935 women. The National Center for Health Statistics Institutional Review Board at the Centers for Disease Control and Prevention approved collection of the NHANES data and the posting of the NHANES files for public-use. Informed consent was acquired before data were collected from participants.

Measures

Data were collected on 10 variables for the present investigation: leukocyte telomere length, nuts and seeds consumption, age, gender, race, education, body mass index (BMI), smoking, physical activity, and alcohol use.

Telomere Length

For the present investigation, the outcome variable was telomere length. According to NHANES (12), "The telomere length assay was performed in the laboratory of Dr. 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 (13, 14). Each 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 standard deviations 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 standard deviation of the T/S ratio were then calculated normally. The interassay coefficient of variation was 6.5%."

Additionally, "Five 96-well quality control plates which represent 5% of the complete set were provided. These duplicate samples are blinded to the investigators. If more than 5% of the duplicate samples on the quality control plates are discordant with their pair in the complete set, the variant fails quality control (i.e., >95% duplicate concordance required)"(12). Mean T/S ratio values were converted to base pairs using the formula: 3274 +2413 x (T/S).

Nuts and Seeds Intake

According to NHANES (15), a computer-assisted dietary interview system, administered by a trained NHANES interviewer, was used to collect dietary data from each participant. Numerous investigations have used data collected via the NHANES 24-h dietary recall system (16-18). Each dietary assessment pertained to foods and beverages consumed during the previous 24 hrs (midnight to midnight). Interviewers were bilingual, and each had a B.S. degree in nutrition or home economics, with 10 credits or more focusing on nutrition. An NHANES Mobile Examination Center was used so that the interviews could be conducted in a private setting. A multi-pass format was used for the dietary interviews. The computer-assisted dietary assessment procedure afforded a standardized interview format. Scripts were used by the interviewers to explain the dietary interview component to the participant (19).

As employed in previous USDA and NHANES surveys, food probes were part of the built-in features of the system.

For the present investigation, the exposure variable was nuts and seeds. Consumption of nuts and/or seeds was recorded and quantified using three methods: 1) grams of nuts and seeds, 2) energy value of the nuts and seeds, and 3) energy value attributed to nuts and seeds divided by total energy intake, resulting in the percentage of each participant's total energy derived from nuts and seeds.

Sociodemographic Covariates

The sociodemographic covariates were: age, gender, race, and education. To define races and ethnicities, NHANES used the following categories: Non-hispanic White, Non-hispanic Black, Mexican American, Other race or Multi-racial (Other), and Other Hispanic. Three categories were used to differentiate based on education level: Less than high school, high school diploma (including GED), and more than high school.

Lifestyle Covariates

In the present investigation, several lifestyle variables were employed as potential mediating factors. Smoking was indexed using pack-years, reflecting cumulative exposure to tobacco smoke. Pack-years was defined as the number of cigarettes smoked per day multiplied by the number of years smoked, divided by 20.

Three categories were employed to define alcohol use: abstainers, moderate drinkers, and heavy drinkers. Participants who reported consuming no alcohol in the past 12 months were abstainers. Moderate drinkers were men who reported drinking more than 0 and less than 3 alcoholic beverages per day during the past 12 months, or women who reported drinking more than 0 but less than 2 drinks per day over the past 12 months. Men who reported drinking 3 or more alcoholic drinks per day over the past 12 months, or women who reported drinking 2 or more alcoholic beverages per day over the past 12 months, were defined as heavy drinkers.

MET (metabolic equivalent) minutes of activity per week during the past 30 days were used to quantify involvement in leisure time physical activity. Participants reported which of 62 physical activities, if any, they participated in, whether the activity was moderate or vigorous, the number of times in the past 30 days they engaged in the activity, and the average duration of the activity. Less than 10-minutes of participation was not recognized by NHANES. For each activity, a MET score was calculated and total MET-minutes per week were estimated by NHANES using the compendium of physical activities (20). Non-sedentary adults were divided into sex-specific tertiles. When considered with sedentary participants, those reporting no activity in the past 30 days, four categories of physical activity resulted: high, moderate, low, and sedentary.

