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

Dóra Révész,[1,2] Josine E. Verhoeven,[1] Martin Picard,[3] Jue Lin,[4] Stephen Sidney,[5] Elissa S. Epel,[6] Brenda W. J. H. Penninx,[1] and Eli Puterman[6,7]

[1]Department of Psychiatry, VU University Medical Center, Amsterdam Public Health Research Institute, 1081 BT Amsterdam, The Netherlands; [2]Department of Epidemiology, GROW School for Oncology and Developmental Biology, Maastricht University, 6200 MD Maastricht, The Netherlands; [3]Division of Behavioral Medicine, Department of Psychiatry, Department of Neurology and CTNI, College of Physicians and Surgeons, Columbia University Medical Center, New York, New York 10032; [4]Department of Biochemistry and Biophysics, University of California San Francisco, San Francisco, California 94158; [5]Kaiser Permanente Division of Research, Oakland, California 94612; [6]Department of Psychiatry, University of California San Francisco School of Medicine, San Francisco, California 94143; and [7]School of Kinesiology, University of British Columbia, Vancouver, British Columbia V6T 1Z1, Canada

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)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received 19 July 2017. Accepted 13 October 2017.
First Published Online 18 October 2017

Abbreviations: B, unstandardized β; BP, blood pressure; CARDIA, Coronary Artery Risk Development in Young Adults; CP, crossing point; CV, coefficient of variation; GEE, generalized estimating equations; HDL, high-density lipoprotein; MetS, metabolic syndrome; mtDNA, mitochondrial DNA; mtDNAcn, mitochondrial DNA copy number; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; ROS, reactive oxygen species; SD, standard deviation; SE, standard error; TL, telomere length; T/S, ratio of telomere repeat copy number to single-copy gene copy number; Y, study year.

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 bidirectional: 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, Y5, 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. Fornage 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 H_2O 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 converted into base pairs with the following formula: bp = 3274 + 2413 × (T/S). The interassay coefficient of variation (CV) was 3.5% [standard deviation (SD) = 2.1%].

Primers for the telomere polymerase chain reaction (PCR) (T runs) are tel1b [5′-CGGTTT(GTTTGG)5GTT-3′], used at a final concentration of 100 nM, and tel2b [5′-GGCTTG(CCTTAC)5CCT-3′], used at a final concentration of 900 nM. The primers for the single-copy gene (human β-globin) PCR (S runs) are hbg1 (5′ GCTTCTGACACAACTGTGTTCACTAGC-3′), used at a final concentration of 300 nM, and hbg2 (5′-CACCAACTTCATCCACGTTCACC-3′), used at a final concentration of 700 nM.

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:

ND1-forward 5′-CCCTAAAACCCGCCACATCT-3′
ND1-reverse 5′GAGCGATGGTGAGAGCTAAGGT-3′
ND1-FAM probe 5′ FAM-CCATCACCCTCTACATCACCGCCC-TAMRA-3′

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[2,(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-R1Buffer/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 or medication for low HDL cholesterol; 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. Sociodemographic 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.

Last, 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 were 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

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

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

Abbreviations: IQR, interquartile range; M, mean.

[a]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.

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 2. Associations With GEE Analyses Between MetS Components (3 Measurements), mtDNAcn (2 Measurements), and TL (3 Measurements) (n = 989)

	mtDNAcn, Y15 and Y25			TL, Y15, Y20, and Y25		
MetS Components, Y15, Y20, and Y25 (per SD)	B	SE	*P*	B	SE	*P*
Waist circumference (per 13.8 cm)	−1.54	3.57	0.67	−13.40	11.63	0.25
Triglycerides (per 1.21 mmol/L)	−4.35	2.20	0.05	4.70	8.92	0.60
HDL cholesterol (per 0.36 mmol/L)	4.25	3.65	0.25	23.28	11.51	0.04
Systolic BP (per 13.7 mm Hg)	−0.16	3.20	0.96	18.61	10.74	0.08
Fasting glucose (per 0.72 mmol/L)	−2.28	4.55	0.62	9.66	11.15	0.39
Number of metabolic dysregulations[a]	−1.88	2.64	0.48	2.90	8.77	0.74
MetS diagnosis[b]	1.54	8.37	0.85	44.47	27.11	0.10

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

[a]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.

