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Association between the anthropometric measurements and dietary habits on telomere shortening in healthy older adults: A-cross-sectional study

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Aim: This study aimed to evaluate the effect of anthropometric measurements and dietary habits on telomere length in healthy older residents in rural and urban areas.

Methods: This was a cross-sectional study. The study population included 81 healthy older individuals aged ≥ 80 years. A quantitative food frequency questionnaire was used to determine dietary habits. Anthropometric measurements were taken by researchers. The telomere length of individuals was determined from leukocytes using quantitative polymerase chain reaction.

Results: Urban women had longer telomeres than rural women ($P < 0.05$). Rural men had significantly higher hip circumference, middle-upper arm circumference and fat-free mass than urban men ($P < 0.05$). It was shown that while fresh vegetable consumption was higher in rural areas, carbonated drink consumption was higher in urban areas ($P < 0.05$). In women, homemade bread and sugar consumption were higher in rural areas, and honey consumption was higher in urban ($P < 0.05$). Red meat, milk-based dessert and pastry consumption explain telomere shortening by 22.5%, 24.8% and 17.9%, respectively. In addition, the model based on anthropometric measurements also contributes to explaining telomere shortening by 42.9%.

Conclusion: Red meat, milk-based dessert and pastry consumption, and waist circumference, hip circumference, waist-to-hip ratio and waist-to-height ratio are associated with telomere length. Longer telomeres are associated with a healthy, balanced, adequate diet and maintaining a healthy body weight/proportion, and they are crucial for achieving healthy aging. *Geriatr Gerontol Int* 2023; 23: 565–572.

Keywords: healthy aging, nutrition, rural, telomere length, urban.

Introduction

Globally, the geriatric population was 727 million in 2020, and it is projected to rise to 1.5 billion by 2050.¹ Aging is a progressive and irreversible process in the human lifecycle.^{2,3} For the first time, the prospect of individuals living beyond the age of 100 years has been considered.⁴ It should be kept in mind that the importance lies not only in longevity, but also in the quality of the aging experience. Due to expectations of longer life, healthy aging has attracted significant interest.^{5,6} In response to the aging global populace, successful aging practices are emerging to reach older adults who are independent, community-dwelling, and are physiologically and psychologically healthy.^{5,7–9}

Telomeres, comprised of the TTAGGG genetic sequence, are located at the end of DNA, protecting DNA during cell division.¹⁰ Telomere shortening is one of the hypotheses for the mechanisms of aging.¹¹ Telomeres undergo reduction with each cellular division, and are influenced by the number of cell divisions and various external factors, including stress, nutrition, physical activity and socioeconomic status.^{12–14} Furthermore, shortened telomeres are correlated with a reduced life expectancy and an increased risk of various age-related diseases.^{15–17}

A healthy, balanced and sufficient diet can enhance an individual's psychological, biological, social and physiological health, contributing to overall well-being.^{18,19} In older adults, nutrition is essential for an independent, social, healthy and disease-free life.^{20,21} Healthy dietary habits also affect telomere shortening.

Research shows that processed meats, carbonated beverages and table sugar are linked to shorter telomeres, whereas a plant-based diet is associated with longer telomeres.^{22–26} The present study investigated the correlation between telomere length, anthropometric measurements and dietary habits in healthy, community-dwelling, urban and rural residential older adults.

Methods

Participants

Participants aged ≥ 80 years, non-disease, non-drug users, community-dwelling, urban and rural residents were included in the present study. Rural and urban residents were chosen according to Turkish Statistical Institute (TurkStat) criteria and disease status determined by a family health clinic. Exclusion criteria were defined as age < 80 years, physical or mental problems (e.g. schizophrenia, bipolar disorder, Alzheimer's disease), inability to move physically, any disease other than hypertension, cachectic or obese, multiple drug use and smoking. Suitable individuals were called and invited to participate in the study. Informed consent was obtained from participants. The study was carried out in accordance with the Declaration of Helsinki protocol, and approved by the Istanbul Medipol University Non-Interventional Studies Ethics Committee (Decision No: 170/2019). The study was carried out from July 2019 to August 2020.

