Associations Between Cellular Aging Markers and Metabolic Syndrome: Findings From the CARDIA Study

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Background: Metabolic syndrome (MetS) is thought to promote biological aging, which might lead to cardiovascular and aging-related complications. This large-scale study investigated longitudinal relationships between MetS, its components, and cellular aging markers: leukocyte mitochondrial DNA copy number (mtDNAcn) and telomere length (TL).

Methods: We included 989 participants from the Coronary Artery Risk Development in Young Adults Study. MtDNAcn [study year (Y) 15, Y25] and TL (Y15, Y20, Y25) were measured via quantitative polymerase chain reaction. MetS components [waist circumference, triglycerides, high-density lipoprotein (HDL) cholesterol, systolic blood pressure, and fasting glucose] were determined (Y15, Y20, Y25). Generalized estimated equation and linear regression models, adjusting for sociodemographics and lifestyle, were used to examine associations between MetS and cellular aging at all time points, baseline MetS and 10-year changes in cellular aging, baseline cellular aging and 10-year changes in MetS, and 10-year changes in MetS and 10-year changes in cellular aging.

Results: MtDNAcn and TL were negatively associated with age [mtDNAcn unstandardized β (B) = −4.76; P < 0.001; TL B = −51.53; P < 0.001] and positively correlated (r = 0.152; P < 0.001). High triglycerides were associated with low mtDNAcn and low HDL cholesterol with short TL. Greater Y15 waist circumference (B = −7.23; P = 0.05), glucose (B = −13.29; P = 0.001), number of metabolic dysregulations (B = −7.72; P = 0.02), and MetS (B = −28.86; P = 0.006) predicted greater 10-year decrease in mtDNAcn but not TL. The 10-year increase in waist circumference was associated with 10-year telomere attrition (B = −27.61; P = 0.04).

Conclusions: Our longitudinal data showed that some metabolic dysregulations were associated with mtDNAcn and TL decreases, possibly contributing to accelerated cellular aging but not the converse. (J Clin Endocrinol Metab 103: 148–157, 2018)
Aging-related diseases, such as cardiovascular disease and diabetes, account for a large proportion of the incidences of all-disease morbidity and mortality in developed nations. Metabolic syndrome (MetS) is a precursor to cardiovascular disease and diabetes, receiving extensive attention as an early deterioration state before disease development in younger adults (1). MetS is defined as having at least three of the following risk factors: abdominal obesity, dyslipidemia [low high-density lipoprotein (HDL) cholesterol and high triglycerides], hypertension, and hyperglycemia (2). MetS components are suggested to play a role in accelerated biological aging, perhaps reinforcing the downward spiral toward aging-related diseases (3–5).

In the last several decades, telomere length (TL) has been suggested as an important marker for cellular aging (6). Telomeres are DNA–protein complexes that cap and stabilize chromosomal ends (6). During each somatic cell division, DNA loses telomeric repeats at an estimated rate of 22 to 41 bp per year (7), eventually causing replicative cell senescence or apoptosis (6). Normal telomere maintenance requires the cellular enzyme telomerase, which adds telomeric DNA, preserving healthy cell function (6). Approximately 64% to 70% of TL is explained by genetic factors (8, 9), although attrition is thought to be accelerated by exposure to oxidative stress, proinflammatory mediators, and endocrine and autonomic dysfunction (10, 11). Leukocyte TL has been associated with aging-related diseases and mortality (12, 13).

Another marker of cellular aging is the decrease in mitochondrial DNA copy number (mtDNAcn) (14). Mitochondria are cellular energy-generating organelles that play an important role in metabolic homeostasis, proliferation, differentiation, and apoptosis. Cells contain numerous mitochondria in their cytoplasm, each containing multiple copies of mitochondrial DNA (mtDNA) (15). With advancing age, mitochondria produce more reactive oxygen species (ROS) and accumulate mtDNA damage and mutations (16). The free radical theory of aging postulates that this process is bi-directional: increased ROS cause oxidative damage to mtDNA, which negatively affects mitochondrial function, leading to cellular dysfunction and senescence (17). MtDNAcn decline seems to be a valid marker of cellular aging, although the literature remains inconsistent. Some studies suggest that a higher mtDNAcn may be a marker of poor mitochondrial health or mitochondrial allostatic load (18), because the copy number might be increased to compensate for DNA damage or mitochondrial dysfunction (19). Nevertheless, TL and mtDNAcn are reported to be positively correlated (20), suggesting that telomeres and mitochondria are functionally linked and associated with the aging process (21). Moreover, lower mtDNAcn has been associated with health outcomes and mortality (14).

