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Original article

Effect of a 3-year lifestyle intervention on telomere length in participants from PREDIMED-Plus: A randomized trial

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SUMMARY

Background & aims: Short telomeres have been observed in chronic disease patients. Identifying environmental and lifestyle factors that could reduce telomere attrition is crucial for disease prevention. The aim of this work was to determine whether weight-loss induced by an energy-reduced Mediterranean diet (erMedDiet) and physical activity (PA) could modify telomere length (TL).
Methods: In 317 randomized non-smoker participants (mean age, 65.8 ± 4.98 years) with metabolic syndrome from two "Prevención con Dieta Mediterránea-Plus" (PREDIMED-Plus) trial centers, we evaluated MedDiet adherence, PA, anthropometric variables and TL at baseline and after a 3-year intervention using an intensive lifestyle program (IG) with an erMedDiet and PA or an unrestricted MedDiet without PA promotion (CG).
Results: Participants in the IG displayed greater 3-year weight reductions (−3.7 ± 4 kg, $P < 0.001$) compared to those in the CG. No differences in TL changes between groups were observed in the cohort as a whole. However, an interaction was observed between the intervention group and sex for TL changes ($p_{interaction} = 0.039$). Women in the IG showed an increase in TL after 3-y (+0.25 ± 0.9, relative units) compared to women in the CG (−0.07 ± 1.0) ($p_{ANCOVA} = 0.036$), whereas no differences between groups were observed in men. Women in the IG had a lower risk of telomere shortening after the intervention (OR = 0.17, 95%CI: 0.05–0.64, p = 0.008) compared to women in the CG.
Conclusions: A 3-year lifestyle intervention based on an erMedDiet and PA slowed telomere shortening in women but not in men.

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Abbreviations

CG	Control group
ErMedDiet	Energy-reduced Mediterranean Diet
IG	Intervention group
MedDiet	Mediterranean Diet
MMqPCR	Monochrome multiplex real-time quantitative PCR metho
PA	Physical activity
PREDIMED	Prevención con Dieta Mediterránea
RCT	Randomized controlled trial
ROS	Reactive oxygen species
SD	Standard deviations
SEM	Standard error of the mean
TL	Telomere length

1. Introduction

Telomeres are the structures responsible for maintaining genomic integrity, and changes in telomere length are linked to several aging processes [1]. Reduced telomere length (TL) has been observed in chronic disease patients and has been associated with an increased risk of disease-related mortality [2]. Therefore, identifying environmental and lifestyle factors that could reduce telomere attrition is crucial for disease prevention. In this regard, a systematic review and meta-analysis of cross-sectional studies has reported an association between Mediterranean Diet (MedDiet) adherence and longer telomeres [3]. Another recent meta-analysis evaluating the effect of lifestyle interventions on TL showed beneficial effects on telomere attrition after promoting physical activity (PA) and dietary counseling [4].

Therefore, the aim of the present research was to determine whether a lifestyle intervention consisting of an energy-reduced MedDiet (erMedDiet) together with PA promotion might play a role on the prevention of telomere shortening in older subjects with metabolic syndrome, compared to participants following an unrestricted caloric MedDiet without weight-loss encouragement.

2. Material and methods

2.1. Study design

The PREDIMED-Plus study is an ongoing 6-year parallel-group, multicenter randomized trial (RCT) involving 6874 participants recruited in 23 Spanish centers, aimed at assessing the effect of a lifestyle intervention on the primary prevention of cardiovascular disease. Eligible participants were women and men of 55–75 years old with no documented history of cardiovascular disease at enrolment, with overweight/obesity, and who had ≥3 components of metabolic syndrome. Participants were randomly assigned (1:1) to an intensive weight-loss lifestyle intervention with an erMedDiet and PA promotion or usual-care advice to follow an energy-unrestricted MedDiet (control). The PREDIMED-Plus protocol (Supporting information 1) has been detailed elsewhere [5,6] and was registered at (http://www.isrctn.com/ISRCTN89898870).