Questionnaire

The questionnaire consisted of two parts. It was prepared by the researcher and applied face-to-face with participants. The first part of the questionnaire included demographic variables (age, marital status, educational status, stress status [have you ever experienced any stressful situation {illness, accident, loss etc.} in your entire life] and longevity of family members etc.). The second part was a 79-item quantitative food frequency questionnaire. Portion size was checked using portion images from Appendix 2 of the Turkey Nutritional Guide.

Anthropometric measurements

Participants' height, waist circumference (WC), hip circumference (HC), upper-middle arm circumference and calf circumference (CC) were measured with inelastic tape by the researcher. Measurements were made from the participants' right side. Height was measured while the vertical axis of the head was perpendicular to the Frankfort plane, the feet were together and the individuals were leaning against a wall. WC was measured while the individual was standing; the abdomen was in a normal relaxed position, the arms were hanging at the sides and the legs were together. Standing in front of the individual, measurements were made at the midpoint between the lowest rib and the crista iliac. The measurement was taken at the end of normal exhalation. The maximum point on the hip was determined by standing on the right side of the individual for HC. While the vertical axis of the head was perpendicular to the Frankfort plane, a measuring tape was placed over the ears and behind the head, over the eyebrows, and head circumference was measured. The midpoint between the shoulder acromion and the elbow olecranon process was determined and measured for middle-upper arm circumference (MUAC). The midpoint between the kneecap and the heel was measured for CC. Waist, hip, MUAC and calf measurements were repeated three times for reliability, and all results were recorded in centimeters. The weight, fat, fat-free mass (FFM), water and basal

metabolic rate were measured using a TANITA BC730 bioelectrical impedance analysis device (Arlington Heights, IL, USA). The researcher calculated body mass index (BMI), waist-hip ratio (WHR) and waist-to-height ratio (WHtR).

Cell separation

The red blood cell lysis method was used for cell separation.²⁷ After the plasmas were separated, the remaining blood cells in the tubes were mixed by pipetting. Next, 10 mL of red blood cell lysis buffer was added to 1 mL of blood sample and shaken for 10 min. Shaken samples were centrifuged at $+4^{\circ}\text{C}$ at 400 g for 5 min using high-speed centrifuge (ECEN-15 K, MRC, Israel). As a result of centrifugation, the cells collapsed to the bottom, and the supernatant was spilled. The remaining cells were washed by adding 2 mL of phosphate-buffered saline and centrifuged again under the same conditions. After centrifugation, the supernatant was discarded. Next, 600 μL of Roswell Park Memorial Institute medium was added to the cells and transferred to 1.5-mL Eppendorf tubes for cell growth. Samples in Eppendorf tubes were kept at $+4^{\circ}\text{C}$ for 15–20 min. Next, 600 μL of dimethyl sulfoxide-fetal bovine serum was added to maintain cell integrity after incubation and nourish the cells during freezing. After waiting 1 h at -20°C , the samples were stored in a -80°C refrigerator.

Genomic DNA analysis and determination of leukocytes telomere length

Genomic DNA analyses were carried out in Muğla University Research Laboratory Center, Regenerative Medicine Laboratory. QIAGEN Tissue and Blood DNA Kit (Hilden, Germany) protocol were followed for the DNA analysis. DNA purity (A260/230) and yield (A260/280) were determined by using the Nanodrop spectrophotometer (N50; Implen, Westlake College, CA, USA).

Leukocyte telomere length (LTL) was measured from the isolated DNAs with a commercial KIT (Absolute Human Telomere Length Quantification qPCR Assay Kit; ScienCell, San Diego, CA, USA). The mean telomere length was determined with the validated quantitative polymerase chain reaction method. Relative mean telomere length in genomic DNA was determined with the ratio (T/S) of telomere repeat copy number (T) to single gene copy number (S).²⁸ The reaction was carried out with LightCycler[®] 96 (Roche, Basel, Switzerland) instrument.