In cross-sectional studies, MetS and its components have been associated with short TL (22–24) and reduced mtDNAcn (25, 26). Longitudinal studies are scarce. Some studies showed that short baseline TL is associated with worse MetS outcomes at follow-up (22, 27) and, conversely that baseline MetS components predict shorter TL over time (3, 5). Few studies that repeatedly measured both MetS and TL reported that telomere attrition paralleled deterioration in obesity measures (5, 22). No longitudinal study has yet examined MetS components and mtDNAcn.

This large-scale study investigated the longitudinal relationships between MetS components and mtDNAcn and TL in the Coronary Artery Risk Development in Young Adults (CARDIA) study. We first examined whether MetS components were consistently associated with mtDNAcn and TL throughout a 10-year period. Then, we investigated whether baseline MetS would predict 10-year changes in cellular aging markers or, conversely, whether baseline cellular aging would predict 10-year changes in MetS. Finally, we correlated 10-year changes in MetS components with 10-year changes in mtDNAcn and TL. We hypothesized that a disadvantageous metabolic state at baseline would be associated with decreased mtDNAcn and shorter TL at follow-up and that larger metabolic deterioration would be accompanied by accelerated cellular aging.

Methods

Study sample

The sample population is from the CARDIA study. Details of study design, recruitment, and procedures have been published elsewhere (28). In short, in 1985 and 1986, CARDIA performed community-based recruitment of 5115 research study participants in Birmingham, Alabama, Chicago, Illinois, and Minneapolis, Minnesota and from the membership of a prepaid health care plan in Oakland, California. The study sample was balanced by race, sex, and education. Follow-up examinations were conducted in study year (Y) 2, Y3, Y7, Y10, Y15, Y20, and Y25. All participants signed consent forms at each examination, with all aspects reviewed and approved by the institutional review board of each participating institution. The current study reports data from a substudy in CARDIA Y15, Y20, and Y25, for which participants were selected if two conditions were satisfied: stored DNA from whole blood was available from Y15, Y20, and Y25 inclusive, and coronary artery calcification data from all years inclusive were also available. Within this substudy, TL was measured at Y15, Y20, and Y25, whereas mtDNAcn was measured at Y15 and Y25. Overall, we selected participants with complete data on the cellular aging measured at Y15 (n = 989). MetS had missing values on the different
components at Y15 (n = 1 to 46), Y20 (n = 0 to 60), and Y25 (n = 4 to 40).

**Blood draw and sample preparation**

Blood samples were drawn by venipuncture into using EDTA-containing tubes, according to a standard protocol (28), in the morning after an overnight fast (>8 hours). Participants were asked not to smoke or perform heavy physical activity for 2 hours before their examination visit. The blood was centrifuged, with aliquots stored at −70°C until shipped on dry ice to the genetics laboratory of Dr. Dornage at the University of Texas Health Sciences Center (Houston, TX). DNA for leukocyte TL was prepared with the Gentra Puregene Cell Kit (QIAGEN, Valencia, CA).

**TL**

DNA samples were shipped in 96-well plates to the University of California San Francisco Blackburn laboratory. All DNA samples were stored in their original plates in a −80°C freezer upon arrival. Of the original DNA samples, 5 µL was diluted with 10 µL of sterile Millipore H₂O to reach final concentration of 33.3 ng/µL. The diluted DNA samples were plated in 96-well plates and stored in a −80°C freezer. DNA samples were thawed on ice on the day of the assay and transferred to 384-well plates. TL measurement assay was adapted from the published original method (29). TL values were measured from DNA by a quantitative polymerase chain reaction (qPCR) assay that determines the ratio of telomere repeat copy number to single-copy gene copy number (T/S) in experimental samples as compared with a reference DNA sample. A higher T/S signifies a longer mean TL. T/S was calculated with the following formula: relative mtDNAcn/diploid genome = POWER[(CPND1-CPRnaseP)]². Interassay CV based on 85 CARDIA samples was 3.4%, and intra-assay CV based on four control DNA samples, averaged from all 21 CARDIA sample plates, was 1.7%.

