



## Higher adherence to an empirically derived Mediterranean dietary pattern is positively associated with telomere length: the Seguimiento Universidad de Navarra (SUN) project

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### Abstract

Telomere integrity is influenced by oxidative stress. Also, inflammation-related factors, including nutritional factors, could modulate telomere integrity. The relationship between *a posteriori*-derived dietary patterns and telomere length (TL) has been scarcely investigated. Thus, our objective was to examine the association between empirically derived dietary patterns ascertained through principal component analysis (PCA) and TL in an older adult Spanish population. A total of 886 older adults (>55 years old; 645 males and 241 females) from the Seguimiento Universidad de Navarra (SUN) cohort were included in the study. TL was measured by monochrome multiplex real-time quantitative PCR. Age-adjusted TL was used for all analyses. Dietary patterns were identified by PCA based on thirty predefined candidate food groups collected from a validated 136-food items frequency questionnaire. Generalised linear models were fitted to obtain  $\beta$ -coefficients and their 95 % CI evaluating differences in TL between each of the four upper quintiles of adherence to dietary patterns and the lowest quintile. Sensitivity analyses by rerunning all multiple linear models under different stratifications were performed to evaluate the robustness of our results. Two major dietary patterns were empirically identified, Western dietary pattern (WDP) and Mediterranean dietary pattern (MDP). After adjustment for potential confounders, longer TL was found among subjects in the highest quintile of MDP ( $\beta = 0.064$ ; 95 % CI 0.004, 0.123). The WDP showed no significant association with TL. In conclusion, higher adherence to *a posteriori*-derived MDP was independently associated with longer telomeres in an older adult Spanish population of the SUN project.

**Key words:** Telomere length: Dietary patterns: Principal component analysis: Seguimiento Universidad de Navarra (SUN) cohort: Mediterranean dietary pattern

Telomeres are specific DNA structures formed by short guanine-rich repeats (TTAGGG) associated with a protein complex at the ends of chromosomes<sup>(1)</sup>. In each cell division, these repetitive sequences are shortened due to the fact that DNA polymerase is not able to complete the replicate telomeres (end replication problem), leading to senescence and apoptosis<sup>(2)</sup>. In consequence, telomere length (TL) has been considered a biological marker of ageing and cell turnover, and shorter telomeres have

been associated with higher incidence of age-related diseases and shorter lifespan<sup>(3,4)</sup>. The inter-individual variability in the rate of telomere shortening suggested that this is a modifiable process linked to agents that promote process of oxidative stress or inflammation<sup>(5,6)</sup>.

In fact, epidemiological studies have reported that dietary components are able to modulate oxidative and inflammatory processes, which are underlying mechanisms of TL maintenance

**Abbreviations:** MMqPCR, monochrome multiplex real-time quantitative PCR; PCA, principal component analysis; SUN, Seguimiento Universidad de Navarra; TL, telomere length; WDP, Western dietary pattern.

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and ageing<sup>(7–9)</sup>. Previous studies on the relationship between TL and diet show a variety of approaches examining dietary patterns. Most of them have evaluated dietary patterns using an *a priori* approach<sup>(10–19)</sup>, and a predefined Mediterranean dietary pattern (MDP) has been positively associated with TL<sup>(10–14)</sup>. In older adults, several studies did not find any association between TL and Mediterranean diet scores<sup>(15–18)</sup>. In contrast, we observed a direct association between the risk of having short telomeres and predefined diet quality scores, including a 14-point Mediterranean diet adherence screener<sup>(19)</sup>.

However, some dietary components that are not included in predefined scores could be important when exploring the association between diet and TL. Interestingly, a more exploratory approach would be required for an in-depth analysis<sup>(20)</sup>. *A posteriori* analyses, for example, through the principal component analysis (PCA), are aimed to identify dietary factors specific for the population under study<sup>(21,22)</sup>.

Until now, to our knowledge, only five epidemiological studies have identified dietary patterns using PCA and subsequently assessed its relationship with TL in adults subjects<sup>(17, 20,23–25)</sup>. In these studies, empirically identified dietary patterns commonly considered as healthy were labelled as 'Prudent', 'Mediterranean', 'Vegetarian', 'Healthy' or even 'Vegetables-Fruits', while unhealthy dietary patterns were named as 'Western', 'High energy-density' or even 'Snacks-drinks' among others. In regard to the relationship between TL and dietary pattern evaluated, only two studies found a positive association between TL and the 'Prudent pattern' (in the remote past) in Korean middle-aged and older adults<sup>(24)</sup>, or between TL and a 'Vegetable-rich pattern' in Chinese women (553 adults)<sup>(20)</sup>.

Finally, there is no clear evidence on the association between the adherence to *a posteriori*-derived dietary pattern by PCA and TL. For this reason, our study aimed to evaluate the association of dietary patterns, through this statistical approach, and TL in an older adult Spanish population of the Seguimiento Universidad de Navarra (SUN) study.

## Subjects and methods

### Study population

This is a cross-sectional study with 886 participants of the SUN project. The SUN cohort is a multipurpose, ongoing and dynamic cohort of Spanish university graduates started in December 1999, and it is permanently open. The design and methods used in the SUN project have been published in detail elsewhere<sup>(26)</sup>. In brief, baseline and follow-up information are collected through postal or web-based questionnaire every 2 years. The study protocol was supported by the Institutional Review Board of the University of Navarra and registered at [clinicaltrials.gov](https://clinicaltrials.gov) as NCT02669602.

Voluntary completion of the baseline epidemiological survey implied informed consent, as participants received detailed information about the whole study.

