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Chapter 3

Telomere length and mental well-being in elderly men from the Netherlands and Greece

Behavior Genetics

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Abstract

Telomeres, repetitive DNA sequences that promote chromosomal stability, have been related to different measures of mental well-being and self-rated health, but mainly in women during adulthood. We aimed to investigate whether accelerated telomere shortening is associated with poor mental well-being and poor self-rated health in community-dwelling elderly men. Leukocyte telomere length was measured using quantitative PCR in two different samples of 203 elderly men (mean age 78 years) from the Netherlands in 1993, and 123 elderly men (mean age 84 years) from Greece in 2000. We also obtained follow-up data in 2000 from 144 Dutch subjects, of whom 75 had paired telomere length data in 1993 and 2000. Mental well-being was conceptualized as dispositional optimism, depressive symptoms, cognitive functioning, and loneliness. Linear regression analyses were used to study the association between telomere length, measures of mental well-being, and self-rated health, while adjusting for potential confounders. In cross-sectional analyses, leukocyte telomere length was not associated with measures of mental well-being and self-rated health, neither in the Netherlands nor in Greece. Also, the rate of leukocyte telomere shortening (mean decrease: 0.28 kbp over 7 years) in the 75 Dutch participants with longitudinal data was not associated with changes in different measures of mental well-being and self-rated health. Thus, our results provide no support for a relationship between leukocyte telomere length and mental well-being in elderly community-dwelling men.

Introduction

Telomeres are repetitive DNA sequences that cap chromosomal ends and play a critical role in promoting chromosomal stability (Blackburn, 2001). Telomeres shorten with every cell division and, after reaching a critical length, eventually induce cellular senescence and apoptosis (Allsopp *et al.*, 1992). Therefore, the capacity of cellular replication is modulated by processes that either accelerate or counter telomere shortening; for instance through the elongating effect of the enzyme telomerase (Greider, 1996). Cellular environment also plays an important role in regulating telomere length. It has been shown that exposure to physical stressors –most convincingly shown for oxidative stress by reactive oxygen species– can hasten telomere shortening and lead to premature cell senescence (Gilley *et al.*, 2008; Richter & Von Zglinicki, 2007). Thus, telomere length reflects the cellular replicative history and the presence of many stressors at the cellular level. Besides replication timing, telomeres also participate in nuclear processes such as chromosome positioning in the nucleus, transcriptional repression, and heterochromatin formation (Taddei & Gasser, 2004). Therefore, telomeres have been considered markers of biological aging, age-related diseases, and mortality (Benetos *et al.*, 2001; Cawthon *et al.*, 2003; Epel *et al.*, 2009).

Several components of mental well-being decline with aging (Reiche *et al.*, 2004) and are associated with an excessive activation of different stress systems. It has been hypothesized that oxidative stress through free radicals may be involved in accelerated telomere shortening in case of poor mental health states (Hovatta *et al.*, 2010), although the detailed molecular mechanisms involved are still poorly understood. Environmental factors involved are stress, diet, smoking, substance abuse, and physical exercise (Nordfjall *et al.*, 2008; Valdes *et al.*, 2005). There is a rapidly growing literature on the link between poor mental well-being and telomere shortening. Several cross-sectional studies that included middle-aged (mean age ranging from 27 to 65) subjects documented a shorter mean telomere length in relation to different measures of poor mental well-being such as in case of unipolar and bipolar mood disorders (Damjanovic *et al.*, 2007; Simon *et al.*, 2006), dispositional pessimism (O'Donovan *et al.*, 2009), cognitive impairment and decline (Valdes *et al.*, 2010; Yaffe *et al.*, 2009), Alzheimer's disease (Honig *et al.*, 2006; Panossian *et al.*, 2003), and childhood trauma (Kananen *et al.*, 2010; Tyrka *et al.*, 2009). In addition, increased psychological stress and poor self-rated mental health were also been related to shorter telomeres (Epel *et al.*, 2004; Parks *et al.*, 2009). Studies among elderly subjects, however, did not find a clear association between telomere length with depressive symptoms or personality traits (i.e. type D personality) among 890 older patients with chronic heart failure (Huzen *et al.*, 2010). We are not aware of studies that prospectively studied changes in telomere length over time in relation to changes in measures of mental well-being, as prior studies had a case–control design, a cross-sectional design, or did not measure telomere length sequentially.