**Mitochondrial DNA copy number**

Leukocyte mtDNAcn was measured by qPCR. A 69-bp fragment within the ND1 gene in mtDNA (nucleotides 3485 to 3553) and an 87-bp fragment within the RNase P gene in the nuclear DNA were simultaneously amplified by an adapted multiplex TaqMan-based qPCR reaction (30). This method was used to determine the relative number of mtDNA copies per diploid nuclear genome (i.e., per cell). The VIC-labeled probe and primer set for RNase P were obtained from Thermofisher Scientific (Waltham, MA) (catalog #4403328). The primer and probe sequences for ND1 (Integrated DNA Technologies, Coralville, IA) are as follows:

<table>
<thead>
<tr>
<th>Primer/Probe</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND1-forward</td>
<td>5’-CCCTAAAACCGCCACATCT-3’</td>
</tr>
<tr>
<td>ND1-reverse</td>
<td>5’-GGCGGCTGTTGCCGACTAATTG-3’</td>
</tr>
<tr>
<td>ND1-FAM probe</td>
<td>5’-FAM-CCCTACCCCTCTACTACA-CCGCC-TAMRA-3’</td>
</tr>
</tbody>
</table>

The reaction contained 12.5 ng of genomic DNA, 100 nM of ND1 probe, 300 nM of ND1-forward primer and ND1-reverse primer each, 1X RNase P copy number Reference Assay, and 1X LightCycler® 480 Probe Master (Roche, Indianapolis, IN) (catalog #04902343001) in a 10-µL reaction. All samples were run in triplicate wells in 384-well plates in a Roche LightCycler 480. PCR conditions were 95°C for 10 minutes, 45 cycles of 95°C for 10 seconds, 60°C for 30 seconds, and 72°C for 1 second, with data acquisition at 72°C. The crossing point (CP) for each well was derived from the LightCycler 480 program via the second derivation method. Relative copy number was calculated with the following formula: relative mtDNAcn/diploid genome = POWER[(CPND1-CPRnaseP)]². Interassay CV based on 85 CARDIA samples was 3.4%, and intra-assay CV based on four control DNA samples, averaged from all 21 CARDIA sample plates, was 1.7%.

**Metabolic syndrome components**

All five MetS components were measured at CARDIA Y15, Y20, and Y25. Waist circumference was measured as the average of two measures at a level midway between the lowest rib and the iliac crest. Seated blood pressure (BP) was measured after a 5-minute rest, taking an average of second and third readings of the first- and fifth-phase Korotkoff sounds. Fasting blood glucose was measured by Roche Modular P hexokinase method. Plasma lipids were measured at the University of Washington Northwest Lipid Research Clinic Laboratory (Seattle, WA). Triglycerides were measured by ultraviolet method and determined enzymatically on the Abbott Spectrum (with Hitachi 917-RIBuffer/4-chlorophenol/enzymes), and HDL cholesterol was measured by Trinder method and determined enzymatically after dextran sulfate–magnesium precipitation on the Abbott Spectrum.

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 (31). In agreement with these standards, for participants using antidiabetic medication (baseline n = 17, 5-year n = 44, 10-year n = 72) when glucose level was <7.0 mmol/L, a value of 7.0 mmol/L was assigned. For participants using antihypertensive medication (baseline n = 64; 5-year n = 161; 10-year n = 266), 10 mm Hg was added to the systolic BP according to the average decline in BP in antihypertensive trials (32).

Based on the recommendations of the National Cholesterol Education Program Expert Panel, MetS diagnosis was defined as having at least three of these dysregulations: abdominal obesity, or waist circumference ≥102 cm in men and ≥88 cm in women; hypertriglyceridemia, or triglycerides ≥1.7 mmol/L; or medication for hypertriglyceridemia; low HDL cholesterol, <1.03 mmol/L in men and <1.30 mmol/L in women; hypertension, or systolic BP ≥130, diastolic BP ≥85 mm Hg, or antihypertensive medication; hyperglycemia, or fasting plasma glucose ≥5.6 mmol/L or antidiabetic medication (33). Furthermore, we calculated a summarizing variable from the number of metabolic dysregulations (range 0 to 5), reflecting MetS severity (22).

**Covariates**

All covariates were recorded at the three time points. Socio-demographic factors included age, sex, race (white or black),
and educational achievement (less than high school or high school and college or postcollege education) and were based on standardized questionnaires. Lifestyle factors included smoking (never, former, or current), alcohol consumption (nondrinker; mild to moderate, defined as 1 to 14 drinks per week (women) or 1 to 21 (men); or heavy, defined as >14 drinks per week (women) or >21 (men)), and habitual physical activity as measured by the CARDIA Physical Activity History, a simplified version of the Minnesota Leisure Time Physical Activity Questionnaire (34).