In May 2008, 1921 participants (>55 years old at baseline questionnaire) of the SUN project were invited to participate in a genetic sub-study<sup>(27)</sup>. Among them, 1085 participants accepted the invitation and received a kit to collect saliva, and 953 of them correctly returned saliva samples. In our analysis,

we excluded sixty-seven participants because they were outside of Willett predefined limits for energy intake (<3347 or >16 736 kJ/d for men, and <2092 or >14 644 kJ/d for women)<sup>(28)</sup>. Thus, the final available sample included 886 older adults (645 males and 241 females) (online Supplementary Fig. S1).

### Dietary assessment

In our study, dietary intake was assessed at baseline with a semi-quantitative FFQ of 136 food items validated and re-evaluated<sup>(29,30)</sup> in Spain, which assessed food habits in the previous year. This questionnaire had the following nine possible response categories: never/almost never, 1–3/month, 1/week, 2–4/week, 5–6/week, 1/d, 2–3/d, 4–6/d and >6/d, and standard portion sizes were specified. Nutrient intake was calculated by multiplying the frequency of consumption by the nutrient content of the specified portion, using data from Spanish food composition tables<sup>(31,32)</sup> by a trained dietitian.

### Telomere length assessment

Genomic DNA was extracted from each participant's saliva samples collected in the kit Orange® DNA Self-Collection kit-OG250, according to the manufacturer's instruction. DNA samples were stored at –80 °C. A monochrome multiplex real-time quantitative PCR was used for TL measurements assay according to the method of Cawthon<sup>(33)</sup>. In brief, telomere repeat amplification (T) is compared with a single copy gene product (S) in a single reaction well on clear, 384-well plates in a CFX384 Touch-Real-time PCR system (BioRad). Then, average TL is correlated with T/S ratio. PCR reactions were run adding 10 µl master mix containing QuantiTectSybr Green PCR kit (Qiagen); the primers (Sigma Aldrich; purified by HPLC) which sequences were telg (5'-ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT-3'), telc (5'-TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACAA-3'), albu (5'-CGGCGGCGGGCGGCGGGCTGGGCGGA AATGCTGCACA-GAATCCTTG-3') and albd (5'-GCCCGGCCCG CCGCGCCCGTCCC GCCGAAAAGCATGTCGCTGTT-3'); followed by 2 µl of each experimental DNA samples (10 ng); nuclease-free water to complete the final volume. Final concentration of combined telomere primers (telc and telg) and albumin primers (albu and albd) was 900 nM each.

For quality control, each sample was run in triplicate. In addition, a seven-point standard curve made from reference DNA samples (point 1, 150 ng/ml; point 2, 75 ng/ml; point 3, 37.5 ng/ml; point 4, 18.75 ng/ml; point 5, 9.38 ng/ml; point 6, 4.69 ng/ml; point 7, 2.34 ng/µl) was included for each plate using a 2-fold dilution series of DNA ranging from 150 to 2.34 ng/ml. TL was expressed as a T:S ratio using the calibration curve (linearity agreement  $R^2 > 0.99$ ) to relative quantification.

### Assessment of other variables

The baseline questionnaire collected information on socio-demographic (e.g. sex, age, college degree, employment status), anthropometric variables (e.g. BMI, weight change during the past 5 years) and health-related habits (e.g. smoking status, alcohol intake, special diets, time spent watching television and sitting, physical activity) and history of illnesses and medical



conditions. BMI was calculated dividing self-reported weight by the square of self-reported height. Physical activity data (metabolic equivalents of task (MET) h/week) were reported from a previously validated questionnaire that answers the time spent on seventeen activities, multiplying by a multiple of the RMR (MET score) according to previously published guidelines<sup>(34)</sup>.

### Assessment of dietary patterns

The 136 food items included in the FFQ were classified into thirty predefined candidate food groups.

The grouping scheme was based on the similarity of nutrients profile or culinary usage among the foods, and it was somewhat similar to that used in previous studies with participants of the SUN project<sup>(35,36)</sup>.

The PCA was applied to these food groups in order to identify the major dietary patterns that could explain the maximum proportion of the variance from the original groups. Sampling adequacy was supported by Kaiser–Meyer–Olkin value  $> 0.6$ <sup>(37)</sup>. The number of factors retained was based on eigenvalues  $> 1$ , the inflexion point on the scree plot, interpretability and variance explanation of each pattern solution. Food groups which received absolute loadings factors  $\geq 0.30$  were considered relevant components of the dietary patterns (Table 1). Absolute values  $< 0.30$  are not listed in Table 1.

Two factors were retained based on the factor loadings of the pre-selected candidate food groups, the first factor was named ‘Western dietary pattern (WDP)’ and the second factor was named ‘MDP’. Factor scores were calculated for each subject as the sum of standardised consumption of each food group weighted by the coefficient of each factor score. Then, WDP and MDP were categorised into quintiles. The second, third and fourth quintiles were grouped to simplify the results. Supplementary analyses of the main result were performed with ungrouped quintiles (online Supplementary Tables S1 and S2).

### Statistical analysis

Sample size was estimated for the comparison between extreme quintiles, assuming a minimum of 150 subjects in each quintile (total 300 subjects in the comparison), with the aim of detecting a mean between-quintile difference ( $\beta$ -coefficient) of 0.03 in TL, assuming a SD of 0.08 for this difference. Under these assumptions, the statistical power will be 0.90 if a two-tailed  $\alpha$  error = 0.05 is assumed.

Inverse probability weighting was used to evaluate age- and sex-adjusted baseline characteristics of participants distributed into quintiles of adherence to WDP and MDP (Table 2). We used percentages for categorical variables and means and standard deviations for continuous variables. TL variable was adjusted for age through the residual method<sup>(28)</sup>.

Generalised linear models were used to evaluate the associations between each of the four upper quintiles of adherence to each dietary pattern and TL in comparison with the lowest quintile.  $\beta$ -Coefficients and their 95% CI were calculated to evaluate this association, and they represent the multivariable-adjusted differences between TL in each of the four upper quintiles and the lowest quintile (reference category).