Although there is evidence of a relationship between telomere shortening and poor mental well-being during adulthood, it is unclear whether such an association persists in late life. We tested the hypothesis that shorter telomeres would be associated with poor well-being in data collected in the Netherlands and Greece.

Methods

Study sample

For the present study we used data from the Zutphen Elderly Study (the Netherlands) and the Cretan Elderly Study (Greece). The Zutphen Elderly Study consisted of 553 men in 1993, of whom 390 were willing to participate (response rate 71%), and 345 had a blood sample. However, only 203 buffy coats could be used for DNA isolation since 142 samples were not available for testing. These data were missing at random, and participants with unavailable DNA did not differ in any baseline characteristics from the participants included in the analyses. For the follow-up examination in 2000, 176 men enrolled in the study in Zutphen. Blood samples were available from 146 individuals. Because of the insufficient quality of the DNA of two of the participants, 144 men (82%) were finally included in the present analysis. Of these 144 Dutch men who were included in the analysis in 2000, 75 men had also a blood sample available in 1993. We only had data of the Cretan sample in 2000, and not in 1993. The Cretan sample consisted of 165 men in 2000. Of these men, 152 were willing to participate (response rate 92%) and blood samples were drawn from 129 individuals. In six men, the concentration of DNA was not sufficient to determine telomere length, yielding information on 123 Cretan men for subsequent analysis. The study was approved by local medical ethics committees.

Leukocyte telomere length measurement

Non-fasting blood samples were collected in the morning into citrate tubes. Buffy coats were obtained for subsequent DNA isolation. Samples from Zutphen were stored at -30°C before transport within a few days of collection to the National Institute for Public Health and Environment (RIVM), the Netherlands. Samples from Crete were firstly stored at -80°C and subsequently transported to the Netherlands on dry ice by plane. After arriving at the RIVM, all samples were stored at -80°C . The change in temperature storage did not affect the DNA quality, since DNA is stable at a temperature below -20°C (Kohler *et al.*, 1997). The DNA used for measurement of telomere length was extracted from a 200 μl buffy coat sample by a single person, within a 4-week period, using QIAamp DNA Mini Kit (Qiagen, Venlo, the Netherlands) according to the manufacturer's protocol. The DNA was quantified using a Nanodrop instrument (Isogen Life Science, Belgium). The samples from Crete and Zutphen were processed simultaneously.

Leukocyte telomere length was determined by quantitative polymerase chain reaction (qPCR) as described by Cawthon (Cawthon, 2002). The qPCR assay for the telomere length measurement was done within a 4-week period following the extraction of DNA. Two master mixes were prepared, one with telomere primers and one with Human β globin (HBG) primers (1x IQ SYBRgreen supermix from BioRad, CA, USA). To confirm reproducibility of the applied method, we repeated for 20 samples the leukocyte telomere length measurement by using an additional reference gene, acidic ribosomal phosphoprotein PO (36B4), 300 nM forward primer (5'-CAGCAAGTGGGAAGGTGTAATCC -3'), 500nM reverse primer (5'- CCCATTCTATCATCAACGGGTACAA -3'). The estimated R-square for the measurement with the extra reference gene was 0.76 ($p < 0.001$). This confirmed the reproducibility of the leukocyte telomere length measurement, with a coefficient of variation for the triplicates of the telomere reaction being 4.1% and for the reference gene was 3.0%. Sample DNA was pipetted into a 96-wells plate at a final concentration of 10 ng/ μl . Subsequently, 20 μl of the mastermix was added and the plate was briefly centrifuged. Each sample was run

