Title: Longitudinal association of telomere length and obesity indices in an intervention study with a Mediterranean diet: The PREDIMED-NAVARRA trial.

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ABSTRACT

Background: Telomeres are nucleoprotein structures that protect the ends of eukaryote chromosomes. Shorter telomere length (TL) is associated with some age-related human disorders, but its relationship with obesity or adiposity parameters remains unclear.

Objective: The aim of this study was to assess the relationship between TL and changes in adiposity indices after a 5-year nutritional intervention.

Design and subjects: TL was measured by quantitative real-time PCR in 521 subjects (55-80 years, 55% women). Participants were randomly selected from the PREDIMED-NAVARRA centre after they completed a 5-year intervention programme. Anthropometric parameters were directly measured by trained personnel at baseline and on a yearly basis thereafter. TL at baseline and changes in TL after 5-year intervention were assessed.

Results: Higher baseline TL significantly predicted a greater decrease in body weight (B= -1.09 kg, 95%CI: -2.01 to -0.16), BMI (B= -0.47 kg/m^2, 95%CI: -0.83 to -0.11), waist circumference (B= -1.15 cm, 95%CI: -2.28 to -0.01), and waist to height ratio (B= -0.008, 95%CI: -0.010 to -0.001) in multiple-adjusted models. In addition, changes in TL during the 5-year intervention were inversely associated with changes in the four anthropometric variables. The reduction in adiposity indices during the intervention, associated with increasing TL, was even higher among subjects with the longest telomeres at baseline. Logistic regression analysis showed that the risk of remaining obese after 5 years was lower in those participants who initially had the longest telomeres and increased their TL after intervention (OR=0.27, 95%CI: 0.03 to 2.03).

Conclusions: Our research suggests that TL is inversely associated with changes in obesity parameters. The assessment of TL can provide further insights for biological pathways leading to adiposity. We show for the first time an improvement of obesity indices when an increase in TL is observed after a 5-year Mediterranean diet intervention.

Key words: Telomere length, adiposity, aging, nutritional intervention, obesity.

INTRODUCTION

Telomeres are nucleoprotein structures that cap and protect the ends of eukaryote chromosomes maintaining genome stability.[1] Because telomeres shorten during mitosis, they are thought to reflect biological age. Thus, a slow and gradual loss of telomere length (TL) with increasing age in human peripheral blood cells has been reported.[2] However, it is also well known that telomere attrition is likely to be a modifiable factor as there is substantial variability in the rate of telomere shortening that is independent of chronological age.[3] Therefore, short telomeres in peripheral blood cells have been described in patients with type 2 diabetes,[4] cancer,[5] metabolic syndrome[6] and in individuals with increased cardiovascular disease risk factors, such as obesity.[7]

Obesity is a common disease characterized by increased oxidative stress and a low-grade systemic inflammation.[8,9] These underlying mechanisms have been suggested to be the link for the association between shorter telomeres and obesity.[10,11] Lee *et al.*[12] showed that high general and abdominal adiposity are directly related to lower TL, suggesting obesity may hasten the aging process. They found an inverse cross-sectional association between TL and body mass index (BMI), waist to hip ratio, total body fat and waist circumference (WC), independent of some metabolic risk factors. Other cross-sectional studies found that TL, measured in blood cells, is associated with an obesity phenotype but only among women.[10,13,14]

Only a small number of studies have investigated the relationship between TL and changes in adiposity or anthropometric variables after an intervention. One prospective study reported that weight loss led to an increase in TL in rectal mucosa of obese men.[15] In the Bogalusa Heart Study, it was observed that weight gain and obesity accelerated

telomere attrition regardless of age.[16] Similar findings were recently reported for Chinese women.[7] Moreover, the single study conducted in the elderly found that shorter TL may be a risk factor for increased adiposity and weight.[17]

To our knowledge, no prospective studies have assessed the association between changes in TL and changes in adiposity indices following a long-term dietary intervention. Thus, the aim of our study was to assess the relationship between TL and changes in anthropometric parameters after 5 years of a nutritional intervention, in the frame of a dietary trial: The PREDIMED-NAVARRA study. Our hypothesized expected change was an improvement in adiposity indices in the subjects who had higher TL at baseline or increased their TL during the 5-year period of dietary intervention.