**Table 1.** Factor loadings for the two major dietary patterns ( $n$  886)\*

Food items	Factor 1 Western	Factor 2 Mediterranean
Refined grains	+0.52	+†
Processed meals	+0.46	+‡
Fast food	+0.45	+‡
Red meat	+0.44	+†
Sauces	+0.41	+‡
Commercial bakery	+0.40	+†
Potatoes	+0.36	+‡
Whole-fat dairy products	+0.36	-†
Processed meats	+0.35	+‡
Sugar-sweetened sodas	+0.34	+†
Eggs	+0.33	+†
Butter	+0.33	-‡
Chocolates	+0.33	+‡
Vegetables	-‡	+0.58
Fruits	-‡	+0.54
Fish and seafood	-‡	+0.43
Olive oil	-‡	+0.37
Fruit juices	+†	+0.34
Nuts	-‡	+0.33
Non-energetic sodas	-†	+0.31
Low-fat dairy products	-‡	+‡
Whole-wheat bread	-‡	+‡
Poultry	-†	+‡
Legumes	+†	+‡
Margarine and other vegetable oils not including olive oil	+‡	+†
Sugar	+‡	-†
Biscuits	+‡	-†
Wine	+‡	+‡
Beer	+‡	+†
Liqueurs	+‡	-†

\* The first factor (Western dietary pattern) and the second factor (Mediterranean dietary pattern) explained 8.4 and 6% of the total variance, respectively. Dietary patterns are labelled on the basis of factor loadings with 0.3 or greater.

†  $|r| < 0.10$ .

‡  $|r| \geq 0.10$  but  $\leq 0.29$ .

We defined short telomeres as a residual TL below the 20th percentile<sup>(19,38)</sup>. Logistic regression models were performed to assess the relationship between adherence to the four upper quintiles of each dietary pattern and the risk of having short telomeres, keeping the lowest quintile as the reference category.

In addition, tests of linear trend across successive quintiles were performed by assigning to each participant the value of the median in his/her quintile and considering these variables as continuous variables.

Potential confounders have been included as covariates: age, sex, year of cohort entry (four categories: 1999–2001, 2002–2004, 2005–2007, 2008), energy intake (continuous), BMI ( $\leq 20$ , 20–21, 22–25,  $\geq 25$  kg/m<sup>2</sup> and a quadratic term for BMI), smoking status (never/ever), alcohol intake (abstainer,  $< 5$  or  $< 10$  g/d, 5–25 g/d or 10–50 g/d,  $> 25$  g/d or  $> 50$  g/d for women and for men, respectively), leisure-time physical activity (MET task h/week, continuous), average daily time of television watching (h/d, continuous), average daily time spent sitting (h/week, continuous), following special diet at baseline (yes/no), weight gain in the past 5 years previous to entering the cohort ( $< 3$  kg/ $\geq 3$  kg), years of education (continuous), prevalence of dyslipidaemia and CVD (yes/no), dietary fibre intake, MUFA intake, PUFA intake, SFA intake and *trans*-fat intake (continuous).

To evaluate the robustness of our results, several sensitivity analyses were conducted by rerunning all multiple linear models under different stratifications: sex, age at baseline (<75 / ≥75 years old), BMI (≤25 / >25 kg/m<sup>2</sup>) and smoking status (never smokers and former or current smokers). Likelihood ratio tests were used to calculate *P* values for interaction.

Finally, several sensitivity analyses were carried out to assess possible sources of bias in the estimation of the association between adherence to the WPS or the MPS and the TL. Thus, we repeated the analyses after including only men or only women, only health professionals or only non-health professionals, excluding participants with dyslipidaemia or CVD at baseline, using the 5th and 95th percentiles as allowed limit for total energy intake, excluding participants with special diet at baseline, excluding participants with nine or more missing values in the baseline FFQ and finally excluding ever smokers.

We used STATA 12.0 for Windows (version 12.0; StataCorp LP) for statistical analyses. The statistical significance level was *P* < 0.05.

## Results

PCA revealed two major dietary patterns (WDP and MDP) that explained 14.3 % of the total variance in the consumption of the predefined thirty food groups. The first factor, WDP, was characterised by a high consumption of refined grains, processed meals, fast foods, red meats, sauces, commercial bakery, potatoes, whole-fat dairy products, processed meats, sugar-sweetened sodas, eggs, butter and chocolates. In contrast, the second factor, MDP, was characterised by a high consumption of vegetables, fruits, fish and seafood, olive oil, fruit juices, nuts and non-energetic sodas (Table 1).

Age- and sex-adjusted baseline characteristics of the 886 older adults (645 men and 241 women, 66.9 (sd 6.1) years old) according to their adherence to a WDP and to a MDP are shown in Table 2. We observed that subjects included in the highest quintile of WDP adherence were less physically active and had a higher intake of total energy intake specifically from SFA. In contrast, subjects in the highest MDP quintile were more physically active and to present lower intakes of SFA.

Table 3 shows the  $\beta$ -regression coefficients and 95 % CI for TL by quintiles of adherence to WDP and MDP, using the lowest quintile as the reference category. In the most adjusted model (model 3), we found only a significant positive association for the difference in TL between the highest and the lowest quintile of MDP ( $\beta = 0.064$ , 95 % CI 0.004, 0.123,  $P_{\text{for trend}} = 0.025$ ), which remained significant when we repeated the main analysis with ungrouped quintiles (online Supplementary Table S1,  $\beta = 0.067$ , 95 % CI 0.003, 0.130,  $P_{\text{for trend}} = 0.043$ ). However, no statistically significant association was found between adherence to the WDP and TL (Table 3 and online Supplementary Table S1).