Statistical analyses

Sample characteristics were described as percentages or means and SDs. For not-normally distributed factors, medians and interquartile ranges were calculated. Associations between MetS and cellular aging markers at the three time points were analyzed via generalized estimating equations (GEEs) with an exchangeable correlation structure, taking into account the within-person correlations at repeated measurements and handling missing observations (35). Separate GEE models were run with mtDNAcn (two time points) and with TL (three time points) as the outcomes. First, to examine the effects of age on the two cellular aging markers, only time-varying age was entered into the model. Then the MetS components, the number of metabolic dysregulations, and MetS diagnosis were entered as separate predictors, adjusted for all covariates: sex, race, and time-varying age, education, smoking, alcohol, and physical activity. To examine whether the associations between MetS and TL or mtDNAcn was driven by either sex or race, we tested interactions between each component with these factors. We completed 28 analyses (five MetS components, the number of metabolic dysregulations, and MetS diagnosis interactions times sex or race), and corrected for multiple testing by using the false discovery rate method (36).

Next, we calculated change scores of MetS components, mtDNAcn, and TL by subtracting baseline values from the 10-year follow-up for all participants with available data at both time points. We tested the associations between baseline mtDNAcn and TL and 10-year changes in MetS components, adjusting for sociodemographic and lifestyle factors and Y15 cellular aging values by using linear regression models. Conversely, we tested fully adjusted associations between baseline MetS and 10-year change in mtDNAcn and TL, adjusting for Y15 values of the cellular aging marker.

Lastly, we conducted linear regression analyses to examine the associations between 10-year changes in MetS components (per 1-SD increase) or the number of MetS dysregulations with 10-year changes in mtDNAcn and TL. Changes in MetS components and changes in the number of MetS dysregulations were entered into separate models as predictors, with 10-year mtDNAcn/TL as the outcome, adjusting for Y15 covariates, Y15 mtDNAcn or TL, and Y15 MetS. All analyses were conducted in SPSS version 20.0 (IBM Corp., Armonk, NY). Statistical significance level was set at P < 0.05, two-tailed.

Results

Table 1 shows the sample characteristics at CARDIA Y15, Y20, and Y25 (n = 989). Overall, more subjects met the criteria for MetS over time, increasing from 13% at baseline to 20% at the 10-year follow-up. Both cellular aging markers significantly decreased over time (P < 0.001) and were significantly associated with age in GEE analyses: mtDNAcn [unstandardized β (B) = −4.76; standard error (SE) = 0.40; P < 0.001] and TL (B = −51.53; SE = 1.18; P > 0.001). MtDNAcn and TL were correlated with their own follow-up measures (r > 0.45, all Ps < 0.001) and positively intercorrelated at baseline (r = 0.15; P < 0.001).

Table 2 shows that higher levels of triglycerides (P = 0.05) were consistently associated with lower mtDNAcn in GEE analyses and that lower levels of HDL cholesterol was associated with shorter TL (P = 0.04). No significant interactions were found between sex or race in the associations between MetS and cellular aging, after correction for multiple testing. These associations were consistent when we incorporated antidiabetic or antihypertensive medications as covariates within the models with glucose and BP, respectively, rather than adjusting the values for medication use (data not shown).

Next, we found that Y15 cellular aging markers did not predict changes in MetS (Table 3). Conversely, higher Y15 waist circumference (P = 0.05), glucose (P = 0.001), number of metabolic dysregulations (P = 0.02), and MetS diagnosis (P = 0.006) predicted larger 10-year decrease in mtDNAcn (Table 4). No significant associations were found between Y15 MetS components and 10-year TL change.

Finally, metabolic changes were not associated with changes in mtDNAcn, but an increase in waist circumference was associated with significant telomere attrition over the 10 years (P = 0.04, Table 5).

Discussion

This large-scale study investigated the longitudinal relationships between MetS components and mtDNAcn and TL throughout a 10-year period. First, mtDNAcn and TL were positively associated with each other and decreased with age. Next, at the three time points, higher triglycerides were associated with lower mtDNAcn, and lower HDL cholesterol was associated with shorter TL. Furthermore, large Y15 waist circumference, low HDL cholesterol, high glucose, and a high number of metabolic dysregulations were associated with a larger 10-year decrease in mtDNAcn, whereas they did not predict the 10-year telomere attrition. Conversely, Y15 cellular aging markers did not predict metabolic deterioration. Lastly, a 10-year increase in waist circumference was associated with 10-year telomere attrition, but none of the other changing MetS components changed in parallel with 10-year changes in cellular aging markers.

