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2019-10

Meinilä , J , Perälä , M-M , Kautiainen , H , Männistö , S , Kanerva , N , Shivappa , N , Hebert , J R , Iozzo , P , Guzzardi , M A & Eriksson , J G 2019 , ' Healthy diets and telomere length and attrition during a 10-year follow-up ' , European Journal of Clinical Nutrition , vol. 73 , no. 10 , pp. 1352-1360 . <https://doi.org/10.1038/s41430-018-0387-4>

<http://hdl.handle.net/10138/321662>

<https://doi.org/10.1038/s41430-018-0387-4>

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Healthy diets and telomere length and attrition during a 10-year follow-up

Running head: Healthy diets and telomere attrition

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1 Abstract

2 **Background:** Telomeres are repeats of DNA that contain the sequence TTAGGG at the ends
3 of each chromosome, and their function is to protect DNA from damage. Little evidence exists
4 regarding the relationship between dietary patterns and telomere length, especially derived
5 applying longitudinal design. The aim was to study if overall dietary pattern associated with
6 leucocyte telomere length (LTL) or faster telomere attrition or both.

7 **Methods:** The setting was longitudinal and observational. Participants were 456 men and 590
8 women whose birth settled in between 1934 and 1944 and who participated in the Helsinki
9 Birth Cohort Study. Baltic sea diet score (BSDS), modified Mediterranean diet score (mMED),
10 and dietary inflammatory index (DII[®]) were calculated based on a 128-item food frequency
11 questionnaire (FFQ) collected in 2001-2004. LTL was measured twice, in 2001-2004 and in
12 2011-2013 by quantitative real-time polymerase chain reaction. Association between the
13 dietary patterns and LTL were analyzed by general linear models with appropriate contrasts.

14 **Results:** BSDS, mMED, and DII did not associate with LTL in the cross-sectional analysis in
15 men or women. Higher mMED at baseline (2001-2004) was associated with slightly faster LTL
16 shortening during the follow-up (standardized β -0.08, 95% CI -0.15, -0.01). No association
17 between mMED and LTL change was found in men. Adherence to BSDS and DII did not
18 associate with LTL change in men or women.

19 **Conclusion:** Baltic sea diet, Mediterranean diet, and diet's inflammatory potential seem to have
20 only little impact on telomere length and telomere attrition in elderly Finnish men and women.

21 **KEY WORDS:** aging, dietary index, diet quality, elderly, nutrition

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INTRODUCTION

Telomeres are DNA sequences at the ends of chromosomes repeating the sequence ‘TTAGGG’. Telomeres shorten when a cell replicates, and their function during replication of DNA is to prevent DNA damage. Shortening of telomeres is considered a biomarker of aging (1). Known risk factors for telomere length shortening are male gender (2), European race (3), smoking (4), and physical inactivity (5). Associations have been found for short telomere length and cancers (6), cardiovascular diseases (7) as well as shorter life expectancy. Inconsistent findings of association with telomere length have been found for body mass index (BMI) (8), psychological stress (9) and dietary factors (10-12).

Current data provide insufficient evidence for firm conclusions of association between dietary parameters and telomere length (13). Some evidence exists for inverse association with high consumption of total and saturated fat (and their food sources) (14), red and processed meats (15), and sugary beverages (16). Some evidence of positive association with telomere length exists for dietary antioxidants (17), whole grains (18), seeds and vegetables (19), and a dietary pattern high in foods that are plant-based and low in highly processed foods (19).

Increasing dietary inflammatory potential, assessed by dietary inflammatory index (DII[®]), has cross-sectionally been associated with shorter telomeres in a large NHANES cohort (20), and also longitudinally in a PREDIMED-NAVARRA study (n=520) (20). Mediterranean Diet (MedDiet) has been associated to longer telomeres in Nurses’ Health Study (NHS) (21), but overall the results have been inconsistent (10,12,22,23). The Healthy Nordic diet, a dietary pattern reflecting food availability in Northern European countries, is a source of antioxidants and has been associated with lower hs-CRP, a biomarker of inflammation (24). However, its relation with telomere length has not been assessed yet.