The current report aimed to evaluate the effects of the PREDIMED-Plus interventions on TL after a 3-year follow-up in participants from 2 of the 23 PREDIMED-Plus centers. This analysis represents a nested sub-study performed in the Reus and Pamplona centers including 317 non-smokers randomly selected with available DNA and information at baseline and after the 3-year intervention. The participant flowchart is shown in Fig. 1. The local institutional review board approved the study protocol. All participants provided written informed consent.

2.2. Telomere length determination

TL was analyzed in participants from the Reus and Pamplona centers based on our previous expertise in TL assessment in human samples [3,7–9]. These two centers were the only ones who offered to participate in this substudy. TL was measured in a subsample of 317 individuals at baseline and after 3 years of follow-up, due to limited resources.

Genomic DNA was isolated from frozen buffy coats using the Maxwell RSC Blood DNA kit (Promega, Madison WI, USA) with the Maxwell RSC Instrument. TL was measured by a monochrome multiplex real-time quantitative PCR method (MMqPCR) based on the Cawthon's method as previously described [7]. In a single reaction, the quantification of the relative copy numbers of telomeres (T) and a single copy gene (albumin; S) was performed in triplicates. TL is expressed as a ratio of these two parameters (T/S ratio).

2.3. Statistical analysis

A *P*-value <0.05 was considered statistically significant. R-Studio was used for all analyses. Means and standard deviations (SD) or percentages are shown for the description of baseline characteristics according to the intervention group. Analyses stratified by sex were performed as a pre-stated analysis, and therefore no other subgroup analyses were conducted. The primary outcome was TL change (3-year minus baseline values). First, to examine interactions between the intervention group and sex for TL changes, likelihood ratio tests were performed. Then, ANCOVA models were run to test the effect of the intervention groups on TL changes in women and men separately. Moreover, to estimate the risk for accelerated telomere shortening ($\Delta TL \leq$ percentile 20; $\Delta TL \leq$ p20) during the 3-year follow-up by intervention group, multivariable-adjusted logistic regression models were performed in analyses stratified by sex. All analyses were adjusted for potential confounders (see figure footnotes). The likelihood ratio test was used to examine interactions between the intervention group and sex for TL changes after the intervention in both the linear (ANCOVA) and the logistic models.

3. Results

A total of 317 participants (mean age, 65.8 ± 4.98 years; 47% women) with metabolic syndrome and a high prevalence of cardiovascular risk factors (62% obesity, 84% hypertension, 63% hypercholesterolemia, 28% diabetes) were included. No significant