The risk of having short TL (< percentile 20th) according to the adherence to both dietary patterns is shown in Table 4. After adjustment for potential confounders (model 3), no significant association between the highest quintile (*v.* the lowest quintile)

of adherence to the WDP (OR 1.04, 95 % CI 0.47, 2.30) or between extreme quintiles of the MDP (OR 0.75, 95 % CI 0.34, 1.65) and the risk of short TL was found. The *P* values for trend were 0.237 for the assessment of the WDP and 0.479 for the MDP. Supplementary analyses with ungrouped quintiles also showed no statistically significant findings (online Supplementary Table S2).

Finally, we carried out several sensitivity analyses to verify the robustness of our findings. According to subgroup analyses, the associations between adherence to each dietary pattern and TL were analysed within the different scenarios (Table 5), and no significant interactions were found. On the other hand, after several sensitivity analyses, the main results did not change substantially: no association was found between WDP and TL (Fig. 1) while the positive association between MDP and TL remained significant in most sensitivity analyses (Fig. 2).

## Discussion

In the present study, we identified two dietary patterns, WDP and MDP, using a PCA in an older adult Spanish population within the SUN cohort. The labelled dietary patterns explained 14.3 % of total variance in dietary intake, although it may be considered as a reduced proportion, it is similar to those values reported in previous studies<sup>(17,39)</sup>.

A highest adherence to a MDP, characterised by a higher consumption of vegetables, fruits, fish and seafood, olive oil, fruit juices, nuts and non-energetic sodas, was positively associated with TL. However, adherence to the WDP did not show any association with TL.

The interpretation of patterns using PCA might involve a degree of subjectivity, and the identified dietary patterns could reveal not a typical WDP or MDP. In fact, consumption of fast food or processed meat was higher, not lower, in the highest quintile of adherence to MDP. Despite the limitations derived from the statistical method used in this *a posteriori* approach, such as factor components derived from predefined food groups, the number of extracted factors or the subjectivity of the interpretation, we observed for the first time a positive association between adherence to an empirically obtained MDP and TL. The protective effects of the MDP and its food groups on oxidative stress and inflammation may explain the positive influence of the MDP on TL. Synergistically, at a molecular level, the interaction between phytochemical compounds may exert a multifactorial protective effect that is capable of reducing disease risk through attenuating specific ageing mechanisms (i.e. inflammation and oxidative stress). MUFA and PUFA, especially *n*-3 PUFA, are able to modulate the production of inflammatory molecules and lipid mediators, resulting in greater metabolic health and lower DNA damage<sup>(7)</sup>. Del Bo *et al.*<sup>(40)</sup> in a review article evaluating the impact of MDP on markers of DNA damage and TL reported that there is a reduction in the levels of 8-hydroxy-2'-deoxyguanosine and a modulation of DNA repair gene expression and TL. Similarly, Wysocki & Seibert suggest that the adherence to a MDP that contains fruits and whole grains along with fibre, antioxidants (e.g.  $\beta$ -carotene, vitamins C and E), *n*-3 fatty acids and soya protein may reduce DNA adducts and





**Table 2.** Baseline characteristics\* of the 886 older adults of the Seguimiento Universidad de Navarra project according to quintiles (Q) of the Western and Mediterranean dietary patterns (Percentages; mean values and standard deviations)