Because of the unclarity of current knowledge, we studied the association between dietary patterns and telomere length in a longitudinal setting. To avoid reflecting possible inappropriateness of a dietary index for the population concerned, we included several dietary indices. This approach also provides insight on whether dietary patterns associate in different manners with telomere length.

PARTICIPANTS AND METHODS

The Helsinki Birth Cohort Study (HBCS) included 13,345 singletons born in Helsinki in 1934–1944 and were alive at the time when all Finns, for the first time, received a unique social security number in 1971. A subsample of 2902 (to reach a final target number of 2000) from the 8760 individuals born in the Helsinki University Central Hospital was randomly selected and asked to participate in a clinical study in 2001–2004. At the end, 2003 participants attended the clinical visits (25). In 2011–2013, 1404 individuals could be traced and were invited to a follow-up visit, and 1094 individuals participated (26). The number of participants who provided data of both, diet at baseline and LTL at baseline and at follow-up, was 1046.

Dietary patterns (i.e. scores of dietary indices) were considered as exposures, LTL and its change as outcomes, and age, body mass index (BMI), smoking, years of education, leisure-time physical activity (LTPA), and follow-up time as potential confounders, and inflammatory markers as potential mediating factors.

In 2001–2004, height of the participants was measured in precision of 0.1 cm, and weight in precision of 0.1 kg, both measured by a trained study nurse in light indoor clothing wearing no shoes. A questionnaire served for obtaining information on smoking (quit or never smoked/smoker), educational attainment (years), and LTPA (times per week).

In 2001-2004, collection of blood samples occurred after overnight fast and samples were preserved as plasma, serum and whole blood in -70°C until analyzed. Research laboratories measured serum lipid fractions (total cholesterol, HDL-C and TAG concentrations) and hs-CRP concentration using routine methods (27-29). Friedewald's formula (30) served for calculating LDL-cholesterol concentrations. Analysis of IL-6 and TNF- α was performed by multiplex sandwich immunoassays. Maximum values for between-run variation were 13% for IL-6 and TNF- α .

Dietary assessment

At baseline (2001-2004), the participants filled in a validated self-administered semiquantitative food-frequency questionnaire (FFQ) including 12 food groups with 128 items for assessment of habitual dietary intake during the past 12 months (31,32). The questionnaire included nine frequency options ranging from never or seldom to greater than or equal to 6 times per day. A study nurse checked each FFQs. The data from the FFQs were entered and processed at the National Institute for Health and Welfare using the National Food Consumption Database FINELI (National Institute for Health and Welfare, Helsinki, Finland) (33).

The healthy Nordic diet illustrated by the Baltic Sea diet score (BSDS) includes nine components (34). For Nordic fruits (apples, pears, and berries), Nordic vegetables (tomatoes, cucumbers, leafy vegetables, roots, cabbages, peas), Nordic cereals (rye, oat, and barley), low-fat and fat-free milk, Nordic fish (salmon and freshwater fishes), ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) and trans-fatty acids, intake above the sex-specific quartiles served as cut-offs. Sex-specific quartiles assigned 0-3 points from lowest to the highest quartile. For red and processed meat, as well as for total fat (E%) the quartiles were scored in a reverse order. For alcohol (ethanol) consumption, moderate alcohol intake (less than 20 g/d

ethanol for men and less than 10 g/d ethanol for women) produced 1 point and higher intakes 0 points. The final BSDS had a range of 0 to 25. Higher score reflects higher adherence to the diet.

Modified Mediterranean diet score (mMED) consists of 9 components (35). For vegetables (excluding potatoes), legumes, fruit and nuts, cereals, fish and fish products, and the ratio of MUFA plus PUFA to SFA, intake above the median (defined separately for sexes) yielded 1 point, other intakes 0 points. For dairy and meat products, intake below the median (defined separately for sexes) yielded 1 point, and otherwise 0 points. For alcohol, intake between 5–25 g/day for women and 10–50 g/day for men yielded 1 point and other intakes 0 points. The total mMED score ranges from 0–9, and higher score reflects higher adherence to MedDiet.