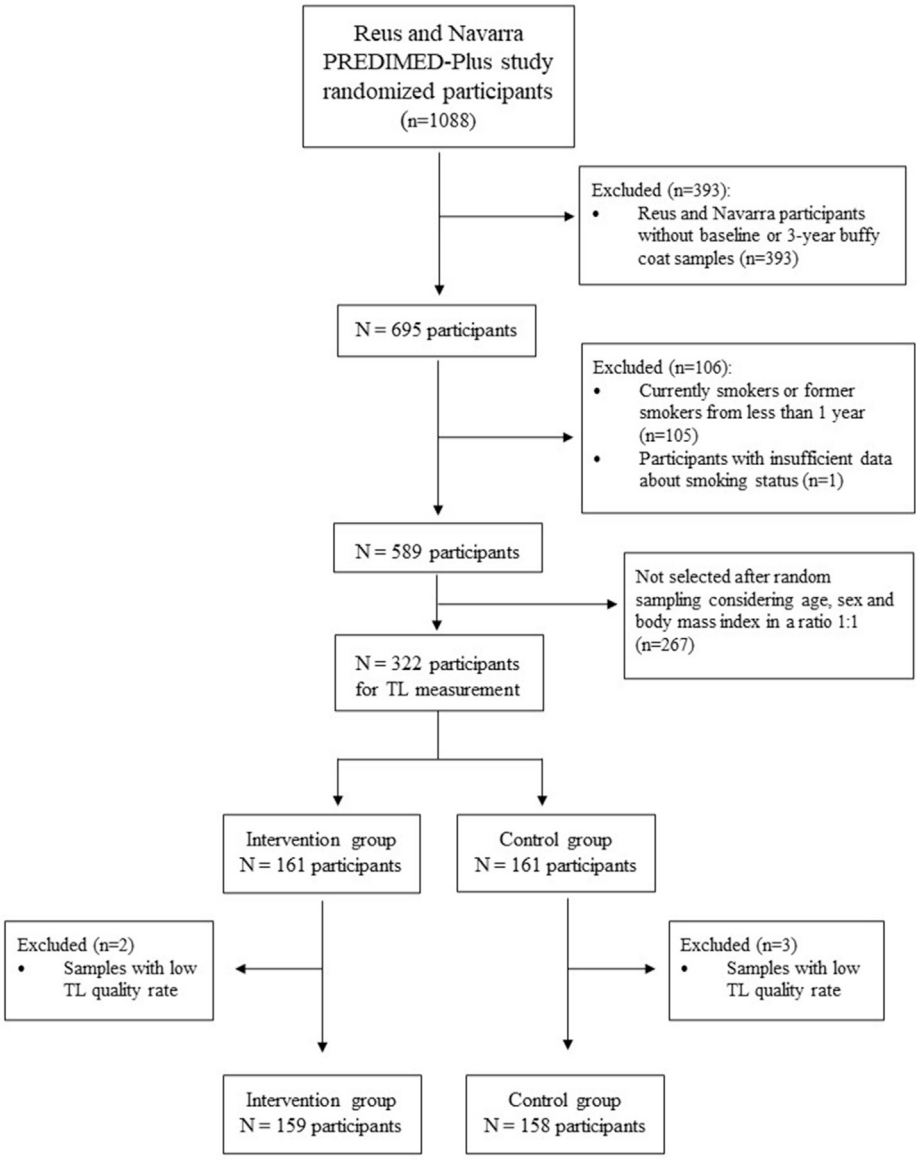

Fig. 1. Flowchart of participants included in the present analysis from the PREDIMED-Plus Study.

differences in the general characteristics between intervention groups at baseline were found, except for insulin treatment (Table 1 and Table S1). As expected, participants in the intensive lifestyle intervention (IG) increased their MedDiet adherence ($p < 0.001$) and PA ($p = 0.004$), achieving greater weight reductions (-3.7 kg $\pm$ 4.0; $p < 0.001$) compared to the control group (CG) participants after the 3-year intervention (Table S2). No differences were found between groups regarding therapy response after the intervention except from metformin, where an increase in the prevalence in the use of this drug was found in the CG compared to the IG (Table S3). No associations between changes in telomere length and changes in cardiovascular risk factors were found (Table S4).

No significant between-group differences in TL changes were found in the cohort as a whole. However, an interaction was observed between the intervention group and sex on TL changes ($p_{interaction} = 0.039$) (Fig. 2). Notably, after the 3-year intervention, a significant TL increase ($+0.25 \pm 0.9$, relative units) was observed in women in the IG *versus* the CG (-0.07 ± 1.0) in fully-adjusted models ($p = 0.036$). No between-group differences in TL were found in men (Fig. 2). Likewise, women in the IG showed an 83% lower risk (OR = 0.17, 95%CI: 0.05 to 0.64; $p = 0.008$) of having a greater telomere shortening rate than women in the CG (Table 2).

4. Discussion

In this PREDIMED-Plus sub-study we found a significant TL increase in women in the IG compared to those in the CG, after 3 years of intervention. Furthermore, women in the IG showed an 83% lower risk of telomere shortening compared to those in the CG. No between-group differences in TL were observed either in men or in the cohort as a whole.