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

Baseline Cellular Aging (1 SD Decrease)	10-y Δ Waist Circumference			10-y Δ Triglycerides			10-y Δ HDL Cholesterol			10-y Δ Systolic BP			10-y Δ Fasting Glucose			10-y Δ Number of Dysregulations[a]		
	B	SE	P	B	SE	P	B	SE	P	B	SE	P	B	SE	P	B	SE	P
MtDNA (per 141 ↓)	0.327	0.245	0.18	−0.023	0.022	0.30	0.017	0.010	0.09	0.452	0.460	0.33	−0.037	0.036	0.31	−0.006	0.033	0.86
TL (per 481 ↓)	−0.304	0.231	0.19	−0.036	0.021	0.09	−0.003	0.009	0.74	−0.147	0.437	0.74	−0.013	0.034	0.70	−0.015	0.031	0.64

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

[a]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.

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 Müezzinler *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 explanation 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 procedures, 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.

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

Baseline Predictors (per SD Increase)	10-y Δ mtDNAcn			10-y Δ TL		
	B	SE	P	B	SE	P
Waist circumference (per 13.8 cm)	−7.23	3.71	0.05	−3.02	7.77	0.70
Triglycerides (per 1.21 mmol/L)	−4.02	3.50	0.25	−1.27	7.27	0.86
HDL cholesterol (per 0.36 mmol/L)	7.18	3.83	0.06	3.58	7.99	0.65
Systolic BP (per 13.7 mm Hg)	−2.23	3.68	0.54	3.45	7.67	0.65
Fasting glucose (per 0.72 mmol/L)	−13.29	3.98	0.001	−1.33	8.34	0.87
Number of metabolic dysregulations[a]	−7.72	3.19	0.02	−0.07	6.66	0.99
MetS diagnosis[b]	−28.86	10.41	0.006	−0.81	21.79	0.97

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

[a]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 >85 mm Hg or antihypertensives; fasting plasma glucose >6.1 mmol/L or antidiabetic medication.

[b]MetS defined as ≥3 dysregulations.

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

	10-y Δ mtDNAcn			10-y Δ TL		
10-y Δ Predictors (per SD Change)	**B**	**SE**	***P***	**B**	**SE**	***P***
Waist circumference (+13.8 cm)	−5.63	6.58	0.39	−27.61	13.71	0.04
Triglycerides (+1.21 mmol/L)	−4.67	6.27	0.46	−8.50	13.08	0.52
HDL cholesterol (+0.36 mmol/L)	−3.74	4.24	0.38	3.38	8.82	0.70
Systolic BP (+13.7 mm Hg)	−4.64	3.48	0.18	−2.48	7.26	0.73
Fasting glucose (+0.72 mmol/L)	−0.38	2.50	0.88	−7.10	5.27	0.18
Number of metabolic dysregulations[a]	−1.27	3.53	0.72	−7.15	7.37	0.33

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

[a]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 >85 mm Hg or antihypertensives; fasting plasma glucose >6.1 mmol/L or antidiabetic medication.

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 more 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

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.

Acknowledgments

Financial Support: The work of B.W.J.H.P., D.R., and J.E.V. was supported by a Netherlands Organisation for Scientific Research Vici grant (number 91811602). Cell aging assays were supported by grants to E.P. and E.S.E. by the John & Catherine MacArthur Foundation Research Network on Socioeconomic Status and Health and to E.P. by the National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health under award number K99/R00 HL 109247. This research was also supported in part by the Canada Research Chairs program to E.P. CARDIA is conducted and supported by the NHLBI in collaboration with the University of Alabama at Birmingham (HHSN268201300025C and HHSN268201300026C), Northwestern University (HHSN268201300027C), the University of Minnesota (HHSN268201300028C), the Kaiser Foundation Research Institute (HHSN268201300029C), and the Johns Hopkins University School of Medicine (HHSN268200900041C). CARDIA is also partially supported by the Intramural Research Program of the National Institute on Aging (NIA) and an intra-agency agreement between NIA and NHLBI (AG0005). This manuscript has been reviewed by CARDIA for scientific content.