For the DII, the association of several micronutrients, macronutrients, and food items (termed food parameters) with inflammation was evaluated by a literature review of 2000 articles for deriving inflammatory effect scores for dietary parameters (36,37). DII scores were standardized using a worldwide database, which contained the means and standard deviations of food parameter intakes including 11 populations worldwide (37). The original DII consists of 45 components. In this study, the available components that were used for calculating DII were energy, carbohydrate, protein, fat, MUFA, PUFA, SFA, trans fat, cholesterol, omega 3, omega 6, fiber, alcohol, vitamin A, beta carotene, niacin, thiamin, riboflavin, vitamin B6, vitamin B12, folic acid, vitamin C, vitamin D, vitamin E, iron, magnesium, selenium, zinc, flavan3ol, flavones, flavonols, isoflavones, onion, and tea. The mean value from the world database for each food parameter was subtracted from the reported intake value for that food parameter and then divided by the standard deviation from the world database to obtain a z-score. In the next step z-scores were converted to proportions by the probnorm function in SAS. A centered value was calculated by doubling the value and subtracting one. Multiplication of this value by the inflammatory effect score was done for each food parameter. For calculating

the overall DII score individual item scores were then summed across all food parameters. Higher DII score reflects a more pro-inflammatory diet, whereas lower score indicates a more anti-inflammatory diet. It should be noted that for results of DII, mMED and BSDS to be comparable, we have reversed DII for some of the analysis (marked as “reversed DII” when applied).

Measurement of telomere length

Relative LTL measurement in 2001–2004 and again in 2011–2013 were done by quantitative real-time polymerase chain reaction (RT-PCR) (38). Peripheral whole blood served for DNA extraction by commercially available kits with manufacturer’s instructions (QIAamp blood Maxi kit and DNeasy blood and tissue kit, respectively; Qiagen srl). Absorbance at 260 and 280 nm indicated concentration and purity of DNA. Electrophoresis in a 0.8% agarose gel served for testing DNA integrity.

In 2001-2004, relative LTL was determined as the ratio of telomere DNA to hemoglobin- β single-copy gene-signal intensities (T/S) (39). In 2011-2013, telomere and single-copy gene underwent separate quantitative PCR reactions (39). LTL was measured using multiplex real-time PCR assays facility in a 384-well plate system (CFX384 Touch Real-Time PCR detection system; Bio-Rad Laboratories), according to the method developed by Cawthon (40) and modified as previously reported by Guzzardi et al. (38). Triplicate runs were performed of each sample, and all plates included 2-4 genomic DNA control samples for calibration of the plate effect and for monitoring the coefficient of variation (CV), which were 21.0% and 6.2%, respectively.

All the procedures were conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Committee

of the Helsinki and Uusimaa Hospital District. All participants provided written informed consent.

Statistical methods

Results of demographic and clinical measures are given in means and standard deviations or frequencies and proportions (%). For describing the characteristics of the population, dietary indices were divided in quartiles (later in the analysis the dietary indices are treated as continuous). Because of the small number of available scores (small range and only integers) in BSDS (0-25) and mMED (0-9) the quartile cut-offs included large proportion of the population and resulted in uneven group sizes which can be seen in **Tables 1** and **2**. At baseline (2001-2004), LTLs were standardized, separately for men and women, and divided in 5 categories according to normal distribution, each category including I: 12.5%, II: 25%, III: 25%, IV: 25%, and V: 12.5% of the participants.