in triplicates. The triplicates were located on the same qPCR-plate and for the reference gene the triplicates were located on another qPCR-plate but in the same positions. For the standard curve a reference DNA sample was diluted serially to produce three concentrations of 1.25, 5, and 10 ng/μl. In every run, negative controls (MQ + mastermix) and reference samples were included. The references were derived from two different HeLa cell lines, one with relatively short telomeres (HeLa S3: 5.5 kb) and one with long telomeres (HeLa 229: 14-15 kb). By adding reference DNA of controls to each qPCR, a standard curve could be created and the absolute telomere length of the samples could be calculated as kilo-base pairs (kbp). The matched 75 samples of the Zutphen Elderly Study were run in the same plate. HeLa cell lines were kindly provided by Prof. Alexander Bürkle, University of Konstanz, Germany. The quantitative polymerase chain reaction (qPCR) was performed using a BioRad MyiQ iCycler Single Color RT-PCR detection system using iQTM SYBR® Green Supermix, containing iTaq Polymerase, dNTPs, SYBRGreen I, and buffers (BioRad, CA, USA).

Measures of mental well-being

Depressive symptoms were assessed in both samples in 2000 using the 15-item version of the Geriatric Depression Scale (GDS-15). The GDS-15 is a validated instrument to screen for depressive symptomatology in older people as it excludes somatic symptoms that might also be related to physical illnesses (Yesavage *et al.*, 1982). The GDS-15 score ranges from 0 to 15 points. Depressive symptoms were additionally assessed in 1993 and 2000, only in Zutphen (The Netherlands), using the Dutch translation of the Zung self-rating depression scale (Zung SDS). The index score of the Zung SDS (i.e., [sumscore/80] x 100) varies from 25 to 100 points (Kitchell *et al.*, 1982; Okimoto *et al.*, 1982; Zung, 1965).

Dispositional optimism was assessed in all participants of both samples in 1993 and 2000 using a four-item questionnaire (Giltay *et al.*, 2006) consisting of the following statements – “I still expect much from life”, “I do not look forward to what lies ahead for me in the years to come”, “My days seem to be passing by slowly”, and “I am still full of plans” (our translations). Within the four score items, two negatively stated items required reversed coding. The additional answer category “do not know” was also coded as the midpoint (score 1). The optimism questionnaire score ranges from 0-8 points, higher scores being indicative of higher optimism levels. For the computation of the optimism score only one missing item per subject was allowed, being subsequently imputed with the mean of the remaining three items. Internal consistency was of moderate strength (Cronbach’s alpha was 0.63 in 2000).

Global cognitive function was tested in 1993 and 2000 only in the Zutphen sample (The Netherlands). The assessment was performed by a trained nurse or interviewer using the 30-point Mini-Mental State Examination test (MMSE) (Folstein *et al.*, 1975). This occurred in a protocolized way in which care was taken to prevent other persons (e.g. wife) to assist the participant during the MMSE testing.

Feelings of loneliness were assessed in both samples in 1993 and 2000 using the Loneliness Scale of De Jong Gierveld. The loneliness scale comprises 11 items, with responses coding on a 0-5 Likert scale. A sample questionnaire item is “I find my circle of acquaintances too limited”. The loneliness score ranges from 0 to 11 points (Scharf & De Jong-Gierveld, 2008). This scale has been shown to have good internal consistency (Cronbach’s alpha 0.82) (De Jong-Gierveld & Kamphuis, 1985).

Participants were asked to report their health status through a single-item question: “How would you describe your current health status”. There were five possible answers to this question, ranging from excellent to bad health.

All questionnaires were translated to the participant’s native language (Dutch or Greek).

Possible confounders

Sociodemographic, lifestyle, and morbidity parameters were obtained using protocolized structured questionnaires (Keys *et al.*, 1966). Living arrangement was dichotomized as living alone or with others; smoking status as smokers and non smokers; and alcohol consumption as users and non-users. Body mass index (BMI) was calculated by dividing body weight (kg) by the squared height (m) of the participant. Physical activity was assessed with a validated questionnaire designed for retired men to calculate the total minutes per week spent in different physical activities (i.e. walking, cycling, gardening, odd jobs, sports, hobbies, and work) (Caspersen *et al.*, 1991). Presence of chronic diseases, defined as having either cardiovascular diseases (i.e. stroke, myocardial infarction, or heart failure), diabetes mellitus or cancer, was determined by a survey questionnaire and confirmed with hospital discharge data and written information from the general practitioner.