SUBJECTS AND METHODS

Study design

The PREDIMED study is a large, parallel-group, multicentre, randomized, controlled, clinical trial designed to assess the effects of the Mediterranean diet on the primary prevention of cardiovascular disease. The design and methods of this trial have been reported in a specific publication.[18] Further details are also available at www.predimed.es.

The study population consisted of women (60 to 80 years) or men (55 to 80 years) with no previously documented history of cardiovascular disease, but at high cardiovascular risk. They had either type-2 diabetes mellitus or at least three of the following major cardiovascular risk factors: current smoking, hypertension, elevated low-density

lipoprotein cholesterol, low high-density lipoprotein cholesterol, overweight/obesity or family history of premature coronary heart disease.

The present analysis deals with a subsample from one of the eleven recruitment centres (PREDIMED-NAVARRA). The PREDIMED-NAVARRA recruitment centre included 1055 of the 7447 subjects participating in the trial, being the first of the eleven centres to complete the enrolment of participants. Recruitment in the PREDIMED-NAVARRA centre took place between June 2003-May 2005, while in the rest of the centres it took place between October 2003- March 2009. Participants were randomly allocated to one of three arms: Mediterranean diet supplemented with extra virgin olive oil, Mediterranean diet supplemented with mixed nuts or a control group (low-fat diet). They were interviewed annually by a dietician, obtaining information about lifestyle, diet and incident diseases. Further aspects of the methods and design of the PREDIMED trial have been reported in detail elsewhere.[18,19]

All subjects provided informed consent and the protocol was approved by the institutional review boards according to the Principles of Helsinki Declaration. This trial is registered at http://www.controlled-trials.com/ISRCTN 35739639.

The present study assessed TL in 521 participants at baseline and after 5 years of recruitment, because the intervention was stopped in 2011 due to a decision of the Data Safety and Monitoring Board to stop the trial. The subjects were randomly selected, within those who had already completed 5 years in the intervention programme, from the three arms of the trial: Mediterranean Diet supplemented with Olive oil (n=211), Mediterranean Diet supplemented with mixed nuts (n=170) and a Control group which consisted in a low-fat diet (n=140).

Telomere length assessment

TL was measured in genomic DNA extracted from human peripheral blood samples with a real-time quantitative PCR approach.[20] This method uses Ribosomal Protein Large PO (RPLPO) single-copy gene as a reference for each sample.

QuantiTect Syber Green PCR kit (Qiagen, Valencia, CA, USA) was used as master mix. The total reaction volume was 10 μL containing 10 ng of genomic DNA. PCRs for telomere (T) and single copy gene (S) expression were performed on white 384-well plates on an ABI-Applied Biosystems 7900 HT thermal cycler (Applied Biosystems, CA, USA). The final telomere primer concentrations were as follows: for telomere amplification tel1, 675 nmol/L and for tel2, 1350 nmol/L; and for the amplification of the single copy gene RPLPO: hRPLPO1, 800 nmol/L; hRPLPO2, 800 nmol/L. The primer sequences were tel1 (5'-GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT-3'), tel2 (5'-TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA-3'), hRPLPO1 (5'-CCCATTCTATCATCAACGGGTACAA -3') and hRPLPO2 (5'-CAGCAAGTGGGAAGGTGTAATCC -3'). All primers were purchased from Sigma-Aldrich, St.Louis, MO, USA. This method expresses TL as a T/S ratio calculated as $2^{-\Delta CT}$. The work of Cawthon[20] confirms that the T/S ratio is approximately $[2^{CT(telomeres)}/2^{CT(single\ copy\ gene)}] = 2^{-\Delta CT}$, since the amount of the PCR product approximately doubles in each cycle of the PCR.