Difference of demographic and clinical measures between the lowest and highest quartiles of the dietary indices were tested by Student's t-test and Pearson's Chi-square test. Correlation coefficients between the dietary indices were calculated by the Pearson method (Supplemental Table 1). Linearity of dietary indices (BSDS, mMED, and reversed DII) across LTL categories was analyzed by ANOVA with continuous dietary index scores as dependent variables and categorized LTL as independent variable, and linear contrasts were set to LTL categories. The analyses were adjusted for age, energy intake, BMI, smoking, LTPA and educational attainment. The association between standardized energy-adjusted (residual method (41)) dietary indices and continuous LTL was tested by partial correlation adjusted for age (Supplemental Figure 1). Equality of variance between quartiles of dietary indices as well as telomere length categories was tested by Levene's test.

The association between standardized dietary indices (BSDS, mMED, and reversed DII) and continuous LTL change from 2001-2004 to 2011-2013 were analyzed by multivariate linear regression analysis using standardized regression coefficients Beta (β). Cohen's standard for Beta value interpretations are as follows; small relationship: > 0.10 , moderate relationship: > 0.30 , and large relationship: > 0.50 (42). The first model was adjusted for energy intake and the second model was adjusted for baseline LTL, age, energy intake, BMI, smoking, LTPA, educational attainment, and follow-up time. Additional adjustments for alcohol intake, blood pressure affecting medication, and diabetes medication did not substantially change the results (results not shown). In case a dietary index was significantly associated with LTL or its change we tested whether any of the individual components was driving the association.

To minimize the effect of statistical phenomenon "regression to the mean" we performed, as a sensitivity analysis, adjustment for baseline LTL by replacing the dependent variable by participant-specific residuals of regression of LTL change by baseline LTL. This did not change the results.

In case the variables did not meet the assumptions (non-normality), we used a bootstrap-type test. Shapiro-Wilk W-test was used for evaluation of normality of variables. Data was analysed by STATA 14.1. Only complete cases were included in the analysis.

RESULTS

At baseline (2001-2004), LTL was not different between those who did not participate from the original study cohort ($n=918$) and the participants of the present study ($n=1046$) (LTL (T/S) in present vs. non-participants: 1.40 (SD 0.01) vs. 1.38 (SD 0.01), $p=0.20$). At baseline (2001-2004) all dietary indices were positively associated with energy intake (reversed DII) (Tables 1 and 2). In women higher scores in all indices (reversed DII) were associated with higher educational attainment (Table 1). In women, higher scores of mMED were associated with

lower BMI whereas in men higher scores of mMED were associated with higher BMI. BSDS, mMED, and DII were highly correlated to each other (Supplemental Table 1).

In the cross-sectional analyses including women, none of the dietary indices were associated with either continuous LTL or LTL categories, neither in women (Figure 1 and Supplemental Figure 1) nor in men (Figures 2 and Supplemental Figure 1).

The mean follow-up time was 9.9 (SD 1.0) years in women and 9.7 (SD 0.9) years in men. In the longitudinal analyses, baseline (in 2001-2004) BSDS, mMED, and reversed DII were not associated with LTL change in the analysis adjusted for energy intake or in a fully adjusted model (baseline LTL, age, energy intake, BMI, smoking, LTPA, educational attainment, and follow-up time), except for the fully adjusted model in women where mMED was associated with faster LTL shortening (Table 3). This association was mainly driven by fruits and nuts component of mMED (adjusted standardized $\beta=0.096$, $p=0.017$).

DISCUSSION

We did not observe any associations between BSDS, mMED, and DII and LTL in either men or women in the cross-sectional analyses. High scores of mMED in women were associated with faster LTL shortening whereas other indices were not associated with LTL shortening. The results are partly in contrast with the few previous studies that have reported association between mMED and DII and LTL and LTL change. This is the first study on the association between BSDS and LTL and LTL change.

Despite the significant association in an unexpected direction between higher mMED and faster telomere shortening the effect estimate was small (42) and thus clinically insignificant. Cross-sectional studies focusing upon MedDiet and LTL in elderly populations, including a large cohort from the Nurses' Health Study ($n=4676$), have reported on positive associations (21,43). In other studies the association has been found only in subgroups of participants, such as non-

Hispanic whites (12), in women but with opposite results in men (22), or only in certain genotype carriers (23). In a randomized controlled trial on the effect of MedDiet on LTL in 55-80-year olds, no association was found between MedDiet and LTL change over a 5-year follow-up (22). This agrees with our results.