Body mass index (BMI) was used to compare body weight independent of height. BMI was calculated using the standard formula: weight in kilograms divided by height in meters squared, kg/m^2 (21). Categories based on standard cut-points were used: underweight (< 18.5), normal weight (≥ 18.5 and < 25.0), overweight (≥ 25.0 and < 30.0), obese (≥ 30.0), or missing (21).

Data Analysis

All participants in NHANES are assigned individual sample weights (22). Each weight reflects the number of individuals in the United States represented by that sample person in NHANES. The sample weights reflect the unequal probability of selection, nonresponse adjustment, and adjustment to independent population controls (22). Using the sample weights in analyses produce unbiased national estimates when unequal selection probability is applied (22). For the present study, sample weights were based on diet records from 1999-2002, which included the nuts and seeds consumption data.

In the present investigation, each statistical result included adjustments based on the complex sampling design of NHANES by incorporating strata, primary sampling units, and sample weights (22). SAS SurveyFreq was used to estimate weighted frequencies and SAS SurveyMeans was used to calculate weighted means, each generalizable to the U.S. population.

Nuts and seeds consumption was indexed using three variables, as described in the Methods section. The extent of the linear relationship between nuts and seeds intake and telomere length was evaluated using regression analysis and the SAS SurveyReg procedure. To determine the extent of curvilinear associations between nuts and seeds intake and telomere length, beyond a possible linear association, quadratic and cubic terms for the exposure variable were evaluated. Regression estimates for each analysis were based on the multistage, probability sampling weights of NHANES. Partial correlation was employed using the SAS SurveyReg procedure to determine the extent to which the association between nuts and seeds consumption and telomere length was affected by differences in age, gender, race, education, smoking, alcohol use, BMI, and physical activity. Because telomere length deviated significantly from a normal distribution, the variable was transformed by natural logarithm prior to regression modeling.

When investigating the relationships between telomere length and each covariate, differences in age were controlled statistically, and the same diet-defined sample weights were employed as when studying the associations between nuts and seeds intake and telomere length. All P-values were two-sided and statistical significance was accepted when alpha was < 0.05. All of the statistical analyses were performed using SAS Version 9.4 (SAS Institute, Inc., Cary, NC).

Table 1
Descriptive characteristics of the sample and mean telomere length across the covariates (n=5582)

Variable	N	Weighted %	Age-adjusted Telomere Mean	SE	F	P
Gender					3.5	0.0708
Men	2647	48.0	5776	41		
Women	2935	52.0	5817	44		
Race					3.3	0.0229
Non-Hispanic White	2729	72.5	5794[a]	46		
Non-Hispanic Black	1027	10.6	5880[b]	52		
Mexican American	1398	7.5	5654[c]	39		
Other Race	140	3.2	5804[a,b]	57		
Other Hispanic	288	6.2	5850[a,b]	73		
Education					7.6	0.0023
< High School	2008	23.0	5723[a]	44		
High School Diploma	1292	25.9	5792[a,b]	54		
> High School	2282	51.1	5834[b]	39		
Physical Activity					9.1	0.0002
Sedentary	3248	49.5	5751[a]	41		
Low Activity	778	15.6	5785[a]	52		
Moderate Activity	775	16.9	5805[a]	54		
High Activity	781	17.9	5933[b]	53		
Body Mass Index					4.1	0.0098
Underweight	81	1.8	5881[a,b,c]	83		
Normal Weight	1569	31.1	5846[a,b]	39		
Overweight	1981	33.6	5782[a,c]	48		
Obese	1808	31.2	5754[c]	46		
Missing	143	2.3	5889[a]	77		
Alcohol Use					0.3	0.7462
Abstainer	2214	36.0	5782	40		
Moderate Drinker	1695	31.6	5804	48		
Heavy Drinker	1673	32.3	5809	49		

Telomere means for different levels of the same variable with the same superscript letter are not significantly different (P > 0.05). Age (yrs) and smoking (pack-years) were treated as continuous variables and are not listed. Mean (± SE) age of the sample was 46.5 ± 0.5 years and mean pack-years was 2.9 ± 0.2.

