Chapter 3

Telomere length as a marker of cellular ageing is associated with prevalence and progression of metabolic syndrome

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Abstract

**Context.** Metabolic Syndrome (MetS) clusters risk factors for age-related conditions including cardiovascular disease and diabetes. Shorter telomere length (TL), a cellular marker for biological age, may predict an individual's deteriorating metabolic condition.

**Objective.** We examined whether shorter baseline TL is associated with a worse metabolic profile and with less favorable trajectories of MetS components over a six-year follow-up.

**Design and setting.** Participants were part of the Netherlands Study of Depression and Anxiety, an ongoing prospective cohort study with six years follow-up.

**Participants.** This study included 2848 participants aged 18-65 years.

**Main outcome measures.** Baseline TL from leukocytes was determined using quantitative polymerase chain reaction, and MetS components (waist circumference, triglycerides, high-density lipoprotein (HDL) cholesterol, systolic blood pressure (SBP) and fasting glucose) were determined at baseline, and after two and six years. Cross-sectional and longitudinal analyses were adjusted for relevant sociodemographic, lifestyle and health factors.

**Results.** Shorter baseline TL was cross-sectionally associated with HDL ($\beta=-0.016$, $SE=0.008$, $p=.05$), waist circumference ($\beta=0.647$, $SE=0.238$, $p=.007$), triglycerides ($\beta=0.038$, $SE=0.009$, $p<.001$), and fasting glucose ($\beta=0.011$, $SE=0.003$, $p<.001$), as well as with the total number of MetS components ($\beta=0.075$, $SE=0.003$, $p=.001$) and the presence of MetS (OR=1.19, 95% CI=1.07-1.33; $p=.002$). Although baseline differences progressively reduced over time, shorter baseline TL was still significantly associated with unfavourable scores of most MetS components at the two- or six-year follow-ups.

**Conclusions.** Cellular aging, as assessed by TL, is associated with a higher metabolic risk profile which maintains to be unfavorable even after a period of six years. These findings suggest that cellular aging might play a role in the onset of various aging-related somatic diseases via its effect on metabolic alterations.

**Keywords.** Telomeres, metabolic syndrome, cellular aging, glucose, HDL cholesterol, blood pressure, abdominal obesity, triglycerides, longitudinal
1. Introduction

Metabolic Syndrome (MetS) is a constellation of interrelated factors (namely abdominal obesity, dyslipidemia including low HDL cholesterol and high triglycerides, hypertension and hyperglycemia), known to be major risk factors for the development of aging-related diseases, such as cardiovascular diseases (CVD) and diabetes. Telomere length (TL), as generally assessed in leukocytes, is a novel marker of cellular aging, and has been associated with increased risks of morbidity and mortality. Telomeres are DNA-protein complexes that cap chromosomal ends and promote chromosomal stability. Normal telomere maintenance requires the cellular enzyme telomerase that adds telomeric DNA, thus preserving TL and healthy cell function. During each cell division, DNA loses telomeric repeats with an estimated shortening rate of 25 base pairs per year, eventually causing replicative cell senescence. Further, accelerated shortening might be the consequence of increased exposure to oxidation and inflammatory mediators. Mean TL is therefore often used as cellular marker for biological age, with shorter telomeres indicating increased biological age. However, it is still largely unknown how telomere maintenance might influence disease processes.

Several studies have shown significant associations between shorter TL and dysregulated MetS components, although some studies did not confirm this. Previous research has often been performed with small samples (N<150), or were restricted to males or females, a somatic diseased population or a small age range. Above all, several studies did not adjust for important confounding factors, such as lifestyle and clinical factors, and have investigated only cross-sectional associations. To date only two studies showed a prospective association between TL and obesity-related measures and insulin, but not with the other MetS components. Furthermore, three studies also looked at TL and investigated the link with anthropometric properties measured earlier in life, and reported an inverse relation between TL at the time of measurement and weight gain throughout the reported period.