As a standard, a calibration curve of the same DNA sample of reference (64-0.25 ng in 2-fold dilutions) was included for each measurement to control the day-to-day variations. Standard curve with linearity $R^2 > 0.98$ was accepted. For quality control, all

samples were run in duplicate and checked for concordance between duplicate values. For obtaining stronger consistency, samples showing a high variation (more than 10%) were rerun and reanalyzed. The lower variation coefficient in the samples (2.68%), as compared with the change in telomere length in all the participants after 5 years of the nutritional intervention, supports the strength of the present methodology.

Anthropometric variables assessment

Anthropometric data were obtained by a trained nurse using standardized methods[19] and they were measured in each of the yearly follow-up visits. The anthropometric variables assessed were weight, BMI, WC and waist to height ratio (WHtR). For this analysis, the change in adiposity indices was calculated as the variable after 5 years of intervention minus the variable at baseline.

Confounders assessment

The baseline interview included the assessment of cardiovascular risk factors and physician diagnoses of hypertension, diabetes and hypercholesterolemia. We also obtained the information about medical, socio-demographic, anthropometric, and lifestyle variables. Physical activity was assessed through a validated physical activity questionnaire[21,22] and dietary habits were evaluated using a semi-quantitative 137-item Food frequency Questionnaire previously validated in Spain.[23]

Statistical analysis

We compared baseline characteristics of participants according to tertiles of TL. We calculated means and standard deviations (SD) or percentages for each variable across

the TL tertiles and assessed the statistical significance of the differences among them with one-way ANOVA and chi-squared tests, respectively.

Pearson correlations analyses were performed to calculate the association between changes in obesity parameters and baseline and follow-up TL. Moreover, we fitted multivariable linear regression models to assess changes in four anthropometric variables (body weight, BMI, WC and WHtR) according to tertiles of TL at baseline. We also assessed differences in each of the four variables for losing or gaining TL after 5 years of follow-up, according to tertiles of baseline TL. B coefficients and 95% Confidence Intervals (CI) were calculated, using those who had the lowest baseline TL as the reference group. For the multiple-adjusted model, the following potential confounders (all of them measured at baseline) were considered: age, sex, BMI (kg/m^2), WC (cm), smoking habit (current smoker, former smoker or never smoker), diabetes status, hypertensive status, dyslipidaemia status, physical activity (METS-min/day), total energy intake (Kcal/day) and group of intervention (Olive oil, nuts and control group). We additionally mutually adjusted for each basal anthropometric variable, depending on the analysis, and multiple testing correction (Benjamini-Hochberg) analysis were performed. Finally, we also fitted multivariable logistic regression models to estimate the association between losing or gaining TL and the odds of remaining obese after 5 years of the intervention, according to tertiles of baseline TL, calculating odds ratios (OR) and 95% CI. A p value<0.05 was considered statistically significant. Analyses were performed using STATA version 12.0 (StataCorp, College Station, TX, USA).

RESULTS

This PREDIMED-NAVARRA subsample assessed in our study included a total of 521 participants (45% males). Baseline characteristics of these participants according to tertiles of TL are presented in Table 1. The three groups were well balanced but small though significant differences were found for age, body weight and total energy intake. Regarding average age, as expected, the greater the age, the lower the TL (p=0.047). Although baseline body weight was significantly different between groups, when analysing by sex no differences were found within either men or women. Energy intake was also different among categories of TL, but no differences were observed according to the macronutrient distribution.