Different RCTs have analyzed the effect of lifestyle interventions on TL with contradictory results. In the Finnish Diabetes Prevention study, no differences were found in the yearly TL change rate between the usual-care group and the IG, following a healthy diet and exercise [10]. Similarly, in a pilot PREDIMED-Plus sub-study

Table 1
Baseline characteristics of the subjects at randomization before the start of the study.

Characteristics	Control group (n = 158)	Intervention group (n = 159)	p-value
Age (years)	65.2 (4.9)	65.6 (5.1)	0.939
Women, n (%)	71 (44.9)	77 (48.4)	0.533
Weight (kg)	85.8 (12.3)	85.9 (14)	0.902
Waist circumference (cm)	106.5 (9.7)	105.9 (9.1)	0.624
BMI (kg/m^2)	32 (3.5)	32.2 (3.8)	0.633
Number of MetS components, n (%)			0.491
3	78 (49.4)	89 (56)	
4	56 (35.4)	48 (30.2)	
5	24 (15.2)	22 (13.8)	
Obesity (BMI≥30 kg/m^2), n (%)	98 (62)	99 (62.3)	0.965
Diabetes[b], n (%)	47 (29.8)	43 (27)	0.594
Cholesterol[a], n (%)	105 (66.5)	96 (60.4)	0.528
Hypertension, n (%)	129 (81.7)	137 (86.2)	0.274
Depression, n (%)	30 (19)	35 (22)	0.505
Smokers, n (%)			0.956
Former	77 (48.7)	77 (48.43)	
Never	81 (51.3)	82 (51.6)	
Educational level, n (%)			0.143
Higher degree or similar	12 (7.6)	13 (8.2)	
University school technician	23 (14.6)	11 (6.9)	
Secondary school	47 (29.8)	58 (36.5)	
Primary school	76 (48.1)	77 (48.4)	
Medication use, n (%)			
Lipid-Lowering drugs			
Statin	79 (50)	70 (44)	0.287
Other lipid-lowering drugs	9 (5.7)	10 (6.3)	0.824
Hypotensive drugs			
Renin direct inhibitor	0	1 (0.6)	0.918
Angiotensin receptor blocker	37 (23.4)	46 (28.9)	0.264
Angiotensin converting enzyme inhibitor	72 (45.6)	57 (35.9)	0.078
Thiazide drugs[c]	56 (35.4)	53 (33.3)	0.693
Antidiabetic drugs			
Insulin treatment	13 (8.2)	3 (1.9)	0.01
Metformin	35 (22.2)	35 (22)	0.976
Telomere length (T/S ratio)	1.10 (0.4)	1.17 (0.5)	0.177

Data are shown as means (SD) for continuous variables or number (%) for categorical variables.
P values for differences between groups by ANOVA or chi-squared test, as appropriate.
Abbreviations: BMI, body mass index; MetS, Metabolic syndrome.
[a] There were missing data for cholesterol in 2 participants (0.6%), one from each group.
[b] Diabetes was defined as previous diagnosis of diabetes or HbA1c ≥ 6.5% (48 mmol/mol), use of antidiabetic medication or having fasting glucose >126 mg/dL (7.0 mmol/L) in the screening visit plus fasting glucose >126 mg/dL (7.0 mmol/L) at baseline visit.
[c] Thiazide drugs include thiazides and thiazide-like diuretics.

performed with 69 non-diabetic participants, no differences in TL were found between intervention groups after 1 year of follow-up, whereas a significant 1-year TL increase in both intervention groups was observed [8]. After 5-year MedDiet interventions supplemented with virgin olive oil or nuts no beneficial effect on TL attrition compared to a low-fat diet was shown in 521 participants from the PREDIMED-Navarra study [9]. In that study, in women but not in men, cross−sectional associations between greater MedDiet adherence and longer telomeres were reported, showing that women had a lower risk of having short telomeres [9]. Finally, two RCTs aiming to evaluate the effect of exercise interventions on TL found a lengthening in the IG participants compared to the controls after 24 and 26-week interventions [11,12].