Whether TL predicts a wider range of metabolic alterations such as in the lipid, glucose or hypertension spectra over an extensive period of time is unknown. The present study examined the cross-sectional and longitudinal relationship between TL and MetS components in a large-scale cohort study, while adjusting for important time-varying confounders. We hypothesized that shorter TL at baseline is cross-sectionally associated with a worse metabolic profile and with less favorable trajectories of MetS components over a six-year follow-up.
2. Methods

2.1. Study sample

Participants were part of the Netherlands Study of Depression and Anxiety (NESDA), a large on-going longitudinal cohort study among 2981 adults (18–65 years); a detailed description of the study rationale, design and method is given elsewhere. Briefly, respondents were recruited between September 2004 and February 2007 from community, primary care, and specialized mental health care. Baseline data collection consisted of a medical examination, a blood draw, self-report questionnaires and a detailed interview. Participants were evaluated again at two-year (2006-2009) and six-year (2010-2013) follow-up visits. Of the entire cohort, 2842 subjects (95%) had complete data on TL and baseline MetS indicators, and were included in our cross-sectional and longitudinal analyses. Participants with available baseline data did not differ in sociodemographic characteristics with those not included in the current analyses. Of the selected participants, 2098 (74%) had complete data at the two-year follow-up, and 1802 (63%) at the six-year follow-up. The research protocol was approved by the ethical committee of participating universities and all respondents provided written informed consent.

2.2. Predictor

2.2.1. Telomere length

Telomere length (TL) was determined at baseline. Fasting blood was drawn from participants in the morning between 8:30 and 9:30 am. Peripheral blood mononuclear cells from all samples were isolated from whole blood using density-gradient centrifugation (with Ficoll-Paque PLUS) and stored in -80°C freezers. Subsequently, early 2012, TL was determined at the laboratory of Telomere Diagnostics, Inc. (TDx, Menlo Park, CA, USA), using quantitative polymerase chain reaction (qPCR) as described elsewhere. All qPCRs were carried out on a Roche Lightcycler 480 realtime PCR machine with 384-tube capacity (Roche Diagnostics Corporation, Indianapolis, IN). Telomere sequence copy number in each patient's sample (T) was compared to a single-copy gene copy number (S), relative to a reference sample. The resulting T/S ratio is proportional to mean TL. To control for inter-assay variability, eight control DNA samples were included in each run. In each batch, the T/S ratio of each control DNA was divided by the average T/S for the same DNA from 10 runs to obtain a normalizing factor. This was done for all eight control samples and the average normalizing factor for these samples was used to correct the participant DNA samples to obtain the final T/S ratio. The T/S ratio for each sample was measured twice. If the duplicate T/S value and the initial value varied by more than 7%, the sample was run a third time and the average of the two closest values was reported. The reliability of the assay was adequate: the included quality control DNA samples on each PCR run illustrated a small intra-assay
coefficient of variation (CV=5.1%), and the inter-assay CV was also sufficiently low (CV=4.6%), as well as for the telomere (CV=2.04%) and the single-gene assays (CV=1.58%) separately.

To compare T/S ratios to telomere restriction fragments (TRF) reported by other studies using Southern blot analysis, we used the conversion formula to convert T/S ratios to kilo base pairs: kilo base pairs = 3.274 + 2.413 x ((T/S-0.0545)/1.16), as previously described 31. A continuous measure of telomeric base pairs (per SD decrease; SD= 0.61 kilo base pairs) was used in analyses; TL was also divided into quartiles in specific analyses explore linearity in trends (TL lowest quartile: 3.85-5.04kbp, second quartile: 5.04-5.35kbp; third quartile: 5.35-5.78kbp; highest quartile: 5.78-7.39kbp).

2.3. Outcome

2.3.1. Metabolic syndrome components

Metabolic indicators were measured at baseline (Y0) and at two- (Y2) and six-year (Y6) follow-up. Waist circumference was measured with a measuring tape at the central point between the lowest front rib and the highest front point of the pelvis, over light clothing. HDL cholesterol, triglycerides, and glucose levels were determined from the fasting blood samples using routine standardized laboratorial methods. The continuous measures were adjusted for medication use based on the estimated effects of the medication. According to the standards of medical care in diabetes, the goal of antidiabetic medication should be to lower the fasting glucose level to <7.0 mmol/L 32. In agreement with these standards, for persons using antidiabetic medication (Y0 N=93; Y2 N=88; Y6 N=85) when glucose level was < 7.0 mmol/L, a value of 7.0 mmol/L was assigned. According to the average decline in triglycerides and increases in HDL cholesterol in fibrate trials 33, 0.10 mmol/L was subtracted from the HDL cholesterol level and 0.67 mmol/L was added to the triglyceride level of persons using fibrates (Y0 N=6; Y2 N=5; Y6 N=5). Since none of the participants was using nicotinic acid, no adjustments were needed for this. Systolic and diastolic blood pressure (SBP, DBP) were measured twice during supine rest on the right arm with the Omron M4-I, HEM 752A (Omron, Healthcare Europe BV, Hoofddorp, The Netherlands) and were averaged over the two measurements. For persons using antihypertensive medication (Y0 N=419; Y2 N=383; Y6 N=384), 10 mm Hg was added to the SBP and 5 mm Hg to the DBP according to the average decline in blood pressure in antihypertensive trials 34.