Mean TL showed a slight average increase of 17 bp per year from baseline to the 5-year follow-up and a larger
decrease by the 10-year follow-up, with an average decrease of 142 bp per year. The slight increase from baseline to the 5-year follow-up is puzzling because most longitudinal telomere studies show TL shortening on average, with some between-person variation (7), including a large portion of subjects who have stable

### Table 1. Sample Characteristics at All Time Points of Subjects With Complete Data on TL and mtDNAcn at Baseline (n = 989)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Year 15</th>
<th>Year 20</th>
<th>Year 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y, M (SD)</td>
<td>40.5 (3.6)</td>
<td>45.4 (3.6)</td>
<td>50.4 (3.6)</td>
</tr>
<tr>
<td>Sex, % female</td>
<td>65.5</td>
<td>65.5</td>
<td>65.5</td>
</tr>
<tr>
<td>Race, % black</td>
<td>40.8</td>
<td>40.8</td>
<td>40.8</td>
</tr>
<tr>
<td>Education completed, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school</td>
<td>18.8</td>
<td>21.3</td>
<td>20.0</td>
</tr>
<tr>
<td>High school and college</td>
<td>60.4</td>
<td>56.4</td>
<td>55.1</td>
</tr>
<tr>
<td>At least some postcollege education</td>
<td>20.7</td>
<td>22.4</td>
<td>24.9</td>
</tr>
<tr>
<td>Lifestyle factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>62.2</td>
<td>63.0</td>
<td>63.1</td>
</tr>
<tr>
<td>Former</td>
<td>18.7</td>
<td>21.3</td>
<td>22.9</td>
</tr>
<tr>
<td>Current</td>
<td>19.0</td>
<td>15.7</td>
<td>14.0</td>
</tr>
<tr>
<td>Drinking, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nondrinker</td>
<td>27.5</td>
<td>24.7</td>
<td>23.0</td>
</tr>
<tr>
<td>Mild to moderate drinker</td>
<td>63.2</td>
<td>65.1</td>
<td>66.1</td>
</tr>
<tr>
<td>Heavy drinker</td>
<td>9.3</td>
<td>10.2</td>
<td>10.9</td>
</tr>
<tr>
<td>Physical activity (total intensity score), M (SD)</td>
<td>336 (273)</td>
<td>335 (275)</td>
<td>335 (274)</td>
</tr>
<tr>
<td>Cellular aging markers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TL, bp, M (SD)</td>
<td>5594 (481)</td>
<td>5679 (451)</td>
<td>4971 (276)</td>
</tr>
<tr>
<td>mtDNAcn, M (SD)</td>
<td>513 (141)</td>
<td>459 (124)</td>
<td></td>
</tr>
<tr>
<td>MetS components</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist circumference, cm, M (SD)</td>
<td>88.4 (13.8)</td>
<td>91.1 (14.5)</td>
<td>93.3 (14.8)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L, median (IQR)</td>
<td>0.94 (0.67)</td>
<td>1.01 (0.79)</td>
<td>1.05 (0.70)</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L, M (SD)</td>
<td>1.32 (0.36)</td>
<td>1.41 (0.43)</td>
<td>1.53 (0.46)</td>
</tr>
<tr>
<td>Systolic BP, mm Hg, M (SD)</td>
<td>111.9 (13.7)</td>
<td>115.4 (14.6)</td>
<td>117.9 (15.4)</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L, median (IQR)</td>
<td>2.34 (0.31)</td>
<td>2.48 (0.33)</td>
<td>2.41 (0.36)</td>
</tr>
<tr>
<td>Number of metabolic dysregulations, M (SD)</td>
<td>1.10 (1.12)</td>
<td>1.27 (1.25)</td>
<td>1.35 (1.28)</td>
</tr>
<tr>
<td>MetS diagnosis, %b</td>
<td>12.7</td>
<td>18.2</td>
<td>20.2</td>
</tr>
</tbody>
</table>