Previous analyses of the association between DII and LTL in cross-sectional settings have shown inconsistent results. In a study of NHANES (n=7215) higher DII was associated with shorter telomeres (20). In contrast, analysis of middle-aged Askleipos population (n=2509) showed no association between DII and LTL, which agrees with our cross-sectional results (44). In the only previous longitudinal study of DII and LTL attrition, in PREDIMED-NAVARRA study, DII was inversely associated with LTL (45). This is in contrast with our results.

One reason for different results compared to other studies could be different clinical characteristics of our study population compared to studies that found association. Participants in the large studies had wider range of age distribution and thus possibly wider range of telomere lengths (20,21,44). The population of PREDIMED-NAVARRA study was also at high risk of cardiovascular disease with higher BMI, more dyslipidemia, hypertension and diabetes compared to our study group.

It is also possible that not all indices are suitable for all populations. In our population DII was not associated with available inflammatory markers, which may explain the lack of association between DII and LTL and its attrition. In addition, mMED might not rank individuals by healthfulness of their diet in a non-Mediterranean population such as the Finnish one. For example, mMED provides points for not consuming dairy, whereas in Finland, dairy products are supplemented with vitamin D, and are a major source of total vitamin D intake in the Finnish

population (46). In addition, the most commonly consumed dietary oil in Finland is rapeseed oil, high in PUFA, while in Mediterranean population it is olive oil, high in MUFA.

Several studies suggest that change in telomere length tends to be correlated with the baseline telomere length (47,48), resulting in greater attrition rate in longer telomeres. However, this may result from a statistical artifact, regression to the mean (49). We have addressed this by performing a sensitivity analysis in which the dependent variable was replaced by participant-specific residuals of regression of LTL change by baseline LTL, which did not change the results. Greater attrition rate in longer telomeres may also be a physiological phenomenon. Telomerase, enzyme that repairs telomeres, may prefer acting on short telomeres compared to longer telomeres (50). Another mechanism could be that old cells divide more rarely and therefore may not lose as much telomere length compared to younger cells (48). These phenomena may reduce the power to detect changes in telomere shortening in elderly populations such as ours and others that observed null.

Strengths of the present study include longitudinal design and two measurements of LTL at different time points. The study population included over 1000 well-characterized participants, reflecting the general urban population. The study also included a validated FFQ (31,32).

In women, the baseline BMI was lower and energy intake higher in the highest quartile compared to the lowest quartile of mMED. This probably reflects higher degree of under-reporting of women with higher BMI, a commonly seen phenomena in nutrition research (51). To diminish this bias the analyses were adjusted for energy intake (51). Because of indices as dietary components this may not have completely overcome the problem because the scoring was done before the energy adjustment. We cannot rule out some unknown relevant confounders. It should be reckoned that qPCR is not the most optimal method for measuring LTL (52). The original method for telomere length measurement is Southern blot (53).

Technical differences in LTL measurement may have affected our power to detect statistically significant results, which must be acknowledged. In addition, the greater variability in the measurements of LTL at age 60 might have obscured associations with dietary indices. Additionally, because of the long follow-up time the participants may have changed their diets in between the LTL measurements, which was not considered. Survivor bias may be present because some of the original participants of HBCS had died during the ten-year follow-up period. However, LTLs in 2001-2004 measurement were not different between those who did not provide follow-up measurement and who did. The results can be generalized to urban Finnish elderly population.

Healthy Nordic diet, MedDiet and diet's inflammatory potential may have little or no association with LTL and its attrition in elderly Finnish men or women. Studies with longitudinal assessment of dietary intakes should be performed to confirm the results.