Studies performed only in women evaluating the effect of a lifestyle intervention have also reported inconsistent conclusions. In the Lifestyle Exercise And Nutrition study including breast cancer survivors, participants in the IG experienced a positive change in TL after 6 months, compared to the telomere shortening in the CG [13]. In contrast, 12-month TL changes were not different between control and intervention groups including different dietary and exercise approaches [14]. Another two RCTs in women found no differences between controls and exercise groups in TL after 6-month and 1-year interventions [15,16].

One possibility is that the discrepancies observed between these studies might be explained by differences in study design regarding the populations studied (men and women or only women, i.e. breast cancer survivors); type and length of the interventions (studies focused on the evaluation of changes in diet and/or physical exercise; the duration ranging from 6 months to 5 years); cell/tissue type and method used to measure TL determination. Further research is warranted to resolve these discrepancies, but this goes beyond the scope of our study.

Differences in TL between women and men have been previously reported [17,18]. A systematic review and meta-analysis of observational studies reported longer telomeres in women than in men [17]. Telomeres have a high sensitivity to oxidation processes [19], and levels of reactive oxygen species (ROS) in women are lower than in men to some extent because of the regulatory action of estrogen in the premenopausal stage [20,21], which might remain even during the postmenopausal period. On the other hand, the mechanisms underlying the observed response to the intervention could be partially explained by the influence of oxidative stress on telomere dynamics. Thus, the dietary pattern followed by the IG, based on the traditional MedDiet, has been proposed as an effective strategy to prevent telomere shortening due to its antioxidant ability [3]. Why this is only observed in women and not in

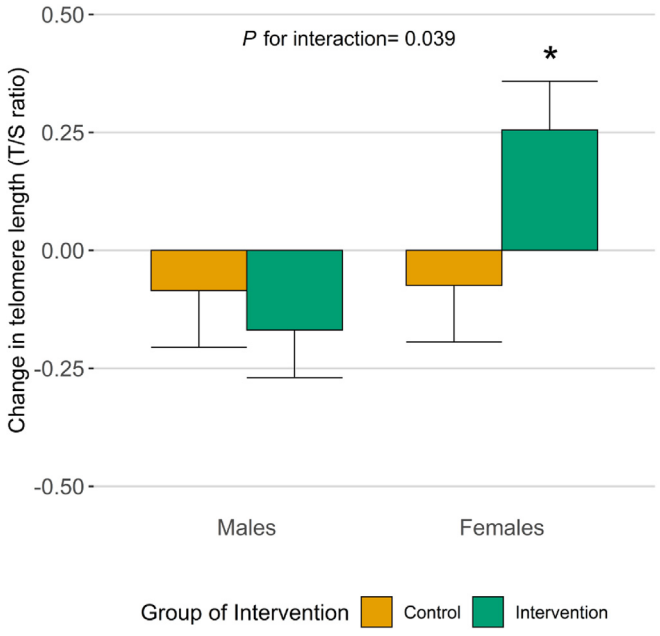

Fig. 2. Changes in Telomere length after 3 years of the PREDIMED-PLUS trial interventions in women and men separately. Interaction between the group of intervention and sex in determining 3-year changes in telomere length (*P* for interaction = 0.039). Mean and SEM changes in telomere length after 3 years of follow-up in women and men by intervention group. *$P = 0.036$ between intervention groups for women. All analyses are adjusted for the following confounding factors: age, BMI (in kg/m^2), baseline levels of TL (T/S ratio, relative units), physical activity (metabolic equivalent tasks in min/week), total energy intake (kcal/day), smoking status (never, former), adherence to MedDiet (17 point-score), diabetes status (yes/no), hypertensive status (yes/no), dyslipidemia status (yes/no). The likelihood-ratio test compares the goodness-of-fit of the following two models: 1) TL changes ~ group of intervention + sex + confounding factors and 2) TL changes ~ group of intervention + sex + confounding factors + sex * group of intervention. n = 315 (two individuals had missing data for dyslipidemia status).

men remains unknown. One explanation might be due to the age-dependent TL attrition observed mainly in men [18], which leave telomeres in women to be more prone to be modified by environmental factors. Furthermore, we cannot discard other sex-related potential mechanisms involved, as those attributed to lifestyle and behavioral factors differing in men and women [21]. More studies are needed to further clarify the mechanisms behind this sex-effect on telomere changes.