	Western dietary pattern						Mediterranean dietary pattern					
	Q1 (n 178)		Q2–Q4 (n 531)		Q5 (n 177)		Q1 (n 178)		Q2–Q4 (n 531)		Q5 (n 177)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Men (%)	72.8		72.8		73.1		72.9		72.8		72.8	
Health professionals (%)	58.9		56.5		52.1		55.3		55.9		55.2	
Smoking status (%)												
Never	34.0		32.0		38.0		32.7		31.7		38.5	
Ever	66.1		68.0		62.0		67.3		68.3		61.6	
Following a special diet at baseline (%)	29.5		18.3		9.8		22.9		17.6		19.8	
Diabetes prevalence (%)	14.5		9.1		4.4		9.3		9.3		7.0	
Dyslipidaemia prevalence (%)	27.2		19.1		18.6		17.3		20.0		21.3	
CVD prevalence (%)	27.1		15.8		13.8		15.6		16.8		19.7	
Cancer prevalence (%)	8.9		8.0		10.5		9.0		10.6		5.0	
Family history of diabetes (%)	18.2		20.0		17.6		18.0		18.2		19.5	
Family history of CVD (%)	24.4		25.6		22.4		28.7		23.3		25.4	
Age-adjusted telomere length	0.007	0.209	−0.006	0.195	0.004	0.176	−0.001	0.209	−0.008	0.181	0.018	0.213
Age (years)	67.0	5.9	66.9	6.3	66.7	5.9	66.9	6.2	66.9	6.0	67.0	6.3
Years of university education	5.5	2.0	5.3	1.8	5.6	2.1	5.5	1.9	5.2	1.8	5.5	2.1
BMI (kg/m <sup>2</sup> )	25.6	3.1	25.9	3.2	25.7	3.1	25.8	3.2	25.9	3.2	25.6	3.1
Physical activity during leisure time (MET h/week)	24.0	18.6	23.2	20.5	19.0	17.9	18.8	15.7	22.8	19.4	26.5	24.4
Daily television watching (h/d)	1.6	1.1	1.7	1.1	1.6	1.3	1.6	1.2	1.7	1.2	1.6	1.0
Time spent sitting (h/week)	5.0	2.1	5.2	1.9	5.3	2.0	5.1	1.9	5.2	1.9	5.2	2.0
Total energy intake (kcal/d)†	1825	567	2181	563	2846	534	1625	549	2241	514	2893	498
Carbohydrate intake (% total energy)	44.8	8.7	43.4	8.2	43.9	8.3	43.3	9.7	43.7	8.0	45.3	8.1
Protein intake (% total energy)	20.2	4.1	18.8	3.6	16.8	3.1	19.1	4.7	18.7	3.5	18.1	3.5
Total fat intake (% total energy)	32.1	7.7	34.7	6.4	36.1	7.2	34.2	8.0	34.5	6.4	33.7	7.1
PUFA intake (% total energy)	4.7	1.7	5.1	1.5	5.5	1.9	4.8	2.0	5.1	1.6	5.2	1.6
SFA intake (% total energy)	9.4	3.2	11.7	3.3	13.1	3.3	12.6	4.1	11.4	3.3	10.4	2.9
MUFA intake (% total energy)	14.7	4.7	15.2	3.7	15.4	3.9	14.5	3.8	15.3	3.8	15.0	4.5
Fibre intake (g/d)	34.1	16.4	30.0	13.4	29.8	10.5	19.0	7.1	30.1	9.8	45.5	16.1
Alcohol intake (g/d)	7.0	8.9	9.5	12.4	12.9	17.0	8.2	12.6	9.8	13.1	11.9	14.9
Vegetables (g/d)	640.2	508.8	544.1	398.3	501.0	311.5	305.6	189.6	529.7	244.8	900.2	707.1
Fruits (g/d)	556.3	500.7	387.2	280.8	317.3	187.6	229.3	150.4	384.5	221.8	666.6	537.0
Low-fat dairy products (g/d)	302.1	273.4	232.8	264.2	145.6	207.4	152.7	189.8	235.0	252.8	293.7	322.5
Full-fat dairy products (g/d)	65.8	84.7	140.3	156.6	256.5	235.1	172.4	226.0	144.1	169.8	143.1	149.1
Refined grains (g/d)	59.6	46.4	113.9	76.9	194.7	111.1	102.5	95.4	124.5	92.1	123.1	88.7
Fish (g/d)	129.9	87.2	119.2	67.6	101.5	63.2	76.3	48.5	119.9	58.3	155.5	97.2
Unprocessed red meat (g/d)	42.4	33.1	74.0	40.7	99.1	47.9	66.8	43.4	74.1	43.9	72.5	47.6
Potatoes (g/d)	27.7	27.7	52.4	45.5	78.6	51.8	33.2	32.0	52.3	41.7	73.4	64.6
Poultry (g/d)	50.0	70.3	38.9	32.5	44.7	29.9	25.3	21.3	41.9	31.6	60.5	69.2
Processed meat (g/d)	21.6	18.2	34.8	25.0	49.7	30.5	24.7	24.3	34.0	21.9	48.0	34.3
Processed meals (g/d)	11.0	18.2	27.1	41.3	72.0	69.9	19.8	30.4	33.3	52.2	45.9	56.2
Eggs (g/d)	12.9	11.1	22.4	15.8	29.3	18.6	19.5	16.5	22.2	16.9	23.4	14.6
Sugar-sweetened sodas (g/d)	5.1	18.0	11.5	24.2	40.6	80.7	9.5	22.2	13.8	34.7	28.8	70.3
Olive oil (g/d)	16.5	15.4	13.6	13.7	12.6	13.6	6.7	6.8	14.2	12.9	19.9	18.0
Commercial bakery (g/d)	2.7	5.0	8.9	13.7	23.1	31.3	8.4	16.6	10.8	19.8	10.7	16.9
Fast food (g/d)	3.0	5.2	8.0	10.7	19.2	19.2	5.2	8.7	9.8	14.5	11.2	12.3
Sauces (g/d)	0.8	1.2	1.7	2.1	3.9	5.3	1.2	1.4	1.7	2.3	3.2	5.0

Dietary patterns and telomeres: SUN project

MET, metabolic equivalents of task.

\* Age- and sex-adjusted baseline characteristics through inverse probability weighting except age and sex.

† To convert kcal to kJ, multiply by 4.184.

**Table 3.** Telomere length (TL) by quintiles (Q) of adherence to Western and Mediterranean dietary patterns\* ( $\beta$ -Coefficients and 95 % confidence intervals)

Dietary patterns	Q1 (n 178)	Q2–Q4 (n 531)		Q5 (n 177)		<i>P</i> <sub>for trend</sub>
		$\beta$	95 % CI	$\beta$	95 % CI	
<b>Western</b>						
TL						
Mean	0.020		–0.006		–0.001	
SD	0.224		0.194		0.172	
Crude model	0 (ref.)	–0.026	–0.060, 0.007	–0.021	–0.062, 0.019	0.396
Model 1	0 (ref.)	–0.019	–0.054, 0.016	–0.012	–0.061, 0.036	0.407
Model 2	0 (ref.)	–0.022	–0.058, 0.013	–0.021	–0.070, 0.029	0.467
Model 3	0 (ref.)	–0.034	–0.073, 0.004	–0.047	–0.106, 0.013	0.537
<b>Mediterranean</b>						
TL						
Mean	–0.000		–0.009		0.027	
SD	0.209		0.181		0.227	
Crude model	0 (ref.)	–0.009	–0.042, 0.024	0.027	–0.014, 0.068	0.146
Model 1	0 (ref.)	–0.006	–0.043, 0.031	0.030	–0.022, 0.083	0.194
Model 2	0 (ref.)	–0.002	–0.039, 0.035	0.040	–0.013, 0.092	0.100
Model 3	0 (ref.)	0.009	–0.030, 0.048	0.064	0.004, 0.123	0.025

ref., Reference; MET, metabolic equivalents of task.