At baseline the presence of metabolic syndrome (MetS) was determined based on The US National Cholesterol Education Program–Adult Treatment Panel III guidelines, requiring the presence of three or more of the following criteria: 1) waist circumference ≥102 cm in men and ≥88 cm in women; 2) triglycerides ≥1.7 mmol/L or medication for hypertriglyceridemia; 3) high-density lipoprotein (HDL) cholesterol
<1.03 mmol/L in men and <1.30 mmol/L in women or medication for reduced HDL cholesterol; 4) blood pressure: systolic ≥130 and/or diastolic ≥85 mm Hg or antihypertensive medication; 5) fasting plasma glucose ≥5.6 mmol/L or antidiabetic medication ¹. We also used the number of MetS abnormalities as a summarizing variable that resembles the severity of MetS in subjects ³⁵.

2.4. Covariates

Covariates were assessed at baseline and two- and six-year follow-up. Sociodemographic factors included sex, age, and years of attained education. Lifestyle variables included alcohol consumption (no drinker, mild-moderate drinker 1–14 (women) / 1–21 drinks per week (men), heavy drinker >14 (women) / >21 (men) drinks per week ³⁶), smoking (never, former, current), and physical activity (International Physical Activity Questionnaire ³⁷, expressed in 1000 metabolic equivalent (MET) minutes in the past week). The presence of CVD or diabetes, for which medical treatment was received, was ascertained by self-report.

We considered high levels of distress and the presence of psychopathology as one of the underlying mechanisms that lead to shortened TL, as earlier research has pointed out that depression and anxiety disorders are associated both with shorter TL ³⁸ and with MetS ³⁵. As this is a cohort which over-recruited subjects diagnosed with depression and anxiety disorders, we had the opportunity to check whether the association between TL and MetS components was modified by psychopathology. The presence of psychiatric diagnosis was evaluated with the clinical interview including the lifetime version of the Composite Interview Diagnostic Instrument (CIDI version 2.1) according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria, administered by specially trained research staff.

2.5. Statistical analyses

Means (± standard deviation) and proportions of characteristics of the study sample were described at baseline, two- and six-year assessments. Due to skewed distributions, natural logarithm-transformations were used in analyses for triglycerides and glucose and estimated values were presented back-transformed in tables and figures. In cross-sectional analyses, fully adjusted linear and logistic regression models were used to examine associations between TL (per SD decrease) as a predictor with the five separate MetS components, the overall number of MetS abnormalities (treated as a measure of MetS severity), and the presence of MetS as outcomes.

Additional analyses were performed by testing TL-by-sex interaction terms, as females are often suggested to have longer TL than males ³⁹. Also, since this sample has an overrepresentation of psychiatric patients, the presence of diagnosed anxiety or depressive disorder was evaluated as a potential mediator or effect modifier by, respectively, adding psychopathology in the model and by testing TL-by-
psychopathology interaction terms. Furthermore, in order to explore whether associations were driven by present somatic disease status, patients with baseline CVD or diabetes were excluded in sensitivity analyses.

In longitudinal analyses, associations of baseline TL with changes in MetS components over the six year course were analyzed by generalized estimating equations (GEE) with an exchangeable correlation structure that takes into account the within-person correlations when examining multiple observations per subject and can handle missing subjects. In addition to TL and time variables, appropriate TL-by-year2 and TL-by-year6 interaction terms were included in the models to estimate rates of change in MetS components according to baseline TL at each follow-up point. From the same models, we derived the parameters of the association between baseline TL and MetS component at specific follow-up. All models were adjusted for time-varying covariates. All analyses were conducted using SPSS version 20.0 (IBM Corp., Armonk, NY, USA) and SAS (v. 9.1, SAS Institute, Inc., Cary, NC). Significance level was set at p<0.05, two-tailed.