Statistical analysis

The data were analyzed in the spss 26.0 Statistical Analysis program (IBM Corporation, Armonk, NY, USA). The normality of the data was tested by examining Kolmogorov–Smirnov/Shapiro–Wilk tests, skewness–kurtosis values and histograms. The data were analyzed by splitting with the Split File feature according to sex and place of residence. Values are the percentage for categorical data and mean \pm standard deviation (SD) for quantitative data. Differences between categories were determined using the χ^2 -test. Differences between paired groups were determined using the *t*-test for normal distribution and the Mann–Whitney *U*-test for non-normal distribution. The bivariate correlation method was used to determine the relationship between LTL, anthropometric measurements and dietary habits. Based on the data obtained from the correlation results, the effect of WC, HC, WHR, WHtR, meat consumption, milk dessert consumption and pastry consumption on LTL was determined by linear regression analysis. Four models were created to examine the impact of food consumption and anthropometric measurements on telomere length.

Model 1 was run for red meat consumption in urban men, model 2 was run for milk-based dessert consumption, model 3 was run for pastry consumption in urban women, and model 4 for WC, HC, WHR and WHtR in rural women. Changes were considered significant when $P < 0.05$.

Results

The present cross-sectional investigation entailed no intervention and adhered to the CONSORT flow chart, as shown in Figure 1.

The participants' demographic variables and LTL are presented in Table 1. The average age for all participants was 83.99 ± 4.71 years, with all participants classified as middle-aged older adults. No significant differences were observed between groups regarding age, educational attainment, marital status, cohabitation status, stress levels and the presence of long-lived relatives ($P > 0.05$). Nevertheless, LTL was notably longer in urban women than in rural women ($P < 0.05$).

Table 2 shows the anthropometric variables. HC, upper-middle arm circumference and FFM were higher in rural men than in urban men ($P < 0.05$). No significant differences were identified in anthropometric measurements between rural and urban women.

Daily food consumption, as calculated from the quantitative food frequency questionnaire variables, is shown in Figure 2. Fresh vegetable consumption was higher in rural men, whereas carbonated beverage intake was higher in urban men ($P < 0.05$). Regarding women's dietary patterns, urban women consumed more honey, whereas rural women had higher intakes of homemade bread and sugar ($P < 0.05$). Participants predominantly

favoured consuming homemade foods, legumes, bulgur, yogurt, nuts, vegetables, olive oil, olives and pickles, although no statistically significant difference was detected. Additionally, the consumption of tarhana, a food made from yogurt, wheat flour, water and salt, was observed. Sage was the preferred herbal tea among both rural and urban groups.

The association of LTL, food consumption and anthropometric data were examined, and is shown in Table 3. Urban men's LTL was positively associated with red meat consumption. In urban women, the intake of milk-based desserts and pastries showed a negative association with LTL. Rural women's WC, HC, WHR and WHtR were negatively associated with LTL. Linear regression analysis assessed the impact of food consumption and anthropometric data on LTL. In urban men, red meat consumption accounted for 22.5% of the variance in LTL. In urban women, milk-based dessert and pastry consumption explained 28.3% and 21.8% of the variance in LTL, respectively. Finally, 42.9% of the variance in rural women's LTL was defined by WC, HC, WHR and WHtR.

Discussion

Aging is an irreversible phenomenon for human beings. The focus should not solely be on extending life, but also on enhancing the quality of the aging experience. Consuming a healthy, well-balanced and sufficient diet, and maintaining optimal bodyweight are crucial for promoting healthy aging.²⁹ Telomere length serves as an indicator of aging and can be influenced by various factors, one of which is the residential environment. Research exploring the association between the living area and telomere length is limited.