Abbreviations: IQR, interquartile range; M, mean.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>mtDNAcn, Y15 and Y25</th>
<th>TL, Y15, Y20, and Y25</th>
</tr>
</thead>
<tbody>
<tr>
<td>MetS Components, Y15, Y20, and Y25 (per SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist circumference (per 13.8 cm)</td>
<td>−1.54 2.57 0.67</td>
<td>−13.40 11.63 0.25</td>
</tr>
<tr>
<td>Triglycerides (per 1.21 mmol/L)</td>
<td>−4.35 2.20 0.05</td>
<td>4.70 8.92 0.60</td>
</tr>
<tr>
<td>HDL cholesterol (per 0.36 mmol/L)</td>
<td>4.25 3.65 0.25</td>
<td>23.28 11.51 0.04</td>
</tr>
<tr>
<td>Systolic BP (per 13.7 mm Hg)</td>
<td>−0.16 3.20 0.96</td>
<td>18.61 10.74 0.08</td>
</tr>
<tr>
<td>Fasting glucose (per 0.72 mmol/L)</td>
<td>−2.28 4.55 0.62</td>
<td>9.66 11.15 0.39</td>
</tr>
<tr>
<td>Number of metabolic dysregulationsa</td>
<td>−1.88 2.64 0.48</td>
<td>2.90 8.77 0.74</td>
</tr>
<tr>
<td>MetS diagnosisb</td>
<td>1.54 8.37 0.85</td>
<td>44.47 27.11 0.10</td>
</tr>
</tbody>
</table>

Adjusted for sex and time-varying age, education, race, smoking, alcohol, and physical activity.

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*Metabolic dysregulations defined as waist circumference >102 cm (men) or >88 cm (women); triglycerides >1.7 mmol/L; HDL cholesterol <1.03 mmol/L (men) or <1.30 mmol/L (women); systolic BP >130 or diastolic BP >85 mm Hg or antihypertensives; fasting plasma glucose >6.1 mmol/L or antidiabetic medication.

b*MetS defined as ≥3 dysregulations.
lengths or TL lengthening (37, 38). Additionally, the current study’s 10-year shortening rate is higher than
the averages reported in the systematic review by Muezzinler et al. (7). Most previous longitudinal studies in this review
had only two TL measurements, although one study with 165 participants had three time points (39). McCracken
et al. (39) reported a small (2%) decrease in TL after a 3-year follow-up and a much larger decrease (19%) after a
6-year follow-up. Regardless of the variations observed in TL rates of change in previous studies, we have no ex-
planation for the slight increase observed in our study. The assay was performed with DNA from all three time
points from the same participants, always assayed in the same batch to eliminate batch differences. Yet we
cannot rule out preassay factors that could have affected the assay systematically. Whole blood samples were
collected via the same protocol for Y15, Y20, and Y25, and DNA was extracted with the same kit, but different
lots were used for blood collection tubes and DNA kits. It remains possible that small changes in collection pro-
cedures, reagent lots, or length of time each sample was in storage before assay completion affected the final TL
values. However, we note that the TL values showed the expected relationships at each time point. Specifically, the
associations between age, sex, and race with TL were similar at each time point, as were the TL intercorrelations at each time point, supporting the validity of the values. In the end, it remains unclear why subjects showed different TL change patterns between Y15, Y20, and Y25, but perhaps this finding reflects the nature of TL as a dynamic and fluctuating feature throughout the lifespan (40, 41) or preassay factors that we could not control for. Future studies with more repeated measurements are needed, taking preanalytical and analytical factors into account, to address this question in a more systematic fashion.

MitDNAcn decreased during the 10-year follow-up, with a yearly decrease of approximately five mtDNA copies, similar to the rate seen in other studies (14, 42). Although mtDNAcn decline seems to be a valid marker for cellular aging, the literature is inconsistent. The number of mtDNA copies per cell is shown to decrease with age in various cell types, such as blood leukocytes (14), human pancreatic cells (43), fibroblasts (44), and skeletal muscle cells (45), but not all studies have confirmed these age-related mtDNAcn decreases (46, 47).

Table 3. Linear Regressions With Baseline mtDNAcn and TL Predicting 10-Year Changes in MetS Components (n = 989)

<table>
<thead>
<tr>
<th>Baseline Cellular Aging (1 SD Decrease)</th>
<th>10-y △ Waist Circumference</th>
<th>10-y △ Triglycerides</th>
<th>10-y △ HDL Cholesterol</th>
<th>10-y △ Systolic BP</th>
<th>10-y △ Fasting Glucose</th>
<th>10-y △ Number of Dysregulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>SE</td>
<td>P</td>
<td>B</td>
<td>SE</td>
<td>P</td>
<td>B</td>
</tr>
<tr>
<td>MtdNAcn (per 141 1)</td>
<td>0.327</td>
<td>0.245</td>
<td>0.18</td>
<td>−0.023</td>
<td>0.022</td>
<td>0.30</td>
</tr>
<tr>
<td>TL (per 481 1)</td>
<td>−0.304</td>
<td>0.231</td>
<td>0.19</td>
<td>−0.036</td>
<td>0.021</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Adjusted for age, sex, education, race, smoking, alcohol, and physical activity.