\* Model 1: adjusted for age, sex, year of cohort entry (1999–2001, 2002–2004, 2005–2007, 2008) and energy intake (continuous). Model 2: additionally adjusted BMI ( $\leq 20$ , 20–21, 22–25,  $\geq 25$  kg/m<sup>2</sup> and a quadratic term for BMI), smoking status (never/ever), alcohol intake (abstainer,  $< 5$  or  $< 10$  g/d, 5–25 or 10–50 g/d,  $> 25$  or  $> 50$  g/d for women and for men, respectively), leisure-time physical activity (MET h/week, continuous), average daily time of television watching (h/d, continuous), average daily time spent sitting (h/week, continuous), following special diet at baseline (yes/no), weight gain in the past 5 years previous to entering the cohort ( $< 3$  kg/ $\geq 3$  kg), years of education (continuous), prevalence of dyslipidaemia and CVD (yes/no). Model 3: additionally adjusted for dietary fibre intake, MUFA intake, PUFA intake, SFA intake and *trans*-fat intake (continuous).

**Table 4.** Short telomere length (TL) ( $< 20$ th percentile (P20)) by quintiles (Q) of adherence to Western and Mediterranean dietary patterns (Odds ratios and 95 % confidence intervals)

Dietary patterns	Q1 (n 178)	Q2–Q4 (n 531)		Q5 (n 177)		<i>P</i> <sub>for trend</sub>
		OR	95 % CI	OR	95 % CI	
<b>Western</b>						
Participants with TL $< P20$ (%)	17		21		18	
Crude model	1 (ref.)	1.30	0.84, 2.01	1.05	0.61, 1.80	0.204
Model 1	1 (ref.)	1.28	0.81, 2.04	1.09	0.58, 2.07	0.273
Model 2	1 (ref.)	1.36	0.85, 2.18	1.20	0.62, 2.33	0.273
Model 3	1 (ref.)	1.27	0.76, 2.12	1.04	0.47, 2.30	0.237
<b>Mediterranean</b>						
Participants with TL $< P20$ (%)	22		20		18	
Crude model	1 (ref.)	0.90	0.59, 1.36	0.76	0.45, 1.28	0.296
Model 1	1 (ref.)	0.92	0.58, 1.45	0.80	0.41, 1.56	0.500
Model 2	1 (ref.)	0.87	0.54, 1.38	0.72	0.36, 1.42	0.339
Model 3	1 (ref.)	0.88	0.53, 1.44	0.75	0.34, 1.65	0.479

ref., Reference; MET, metabolic equivalents of task.

\* Model 1: adjusted for age, sex, year of cohort entry (1999–2001, 2002–2004, 2005–2007, 2008) and energy intake (continuous). Model 2: additionally adjusted BMI ( $\leq 20$ , 20–21, 22–25,  $\geq 25$  kg/m<sup>2</sup> and a quadratic term for BMI), smoking status (never/ever), alcohol intake (abstainer,  $< 5$  or  $< 10$  g/d, 5–25 or 10–50 g/d,  $> 25$  or  $> 50$  g/d for women and for men, respectively), leisure-time physical activity (MET h/week, continuous), average daily time of television watching (h/d, continuous), average daily time spent sitting (h/week, continuous), following special diet at baseline (yes/no), weight gain in the past 5 years previous to entering the cohort ( $< 3$  kg/ $\geq 3$  kg), years of education (continuous), prevalence of dyslipidaemia and CVD (yes/no). Model 3: additionally adjusted for dietary fibre intake, MUFA intake, PUFA intake, SFA intake and *trans*-fat intake (continuous).

protect telomeres<sup>(41)</sup>. However, more studies are needed to establish the molecular bases of the beneficial effect of the MDP on DNA damage and telomere integrity.

Five epidemiological studies have developed empirically derived dietary patterns through PCA, in which supposedly healthy dietary patterns similar to MDP were found<sup>(17,20,23–25)</sup>. In line with our results, Lee *et al.*<sup>(24)</sup> observed that higher adherence to the ‘Prudent’ dietary pattern was associated with longer telomeres in 1958 middle-aged and older Korean adults. Moreover, in Chinese women a higher adherence to a ‘Vegetable-rich’ pattern was positively related to TL<sup>(20)</sup>. In contrast to these results, several studies did not find any association

between TL and healthy dietary patterns labelled as ‘Whole grains and fruit’ in 840 multi-ethnic adults aged 40–69 years old<sup>(23)</sup>, ‘Healthy’ and ‘Vegetarian’ in a population of 300 men aged 20–40 years old<sup>(25)</sup> or ‘Vegetables-fruits’ in 1981 elderly Chinese subjects<sup>(17)</sup>.

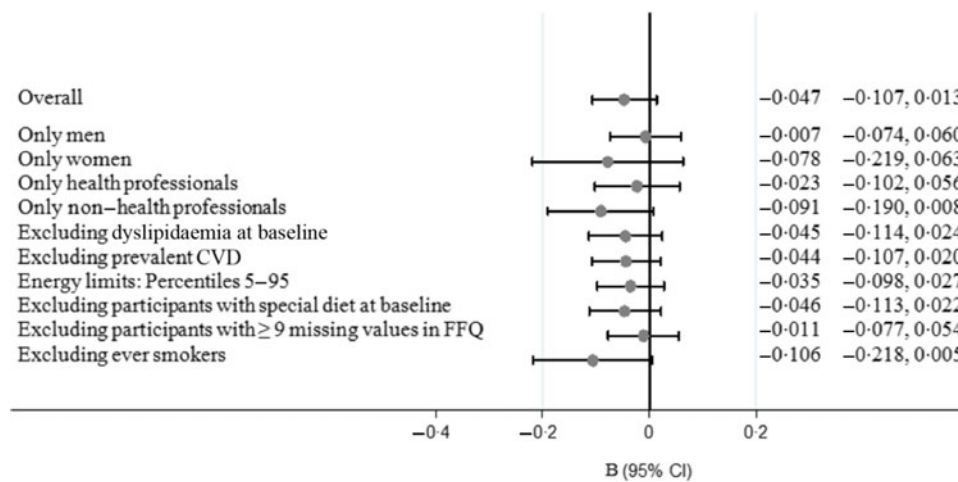
Most of the studies aimed to evaluate the relationship between TL and Mediterranean diet have used an *a priori* approach<sup>(10–19)</sup>. In fact, we previously analysed the adherence to a Mediterranean diet using a 14-points score in the same population of the present study, and we observed a lower risk of having short TL ( $< 20$ th percentile) among older adults in the top tertile<sup>(19)</sup>. In this sense, in 4676 women of the Nurses’ Health