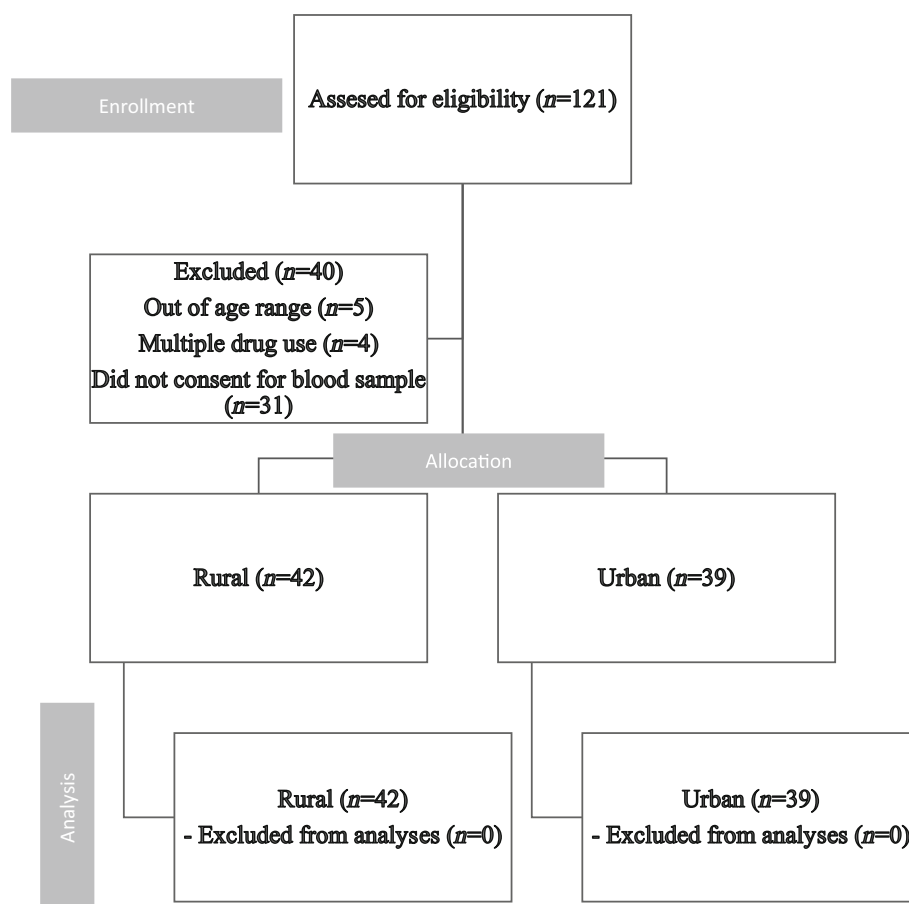


Figure 1 CONSORT flow chart.

Table 1 Participants' demographic variables and parameters associated with leukocyte telomere length

Demographic variables	Men (<i>n</i> = 36)		<i>P</i>	Women (<i>n</i> = 45)		<i>P</i>
	Rural (<i>n</i> = 19) mean ± SD	Urban (<i>n</i> = 17) mean ± SD		Rural (<i>n</i> = 23) mean ± SD	Urban (<i>n</i> = 22) mean ± SD	
Age (years)	83.11 ± 5.24	84.47 ± 3.86	0.384	83.26 ± 3.93	85.14 ± 5.52	0.195
	% (<i>n</i>)	% (<i>n</i>)	<i>P</i>	% (<i>n</i>)	% (<i>n</i>)	<i>P</i>
Education						
Illiterate	5.3 (1)	5.9 (1)	0.157	69.6 (16)	39.3 (11)	0.053
Primary school	94.7 (18)	76.5 (13)		30.4 (7)	60.7 (17)	
High school	0	17.6 (3)		0	0	
Marital status						
Married	84.2 (16)	76.5 (13)	0.558	34.8 (8)	18.2 (4)	0.208
Widow	15.8 (3)	23.5 (4)		65.2 (15)	81.8 (18)	
Co-living status						
Alone	5.3 (1)	11.8 (2)	0.757	26.1 (6)	31.8 (19)	0.843
Live with spouse	78.9 (15)	70.6 (12)		34.8 (8)	27.3 (6)	
Live with child	15.8 (3)	17.6 (3)		39.1 (9)	40.9 (9)	
Stress status						
Yes	42.1 (8)	70.6 (12)	0.086	87 (20)	86.4 (19)	0.953
Does anyone in the family live long?						
Yes	84.2 (19)	58.8 (10)	0.090	82.6 (19)	86.4 (19)	0.728
LTL (kb)	1.35 ± 0.38	1.49 ± 0.64	0.431	1.24 ± 0.47	1.94 ± 0.52	0.000*

Abbreviation: LTL, leukocytes telomere length.