Table 4. Linear Regressions With Baseline MetS Components Predicting 10-Year Changes in mtDNAcn and TL (n = 989)

<table>
<thead>
<tr>
<th>Baseline Predictors (per SD Increase)</th>
<th>10-y △ mtDNAcn</th>
<th>10-y △ TL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>SE</td>
</tr>
<tr>
<td>Waist circumference (per 13.8 cm)</td>
<td>−7.23</td>
<td>3.71</td>
</tr>
<tr>
<td>Triglycerides (per 1.21 mmol/L)</td>
<td>−4.02</td>
<td>3.50</td>
</tr>
<tr>
<td>HDL cholesterol (per 0.36 mmol/L)</td>
<td>7.18</td>
<td>3.83</td>
</tr>
<tr>
<td>Systolic BP (per 13.7 mm Hg)</td>
<td>−2.23</td>
<td>3.68</td>
</tr>
<tr>
<td>Fasting glucose (per 0.72 mmol/L)</td>
<td>−13.29</td>
<td>3.98</td>
</tr>
<tr>
<td>Number of metabolic dysregulations*</td>
<td>−7.72</td>
<td>3.19</td>
</tr>
<tr>
<td>MetS diagnosis**</td>
<td>−28.86</td>
<td>10.41</td>
</tr>
</tbody>
</table>

Adjusted for age, sex, education, race, smoking, alcohol, physical activity, and baseline cellular aging marker.

*Metabolic dysregulations defined as waist circumference >102 cm (men) or >88 cm (women); triglycerides >1.7 mmol/L; HDL cholesterol <1.03 mmol/L (men) or <1.30 mmol/L (women); systolic BP >130 or diastolic BP >85 mm Hg or antihypertensives; fasting glucose >6.1 mmol/L or antidiabetic medication.

**MetS defined as ≥3 dysregulations.
One study also found that the decline in mtDNAcn starts at ~48 years of age (14). In the current study, we show that metabolic dysregulations and increasing age were associated with low mtDNAcn in peripheral blood mononuclear cells (PBMCs) and that mtDNAcn was positively correlated with the more established cell aging marker TL.

Overall, only higher triglycerides were consistently associated with decreased mtDNAcn, and only lower HDL cholesterol was associated with shorter TL. Our results do not fully confirm the associations reported in earlier studies between the other MetS components and mtDNAcn (25, 26, 48) or TL (22–24, 49). Also, we did not find that baseline MetS components predicted 10-year TL attrition, as seen before (5), although our finding is in line with some earlier studies that did not observe this association with MetS components (3), body weight (38), or insulin resistance (50). Verhulst et al. (50) found that shorter TL predicted greater insulin resistance 12 years later, which was not an outcome we included here. It is unclear why these findings differ. We previously showed that subjects with a disadvantageous metabolic profile at baseline had shorter TL over time but not a faster attrition rate, possibly because of a strong homeostatic mechanism (3). However, we found that greater baseline waist circumference, higher glucose, and greater MetS severity did predict the 10-year decline of mtDNAcn.

Finally, we confirmed earlier studies that found that an increase in waist circumference parallels telomere attrition (3, 5). Perhaps TL is a marker of the current metabolic state, whereas mtDNAcn responds with long-term changes. Future studies should be designed to investigate the complex relationship between these two cellular aging markers.

It remains hard to explain why some MetS components are associated with accelerated cellular aging in certain studies but not in others. The most robust association is the one between the abdominal obesity and lipid components and cellular aging. According to the adipocyte overflow hypothesis, enlarged adipocytes reach their fat storage capacities, causing an “overflow” of fatty acids into sites such as the liver and muscle (51). These fatty acids not only promote deterioration of other MetS components (e.g., insulin resistance) but also increase systemic inflammation and oxidative stress, both catalysts of telomeric attrition (51–53). This “metabolic oversupply” in cells is also shown to fragment mitochondria, increasing ROS production and promoting the accumulation of mtDNA damage, whereas an undersupply is shown to promote mitochondrial fusion and limit mtDNA damage (54). Therefore, maintenance of metabolic balance appears to be important to preserve mitochondrial function, and excessive mitochondrial damage may contribute to the deteriorating effects of MetS. The association between fasting glucose and mtDNAcn is also very interesting given the strong dose–response relationship between fasting glycemia and all-cause mortality (55) and the damaging effects of hyperglycemia on mtDNA (54). Measurements of mitochondrial integrity and function, such as mtDNA damage and respiratory chain function, would be informative to probe the functional significance of mtDNAcn changes in the current study and to elucidate the role of mitochondria in MetS and cell aging.