3. Results

At baseline, the 2842 subjects were on average 41.9 years (SD=13.0), 66.4% was female and they had attained 12.2 years (SD=3.3) of education (Table 1). The average T/S ratio was 1.11 (SD=0.30), corresponding to an average TL of 5.46 kilo base pairs (SD=0.61). Of the subjects, 604 (21.3%) had MetS at baseline.

Overall, after two years (n=2098) and six years (n=1802), the levels of MetS components were only slightly deteriorated, but overall there were relatively less current smokers and heavy drinkers throughout the follow-up years. Shorter TL (per SD decrease) was cross-sectionally associated with a higher number of MetS abnormalities (β=0.075, SE=0.023, p=.001), higher waist circumference (β=0.647, SE=0.238, p=.007), triglycerides (β=0.038, SE=0.009, p<.001) and fasting glucose (β=0.011, SE=0.003, p<.001), and with lower HDL cholesterol (β=−0.016, SE=0.008, p=.05) (Table 2). However, systolic blood pressure was not associated with TL (p=.21). Furthermore, at baseline each SD decrease in TL was associated with a 1.19-fold higher odds (95%CI=1.07-1.33; p=.002) of having MetS. We have derived Figure 1 from this model in order to show that the probability of having MetS increased with shorter TL.
Table 1: Sample characteristics in subjects with complete data at baseline, two- or six-years follow-up

<table>
<thead>
<tr>
<th></th>
<th>Baseline (N=2842)</th>
<th>Year 2 (N=2098)</th>
<th>Year 6 (N=1802)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Telomere length</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T/S ratio, M (sd)</td>
<td>1.11 (0.30)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Base pairs, M (sd)</td>
<td>5464 (614)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yrs), M (sd)</td>
<td>41.9 (13.0)</td>
<td>44.5 (13.1)</td>
<td>48.5 (13.0)</td>
</tr>
<tr>
<td>Sex (female), %</td>
<td>66.4</td>
<td>64.1</td>
<td>64.5</td>
</tr>
<tr>
<td>Years of education, M (sd)</td>
<td>12.2 (3.3)</td>
<td>12.5 (3.3)</td>
<td>12.8 (3.3)</td>
</tr>
<tr>
<td><strong>Lifestyle and health factors</strong></td>
<td></td>
<td></td>
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<tr>
<td>Smoking, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>798 (28.1)</td>
<td>698 (33.3)</td>
<td>542 (30.1)</td>
</tr>
<tr>
<td>Former</td>
<td>940 (33.1)</td>
<td>728 (34.7)</td>
<td>752 (41.7)</td>
</tr>
<tr>
<td>Current</td>
<td>1104 (38.8)</td>
<td>667 (31.8)</td>
<td>506 (28.1)</td>
</tr>
<tr>
<td>Drinking, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-drinker</td>
<td>484 (17.0)</td>
<td>319 (15.2)</td>
<td>315 (17.5)</td>
</tr>
<tr>
<td>Mild-moderate drinker</td>
<td>1996 (70.2)</td>
<td>1493 (71.2)</td>
<td>1263 (70.1)</td>
</tr>
<tr>
<td>Heavy drinker</td>
<td>362 (12.7)</td>
<td>239 (11.4)</td>
<td>167 (9.3)</td>
</tr>
<tr>
<td>Physical activity (*1000 MET-min/wk), median (IQR)</td>
<td>2.8 (3.4)</td>
<td>3.1 (4.1)</td>
<td>2.9 (4.0)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>91 (3.2)</td>
<td>88 (4.2)</td>
<td>83 (4.6)</td>
</tr>
<tr>
<td>Cardiovascular disease, n (%)</td>
<td>119 (4.2)</td>
<td>92 (4.4)</td>
<td>96 (5.3)</td>
</tr>
<tr>
<td><strong>Metabolic Syndrome indicators</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist (cm), M (sd)</td>
<td>89.1 (14.0)</td>
<td>89.8 (14.0)</td>
<td>92.5 (13.8)</td>
</tr>
<tr>
<td>Triglycerides, M (sd) *</td>
<td>1.12 (1.68)</td>
<td>1.15 (1.68)</td>
<td>1.15 (1.69)</td>
</tr>
<tr>
<td>HDL cholesterol, M (sd)</td>
<td>1.62 (0.44)</td>
<td>1.54 (0.42)</td>
<td>1.55 (0.44)</td>
</tr>
<tr>
<td>Systolic blood pressure, M (sd)</td>
<td>135.9 (20.2)</td>
<td>134.4 (19.0)</td>
<td>135.3 (20.1)</td>
</tr>
<tr>
<td>Glucose, M (sd) *</td>
<td>5.12 (1.17)</td>
<td>5.28 (1.17)</td>
<td>5.49 (1.17)</td>
</tr>
<tr>
<td>Number of MetS abnormalities, median (IQR)</td>
<td>1.0 (2.0)</td>
<td>1.0 (3.0)</td>
<td>2.0 (2.0)</td>
</tr>
<tr>
<td>Metabolic syndrome (ATP-III), n (%) b</td>
<td>604 (21.3)</td>
<td>532 (25.4)</td>
<td>523 (29.0)</td>
</tr>
</tbody>
</table>