ACKNOWLEDGEMENTS

This study was supported by grants from Finska Läkaresällskapet, the Finnish Special Governmental Subsidy for Health Sciences, Academy of Finland, Samfundet Folkhälsan, Liv och Hälsa, the Signe and Ane Gyllenberg Foundation, EU FP7 [Developmental Origins of Healthy Aging (DORIAN)] project number 278603, and EU H2020-PHC-2014-DynaHealth grant 633595 (all for the Helsinki Birth Cohort Study).

AUTHORS' CONTRIBUTIONS: JM, JGE, SM, MMP, and HK designed research, JGE, MMP, NK, SM, PI, MAG, NS, and JRH provided essential materials, JM and HK performed statistical analysis, JM wrote the paper, JM had primary responsibility of final content, JM, JGE, SM, MMP, NK, PI, MAG, HK, JRH, and NS provided inputs to the paper and approved the final version of the paper.

CONFLICT OF INTEREST

Dr. James R. Hébert owns controlling interest in Connecting Health Innovations LLC (CHI), a company planning to license the right to his invention of the Dietary Inflammatory Index (DII) from the University of South Carolina in order to develop computer and smart phone applications for patient counseling and dietary intervention in clinical settings. Dr. Nitin Shivappa is an employee of CHI. Other authors have no conflicts of interest (JM, HK, MMP, JGE, SM, NK, PI, MAG).

FUNDING

Supported by grants from Finska Läkaresällskapet, the Finnish Special Governmental Subsidy for Health Sciences, Academy of Finland, Samfundet Folkhälsan, Liv och Hälsa, the Signe and Ane Gyllenberg Foundation, EU FP7 [Developmental Origins of Healthy Aging (DORIAN)] project number 278603, and EU H2020-PHC-2014-DynaHealth grant 633595 (all for the Helsinki Birth Cohort Study).

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Figure 1. Mean (95% CI) dietary scores in 2001-04 in level of leucocyte telomere length after adjustment for age, smoking, energy intake, BMI, educational attainment, and leisure-time physical activity in women (n=587). BSDS, Baltic sea diet score; mMED, modified Mediterranean diet score; DII, dietary inflammatory index.

Figure 2. Mean (95% CI) dietary score in 2001-04 in quintiles of leucocyte telomere length after adjustment for age, smoking, energy intake, BMI, educational attainment, and leisure time physical activity in men (n=451). BSDS, Baltic sea diet score; mMED, modified Mediterranean diet score; DII, dietary inflammatory index.