Results

Complete data were collected for 5,582 adults, 2,647 men and 2,935 women, a nationally representative sample of civilians aged 20-84 years living in the United States. Mean (±SE) age of the weighted sample was 46.5±0.5 years. Average daily nuts and seeds consumption was 4.2±0.4 grams, 24.4±2.6 kcal, and 1.0±0.1 percent of total energy intake. A total of 8.9% (n=476) of the sample reported eating nuts and/or seeds during the diet-recall assessment. Among adults reporting nuts or seeds consumption, median intake was 32.7±1.7 grams (1.2 oz) per day, 193.5±8.1 kcal (810.0±33.9 kJ) per day, and 8.3±0.6 percent of total energy intake. Average telomere length for the sample was 5835±41 base pairs for the entire sample. Table 1 displays the weighted descriptive characteristics of the sample, focusing on the sociodemographic and lifestyle categorical variables.

Nuts and Seeds and Telomere Length

In the present study, age and telomere length were strongly and inversely related. Specifically, the model-based estimate of the age-related rate of telomere shortening was 15.4 base pairs per year (F=581.1, R^2=.165, P<0.0001). Beyond the linear term, age-squared was not associated with telomere length (F=0.0, P=0.8431).

Consumption of nuts and seeds was linearly and positively related to telomere length. As shown in Table 2, in U.S. adults of the same chronological age, for each 10 grams (.35 oz) of nuts and seeds reported, telomere length was 9.0 base pairs longer (F=5.9, P=0.0219). With age as the only covariate, the nuts and seeds squared term (F=0.0, P=0.9267), and the cubed term (F=0.1, P=0.8298) were not significant predictors of telomere length, beyond the linear term.

As revealed in Table 2, after adjusting for differences in age, for each 100 kcal of nuts and seeds eaten per day, telomere length was 14.3 base pairs longer, on average (F=5.3, P=0.0287). When expressed as a percentage of total energy, for each 1-percent of total energy derived from nuts and seeds, telomeres were 5 base pairs longer, on average (F=8.6, P=0.0065).

Adjusting statistically for differences in the sociodemographic variables, including age, gender, race, and education, had little effect on the relationship between nuts and seeds and telomeres, as shown in Table 2. Adding the lifestyle covariates to the model (i.e., smoking, BMI, physical activity, and alcohol use), along with the sociodemographic variables, had modest effects on the relationship. Specifically, for each 10 grams (.35 oz) of nuts and seeds consumed, telomeres were 8.5 base pairs longer, on average (F=6.0, P=0.0207). Also, with all of the covariates controlled, for each additional 100 kcal of nuts and seeds consumed per day, telomere length was 13.3 base pairs longer (F=5.2, P=0.0295). Additionally, after adjusting for the sociodemographic and lifestyle covariates together, telomere length was 4.4 base pairs longer for each 1-percent of total energy derived from nuts and seeds (F=8.0, P=0.0082).

Telomere Length and the Covariates

Telomere length was related to several of the potential confounding factors, with age controlled statistically. Among the sociodemographic variables, race and education were associated significantly with telomere length. Specifically, as revealed in Table 1, after adjusting for differences in age, Mexican Americans had the shortest telomeres, significantly shorter than each of the other races and ethnic groups. Additionally, non-Hispanic Whites had significantly shorter telomeres than non-Hispanic Blacks. For education, adults with more than a high school education had significantly longer telomeres than those without a high school education. The relationship between gender and telomere length was borderline significant.

Among the lifestyle covariates, age-adjusted physical activity and BMI were each significant predictors of telomere length. Adults reporting high physical activity had significantly longer telomeres than those who were sedentary or those who had low or moderate activity levels. For BMI, telomere length was significantly shorter for the obese when compared to those of normal weight or those with missing BMI data. Alcohol use was not related to telomere length.

Table 2

Relationship between consumption of nuts and seeds and telomere length (base pairs) in 5582 U.S. adults, independent of key mediating variables