Correspondence and Reprint Requests: Dóra Révész, PhD, GROW School for Oncology and Developmental Biology, Maastricht University Medical Centre, P.O. Box 616, 6200 MD Maastricht, The Netherlands. E-mail: dora.revesz@maastrichtuniversity.nl.

Disclosure Summary: The authors have nothing to disclose.

References

1. Gami AS, Witt BJ, Howard DE, Erwin PJ, Gami LA, Somers VK, Montori VM. Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. *J Am Coll Cardiol*. 2007;**49**(4):403–414.
2. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC, Jr, Spertus JA, Costa F. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. *Curr Opin Cardiol*. 2006; **21**(1):1–6.
3. Révész D, Milaneschi Y, Verhoeven JE, Lin J, Penninx BW. Longitudinal associations between metabolic syndrome components and telomere shortening. *J Clin Endocrinol Metab*. 2015;**100**(8): 3050–3059.
4. Morley JE. Diabetes and aging: epidemiologic overview. *Clin Geriatr Med*. 2008;**24**(3):395–405, v.
5. Huzen J, Wong LSM, van Veldhuisen DJ, Samani NJ, Zwinderman AH, Codd V, Cawthon RM, Benus GF, van der Horst IC, Navis G, Bakker SJ, Gansevoort RT, de Jong PE, Hillege HL, van Gilst WH, de Boer RA, van der Harst P. Telomere length loss due to smoking and metabolic traits. *J Intern Med*. 2014;**275**(2):155–163.
6. Blackburn EH. Switching and signaling at the telomere. *Cell*. 2001; **106**(6):661–673.
7. Müezzinler A, Zaineddin AK, Brenner H. A systematic review of leukocyte telomere length and age in adults. *Ageing Res Rev*. 2013; **12**(2):509–519.
8. Broer L, Codd V, Nyholt DR, Deelen J, Mangino M, Willemsen G, Albrecht E, Amin N, Beekman M, de Geus EJ, Henders A, Nelson CP, Steves CJ, Wright MJ, de Craen AJ, Isaacs A, Matthews M, Moayyeri A, Montgomery GW, Oostra BA, Vink JM, Spector TD, Slagboom PE, Martin NG, Samani NJ, van Duijn CM, Boomsma DI. Meta-analysis of telomere length in 19,713 subjects reveals high heritability, stronger maternal inheritance and a paternal age effect. *Eur J Hum Genet*. 2013;**21**(10):1163–1168.
9. Hjelmborg JB, Dalgård C, Möller S, Steenstrup T, Kimura M, Christensen K, Kyvik KO, Aviv A. The heritability of leucocyte telomere length dynamics. *J Med Genet*. 2015;**52**(5):297–302.