Footnote: M = Mean; sd = Standard deviation; MET= Metabolic Equivalent; IQR = Interquartile range; * natural logarithm-transformed factors presented back-transformed; Abnormalities defined as: 1) waist circumference>102 cm (men) and >88 cm (women); 2) triglycerides >1.7 mmol/L (150 mg/dL) or medication for hypertriglyceridemia; 3) high-density lipoprotein (HDL) cholesterol <1.03 mmol/L (40 mg/dL, men) and <1.30 mmol/L (50 mg/dL, women) or medication for reduced HDL cholesterol; 4) BP: systolic >130 and/or diastolic >85 mm Hg or antihypertensive; 5) fasting plasma glucose >5.6 mmol/L (100 mg/dL) or antidiabetic medication; b Metabolic syndrome defined as having ≥ 3 abnormalities; Metabolic syndrome defined as having ≥ 3 abnormalities.
Table 2: Cross-sectional associations (betas (standard errors)) between telomere length (TL, per standard deviation decrease) and continuous metabolic syndrome (MetS) components (N=2842)

<table>
<thead>
<tr>
<th>TL (per SD decrease) a</th>
<th>Waist (ln)</th>
<th>Triglycerides</th>
<th>HDL cholesterol</th>
<th>Systolic BP (ln)</th>
<th>Glucose</th>
<th>MetS abnormalities b</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL highest quartile c</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>0.075 (0.023)</td>
</tr>
<tr>
<td>3rd</td>
<td>-0.073 (0.642)</td>
<td>0.023 (0.025)</td>
<td>-0.023(0.021)</td>
<td>-0.829(0.931)</td>
<td>0.018 (0.008) *</td>
<td>0.081 (0.061)</td>
</tr>
<tr>
<td>2nd</td>
<td>0.785 (0.651)</td>
<td>0.073 (0.026)</td>
<td>-0.022(0.021)</td>
<td>-0.213(0.944)</td>
<td>0.029 (0.008) ***</td>
<td>0.150 (0.062)</td>
</tr>
<tr>
<td>Lowest</td>
<td>0.937 (0.670)</td>
<td>0.082 (0.027)</td>
<td>-0.031(0.022)</td>
<td>-1.796(0.972)</td>
<td>0.028 (0.008) ***</td>
<td>0.144 (0.064)</td>
</tr>
</tbody>
</table>

Footnote: Adjusted for age, sex, education, smoking, alcohol, physical activity; + p<.10; * p<.05; ** p<.01; *** p<.001; a Standard deviation of TL=614bp; b Abnormalities defined as: 1) waist circumference>102 cm (men) and >88 cm (women); 2) triglycerides >1.7 mmol/L (150 mg/dL) or medication for hypertriglyceridemia; 3) high-density lipoprotein (HDL) cholesterol <1.03 mmol/L (40 mg/dL, men) and <1.30 mmol/L (50 mg/dL, women) or medication for reduced HDL cholesterol; 4) BP: systolic >130 and/or diastolic >85 mm Hg or antihypertensive; 5) fasting plasma glucose >5.6 mmol/L (100 mg/dL) or antidiabetic medication; c LTL lowest quartile: 3.85-5.04kbp, second quartile: 5.04-5.35kbp; third quartile: 5.35-5.78kbp; highest quartile: 5.78-7.39kbp

In sensitivity analyses, no significant TL-by-sex interactions were found, indicating that associations between TL and MetS components were consistent for men and women. Moreover, additional adjustment for the presence of psychopathology did not substantially reduce the association between TL and MetS components and TL-by-psychopathology interaction terms subsequently entered in the models were non-significant, implying that the associations between TL and MetS components were not different when a subject was healthy or had a current or remitted anxiety or depressive disorder. Furthermore, the associations did not change when baseline CVD (N=119) and diabetes (N=91) patients were excluded from the analyses, illustrating that results were not due to the presence of overt clinical diseases. At last, the associations between TL and glucose, lipid and blood pressure components were not altered by incorporating medication information (antidiabetics, fibrates, antihypertensives) in the final analyses, as findings were very similar when we did not incorporate this information.