Statistical analysis

Baseline and follow-up telomere length were normally distributed. As MMSE scores were negatively skewed (due to the ceiling effect of the maximum score of 30), 32 minus the score was log-transformed and inverted to normalize the distribution. Physical activity was positively skewed and logarithmically transformed before the analyses. Geometric means are presented with 10th and 90th percentiles [P10; P90]. Other data are presented as numbers and percentages for categorical variables, and means $\pm$ standard deviations, or medians with interquartile ranges for continuous variables (when appropriate). The decline in telomere length over time in the Zutphen sample was tested by t-test for paired samples.

Linear regression was used to analyze the cross-sectional and longitudinal associations of leukocyte telomere length with self-rated health, and measures of mental well-being (i.e. dispositional optimism, depressive symptoms, cognitive function, and loneliness). Multivariate models were applied to adjust for the effect of potential confounders. Firstly, we tested the association between leukocyte telomere length and mental well-being or self-rated health in a crude model. Subsequently, we adjusted for age. Finally, we additionally adjusted for the following covariates: living arrangement, smoking status, alcohol consumption, physical activity, chronic diseases, and BMI. Sensitivity analyses were conducted after excluding subjects with a history of cardiovascular disease, diabetes mellitus, or cancer.

An additional linear regression analysis was used to assess associations between changes in leukocyte telomere length and changes in measures of poor mental well-being and self-rated health. Again, multivariate models as described above were applied to adjust for the effect of potential baseline confounders.

All p-values are two-tailed and considered statistically significant at the level of $p < 0.05$. Data analyses were performed using SPSS for Windows, version 17.0 (Chicago, IL).

Results

Sociodemographic and clinical characteristics of both samples are summarized in Table 1. The mean age of men from Zutphen in 1993 was 78 years (range 72–91); whereas in 2000, the mean age of men from both Crete and Zutphen was 84 years old (range 79–98). In 2000, Cretan elderly men had longer telomeres (mean telomere length 5.0 kbp) than elderly men from Zutphen (mean telomere length 4.8 kbp; $p < 0.001$). Regarding mental well-being, Cretan elderly men were less optimistic and showed more depressive symptoms than elderly

men from Zutphen ($p < 0.001$). Although more Cretan elderly men rated their health status as poor, they were less likely to have chronic diseases compared to elderly men from Zutphen ($p < 0.001$). Finally, Cretan elderly men reported more physical activity than elderly men from Zutphen ($p < 0.001$).

Table 1. Baseline characteristics of elderly men from the Netherlands (Zutphen) in 1993 and 2000 and Crete (Greece) in 2000

Variables	Zutphen		Crete
	1993	2000	2000
Number of participants	203	144	123
Age (years), mean (range)	77.6 (72.0-91.0)	84.1 (79.5-98.2)	84.1 (79.2-93.4)
Telomere length mean $\pm$ SD	5.0 $\pm$ 0.5	4.8 $\pm$ 0.3	5.0 $\pm$ 0.5
GDS score, median (IQR)	-	2 (1-4)	3 (1-7)
Zung score, median (IQR)	42.5 (26.3-66.3)	46.3 (38.8-50.0)	-
Dispositional optimism, median (IQR)	6 (4-7)	5 (4-6)	4 (2-6)
MMSE, median (IQR)	27 (25-28)	28 (26-29)	-
Loneliness, median (IQR)	1 (0-3)	2 (0-5)	3 (1-5)
Living alone, (%)	45 (22.3%)	56 (38.9%)	23 (19.8%)
Current smoking, (%)	55 (29.1%)	14 (9.7%)	11 (8.9%)
Alcohol users, (%)	141 (76.2%)	110 (76.4%)	66 (55.5%)
Body Mass Index, mean $\pm$ SD	25.6 $\pm$ 2.6	25.6 $\pm$ 3.7	24.9 $\pm$ 4.2
Physical activity, (min/week), median (P10, P90)	513 (125; 1369)	210 (0; 835)	397 (0; 1440)
Chronic diseases, n(%)	59 (29.1%)	70 (48.6%)	29 (23.6%)
Self-rated health, n(%)			
• Excellent	22 (10.9%)	7 (4.9%)	9 (7.6%)
• Very good	93 (46.0%)	19 (13.3%)	13 (11.0%)
• Good	74 (36.6%)	86 (60.1%)	34 (28.8%)
• Poor	10 (5.0%)	29 (20.3%)	39 (33.1%)
• Bad	3 (1.5%)	2 (1.4%)	23 (19.5%)

Data are presented as n(%), mean ($\pm$ SD), or median (IQR) when appropriate.