**Table 5.** Subgroup analyses for the association between dietary patterns and telomere length in older adults of the Seguimiento Universidad de Navarra cohort\* ( $\beta$ -Coefficients and 95 % confidence intervals)

	n	Western dietary pattern			Mediterranean dietary pattern		
		$\beta$	95 % CI	$P_{\text{for interaction}}$	$\beta$	95 % CI	$P_{\text{for interaction}}$
Overall	886						
Subgroup analyses							
Sex				0.960			0.225
Male	645	-0.004	-0.028, 0.020		-0.004	-0.030, 0.022	
Female	241	-0.014	-0.064, 0.036		0.065	0.013, 0.112	
Age at recruitment (years)				0.221			0.216
$\leq 75$	787	-0.009	-0.031, 0.014		0.025	-0.000, 0.050	
$> 75$	99	-0.025	-0.108, 0.102		-0.017	-0.100, 0.065	
BMI (kg/m <sup>2</sup> )				0.981			0.660
$\leq 25$	367	-0.009	-0.039, 0.020		0.020	-0.013, 0.052	
$> 25$	519	-0.018	-0.050, 0.015		0.019	-0.015, 0.053	
Smoking status				0.870			0.054
Never	294	-0.028	-0.067, 0.010		0.059	0.018, 0.100	
Ever	592	-0.004	-0.031, 0.023		-0.003	-0.032, 0.027	

MET, metabolic equivalents of task.

\* Adjusted for age, sex, year of cohort entry (four categories: 1999–2001, 2002–2004, 2005–2007, 2008), energy intake (continuous), BMI ( $\leq 20$ , 20–21, 22–25,  $\geq 25$  kg/m<sup>2</sup> and a quadratic term for BMI), smoking status (never/ever), alcohol intake (abstainer,  $< 5$  or  $< 10$  g/d, 5–25 or 10–50 g/d,  $> 25$  or  $> 50$  g/d for women and for men, respectively), leisure-time physical activity (MET h/week, continuous), average daily time of television watching (h/d, continuous), average daily time spent sitting (h/week, continuous), following special diet at baseline (yes/no), weight gain in the past 5 years previous to entering the cohort ( $< 3$  kg /  $\geq 3$  kg), years of education (continuous), prevalence of dyslipidaemia and CVD (yes/no), dietary fibre intake, MUFA intake, PUFA intake, SFA intake and *trans*-fat intake (continuous).

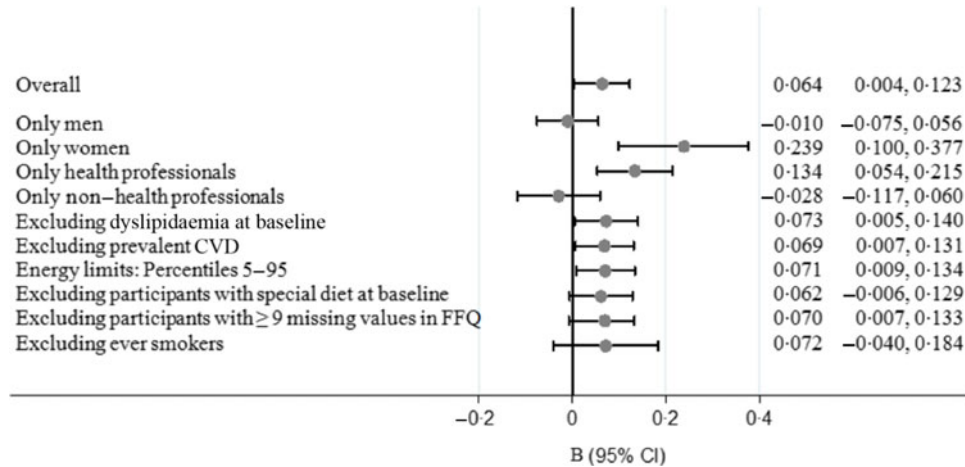


**Fig. 1.** Sensitivity analyses for the association between the adherence to the Western dietary pattern (WDP) and telomere length (high v. low quintile of adherence to WDP). Linear regression models adjusted for age, sex, year of cohort entry (four categories: 1999–2001, 2002–2004, 2005–2007, 2008), energy intake (continuous), BMI ( $\leq 20$ , 20–21, 22–25,  $\geq 25$  kg/m<sup>2</sup> and a quadratic term for BMI), smoking status (never/ever), alcohol intake (abstainer,  $< 5$  or  $< 10$  g/d, 5–25 or 10–50 g/d,  $> 25$  or  $> 50$  g/d for women and for men, respectively), leisure-time physical activity (metabolic equivalents of task (MET) h/week, continuous), average daily time of television watching (h/d, continuous), average daily time spent sitting (h/week, continuous), following special diet at baseline (yes/no), weight gain in the past 5 years previous to entering the cohort ( $< 3$  kg /  $\geq 3$  kg), years of education (continuous), prevalence of dyslipidaemia and CVD (yes/no), dietary fibre intake, MUFA intake, PUFA intake, SFA intake and *trans*-fat intake (continuous).