Significant inverse Pearson correlations of changes in body weight and BMI with both baseline and follow-up TL were found (Table 2). In addition, we fitted a multiple regression model to predict changes in adiposity indices at year 5 according to tertiles of baseline TL (Table 3). Higher baseline TL significantly predicted a greater reduction in body weight (B = -1.11 kg, 95% CI:-2.03 to -0.19 kg for third *vs.* first tertile), BMI (B = -0.49 kg/m^2, 95% CI:-0.85 to -0.13), WC (B = -1.24 cm, 95% CI: -2.39 to -0.09) and WHtR (B= -0.009, 95% CI: -0.020 to -0.001), when adjusted for age, sex and the corresponding anthropometric variable at baseline. These associations remained statistically significant in the multiple-adjusted model.

We also found an association between changes in TL during the intervention period and the anthropometric variables after 5 years of the nutritional intervention, according to tertiles of baseline TL (Table 4). In fully adjusted analyses, participants who increased TL reduced more their weight (*p* trend=0.008), BMI (*p* trend=0.009), WC (*p*

trend=0.044) and WHtR (*p* trend= 0.051) than those who decreased TL after the intervention. Furthermore, we found that changes in TL after 5 years of the nutritional intervention were influenced by the initial TL. In fact, the observed reduction in adiposity markers, associated with increasing TL, was even greater in those who had longer telomeres at baseline.

Interestingly, at the beginning 38% of the volunteers were obese. Nineteen percent of them successfully decreased their BMI under the threshold of 30 kg/m^2 after 5 years of nutritional intervention, whereas 81% remained obese. The OR for remaining obese after 5 years, for those who initially had the longest TL, was 0.27 (95% CI: 0.03 to 2.03) among those who increased TL, and 0.43 (95% CI: 0.10 to 1.89) among those who decreased TL during the follow-up (Table 5). Although these results did not reach statistical significance, a tendency was observed: the higher the baseline TL and the greater the increase of TL, the lower the risk of remaining obese.

DISCUSSION

In this study enrolling 521 elderly individuals, we found a significant inverse relationship between TL and adiposity indices using both initial and follow-up data. TL measured at baseline and changes in TL during follow-up were significantly inversely associated with changes in adiposity indices including body weight, BMI, WC and WHtR after 5 years of a nutritional intervention. In addition, the risk of remaining obese after the intervention was lower in those participants who had the highest baseline TL and subsequently increased their TL during the intervention 5-year period. Tertiles of baseline TL were calculated in order to clarify whether presenting larger or shorter telomeres could be an indicator for adiposity after a 5-year intervention. We also

suggest that telomere attrition rate was influenced by the initial TL, as other studies have already reported.[24,25]

To the best of our knowledge this is the first study analysing the effect of TL changes and baseline TL on changes in adiposity indices in the context of a long-term nutritional intervention. Mostly all of the literature considered obesity as a risk factor for telomere shortening. Recently, just one report proposed the opposite, as they suggested that shorter TL may be a risk factor for increased adiposity in the elderly.[17] Therefore, we suggest that the assessment of TL could be a useful biomarker for a better understanding of the biological pathways implicated in obesity progression.

Although the mean age of our population was 67 years, a significant correlation supporting the well-established age-related telomere loss was observed, regardless of accumulated diseases that could decrease the association between TL and age. Interestingly we detected an increase in TL in about 40% of the participants over the 5-year follow-up period. Increased TL after a period of time has been observed recently in observational or interventional studies.[3,24,26] Hence, TL is a dynamic factor and it can vary to both directions during a lifetime.[24]

There are a few reports on the association between TL and obesity-related parameters, in which obesity has been linked to accelerated telomere attrition. In order to evaluate the role of obesity and abdominal adiposity in TL, several studies have used indirect anthropometric measures. The association between BMI and TL is controversial, in some studies no relation was observed in either elderly[27,28] or middle-aged individuals[29,30] but in other studies an inverse association was found.[7,10,12,14] In the Bogalusa Heart Study, Gardner *et al*.[16] found that a decrease in BMI was strongly

associated with an increase in TL (r=-0.423, p<0.001) in a middle-aged population. This is in agreement with our findings since we found a greater significant decrease in BMI in those subjects who increased their TL during the intervention period and had also higher baseline TL.