† Chronic disease was defined as having prevalent cardiovascular disease (i.e. myocardial infarction, stroke, heart failure) cancer, or diabetes mellitus.

Table 2. Association of telomere length with measures of mental well-being and self-rated health in older men from Zutphen and Crete

Variables	Zutphen				Crete			
	No.	Beta	Test	P-value	No.	Beta	Test	P-value
Depressive symptoms (Zung SDS)								
• Crude association	171	-0.004	t(168)=-0.05	0.96	136	-0.039	t(133)=-0.46	0.65
• Model 1*	171	0.006	t(167)=0.07	0.94	136	-0.020	t(132)=-0.23	0.82
• Model 2†	167	0.023	t(155)=0.28	0.78	135	-0.077	t(123)=-0.90	0.37
Depressive symptoms (GDS_15)								
• Crude association	-	-	-	-	122	-0.099	t(119)=-1.09	0.28
• Model 1*	-	-	-	-	122	-0.051	t(118)=-0.54	0.59
• Model 2†	-	-	-	-	122	-0.071	t(112)=-0.77	0.44
Optimism score (4-item questionnaire)								
• Crude association	201	-0.025	t(198)=-0.35	0.73	142	-0.074	t(139)=-0.87	0.38
• Model 1*	187	-0.041	t(183)=-0.57	0.57	142	-0.148	t(138)=-1.82	0.07
• Model 2†	178	-0.018	t(168)=-0.24	0.81	141	-0.128	t(131)=-1.53	0.13
Cognitive function (MMSE)								
• Crude association	183	0.010	t(180)=0.14	0.89	137	-0.119	t(134)=-1.39	0.17
• Model 1*	183	-0.021	t(179)=-0.30	0.77	137	-0.144	t(133)=-1.66	0.10
• Model 2†	173	0.003	t(163)=0.03	0.97	136	-0.099	t(126)=-1.06	0.29
Loneliness (de Jong Gierveld)								
• Crude association	189	0.032	t(186)=0.44	0.66	140	-0.036	t(137)=-0.43	0.67
• Model 1*	179	0.088	t(175)=1.17	0.24	140	-0.038	t(136)=-1.44	0.66
• Model 2†	170	0.109	t(160)=1.43	0.16	139	-0.040	t(129)=-1.45	0.65
Self-rated health								
• Crude association	202	-0.002	t(199)=-0.03	0.98	143	-0.071	t(140)=-0.84	0.40
• Model 1*	188	0.024	t(184)=0.33	0.74	143	-0.066	t(139)=-0.77	0.44
• Model 2†	178	0.035	t(168)=0.46	0.64	142	-0.089	t(132)=-1.04	0.30

Results are obtained with linear regression analysis and presented as betas with P-values

* Adjusted for age.

† Additionally adjusted for living arrangement, alcohol consumption, physical activity, chronic diseases, smoking status, and BMI.

Table 3. Associations between changes in mental well-being and self-rated health and changes in telomere length in 75 older men from Zutphen from 1993 till 2000

Variables	Δ Telomere length		
	Beta	Test	P-value
Δ Depressive symptoms (Zung SDS)			
Crude association	-0.061	t(57)=-0.47	0.64
Model 1*	-0.058	t(56)=-0.45	0.66
Model 2†	-0.006	t(48)=-0.05	0.96
Δ Optimism score (4-item questionnaire)			
• Crude association	-0.013	t(71)=-0.11	0.92
• Model 1*	-0.008	t(68)=-0.07	0.95
• Model 2†	0.017	t(59)= 0.14	0.89
Δ Cognitive function (MMSE)			
• Crude association	0.084	t(64)= 0.68	0.50
• Model 1*	0.092	t(63)= 0.75	0.46
• Model 2†	0.074	t(54)= 0.55	0.58
Δ Loneliness (de Jong Gierveld)			
• Crude association	0.060	t(66)= 0.49	0.62
• Model 1*	0.070	t(63)= 0.56	0.58
• Model 2†	0.106	t(54)= 0.80	0.43
Δ Self-rated health			
• Crude association	0.022	t(70)= 0.18	0.86
• Model 1*	0.033	t(67)= 0.27	0.79
• Model 2†	0.122	t(58)= 0.96	0.34