The present findings should be interpreted with some caution, and the following limitations should be taken into account. TL and mtDNAcn were measured with qPCR in cellular homogenates, and although this is a widely used and cost-efficient high-throughput technique, one might argue that mean bulk TL does not capture subpopulations of cells with abnormally short telomeres (56) and that various mistakes can occur in measuring the mitochondrial genome to nuclear genome ratio (57). Thus, qPCR may not capture biologically meaningful changes over time in comparison with other methods (56). Also, both mtDNAcn and TL are measured

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**Table 5. Linear Regressions With 10-Year Changes in MetS Predicting 10-Year Changes in mtDNAcn and TL (n = 989)**

<table>
<thead>
<tr>
<th>10-y Δ Predictors (per SD Change)</th>
<th>10-y Δ mtDNAcn</th>
<th>10-y Δ TL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist circumference (+13.8 cm)</td>
<td>-5.63 (6.58) 0.39</td>
<td>-27.61 (13.71) 0.04</td>
</tr>
<tr>
<td>Triglycerides (+1.21 mmol/L)</td>
<td>-4.67 (6.27) 0.46</td>
<td>-8.50 (13.08) 0.52</td>
</tr>
<tr>
<td>HDL cholesterol (+0.36 mmol/L)</td>
<td>-3.74 (4.24) 0.38</td>
<td>3.38 (8.82) 0.70</td>
</tr>
<tr>
<td>Systolic BP (+13.7 mm Hg)</td>
<td>-4.64 (3.48) 0.18</td>
<td>-2.48 (7.26) 0.73</td>
</tr>
<tr>
<td>Fasting glucose (+0.72 mmol/L)</td>
<td>-0.38 (2.50) 0.88</td>
<td>-7.10 (5.27) 0.18</td>
</tr>
<tr>
<td>Number of metabolic dysregulationsa</td>
<td>-1.27 (3.53) 0.72</td>
<td>-7.15 (7.37) 0.33</td>
</tr>
</tbody>
</table>

---

*aMetabolic dysregulations defined as waist circumference >102 cm (men) or >88 cm (women); triglycerides >1.7 mmol/L; HDL cholesterol <1.03 mmol/L (men) or <1.30 mmol/L (women); systolic BP >130 or diastolic >85 mm Hg or antihypertensives; fasting plasma glucose >6.1 mmol/L or antidiabetic medication.

*Adjusted for age, sex, education, race, smoking, alcohol, physical activity, baseline predictor, and baseline cellular aging marker.
in leukocytes, because they are easily accessible and the results are comparable with those of most other studies. For TL, high correlations have been observed between various tissues and leukocytes (58), but these correlations have not yet been examined for mtDNA. Methodologically, one potential explanation for the lack of consistent results with mtDNAcn across studies could lie in differences in the biological material examined, including whole blood, purified total leukocytes, PBMCs, and granulocytes. This is because cellular composition, particularly that of platelets (which contain abundant mtDNA but no nuclear DNA) in whole blood or platelet contamination in isolated PBMCs, may cause or mask apparent differences in mtDNAcn (59). To enable interpretation of findings across studies in this developing field, detailed isolation methods or lack thereof should be reported. Lastly, telomerase was not assessed in this study. Had we measured telomerase, more information would have been available about the telomeric homeostasis system (60). Nevertheless, this study not only looked at two cellular aging markers simultaneously but also described a 10-year follow-up in ~1000 participants. Other major strengths of this study were the extensive measurement of MetS components, medication use, and relevant sociodemographic and lifestyle factors.

Overall, this large-scale longitudinal investigation showed that both mtDNAcn and TL decreased over a 10-year period, consistent with their use as proxies for cellular aging in human population studies. Although baseline MetS did not predict TL attrition, it did predict greater decline in leukocyte mtDNAcn, an effect apparently driven mostly by abdominal obesity and fasting glucose. Reversely, baseline cellular aging did not predict metabolic deteriorations, suggesting that metabolic stress promotes cellular aging but not the converse. When we looked at the parallel deterioration of metabolic dysregulations and cellular aging, increased abdominal obesity was associated with accelerated telomere attrition. Overall, these findings provide the basis for future mechanistic studies aimed at elucidating the effect of metabolic stress on cellular aging and the interplay between mitochondria and telomeres in humans. Ultimately, resolving these mechanisms and identifying modifiers of this relationship should help us discover targets in the prevention or delay of aging-related morbidities and health impairments.

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Disclosure Summary: The authors have nothing to disclose.

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