There is just one study regarding the effect of weight loss on TL in midrectal biopsies of obese men.[15] They suggested that losing weight may contribute to the prevention of telomere shortening and DNA damage, which is in accordance with our findings. On the other hand, other investigations[7,31] reported that weight gain and obesity might accelerate aging since telomere shortening was greater.

A significant inverse association with WC was observed, suggesting that reduced TL may contribute to abdominal obesity. Our finding agrees with the works of Nordfjall *et al*.[14] in similar aged individuals, who showed that a higher WC is linked to shorter telomeres (r =-0.099; p=0.032) after adjusting for age. Several studies also reported a similar negative association between WC and TL,[7,32,33] whereas others did not find a correlation with this parameter.[27,29]

Another contribution of the present work is the use of a novel index for central obesity (WHtR) which is equally fair for short and tall persons, comparing to just measuring WC. Several studies have found that this is a more valid measurement than BMI[34] because it is a better discriminator for cardiovascular risk factors. The recently reported study by Cui *et al*.[7] showed a significant inverse association between relative TL and WHtR (*P* trend=0.004) in Chinese women. Our trial showed for the first time an association between WHtR and TL including men and women, demonstrating the

higher the increase in TL the greater the decrease in WHtR after the nutritional intervention.

Njajou *et al.*[17] recently reported that shorter telomeres may be a risk factor for elevated levels of adiposity in elderly subjects (mean age 73 years). Specifically, this study showed TL to be associated with positive change in BMI and % body fat after 7 years of follow-up. These results are not in line with ours. Nevertheless, due to these discrepancies, it is unclear whether telomere gain is a cause or a consequence of changes in adiposity. Therefore, more studies need to be conducted.

There are several strengths in our research. The prospective nature of our study with a long follow-up period enabled us to measure initial TL and TL after 5 years of the nutritional intervention. Our study is novel in the sense that it is the first time that TL was measured in a large number of subjects, in white blood cells and after a long period of time in the context of a Mediterranean diet intervention. There is just one study in the literature showing that a 4-week intervention with a Mediterranean diet prevents telomere shortening of endothelial cells in 20 elderly subjects.[35] Moreover, we fitted multiple-adjusted models and multiple testing correction to minimize small differences among individuals and potential confounders. In the regression analysis, we controlled a substantial number of potential confounders, which is much more informative to suggest causal associations than simply obtaining correlation coefficients as most previous studies have reported. On the other hand, one limitation of the present study could be that measurements in elderly subjects at high cardiovascular risk may possibly be affected by existing chronic diseases. In fact, several studies did find that shorter telomeres were significantly correlated with smoking[10,13] or other chronic diseases such

as dyslipidaemia, hypertension or diabetes.[4,36] But this limitation was partly solved by doing multiple adjustments.

In conclusion, our study shows an association between TL and obesity related parameters. We suggest that initial TL could predict changes in obesity anthropometric variables, proposing the assessment of TL as a biomarker of adiposity. In addition, we show for the first time that a decrease in obesity risk is linked to higher TL after a 5-year Mediterranean diet intervention. Nevertheless, further research is warranted to confirm these findings and to better understand the possible biological mechanisms explaining these associations.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table 1. Baseline characteristics according to tertiles of telomere length. The PREDIMED-NAVARRA study.