Results are obtained with linear regression analysis and presented as betas with P-values

* Adjusted for age.

† Additionally adjusted for living arrangement, smoking status, alcohol consumption, physical activity, chronic diseases, and BMI.

Table 2 shows the results from the linear regression analyses for the elderly men from Zutphen and Crete. We found no statistically significant associations between leukocyte telomere length with self-rated health or any measure of mental well-being. In an additional analysis in men from Zutphen in 1993, we found no differences in telomere length among groups categorized according to quartiles of the MMSE (mean TL of 4.99 vs. 5.08 in lower versus upper quartile with $MMSE \leq 23$ and ≥ 29 points; $p=0.42$). Sensitivity analyses in elderly men free from chronic diseases confirmed the absence of any tendency towards an association between leukocyte telomere length and self-rated health or mental well-being (data not shown). Multivariate adjustments for potential confounding factors did not alter these results (i.e., Models 1 and 2).

Additional analysis of the paired DNA samples from 75 Dutch participants with data in 1993 and in 2000 showed that the mean telomere length in 1993 was 5.03 kbp, whereas the mean telomere length in 2000 was 4.76 kbp. The mean decline in leukocyte telomere length during 7 years of follow-up was 40.2 bp per year (95% confidence interval [CI]: 26.9-53.5; $p<0.001$), as previously described (Houben *et al.*, 2011). Although most individuals showed a decrease in telomere length, for 12 participants (16%) we found an increase. Pearson's correlation coefficient showed a moderately strong relationship between telomere length in 1993 and 2000 in 75 men ($r=0.51$; $p<0.001$). Results from the linear regression analysis showed no association between absolute changes in leukocyte telomere length and absolute changes in any measure of mental well-being or self-rated health over seven years of follow-up (Table 3). When we used relative changes of telomere length (TL in 2000 minus TL in 1993 divided by TL in 1993) instead of absolute changes (TL in 2000 minus TL in 1993) similar results were found.

Discussion

The current study examined the association between telomere length, mental well-being, and self-rated health in two samples of elderly men (over 70 years old) from two different European countries. In contrast to previous studies among younger adults (Simon *et al.*, 2006; O'Donovan *et al.*, 2009; Panossian *et al.*, 2003; Honig *et al.*, 2006; Tyrka *et al.*, 2009; Epel *et al.*, 2004), our results revealed no cross-sectional associations between leukocyte telomere length and measures of mental well-being or self-rated health, neither in the Netherlands nor in Greece. Furthermore, the longitudinal analysis of data from 75 men with measurements in 1993 and 2000 showed neither an association between the telomere shortening rate and changes in mental well-being or self-rated health.