	Telomere Length (base pairs)			
Exposure variable controlled	**Regression Coefficient**	**SE**	**F**	**P**
Nuts & Seeds (per 10 grams)[a]				
age	9.0	4.0	5.9	0.0219
sociodemographic	8.7	4.0	5.4	0.0280
sociodemographic and lifestyle	8.5	3.7	6.0	0.0207
Nuts & Seeds (per 100 kcal)[b]				
age	14.3	6.7	5.3	0.0287
sociodemographic	13.7	6.9	4.7	0.0394
sociodemographic and lifestyle	13.3	6.4	5.2	0.0295
Nuts & Seeds (per 1 percent of total energy)[c]				
age	5.0	1.9	8.6	0.0065
sociodemographic	4.6	1.9	7.1	0.0127
sociodemographic and lifestyle	4.4	1.7	8.0	0.0082

a. Per 10 grams: The regression coefficient reflects differences in telomere length (base pairs) per 10 grams (.35 ounces) of nuts and seeds consumed; b. Per 100 kcal: The regression coefficient reflects differences in telomere length (base pairs) per 100 kcals of nuts and seeds consumed; c. Percent of total energy: The regression coefficient reflects differences in telomere length (base pairs) per 1 percent difference in energy derived from nuts and seeds; Sociodemographic covariates included: age, gender, race, and education; Lifestyle covariates included: BMI, physical activity (Met-minutes), cigarette smoking (pack years), and alcohol use.

Discussion

The association between nuts and seeds consumption and telomere length in a large, nationally representative sample of U.S. adults, ages 20-84, was the focus of the present investigation. The mediating effects of age, gender, race, education, BMI, smoking, physical activity, and alcohol use were also studied. Findings showed that as consumption of nuts and seeds increased, telomere length also increased, implying reduced cell aging. The relationship was linear and there were no curvilinear components.

The regression model estimate of the age-related rate of telomere shortening in the present sample was 15.4 base pairs per year. In other words, if two groups of the same chronological age differed by 30.8 telomere base pairs, then the group with the shorter telomeres would be estimated to have 2 additional years of cell aging compared to those with the longer telomeres. Hence, interpretation of the findings of the present study suggests the following: U.S. adults of the same age would experience almost 2 years less biologic aging per 30 grams (1 oz) of nuts and seeds consumed per day. The estimated biologic aging advantage would be nearly 1 year for each 100 kcal of nuts and seeds consumed per day. Moreover, when reported as a percentage of total energy, adults deriving 5% of their total energy from nuts and seeds would have approximately 1 2/3 years less cellular aging than non-consumers. Clearly, consumption of nuts and seeds accounts for meaningfully lower levels of biologic aging in U.S. men and women.

Adjusting for differences in the sociodemographic factors weakened slightly the relationship between nuts and seeds consumption and telomere length. Including the lifestyle covariates with the sociodemographic factors, so that all of the potential confounders could be controlled simultaneously, attenuated the association slightly more, but all of the relationships remained significant. Apparently, little of the link between nuts and seeds and telomere length can be attributed to differences in these variables.

Years of increased or decreased cellular aging associated with the consumption of nuts and seeds can be understood better when compared to other lifestyle variables. For example, in the present study, pack-years of smoking was significantly and inversely related to telomere length (F=14.3, P=0.0007), after adjusting for all the covariates. For each pack-year reported, telomeres were approximately 4.2 base pairs shorter. Therefore, adults reporting 10 pack-years of smoking would tend to have about 2.7 years of advanced biologic aging. On the other hand, moderate alcohol drinkers had telomeres that were 22 base pairs longer than abstainers, resulting in a non-significant biologic aging advantage. Given the associations between smoking, alcohol use, and telomere length as a reference, it appears that consumption of nuts and seeds accounts for meaningful decreases in biologic aging and cell senescence in U.S. adults.

Others have studied the association between dietary factors and telomere length. For example, Leung et al determined that sugar-sweetened soda was linearly associated with shorter telomeres, translating into approximately 1.8 years of additional aging for each 8-ounce serving per day. Moreover, as part of the Nurses' Health Study, Cassidy et al determined that dietary fiber was directly and linoleic acid intake was inversely related to telomere length in approximately 2800 women (23). In a sample of Korean adults, Lee et al identified two dietary patterns: prudent and western. The prudent pattern was associated with longer telomeres, as was nut consumption when considered separately (24). Similarly, Zhou et al determined that legumes, nuts, fish, and seaweeds were protective factors associated with longer telomeres in 556 Chinese adults (25). Conversely, Nettleton et al studied 840 Whites, Blacks, and Hispanic adults and found that nuts and seeds intake was not related to telomere length, although processed meats were inversely related.