*Independent samples *t*-test, significant at *P* < 0.05.

Table 2 Participants anthropometric variables

Anthropometric variables	Men (<i>n</i> = 36)		<i>P</i>	Women (<i>n</i> = 45)		<i>P</i>
	Rural (<i>n</i> = 19) mean ± SD	Urban (<i>n</i> = 17) mean ± SD		Rural (<i>n</i> = 23) mean ± SD	Rural (<i>n</i> = 19) mean ± SD	
Weight (kg)	71.20 ± 11.66	63.79 ± 10.86	0.058	59.89 ± 13.09	61.33 ± 11.23	0.695
Height (cm)	161.53 ± 8.25	159.29 ± 7.34	0.399	147.78 ± 5.47	146.86 ± 5.81	0.587
BMI (kg/m ²)	27.22 ± 3.43	25.08 ± 3.85	0.088	27.36 ± 5.64	38.38 ± 4.59	0.511
WC (cm)	102.68 ± 18.94	94.24 ± 11.54	0.121	94.30 ± 13.21	98.64 ± 10.96	0.239
HC (cm)	102.84 ± 6.19	98.06 ± 6.67	0.032*	106.70 ± 12.47	108.50 ± 10.85	0.608
WHC	1.00 ± 0.16	0.96 ± 0.07	0.360	0.89 ± 0.08	0.91 ± 0.73	0.274
WHtR	0.65 ± 0.10	0.59 ± 0.07	0.149	0.64 ± 0.09	0.67 ± 0.08	0.179
MUAC (cm)	29 ± 2.98	26.41 ± 2.74	0.011*	27.89 ± 4.83	28.46 ± 3.79	0.667
CC (cm)	34.53 ± 3.85	34.53 ± 6.19	0.999	33.13 ± 4.82	35.18 ± 4.11	0.133
Fat (%)	26.37 ± 5.84	24.85 ± 4.78	0.402	33.36 ± 8.44	34.98 ± 4.49	0.487
FFM (kg)	49.69 ± 7.19	45.16 ± 6.00		36.97 ± 4.49	37.18 ± 4.24	0.872
Water (%)	51.88 ± 3.63	52.09 ± 2.81	0.845	44.64 ± 4.69	43.66 ± 4.02	0.454
BMR (kcal)	1485.84 ± 235.12	1374.35 ± 181.73	0.124	1179.65 ± 145.99	1181.77 ± 132.82	0.791

Abbreviations: BMI, body mass index; BMR, basal metabolic rate; CC, calf circumference; FFM, fat-free mass; HC, hip circumference; MUAC, middle-upper arm circumference; WC, waist circumference; WHC, waist-to-hip ratio; WHtR, waist-to-height ratio.

*Independent samples *t*-test, significant at *P* < 0.05.

Brown *et al.* found that a good neighborhood is linked to longer telomeres, impacting telomere length through factors, such as aesthetics, availability of green spaces and socioeconomic status.³⁰

Alexeeff *et al.* showed that neighborhood socioeconomic status can influence telomere shortening, with lower socioeconomic status correlating with shorter telomeres.³¹ A study on blackbirds showed that forest-dwelling birds possess longer telomeres compared with their urban counterparts.³² In the present investigation, LTL was longer in urban women. It is postulated that disparate levels of urbanization account for the conflicting results. Other studies have compared developed nations with villages or urban

areas with forests. The present study was carried out in Muğla, Turkey's city with the highest life expectancy. Despite being a developed city, it is hypothesized that factors, such as the presence of green spaces, lower levels of urbanization, and the unique geographical positioning of the city between mountains and the sea might influence telomere attrition.