Telomere length	T1	T2	T3	P
n	175	174	172	
Age	67.8 (5.6)	67.0 (6.1)	66.2 (6.1)	0.047
Sex (% males)	39	49	48	0.157
Intervention Groups				0.098
MeDiet + Olive oil (%)	32	28	61	
MeDiet + Nuts (%)	38	34	26	
Control (%)	30	38	13	
Weight (kg)	73.0 (11.5)	76.0 (10.8)	75.4 (11.0)	0.029
BMI[1] (kg/cm^2)	29.0 (3.4)	29.4 (2.9)	29.1 (3.4)	0.488
WC[2] (cm)	94.1 (10.1)	95.7 (9.8)	95.6 (9.4)	0.243
WHtR[3]	0.59 (0.06)	0.59 (0.05)	0.59 (0.05)	0.945
Physical activity (METS-min/d)	260 (188)	273 (201)	298 (198)	0.186
Total energy intake (Kcal/d)	2184 (529)	2222 (515)	2355 (526)	0.006
Carbohydrates (% Kcal)	40	41	40	0.358
Proteins (% Kcal)	16	16	16	0.142
Lipids (% Kcal)	41	40	40	0.823
Smoking				0.090
Current smokers (%)	10	19	16	
Former smokers (%)	21	26	20	
Dyslipidaemia (%)	64	67	67	0.749
Hypertension (%)	87	80	83	0.264
Diabetes (%)	35	34	40	0.510

The table shows mean (standard deviation), or %.
[1]BMI: body mass index, [2]WC: waist circumference, [3]WHtR: waist to height ratio.
T1= < 78.25; T2= 78.25 to 198.08; T3= > 198.08.

Table 2. Pearson correlations coefficients of baseline and follow-up TL with changes in adiposity parameters after 5 years of the nutritional intervention. The PREDIMED-NAVARRA study.

	Baseline TL[1]	ΔTL[2]
ΔBody Weight (kg)	r= -0.110 (-0.194 to -0.024)	r= -0.088 (-0.172 to -0.002)
ΔBMI (kg/m^2)	r= -0.119 (-0.205 to -0.034)	r= -0.089 (-0.174 to -0.003)
ΔWC (cm)	r= -0.141 (-0.224 to -0.055)	r= -0.052 (-0.138 to 0.035)
ΔWHtR	r= -0.144 (-0.227 to -0.058)	r= -0.053 (-0.138 to 0.034)

TL: telomere length, WC: waist circumference, WHtR: waist to height ratio.
[1] Adjusted for age.
[2] Adjusted for age and baseline TL.

Table 3. Association between baseline telomere length and changes in anthropometric variables after 5 years of a nutritional intervention. The PREDIMED-NAVARRA study.

	Tertiles of baseline TL			
	T1	**T2**	**T3**	***P* for trend**
Weight change (kg)				
Absolute change	0.26	-0.14	-0.82	
Relative Change				
Age, sex and basal weight adjusted	0 (Ref.)	-0.31 (-1.23 to 0.60)	-1.11 (-2.03 to -0.19)	0.015*
Multiple-adjusted model[1]	0 (Ref.)	-0.22 (-1.14 to 0.69)	-1.09 (-2.01 to -0.16)	0.016*
BMI change (kg/m^2)				
Absolute change	0.12	-0.05	-0.33	
Relative Change				
Age, sex and basal BMI adjusted	0 (Ref.)	-0.16 (-0.51 to 0.20)	-0.49 (-0.85 to -0.13)	0.007
Multiple-adjusted model[1]	0 (Ref.)	-0.13 (-0.49 to 0.23)	-0.47 (-0.83 to -0.11)	0.009*
Waist circumference change (cm)				
Absolute change	1.53	1.71	0.22	
Relative Change				
Age, sex and basal WC adjusted	0 (Ref.)	0.34 (-0.80 to 1.47)	-1.24 (-2.39 to -0.09)	0.008*
Multiple-adjusted model[1]	0 (Ref.)	0.21 (-0.91 to 1.32)	-1.15 (-2.28 to -0.01)	0.017*
Waist to height ratio change				
Absolute change	0.009	0.011	0.001	
Relative Change				
Age, sex and basal WHtR ratio adjusted	0 (Ref.)	0.001 (-0.006 to 0.008)	-0.009 (-0.020 to -0.001)	0.005
Multiple-adjusted model[1]	0 (Ref.)	0.001 (-0.006 to 0.008)	-0.008 (-0.010 to -0.001)	0.014*