Among the strengths of the present study is the analysis of a wide range of mental well-being variables in relation to telomere length. The longitudinal study design allowed us to identify the determinants of leukocyte telomere length change in a relatively large cohort of community-dwelling elderly subjects. Furthermore, we used the validated qPCR technique including shorter and longer sized standard samples to assess telomere length instead of the more time-consuming Southern Blot (Cawthon, 2002). Also noteworthy is the international design of this study. Our results were consistent in two different population-based samples of different origin and advanced age. Although we did not find statistically significant associations between telomere length and mental wellbeing, our findings suggest that the rate of telomere shortening varied across countries. The observed longer telomeres in Cretan men compared to men from Zutphen may partly be explained by different lifestyles. It is well-known that inhabitants from Mediterranean countries usually engage in healthier lifestyles than people from Northern European countries (Trichopoulou & Lagiou, 1997). In our study, participants from Crete spent more time in physical activities than participants from Zutphen. Furthermore, it is possible that the Mediterranean diet (more habitual in Crete) incorporates more antioxidants that may buffer telomere shortening through reduction of oxidative stress (Dai *et al.*, 2008; Buijsse *et al.*, 2007). However, several limitations should be considered in the interpretation of our results. First, the elderly men had all survived until old age, meaning that they were aging relatively well. Given that cell aging processes are considered to be related to life expectancy, it is possible that the advanced age of our sample hampered the detection of a relationship between telomere length and markers of mental well-being. A second explanation may be that our study was only composed of men, which limits the generalization of our results to women, while many of the previous studies with positive findings included only women (O'Donovan *et al.*, 2009; Valdes *et al.*, 2010; Epel *et al.*, 2004; Parks *et al.*, 2009), a majority of women (Tyrka *et al.*, 2009; Kananen *et al.*, 2010; Damjanovic *et al.*, 2007), or about 50% women (Simon *et al.*, 2006; Yaffe *et al.*, 2009). Third, associations may exist in clinical samples with neuropsychiatric diseases rather than epidemiological samples from the general population. Fourth, methodological error likely played a minor role, because the measurement method was rigorously tested and calibrated, and both telomere length measurements were in line with previously reported mean telomere length in this age range (Slagboom *et al.*, 1994). Yet, a limitation of our study was that telomere length was assessed in stored buffy coats, whereas it should preferably be measured in peripheral blood mononuclear cells (PBMCs) obtained from fresh buffy coats (Choi *et al.*, 2008). Moreover, the 4-item optimism questionnaire has not been validated yet and we relied on self-report measures rather than semi-structured psychiatric interviews for mental-wellbeing measures (except for the MMSE). Another limitation would be that we did

not measure telomerase activity. Finally, though our sample was relatively large compared to most of the earlier studies among older subjects, it is possible that type II error has arisen and hampered the detection of positive associations. Therefore, future studies in larger samples are needed to confirm our results.

Although our findings contrast with several previously reports, our findings are in line with a study among 890 patients with chronic heart failure (61% male, with a mean age of 73 years), which found no association between telomere length and self-reported depressive symptoms or the type D personality trait (Huzen *et al.*, 2010). Although a relationship seems to exist during adulthood, leukocyte telomere length may no longer be related to mental well-being in the oldest old subjects. There is also evidence that there is less telomere length variation in the healthy elderly subjects versus younger subjects (Halaschek-Wiener *et al.*, 2008). Moreover, there may be a higher degree of telomere length instability in older subjects, which may also explain our null findings (Harris *et al.*, 2006; Martin-Ruiz *et al.*, 2005). The instability of telomere length in the old age is thought to derive from an altered function of the enzyme telomerase, but this hypothesis is yet to be confirmed. In the current Dutch cohort, we previously found that telomere length did not predict mortality at old age (Houben *et al.*, 2011), similar to the subgroup of participants aged 75 years or older, in which telomere length was also no longer a significant predictor of mortality (Cawthon *et al.*, 2003).

Mental well-being is known to deteriorate in old age. Dispositional optimism declines with age (Giltay *et al.*, 2006), whereas self-reported depressive symptoms (Barefoot *et al.*, 2001; Davey *et al.*, 2004), cognitive function (Schonknecht *et al.*, 2005), and loneliness (Cohen-Mansfield *et al.*, 2009) increase over time. It has been suggested that psychosocial or biological stressors might act as mediators between telomere shortening and measures of poor mental well-being or self-rated health, for instance through increased oxidative stress on cellular level (Hovatta *et al.*, 2010; Tyrka *et al.*, 2009). Although some studies did find evidence of an association between higher levels of oxidative stress and measures of poor mental well-being including dementia and depression (Simon *et al.*, 2006; Damjanovic *et al.*, 2007; Valdes *et al.*, 2010; Yaffe *et al.*, 2009; Panossian *et al.*, 2003), the mechanism underlying the transduction of such stressors to oxidative stress on cellular level remains to be further elucidated.

In summary, our results provide no support for the relationship between leukocyte telomere length and mental well-being or self-rated health in elderly men.

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