Study<sup>(10)</sup> and in 217 elderly Italian subjects<sup>(13)</sup>, a direct association between TL and Mediterranean diet score was also observed. However, in the National Health and Nutrition Examination Survey (NHANES) epidemiological study<sup>(14)</sup> and the PREvención con DIeta MEDiterránea (PREDIMED)-NAVARRA study<sup>(12)</sup>, the relationship was observed only in women, and among whites but not among African Americans and Hispanics in a multi-ethnic study<sup>(11)</sup>. On the other hand, pre-defined Mediterranean diet scores did not show any association with TL in three recent studies in elderly populations comprising

647 healthy Australian adults aged 57–68 years old<sup>(15)</sup>, 1046 older Finns adults<sup>(16)</sup> and 1981 Chinese adults aged 65 years and over<sup>(17)</sup>.

Unfortunately, the finding of a WDP in a Mediterranean population (Spanish) is currently not a surprise. Many of the countries bordering the Mediterranean Sea are drifting away from MDP, and, unfortunately, their diets are evolving towards WDP<sup>(42–44)</sup>. In our study, this could be partly explained by the fact that generally the type of work in a highly educated population (cohort of university graduates) often prevents them from



**Fig. 2.** Sensitivity analyses for the association between the adherence to the Mediterranean dietary pattern (MDP) and telomere length (high v. low quintile of adherence to MDP). Linear regression models adjusted for age, sex, year of cohort entry (four categories: 1999–2001, 2002–2004, 2005–2007, 2008), energy intake (continuous), BMI ( $\leq 20$ , 20–21, 22–25,  $\geq 25$  kg/m<sup>2</sup> and a quadratic term for BMI), smoking status (never/ever), alcohol intake (abstainer,  $< 5$  or  $< 10$  g/d, 5–25 or 10–50 g/d,  $> 25$  or  $> 50$  g/d for women and for men, respectively), leisure-time physical activity (metabolic equivalents of task (MET) h/week, continuous), average daily time of television watching (h/d, continuous), average daily time spent sitting (h/week, continuous), following special diet at baseline (yes/no), weight gain in the past 5 years previous to entering the cohort ( $< 3$  kg/ $\geq 3$  kg), years of education (continuous), prevalence of dyslipidaemia and CVD (yes/no), dietary fibre intake, MUFA intake, PUFA intake, SFA intake and *trans*-fat intake (continuous).

preparing their own foods at home and their work environment may influence their choices of dietary patterns<sup>(45)</sup>. Nevertheless, Mediterranean pattern was the second identified dietary pattern in line with other studies in Mediterranean countries<sup>(46–48)</sup>.

In this work, we did not observe any association between WDP and TL, in agreement with previous findings that an unhealthy pattern empirically obtained, such as WDP<sup>(24,25)</sup>, ‘snacks-drinks-milk’<sup>(17)</sup>, ‘fats and processed meat’<sup>(23)</sup> or ‘high energy-density’<sup>(20)</sup>, was not associated with TL. WDP in our Mediterranean population differed in part from typical ‘Western’ pattern; indeed, subjects in the top quintile of adherence to WDP had a high consumption of vegetables (501 g/d), fish (102 g/d), fruits (317 g/d) and a high percentage of monounsaturated fat intake (15 % of total energy). In addition, traditional healthy eating habits related to Mediterranean area such as the use of aromatic herbs or minimally processed dishes following specific culinary techniques prepared with a variety of plant-derived ingredients were not collected in the FFQ used in the SUN cohort, and it is likely that participants with greater adherence to a WDP might be benefiting, at least partially, from some of these healthy dishes and traditional culinary practices<sup>(49)</sup>.

The strengths of the present study included the exploratory identification of dietary pattern with an empirical method (PCA), the technique used (monochrome multiplex real-time quantitative PCR) to determinate TL measurements and the relatively large sample size. On the other hand, the repeated validation studies of the FFQ represent another strength<sup>(29,30)</sup>. The homogeneity of the cohort (Spanish university graduates) in terms of socio-economic and educational status could improve the validity of our study, by increasing the homogeneity of the sample, obtaining more accurate and well-informed self-reports and reducing the potential for confounding by socio-economic factors. In addition, we included three multiple adjusted models for potential confounders, and we performed a variety of sensitivity analyses to support the robustness of our findings.

On the other hand, some limitations must be recognised. First, the cohort is restricted to university graduates; consequently, our participants are not representative of the general population. Second, although the FFQ has been validated in the SUN cohort some degree of misclassification might exist in the dietary. However, the misclassification is more likely to be non-differential, and the bias would be probably towards the null.

Third, DNA was extracted from saliva sample, due to the lower invasiveness of sample collection and the large DNA amount from a simple extraction. This method is vastly used in epidemiological studies<sup>(50)</sup>. Despite TL measurement differs according to cells or tissues, salivary TL and leucocytes TL are positively correlated<sup>(51)</sup>.

In conclusion, our findings show that greater adherence to *a posteriori*-derived MDP is associated with TL in an older adult Spanish population. However, further research is necessary to confirm the beneficial effect of a Mediterranean-diet style on this marker of DNA damage in other larger Mediterranean and non-Mediterranean cohorts.

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The authors' contributions were as follows: A. O.-R. contributed to perform the experiments, the statistical analyses and wrote the manuscript; I. Z. and A. M. participated in the study design, statistical analyses, data interpretation and manuscript drafting; L. A.-P. performed experiments; G. Z. was responsible for experiments design; M. A. M.-G. participated in the study design, statistical analyses, data interpretation and financial management. All the authors actively participated in the manuscript preparation, as well as revised and approved the final manuscript.

The authors declare that there are no conflicts of interest.

### Supplementary material

For supplementary material referred to in this article, please visit <https://doi.org/10.1017/S0007114520004274>

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