A healthy physique is integral to the process of healthy aging. Body composition is also connected to physical capabilities and mental well-being. It has been determined that non-sarcopenic older individuals have a BMI of 28.53 ± 4.81 kg/m² and a MUAC of 29.51 ± 3.70 cm.³³ A study examining the association between

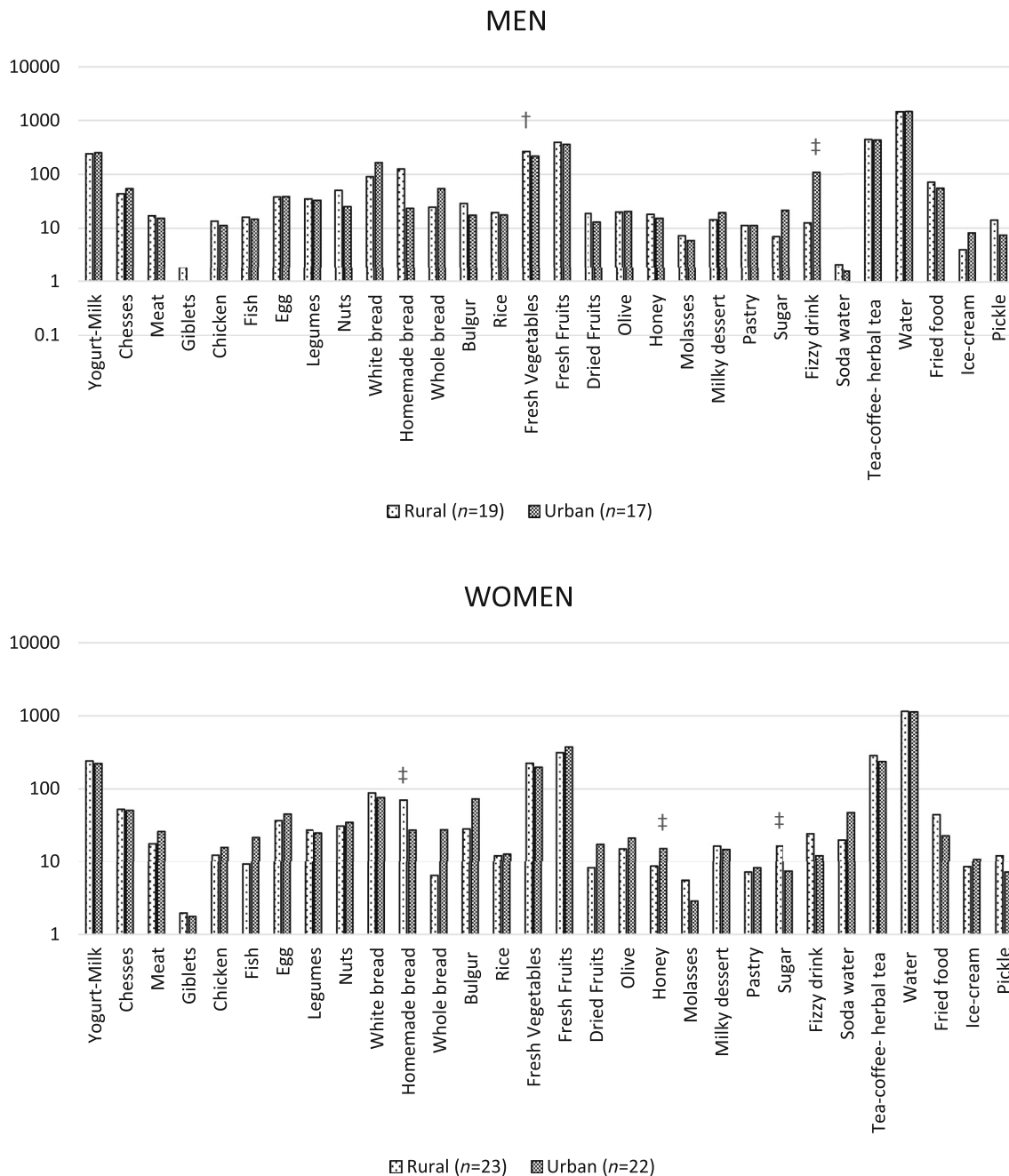


Figure 2 Participants quantitative food-frequency questionnaire variables. †Independent samples *t*-test, significant at $P < 0.05$ level, ‡Mann-Whitney *U*-test, significant at $P < 0.05$ level.