Table 1. Clinical and sociodemographic characteristics of female participants in the lowest (Q1) and highest quartiles (Q4) of BSDS, mMED, and reversed DII.									
	BSDS			mMED			reversed DII		
	Q1 (n=127)	Q4 (n=169)		Q1 (n=107)	Q4 (n=228)		Q1 (n=147)	Q4 (n=147)	
	mean (SD)	mean (SD)	p ¹	mean (SD)	mean (SD)	p ¹	mean (SD)	mean (SD)	p ¹
Age at baseline, y	60.9 (2.7)	61.6 (2.8)	0.05 2	61.3 (3.2)	61.5 (2.7)	0.66	61.1 (2.9)	61.7 (2.7)	0.05 4
Educational attainment, y	11.8 (3.4)	12.8 (3.6)	0.01 2	11.2 (3.2)	12.5 (3.5)	0.00 1	11.5 (3.3)	12.7 (3.8)	0.00 4
Body mass index, kg/m ²	27.2 (4.8)	26.7 (4.7)	0.33	28.0 (5.0)	26.9 (4.6)	0.04 2	27.7 (4.5)	27.1 (4.9)	0.24
Energy intake, kJ/d	7358 (2213)	9293 (2877)	<0.0 01	7071 (2133)	9428 (3074)	<0.0 01	5867 (1462)	10644 (3043)	<0.0 01
Cholesterol, mmol/l	6.1 (1.0)	5.9 (1.0)	0.09	6.0 (1.0)	5.9 (1.1)	0.42	6.0 (1.0)	6.0 (1.1)	0.97
Triglycerides, mmol/l	1.4 (0.6)	1.2 (0.6)	0.02 5	1.4 (0.6)	1.3 (0.7)	0.07	1.5 (0.7)	1.3 (0.6)	0.01 8
HDL, mmol/l	1.7 (0.4)	1.8 (0.5)	0.08	1.7 (0.4)	1.8 (0.5)	0.03 6	1.7 (0.4)	1.8 (0.4)	0.00 8
LDL, mmol/l	3.7 (0.9)	3.5 (0.9)	0.03 6	3.7 (0.8)	3.5 (0.9)	0.17	3.6 (0.9)	3.6 (1.0)	0.69
IL-6, ng/ml	249.1 (994.4)	167.8 (701.8)	0.41	145.3 (317.4)	184.8 (710.4)	0.58	118.3 (299.4)	153.6 (483.2)	0.45
TNF-alpha, pg/ml	16.6 (46.1)	17.5 (56.5)	0.88	19.5 (56.0)	22.7 (133.9)	0.81	18.4 (59.6)	18.4 (59.1)	1
hs-CRP, mg/l	3.4 (4.7)	2.9 (4.7)	0.4	4.2 (7.2)	3.6 (5.8)	0.43	4.0 (7.5)	3.7 (6.2)	0.74
LTPA, times/wk	1.2 (1.8)	1.7 (2.0)	0.03 3	1.4 (1.9)	1.7 (2.0)	0.24	1.2 (1.8)	1.6 (1.9)	0.0 9
	n (%)	n (%)	p ²	n (%)	n (%)	p ²	n (%)	n (%)	p ²
Smokers, n (%)	38 (29.9)	16 (9.6)	<0.0 01	32 (29.9)	30 (13.2)	<0.0 01	40 (27.2)	20 (13.7)	0.00 4

¹difference tested by Student's two-tailed t-test, ²difference tested by Pearson chi-square test. Q, quartile; BSDS, Baltic sea diet score; mMED, modified Mediterranean diet score; DII, dietary inflammatory index; LTPA, leisure-time physical activity. Missing data for educational attainment n=1, LTPA n=1, IL-6 n=2, TNF-alpha n=2, and smoking n=3.

Table 2. Clinical and sociodemographic characteristics of male participants in the lowest (Q1) and highest quartiles (Q4) of BSDS, mMED, and reversed DII.									
	BSDS			mMED			reversed DII		
	Q1 n=(104)	Q4 (n=135)		Q1 (n=50)	Q4 (n=129)		Q1 (n=114)	Q4 (n=114)	
	mean (SD)	mean (SD)	p ¹	mean (SD)	mean (SD)	p ¹	mean (SD)	mean (SD)	p ¹
Age at baseline, y	61.2 (2.6)	61.6 (2.7)	0.22	61.3 (2.7)	61.2 (2.4)	0.74	61.3 (2.7)	61.2 (2.4)	0.84
Educational attainment, y	12.5 (4.1)	13.2 (3.8)	0.13	11.2 (3.2)	12.5 (3.5)	0.018	12.7 (4.1)	13.0 (3.8)	0.53
Body mass index, kg/m ²	27.1 (3.5)	27.4 (3.4)	0.54	26.1 (3.2)	27.3 (3.6)	0.032	26.7 (3.5)	27.8 (3.6)	0.014
Energy intake, kJ/d	9125 (3229)	10915 (3144)	<0.001	8266 (2400)	11685 (3262)	<0.001	7140 (1707)	13511 (3445)	<0.001
Cholesterol, mmol/l	5.8 (1.1)	5.6 (1.0)	0.13	5.6 (0.9)	5.6 (0.9)	0.92	5.8 (1.0)	5.8 (0.9)	0.81
Triglycerides, mmol/l	1.6 (0.8)	1.4 (0.7)	0.21	1.4 (0.5)	1.4 (0.6)	0.42	1.4 (0.6)	1.5 (0.8)	0.54
HDL, mmol/l	1.5 (0.3)	1.4 (0.4)	0.88	1.4 (0.3)	1.5 (0.4)	0.17	1.5 (0.3)	1.5 (0.4)	0.81
LDL, mmol/l	3.7 (0.9)	3.5 (0.9)	0.22	3.6 (0.8)	3.5 (0.8)	0.43	3.7 (0.8)	3.7 (0.9)	0.96
IL-6, ng/ml	146.1 (345.2)	276.1 (1145.9)	0.27	132.4 (227.0)	222.4 (743.0)	0.41	136.0 (277.6)	232.6 (1364.7)	0.46
TNF-alpha, pg/ml	14.2 (30.8)	53.0 (277.9)	0.16	12.1 (16.7)	38.9 (153.2)	0.22	15.3 (43.8)	41.2 (260.8)	0.3
hs-CRP, mg/l	3.2 (4.8)	2.0 (3.7)	0.027	3.0 (4.6)	1.9 (2.3)	0.034	3.0 (4.7)	2.3 (2.7)	0.2
LTPA, times/wk	1.4 (1.8)	2.1 (2.0)	0.004	1.2 (1.8)	1.8 (1.9)	0.058	1.4 (1.9)	2.0 (2.0)	0.024
	n (%)	n (%)	p ²	n (%)	n (%)	p ²	n (%)	n (%)	p ²
Smokers, n (%)	36 (34.6)	16 (12.0)	<0.001	16 (32.0)	20 (15.8)	0.016	30 (26.8)	24 (21.2)	0.33