Longitudinal associations between baseline TL and changes in MetS components over the six-year follow-up period were examined using GEE models using all 2842 subjects and adjusting for time-varying covariates (Table 3). Overall, as illustrated by the significant time effects, an increase in waist circumference, triglycerides, glucose and number of MetS abnormalities, and a decrease in HDL cholesterol and SBP were observed during the follow-up period. When looking at TL-by-year2 interaction terms, we found significantly different rates of change at Year 2 within triglycerides, SBP and the number of MetS abnormalities. For the TL-by-year6 interaction terms, significantly different rates of change at Year 6 were found for waist circumference, triglycerides and fasting glucose. The negative coefficients of
these interaction terms show that shorter LTL was associated with a less steep increase of MetS components, suggesting that the differences found at baseline progressively reduced over the follow-up. However, shorter baseline TL was still associated at Year 2 with higher waist circumference ($\beta=0.530$, 95% CI: 0.041 – 1.018, $p=0.034$), glucose ($\beta=0.008$, 95% CI: 0.002 – 0.014, $p=0.009$) and lower SBP ($\beta=-0.998$, 95% CI: -1.670 – -0.326, $p=0.004$) and at year 6 with lower HDL cholesterol ($\beta=-0.026$, 95% CI: -0.043 – -0.009, $p=0.002$) and a higher number of MetS components ($\beta=0.054$, 95% CI: 0.000 – 0.107, $p=0.049$). These results are listed in Supplemental Table 1 for all components at the different time points.

In order to better illustrate the relationship between baseline TL and trajectories of MetS components over time, we plotted the estimated means of MetS components at each follow-up across different TL values from the GEE models (Figure 2). This figure clearly illustrated that – although differences diminished over time – persons with short baseline TL still had a worse level for many of the MetS components even after an extended period of time.

Table 3: Longitudinal associations (betas (standard error), time-associations and time-interactions) between telomere length (TL, per standard deviation decrease) and continuous metabolic syndrome (MetS) components (N=2842)

<table>
<thead>
<tr>
<th></th>
<th>Waist (ln)</th>
<th>Triglycerides</th>
<th>HDL cholesterol</th>
<th>Systolic BP (ln)</th>
<th>Glucose</th>
<th>MetS abnormalities&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>TL*</td>
<td>0.747 (0.234)</td>
<td>0.040 (0.009)</td>
<td>-0.015 (0.008)</td>
<td>-0.408 (0.334)</td>
<td>0.011 (0.003)</td>
<td>0.070 (0.022)</td>
</tr>
<tr>
<td>Year 2</td>
<td>0.814 (0.141)</td>
<td>0.042 (0.009)</td>
<td>-0.098 (0.006)</td>
<td>-2.408 (0.295)</td>
<td>0.030 (0.003)</td>
<td>0.098 (0.020)</td>
</tr>
<tr>
<td>Year 6</td>
<td>3.408 (0.183)</td>
<td>0.046 (0.010)</td>
<td>-0.083 (0.007)</td>
<td>-0.967 (0.341)</td>
<td>0.009 (0.005)</td>
<td>0.289 (0.025)</td>
</tr>
<tr>
<td>TL*Yr2</td>
<td>-0.217 (0.134)</td>
<td>-0.031 (0.009)</td>
<td>0.004 (0.006)</td>
<td>-0.590 (0.298)</td>
<td>-0.003 (0.003)</td>
<td>-0.038 (0.020)</td>
</tr>
<tr>
<td>TL*Yr6</td>
<td>-0.338 (0.168)</td>
<td>-0.030 (0.010)</td>
<td>-0.011 (0.007)</td>
<td>0.122 (0.330)</td>
<td>-0.006 (0.003)</td>
<td>-0.016 (0.024)</td>
</tr>
</tbody>
</table>