The metabolic and molecular basis of action underlying the benefits of MedDiet on TL may be explained by the recognized potential antioxidant and anti-inflammatory properties associated to the combined consumption of high amounts of vegetables, fruits, nuts and seeds, legumes and virgin olive oil [9,22,23]. In fact, systematic reviews of cohort studies and RCTs concluded that a high adherence to the MedDiet dietary pattern is associated with telomere lengthening [3], whereas a high consumption of red meat and processed meat and sweetened beverages may have the opposite effect [24]. Energy restriction may also partially explain the beneficial effects on TL observed in the intervention group of our study, as it has been shown that energy restriction was associated with telomere lengthening [25] and improved health and lifespan in animal models and humans (reviewed in [26]).

It is worth mentioning that, in a meta-analysis, it has been demonstrated that patients with diabetes had shorter TL compared to healthy individuals [27]. Notably, elevated glucose and oxidative stress levels could interfere with telomerase activity, resulting in telomere shortening [28]. However, in our study, the prevalence of diabetes, baseline glycated hemoglobin levels (as a marker of glucose dysregulation) and changes in glycated hemoglobin levels did not differ between the intervention groups. Nevertheless, models have been adjusted by diabetes status to account for potential confounding.

Among the strengths of our sub-study, the PREDIMED-plus is a RCT which allowed us to explore long-term changes in TL. The technique used to measure TL (MMqPCR) quantifies telomere length and the single copy gene in one well, in a single reaction, thus reducing variability. Lastly, the models fitted were adjusted for several confounders. Among the limitations, our population included non-smoker participants with overweight/obesity and high cardiometabolic risk, thus making it difficult to generalize these results to other populations. Another limitation is that we did not have available the biological variables which might help to explain the underlying mechanisms behind the sex-differential effects on telomere changes in response to the intervention; thus, our results should be interpreted with caution. Nevertheless, the influence of sex on telomere biology is a topic that warrants further exploration.

Table 2
Risk for telomere shortening (ΔTL ≤20th percentile) after 3 years of follow-up by intervention group.

		OR for telomere shortening after 3 years of intervention								
		Crude			Model 1			Model 2		
		OR (95% CI)	p	p for interaction	OR (95% CI)	p	p for interaction	OR (95% CI)	p	p for interaction
All population (n = 317)	**Control**	1 (Ref.)		0.044	1 (Ref.)		0.009	1 (Ref.)		0.012
	Intervention	0.85 (0.49–1.47)	0.557		0.54 (0.26–1.13)	0.102		0.56 (0.26–1.22)	0.142	
Men (n = 169)	**Control**	1 (Ref.)			1 (Ref.)			1 (Ref.)		
	Intervention	1.47 (0.68–3.18)	0.333		1.43 (0.50–4.07)	0.506		1.35 (0.39–4.70)	0.637	
Women (n = 148)	**Control**	1 (Ref.)			1 (Ref.)			1 (Ref.)		
	Intervention	0.47 (0.21–1.05)	0.066		0.21 (0.07–0.66)	0.007		0.17 (0.05–0.64)	0.008	

Model 1: adjusted for sex, age and baseline TL.
Model 2: adjusted for sex, age, baseline TL, BMI (in kg/m2), physical activity (metabolic equivalent tasks in min/week), total energy intake (kcal/day), smoking status (never/former), adherence to MedDiet (17 point-score), diabetes status (yes/no), hypertensive status (yes/no), dyslipidemia status (yes/no). n = 315 (two individuals had missing data for dyslipidemia status).
P for interaction between the group of intervention and sex in determining the risk for telomere shortening after 3 years of intervention.