obesity and mental status concluded that elevated BMI, WHtR and MUAC heighten the risk of dementia, emphasizing the importance of maintaining average bodyweight and proportions for mental health.³⁴ In another investigation assessing the nutritional status of older adults, it was shown that a majority of older individuals showed higher than average bodyweight, BMI, WC, HC and WHR, even when at risk of malnutrition.³⁵ Tsai *et al.* explored BMI, MUAC and CC in relation to functional ability in older adults, establishing that BMI and CC are significant predictors of functional capacity.³⁶ An increase in BMI and a decrease in CC were associated with a decline in functional ability. Lissner *et al.* identified a correlation between larger hip circumferences

and reduced health complications.³⁷ Rural men were found to have greater HC, MUAC and FFM compared with their urban counterparts. In the present study, anthropometric measurements were similar to those of non-sarcopenic older adults. It is hypothesized that rural participants engage in higher levels of physical activity due to the prevalence of agricultural areas, potentially exerting a positive influence on MUAC and FFM. As the study population comprises healthy older individuals, it is believed that the larger HC observed in rural men is indicative of superior health status.

A nutritious diet influences overall well-being and facilitates healthy aging. General recommendations for a healthy diet include

Table 3 Parameters association with leukocytes telomere length

Model No	Sex-living area	LTL (kb)						
		<i>r</i>	<i>R</i> ²	Adj. <i>R</i> ²	B	SE	Beta	<i>P</i>
Model 1	Men-urban (<i>n</i> = 17)							
	Red meat consumption	0.536	0.274	0.225	0.022	0.009	0.523	<i>P</i> < 0.05
Model 2	Women-urban (<i>n</i> = 22)							
	Milk-based dessert consumption	-0.532	0.283	0.248	-0.019	0.007	-0.532	<i>P</i> < 0.05
Model 3	Pastry consumption	-0.467	0.218	0.179	-0.03	0.013	-0.467	<i>P</i> < 0.05
	Women-rural (<i>n</i> = 23)							
Model 4	WC (cm)	-0.693	0.533	0.429	0.043	0.094	1.204	<i>P</i> < 0.01
	HC (cm)	-0.505			-0.057	0.083	-1.517	<i>P</i> < 0.05
	WHR	-0.481			-7.072	9.054	-1.194	<i>P</i> < 0.05
	WHtR	-0.713			-3.194	3.145	-0.604	<i>P</i> < 0.01

Abbreviations: HC, hip circumference; WC, waist circumference; WHC, waist-to-hip ratio; WHtR, waist-to-height ratio.

all food groups, an assortment of vegetables, fruits, wholesome proteins and whole grains. In the Turkish Nutrition and Health Survey, the consumption of fresh vegetables for men over 65 was identified as 252.8 ± 180.31 g.³⁸ The Turkish Nutrition and Health Survey data showed fish consumption at 10.9 ± 41.06 g, sweets (including sugar) at 30.6 ± 31.6 g and sugar consumption at 13.1 ± 16.33 g for the general population aged ≥ years15. The present study showed that rural men consumed more fresh vegetables than their urban counterparts. Vegetable consumption surpassed Turkish Nutrition and Health Survey levels in both residential areas. An examination of women's consumption patterns showed that honey (*P* < 0.05) and fish consumption (*P* < 0.05) were higher among urban women compared with rural women.

In contrast, sugar and homemade bread consumption were greater among rural women. Muğla, a province known for fresh vegetable production, is believed to contribute to higher fresh vegetable consumption. Agricultural regions are predominantly situated in rural areas. Owing to their distance from the sea, rural areas have limited access to perishable seafood, such as fish, which requires cold chain transportation and storage.