**Footnote:** Adjusted for time, baseline age, sex, education, smoking, alcohol, physical activity; + p<.10; * p<.05; ** p<.01; *** p<.001; <sup>a</sup>Standard deviation of TL=614bp; <sup>b</sup>Abnormalities defined as: 1) waist circumference >102 cm (men) and >88 cm (women); 2) triglycerides >1.7 mmol/L (150 mg/dL) or medication for hypertriglyceridemia; 3) high-density lipoprotein (HDL) cholesterol <1.03 mmol/L (40 mg/dL, men) and <1.30 mmol/L (50 mg/dL, women) or medication for reduced HDL cholesterol; 4) BP: systolic >130 and/or diastolic >85 mm Hg or antihypertensive; 5) fasting plasma glucose >5.6 mmol/L (100 mg/dL) or antidiabetic medication.
Figure 1: Cross-sectional association between Metabolic Syndrome at baseline and TL: -2 standard deviations (SD= 4.23 kbp), -1SD (=4.85 kbp), the mean (=5.46 kbp), +1SD (=6.08 kbp) and +2SD (=6.70 kbp). All analyses were adjusted for age, sex, education, smoking, alcohol, physical activity.

4. Discussion

This study has shown that shorter leukocyte telomere length is cross-sectionally associated with abdominal obesity, dyslipidemia, hyperglycemia, and the presence and severity of metabolic syndrome. Although baseline differences progressively reduced over time, shorter baseline TL was still significantly associated with unhealthy MetS state for most components throughout the six-year follow-up. This clearly illustrates that cellular aging is not just a short-term state marker of deteriorated metabolic health, but that short TL is likely reflective of an underlying mechanism illustrating chronic subsequent deterioration of a person's metabolic health.

Within the current study, we have found that cellular aging is associated with abdominal obesity and dyslipidemia (low HDL cholesterol and high triglycerides). Several previous cross-sectional studies have found shorter TL in subjects with a disadvantageous weight \(^9-13;18;24;41\) or lipid profile \(^14-18;24\), whereas few studies have not found these associations \(^5;19;21;22\).
Figure 2: Estimated means of the five MetS components and the number of MetS abnormalities at Y0, Y2 and Y6 (N=2842), calculated for telomere length (TL) -2 standard deviations (SD= 4.23 kbp), -1SD (=4.85 kbp), the mean (=5.46 kbp), +1SD (=6.08 kbp) and +2SD (=6.70 kbp). Betas, 95% CI and p-values of all the associations between TL (per SD decrease) and each of the components at the different time points are listed in Supplemental Table 1.
Shorter TL was also associated with higher fasting glucose levels, as seen in earlier cross-sectional studies \(^{15;24;41}\), whereas others have not found these associations \(^{5;12;19;21;22}\). The discrepancies between studies are probably attributable to differences in the study populations (age, clinical vs population-based) and in the assessment of metabolic parameters and TL. Our study adds to this growing literature by showing that TL is associated with a wide range of metabolic parameters and predicts less favorable trajectories of these factors over time in a large-scale cohort study, while adjusting for important time-varying confounders.

To date, only two studies have investigated the longitudinal association between TL \(^{23;25}\) and obesity-related measures. Njajou et al. have reported that shorter TL assessed at baseline was associated with smaller increases in the percentage change for both BMI and body fat between baseline and a seven-year follow-up in elderly subjects \(^{25}\). This is in line with the pattern that we found of a less steep increase of some MetS components over time for those with shorter baseline TL. This may be partly due to the fact that subjects with longer telomeres at baseline may have more room to worsen, determining therefore an apparently steeper rate of worsening (regression to the mean) during the follow-up period. The availability of repeated measurements of TL may yield a more precise picture of the longitudinal relationship with MetS components as it may also account for change in TL over time. Indeed, the study by Gardner et al. based on 50 participants with two assessments of TL, showed that increase in TL over time was associated with lower insulin resistance and lower BMI over 10 years \(^{23}\). Nevertheless, despite that baseline differences progressively reduced over time in the current study, participants with short TL still had more disadvantageous metabolic outcomes during the six-year follow-up compared to subjects with long TL. This is indeed what has been found also by the study of Njajou et al., as they reported that TL was still negatively associated with BMI and percentage total body fat after seven years \(^{25}\).