¹difference tested by Student's two-tailed t-test, ²difference tested by Chi-square test. Q, quartile; BSDS, Baltic sea diet score; mMED, modified Mediterranean diet score; DII, dietary inflammatory index; LTPA, leisure-time physical activity. Missing data for educational attainment n=2, LTPA n=2, IL-6 n=6, cholesterol n=1, triglycerides n=1, HDL n=1, LDL n=1, TNF-alpha n=6 hs-CRP n=2, and smoking n=4.

Table 3. Standardized Beta values of multivariate linear regression analysis for continuous dietary indices as predictors of LTL change from 2001-2004 to 2013-2014.

	Female			Male		
	n	Beta	95% CI	n	Beta	95% CI
Model 1	560			456		
BSDS		-0.02	-0.1 0.07		-0.05	-0.14 0.05
mMED		-0.07	-0.16 0.02		-0.09	-0.19 0.01
reversed DII		-0.02	-0.13 0.09		-0.11	-0.24 0.03
Model 2	587			451		
BSDS		-0.02	-0.09 0.04		-0.03	-0.10 0.04
mMED		-0.08	-0.15 -0.01		-0.04	-0.10 0.03
reversed DII		-0.04	-0.10 0.03		-0.05	-0.12 0.02

Model 1 adjusted for energy intake. Model 2 adjusted for energy intake, baseline LTL, age, BMI, smoking, leisure time physical activity, educational attainment, and follow-up time. BSDS, Baltic sea diet score; mMED, modified Mediterranean diet index; DII, dietary inflammatory index. Missing covariate data in women: educational attainment n=1, smoking 3; in men: educational attainment n=2, smoking n=4, leisure time physical activity n=2.

Figure 1. Mean (95% CI) dietary scores in 2001-04 in level of leucocyte telomere length after adjustment for age, smoking, energy intake, BMI, educational attainment, and leisure-time physical activity in women (n=587). BSDS, Baltic sea diet score; mMED, modified Mediterranean diet score; DII, dietary inflammatory index.

Figure 2. Mean (95% CI) dietary score in 2001-04 in quintiles of leucocyte telomere length after adjustment for age, smoking, energy intake, BMI, educational attainment, and leisure time physical activity in men (n=451). BSDS, Baltic sea diet score; mMED, modified Mediterranean diet score; DII, dietary inflammatory index.