The table shows B coefficients (95%CI). BMI: body mass index, WC: waist circumference, WHtR: waist to height ratio.
[1]Adjusted for age, sex, basal BMI, basal WC, basal weight or WHtR, smoking (3 categories), diabetes status (dichotomous), hypertensive status (dichotomous), dyslipidaemia status (dichotomous), physical activity (METS-m/d), total energy intake (Kcal/d) and group of intervention.
* p value < 0.05 after correcting for Benjamini-Hochberg multiple comparisons.

Table 4. Association between telomere length changes during follow-up and changes in anthropometric variables after 5 years of a nutritional intervention, according to tertiles of baseline TL. The PREDIMED-NAVARRA study.

	Tertiles of baseline TL			
	T1	**T2**	**T3**	***P* for trend**
Weight change (kg)				
Change in TL during follow-up				
TL[1] decreased (n=314)	0 (Ref.)	0.10 (-1.19 to 1.39)	-0.71 (-1.96 to 0.53)	0.197
TL increased (n=207)	0.29 (-1.03 to 1.61)	-0.34 (-1.80 to 1.13)	-1.58 (-3.21 to 0.06)	0.008
BMI change (kg/m^2)				
Change in TL during follow-up				
TL decreased (n=314)	0 (Ref.)	-0.06 (-0.57 to 0.44)	-0.39 (-0.87 to 0.10)	0.111
TL increased (n=207)	0.01 (-0.51 to 0.52)	-0.24 (-0.81 to 0.33)	-0.71 (-1.35 to -0.08)	0.009
Waist circumference change (cm)				
Change in TL during follow-up				
TL decreased (n=314)	0 (Ref.)	0.68 (-0.89 to 2.25)	-0.88 (-2.41 to 0.64)	0.083
TL increased (n=207)	-0.01 (-1.62 to 1.61)	-0.62 (-2.40 to 1.16)	-1.95 (-3.94 to 0.04)	0.044
Waist to height ratio change				
Change in TL during follow-up				
TL decreased (n=314)	0 (Ref.)	0.003 (-0.007 to 0.013)	-0.007 (-0.016 to 0.003)	0.053
TL increased (n=207)	-0.001 (-0.011 to 0.009)	-0.005 (-0.016 to 0.006)	-0.013 (-0.025 to 0.001)	0.051

The table shows B coefficients (95%CI).
[1]TL: telomere length.
Adjusted for age, sex, basal BMI, basal WC, basal weight or WH ratio, smoking (3 categories), diabetes status (dichotomous), hypertensive status (dichotomous), dyslipidaemia status (dichotomous), physical activity (METS-m/d), total energy intake (Kcal/d) and group of intervention.
T1= < 78.25; T2= 78.25 to 198.08; T3= > 198.08.

Table 5. Odds Ratio and 95% CI of remaining obese (BMI≥30 kg/m^2) after 5 years of a nutritional intervention, according to tertiles of baseline TL and change in TL during follow-up. The PREDIMED-NAVARRA study.

	Tertiles of baseline TL		
	T1	**T2**	**T3**
Change in TL during follow-up			
TL[1] decreased (n=119)	1 (Ref.)	0.50 (0.12 to 2.15)	0.43 (0.10 to 1.89)
TL increased (n=77)	0.91 (0.19 to 4.42)	0.48 (0.10 to 2.31)	0.27 (0.03 to 2.03)

[1]TL: telomere length.
Adjusted for age, sex, basal BMI, basal WC, smoking (3 categories), diabetes status (dichotomous), hypertensive status (dichotomous), dyslipidaemia status (dichotomous), physical activity (METS-m/d), total energy intake (Kcal/d) and group of intervention.
T1= < 78.25; T2= 78.25 to 198.08; T3= > 198.08.