We did not find consistent patterns of association between TL and hypertension, in line with earlier research \(^{5;21;22}\). The finding of lower SBP at 2-year follow-up for shorter baseline TL is unclear. Since this association was found neither at baseline nor at 6-year follow-up, we may hypothesize that this was a spurious finding, or that we did not had reliable and sensitive enough assessments of blood pressure over time. Alternatively, blood pressure may reflect another pathway of metabolic abnormalities, as opposed to abnormalities of obesity-induced insulin resistance and dyslipidemia, as suggested by Melka et al. \(^{42}\).

Altogether, we confirmed that accelerated cellular aging is linked to MetS \(^{5;19}\), and with a higher number of MetS abnormalities, representing a more severe metabolic profile. Shortened telomeres could lead to a worsened metabolic state through various molecular mechanisms, such as increased inflammation, sympathetic nervous system activity, and oxidative damage \(^{9;31;41;43;44}\), conditions known to be
associated with the telomere attrition rate. When cells with short telomeres become senescent, for example, they release inflammatory cytokines, inducing insulin resistance and defective HDL cholesterol. Another mechanism could be that telomeric DNA damage leads to compromised mitochondrial functioning through dampened p53 expression, eventually causing less fatty acid oxidation and glucose utilization, and less protection against oxidative stress. Additionally, shortened telomeres are also associated with adipocyte hypertrophy, which in turn are found to be linked to poor glycemic and lipid control.

Some limitations of this study were the fact that we only determined TL at baseline, whereas repeated measurements would have yielded more information regarding the coherence of TL and the trajectory of MetS. Due to the observational nature of the current study, no conclusions on causation can be directly drawn. In addition, we did not measure the dynamics of the telomere maintenance system, since we did not measure activity of the telomerase enzyme. Previously, Epel et al. found that glucose was not associated directly with TL but with telomerase, which may have acted as a compensatory mechanism within cells with short telomeres. Even though the absence of telomerase has been shown to lead to progressive telomere shortening, it is still under debate how telomerase negative cells, or cells with very low levels of telomerase, can survive and replicate. For instance, a study in mice has shown that mutations in the genes encoding telomerase led to progressive shortening of telomeres. However, restoration of normal telomerase genotypes in offspring of affected individuals did not elongate inherited short telomeres immediately, suggesting that this maintenance system is more complex, and that even though humans and mice both share the same telomere sequence in their DNA (TTAGGG), the telomere – telomerase system does not seem identical in mice and humans. Furthermore, TL was assessed in leukocytes, as they are easily accessible and the results are comparable with the majority of other studies. Although there is an ongoing debate about the comparability of TL in different subtypes of leukocytes, leukocyte TL seems to correlate strongly with other body tissues. Strengths of this study were its large sample size and the detailed measurement of MetS, taking into account important medication that could influence the separate components. Furthermore, MetS components and all covariates were determined longitudinally, giving us the unique opportunity to examine the course over time and take into account changing lifestyle and clinical factors as well.

Future studies should attempt to shed more light on the complex relation between the telomere maintenance system and the MetS components by measuring TL at more time points, and applying an experimental design to test whether TL is a cause, a consequence or an epiphenomenon of age-related metabolic dysregulations. If lifestyle and externally modifiable factors are predicting telomere shortening, there might also be an opportunity to improve maintenance and even lengthen telomeres with behavioral interventions, such as diet, exercise and stress management.
strategies. We believe that the current findings provide an initial input for future research (especially including experimental and bench studies) aimed at testing whether TL may be one of the causal factors, among and in interactions with other biological processes, for age-related metabolic dysregulations. We also want to point out that aging, with or without metabolic dysregulations, is a complex phenomenon, and is accompanied by many biological changes. On the other hand, researchers should also weigh in mind that telomere maintenance is an important homeostasis system for the uncontrolled proliferation of tumor cells, demonstrating the fragile balance within this system.

To conclude, we showed that cellular aging, as assessed by TL, is associated with a higher metabolic risk profile and with less favorable trajectories of metabolic biomarkers over time. These findings suggest that TL might play a role in the onset of various aging-related somatic diseases via its effect on metabolic alterations. Understanding of the possible causal pathways between telomere shortening and metabolic alterations requires further research. If such mechanisms will be shown to be accessible to effective interventions, reduction of telomere attrition may become a target to prevent metabolic dysregulations and their deleterious effect on health.
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