Consequently, as recommended for all age groups, efforts should be made to facilitate access to seafood in rural areas and promote fish consumption at least twice a week. Both sexes show high consumption of sugar or sugar-added/sugar-inclusive foods. This could be attributed to greater physical activity and increased energy demands in rural areas, whereas urban participants have easier access to packaged and prepared foods, potentially raising sugar intake. Excessive sugar consumption is linked to chronic illness, such as diabetes, cardiovascular disease and kidney diseases.^{39,40} However, sugar consumption among all participants remains below the maximum daily amount recommended by the World Health Organization.⁴¹ Nevertheless, higher sugar consumption should be carefully monitored due to its detrimental health effects, particularly for older adults.

The anti-oxidant properties of herbs and foods can potentially influence telomere attrition.^{42,43} Oxidative stress is critical in various aging mechanisms, including telomere shortening.⁴⁴ A total of 83 edible wild herbs are available in Muğla, with the most well-known being dead nettle (*Urtica dioica*), rosemary (*Rosmarinus officinalis* L.), hibiscus (*Malva sylvestris*), chicory (*Cichorium intybus*), ivy (*Tamus communis*, *Tamus cretica*), radish (*Raphanus raphanistrum*) and sage (*Salvia officinalis*).⁴⁵ Olive oil, tarhana, bulgur and yogurt consumption were high in urban and rural areas (*P* > 0.05). Additionally, sage is favored as a herbal tea. Muğla is traditionally renowned for tarhana, pine honey, olive oil and olives, and these customary foods are widespread.^{46,47} The presence of green spaces and agricultural fields also contributes to the consumption

of wild herbs. In the present study, sage consumption is particularly noteworthy. These wild herbs contain various bioactive compounds with anti-oxidant properties.⁴⁸ The healthy and long lives of the participants might be associated with these traditional foods and herbs.

Food kind and telomere attrition were investigated in a few studies. In particular, the inverse relationship between processed meat consumption and telomere length has been shown by other studies.^{26,49} Also, studies have determined that high sugar and refined grains consumption is associated with telomere shortening.^{23,50–52} It was found that red meat consumption and telomere length have a positive correlation in urban men. It was shown that an inverse relationship was found between the consumption of milk-based desserts, pastries and telomere length in women residing in urban. Muğla is in the Mediterranean area, and lamb, goat and sheep consumption are preferred instead of beef. This type of meat is rich in protein and has lower saturated fat. Due to its protein content, it is beneficial primarily to protect older adults from sarcopenia and protein-energy malnutrition.

One study of older adults found no significant association between WC and telomere shortening.⁵³ An increase in WC is linked to an increased risk of chronic diseases.⁵⁴ Similarly, elevated BMI is associated with metabolic syndrome, obesity, diabetes and obese individuals have shorter telomeres than those with average bodyweight.⁵⁵ In another study evaluating the relationship between anthropometric parameters and telomere length in adults, it was shown that thigh circumference and CC were positively associated with telomere length.⁵⁶ The present study showed that WC, HC, WHR and WHtR were negatively correlated with LTL. There are limited findings regarding the relationship between LTL and anthropometric measurements in older adults. Maintaining average bodyweight/proportion is crucial for older adults to achieve independent and healthy aging.

The present study had some limitations. It was carried out in only one province, and future research could be planned with towns, both domestically and internationally. Additionally, telomere length quantitative polymerase chain reaction analysis was carried out only once. Despite these limitations, this research had strengths. To our knowledge, this is the first study carried out on healthy individuals aged >80 years, examining the association between the living area, dietary habits, anthropometric measurements and telomere length. The impact of anthropometric measurements and dietary habits on telomere length in healthy older individuals aged ≥80 years has been shown for the first time. Further research is required to explore the association between telomere length, living area, anthropometric measurements and dietary habits.

Telomere attrition serves as an indicator of biological aging. A multitude of factors can influence telomere shortening. Studies have shown associations between telomere length and the consumption of red meat, milk-based desserts and pastries, as well as WC, HC, WHR and WHtR. Maintaining a healthy, balanced and adequate diet, along with healthy bodyweight and proportion, are essential components for promoting healthy aging.

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Disclosure statement

The authors declare no conflict of interest.

Ethics statement

The study was conducted with the approved ethical standards.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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