5. Conclusions

Our study suggests that an intensive lifestyle intervention based on a MedDiet, PA promotion and weight loss encouragement increased telomere length in older women with high cardiometabolic risk but not in men. Women following this intensive intervention also had a lower risk of telomere shortening. Whether the effect on TL is due to body weight loss, increased adherence to an energy-restricted MedDiet and/or increased in PA needs to be further addressed. Therefore, observational studies and clinical trials replicating these results and exploring possible implicated mechanisms are warranted.

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Authors' contributions

Conceptualization: AM, SG-C, SC and JS-S; methodology: AM, SG-C, SC and JS-S; software: MFdlP, CV-H and SG-C; validation: all authors; data analysis: MFdlP, CV-H and SG-C; statistical analysis and data interpretation: all authors; resources: all authors; writing—original draft preparation: AM, MFdlP, SC, CV-H, SG-C and JS-S; writing—review and editing: all authors; supervision: AM, MFdlP, SC, CV-H, SG-C and JS-S; project administration and coordination: MM-G and JS-S; funding acquisition: MM-G and JS-S. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

All authors declare that they have no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2023.06.030.

References

[1] Chakravarti D, LaBella KA, DePinho RA. Telomeres: history, health, and hallmarks of aging. Cell 2021;184:306–22.
[2] Schneider CV, Schneider KM, Teumer A, Rudolph KL, Hartmann D, Rader DJ, et al. Association of telomere length with risk of disease and mortality. JAMA Intern Med 2022;182:291–300.
[3] Canudas S, Becerra-Tomás N, Hernández-Alonso P, Galié S, Leung C, Crous-Bou M, et al. Mediterranean diet and telomere length: a systematic review and meta-analysis. Adv Nutr 2020. https://doi.org/10.1093/advances/nmaa079.
[4] Buttet M, Bagheri R, Ugbolue UC, Laporte C, Trousselard M, Benson A, et al. Effect of a lifestyle intervention on telomere length: a systematic review and meta-analysis. Mech Ageing Dev 2022;206:111694.
[5] Martínez-González MA, Buil-Cosiales P, Corella D, Bulló M, Fitó M, Vioque J, et al. Cohort profile: design and methods of the PREDIMED-Plus randomized trial. Int J Epidemiol 2019;48:387–388o.
[6] Schröder H, Cárdenas-Fuentes G, Martínez-González MA, Corella D, Vioque J, Romaguera D, et al. Effectiveness of the physical activity intervention program in the PREDIMED-Plus study: a randomized controlled trial. Int J Behav Nutr Phys Activ 2018;15.
[7] Ojeda-Rodríguez A, Zazpe I, Alonso-Pedrero L, Zalba G, Martínez-González MA, Marti A. Higher adherence to an empirically derived Mediterranean dietary pattern is positively associated with telomere length: the Seguimiento Universidad de Navarra (SUN) project. Br J Nutr 2021;126:531–40.
[8] Fernández de la Puente M, Hernández-Alonso P, Canudas S, Marti A, Fitó M, Razquin C, et al. Modulation of telomere length by mediterranean diet, caloric restriction, and exercise: results from PREDIMED-plus study. Antioxidants 2021:10.
[9] García-Calzón S, Martínez-González MA, Razquin C, Arós F, Lapetra J, Martínez JA, et al. Mediterranean diet and telomere length in high cardiovascular risk subjects from the PREDIMED-NAVARRA study. Clin Nutr 2016;35:1399–405.
[10] Hovatta I, de Mello VDF, Kananen L, Lindström J, Eriksson JG, Ilanne-Parikka P, et al. Leukocyte telomere length in the Finnish diabetes prevention study. PLoS One 2012;7:34948.
[11] Puterman E, Weiss J, Lin J, Schilf S, Slusher AL, Johansen KL, et al. Aerobic exercise lengthens telomeres and reduces stress in family caregivers: a randomized controlled trial - curt Richter Award Paper 2018. Psychoneuroendocrinology 2018;98:245–52.
[12] Werner CM, Hecksteden A, Morsch A, Zundler J, Wegmann M, Kratzsch J, et al. Differential effects of endurance, interval, and resistance training on telomerase activity and telomere length in a randomized, controlled study. Eur Heart J 2019;40:34.
[13] Sanft T, Usiskin I, Harrigan M, Cartmel B, Lu L, Li FY, et al. Randomized controlled trial of weight loss versus usual care on telomere length in women with breast cancer: the lifestyle, exercise, and nutrition (LEAN) study. Breast Cancer Res Treat 2018;172:105–12.
[14] Mason C, Risques RA, Xiao L, Duggan CR, Imayama I, Campbell KL, et al. Independent and combined effects of dietary weight loss and exercise on leukocyte telomere length in postmenopausal women. Obesity 2013;21:1–15.
[15] Eigendorf J, Melk A, Haufe S, Boethig D, Berliner D, Kerling A, et al. Effects of personalized endurance training on cellular age and vascular function in middle-aged sedentary women. Eur J Prev Cardiol 2019;26:1903–6.
[16] Friedenreich CM, Wang Q, Ting NS, Brenner DR, Conroy SM, McIntyre JB, et al. Effect of a 12-month exercise intervention on leukocyte telomere length: results from the ALPHA Trial. Cancer Epidemiol 2018;56:67–74.
[17] Gardner M, Bann D, Wiley L, Cooper R, Hardy R, Nitsch D, et al. Gender and telomere length: systematic review and meta-analysis. Exp Gerontol 2014;51:15.
[18] Dalgård C, Benetos A, Verhulst S, Labat C, Kark JD, Christensen K, et al. Leukocyte telomere length dynamics in women and men: menopause vs age effects. Int J Epidemiol 2015;44:1688–95.
[19] Lin J, Epel E. Stress and telomere shortening: insights from cellular mechanisms. Ageing Res Rev 2022;73:101507.
[20] Lulkiewicz M, Bajsert J, Kopczynski P, Barczak W, Rubis B. Telomere length: how the length makes a difference. Mol Biol Rep 2020;47:7181.
[21] Needham BL, Diez Roux AV, Bird CE, Bradley R, Fitzpatrick AL, Jacobs DR, et al. A test of biological and behavioral explanations for gender differences in telomere length: the multi-ethnic study of atherosclerosis. Biodemogr Soc Biol 2014;60:156–73.