Many studies show that the Mediterranean diet, which promotes consumption of healthy fats and encourages consumption of nuts and seeds, is related significantly to longer telomeres and decreased cellular aging (26-29). For example, using a 4-week intervention, Marin et al reported that a Mediterranean diet enriched with MUFA prevents telomere shortening in elderly subjects compared to a saturated fat-based diet or a high simple-carbohydrate diet (26). The researchers argue that consumption of healthy dietary fats tempers oxidative stress in human endothelial cells. They conclude that the Mediterranean diet "protects cells from oxidative stress, prevents cellular senescence and reduces cellular apoptosis"(26) (P. 1310).

Using a cross-sectional investigation, Boccardi et al showed that better adherence to a traditional Mediterranean diet was related to longer telomeres and higher telomerase activity in older participants (27). Likewise, studying almost 4700 participants from the Nurses' Health Study, Crous-Bou et al indicated that telomeres tend to be longer as adherence to the Mediterranean diet increases (28). And in a correlational investigation by Gu et al, adherence to the Mediterranean diet was related to longer telomeres in Whites, but not Blacks or Hispanics (29).

The hypothesis that nuts and seeds predict longer telomeres was based on research showing that these foods help reduce oxidative stress and inflammation. Oxidative stress and inflammation are reported as the primary mechanisms responsible for telomere shortening (30). In a comprehensive review, Houben et al provide evidence showing that telomere length is a biomarker of chronic oxidative stress (31). In this context, in the Multi-Ethnic Study of Atherosclerosis (MESA), nut intake was inversely related to C-reactive protein, interleukin-6, and fibrinogen concentrations (32). Moreover, in a randomized cross-over study, Jenkins et al., revealed that nuts significantly reduce oxidized LDL concentrations and other markers of CVD risk and oxidative stress (33). Additionally, Casas-Agustench et al indicate in multiple papers, including a review, that nut intake results in significant reductions in interleukin-6 levels, LDL oxidation, and inflammatory processes, as well as lower fasting insulin and HOMA levels, and improved endothelial function (34-36).

Nuts and seeds have healthy concentrations of unsaturated fats, plant-based amino acids, vitamins, minerals, fiber, tocopherols, phytosterols, and polyphenols (7, 8, 10). They are recommended as part of a nutritious diet in multiple sections of the recently published Dietary Guidelines for Americans (2015-2020). However, the relationship between nuts and seeds and telomere length in U.S. adults could be a function of factors other than the impressive array of nutritional properties of nuts and seeds.

The present study had multiple limitations. First, causal conclusions are not fitting because the investigation was cross-sectional. Second, consumption of nuts and seeds was self-reported. Therefore, the findings could be partly a result of measurement error, although such error would typically dilute the association between nuts and seeds and telomere length, not strengthen it. Third, adults reporting eating nuts and seeds could reflect atypical individuals who practice a lifestyle different from others. Because of this threat, many sociodemographic and lifestyle variables were controlled statistically. They had little effect on the outcome. However, an unidentified lurking variable could explain the relationship.

There were a number of strengths associated with the present study. First, the sample was large, multi-racial, and representative of the U.S. population, 20-84 years of age. Second, telomere length was measured by an independent and reputable lab using well-established methods. Telomere length was highly correlated with chronological age, and was also associated significantly with education, smoking, obesity, and physical activity, consistent with the literature. Third, statistical adjustments were made for differences in a number of potential mediating factors, including age, gender, race, education, smoking, BMI, physical activity, and alcohol use.

In conclusion, consumption of nuts and seeds was linearly and significantly related to telomere length in a random sample of 5,582 men and women representing U.S. adults. Regular consumption of nuts and seeds accounted for meaningful decreases in cellular aging, with greater reductions in biologic senescence with higher intakes. Findings of the current study support the latest Dietary Guidelines for Americans encouraging consumption of nuts and seeds as part of a healthy diet.

Acknowledgments: All of the time and effort expended by the thousands of participants of this study is sincerely appreciated.

Conflicts of Interest: The author has no conflicts of interest to report.

Ethical Standards: This study complied with current laws and ethical standards of the country in which it was conducted. The National Center for Health Statistics Institutional Review Board at the Centers for Disease Control and Prevention approved collection of the NHANES data and the posting of the NHANES files for public-use. Informed consent was acquired before data were collected from participants.

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