[22] Tosti V, Bertozzi B, Fontana L. Health benefits of the mediterranean diet: metabolic and molecular mechanisms. Journals Gerontol - Ser A Biol Sci Med Sci. 2018;73:318–26.
[23] Ojeda-Rodríguez A, Zazpe I, Alonso-Pedrero L, Zalba G, Guillen-Grima F, Martinez-Gonzalez MA, et al. Association between diet quality indexes and the risk of short telomeres in an elderly population of the SUN project. Clin Nutr 2020;39:2487–94.
[24] Galiè S, Canudas S, Muralidharan J, García-Gavilán J, Bulló M, Salas-Salvadó J. Impact of nutrition on telomere health: systematic review of observational cohort studies and randomized clinical trials. Adv Nutr 2020;11:576–601.
[25] Kark JD, Goldberger N, Kimura M, Sinnreich R, Aviv A. Energy intake and leukocyte telomere length in young adults. Am J Clin Nutr 2012;95:479–87.
[26] Ekmekcioglu C. Nutrition and longevity–From mechanisms to uncertainties. Crit Rev Food Sci Nutr 2020;60:3063–82.
[27] Wang J, Dong X, Cao L, Sun Y, Qiu Y, Zhang Y, et al. Association between telomere length and diabetes mellitus: a meta-analysis. J Int Med Res 2016;44:1156.
[28] Cheng F, Carroll L, Joglekar MV, Januszewski AS, Wong KK, Hardikar AA, et al. Diabetes, metabolic disease, and telomere length. Lancet Diabetes Endocrinol 2021;9:117–26.