10. Révész D, Verhoeven JE, Milaneschi Y, de Geus EJ, Wolkowitz OM, Penninx BW. Dysregulated physiological stress systems and accelerated cellular aging. *Neurobiol Aging*. 2014;**35**(6):1422–1430.
11. Perseguini NM, Verlengia R, Milan JC, Minatel V, Rehder-Santos P, Takahashi AC, Santana-Lemos BA, Calado RT, Ferreira Filho P, Porta A, Catai AM. Cardiac autonomic modulation, C-reactive protein or telomere length: which of these variables has greater importance to aging? *Int J Cardiol*. 2015;**178**:79–81.
12. Haycock PC, Heydon EE, Kaptoge S, Butterworth AS, Thompson A, Willeit P. Leucocyte telomere length and risk of cardiovascular disease: systematic review and meta-analysis. *BMJ*. 2014;**349**:g4227.
13. Rode L, Nordestgaard BG, Bojesen SE. Peripheral blood leukocyte telomere length and mortality among 64,637 individuals from the general population. *J Natl Cancer Inst*. 2015;**107**(6):djv074.
14. Mengel-From J, Thinggaard M, Dalgård C, Kyvik KO, Christensen K, Christiansen L. Mitochondrial DNA copy number in peripheral blood cells declines with age and is associated with general health among elderly. *Hum Genet*. 2014;**133**(9):1149–1159.
15. Gilkerson R, Bravo L, Garcia I, Gaytan N, Herrera A, Maldonado A, Quintanilla B. The mitochondrial nucleoid: integrating mitochondrial DNA into cellular homeostasis. *Cold Spring Harb Perspect Biol*. 2013;**5**(5):a011080.
16. Meissner C, Bruse P, Oehmichen M. Tissue-specific deletion patterns of the mitochondrial genome with advancing age. *Exp Gerontol*. 2006;**41**(5):518–524.
17. Picard M. Pathways to aging: the mitochondrion at the intersection of biological and psychosocial sciences. *J Aging Res*. 2011;**2011**:814096.
18. Picard M, Juster RP, McEwen BS. Mitochondrial allostatic load puts the "gluc" back in glucocorticoids. *Nat Rev Endocrinol*. 2014;**10**(5):303–310.
19. Yu-Wai-Man P, Sitarz KS, Samuels DC, Griffiths PG, Reeve AK, Bindoff LA, Horvath R, Chinnery PF. OPA1 mutations cause cytochrome c oxidase deficiency due to loss of wild-type mtDNA molecules. *Hum Mol Genet*. 2010;**19**(15):3043–3052.
20. Tyrka AR, Carpenter LL, Kao HT, Porton B, Philip NS, Ridout SJ, Ridout KK, Price LH. Association of telomere length and mitochondrial DNA copy number in a community sample of healthy adults. *Exp Gerontol*. 2015;**66**:17–20.
21. Sahin E, Colla S, Liesa M, Moslehi J, Müller FL, Guo M, Cooper M, Kotton D, Fabian AJ, Walkey C, Maser RS, Tonon G, Foerster F, Xiong R, Wang YA, Shukla SA, Jaskelioff M, Martin ES, Heffernan TP, Protopopov A, Ivanova E, Mahoney JE, Kost-Alimova M, Perry SR, Bronson R, Liao R, Mulligan R, Shirihai OS, Chin L, DePinho RA. Telomere dysfunction induces metabolic and mitochondrial compromise. *Nature*. 2011;**470**(7334):359–365.
22. Révész D, Milaneschi Y, Verhoeven JE, Penninx BW. Telomere length as a marker of cellular aging is associated with prevalence and progression of metabolic syndrome. *J Clin Endocrinol Metab*. 2014;**99**(12):4607–4615.
23. Monickaraj F, Gokulakrishnan K, Prabu P, Sathishkumar C, Anjana RM, Rajkumar JS, Mohan V, Balasubramanyam M. Convergence of adipocyte hypertrophy, telomere shortening and hypoadiponectinemia in obese subjects and in patients with type 2 diabetes. *Clin Biochem*. 2012;**45**(16-17):1432–1438.
24. Zhao J, Zhu Y, Uppal K, Tran VT, Yu T, Lin J, Matsuguchi T, Blackburn E, Jones D, Lee ET, Howard BV. Metabolic profiles of biological aging in American Indians: the Strong Heart Family Study. *Aging (Albany NY)*. 2014;**6**(3):176–186.
25. Lee JY, Lee DC, Im JA, Lee JW. Mitochondrial DNA copy number in peripheral blood is independently associated with visceral fat accumulation in healthy young adults. *Int J Endocrinol*. 2014;**2014**:586017.
26. Huang CH, Su SL, Hsieh MC, Cheng WL, Chang CC, Wu HL, Kuo CL, Lin TT, Liu CS. Depleted leukocyte mitochondrial DNA copy number in metabolic syndrome. *J Atheroscler Thromb*. 2011;**18**(10):867–873.
27. Njajou OT, Cawthon RM, Blackburn EH, Harris TB, Li R, Sanders JL, Newman AB, Nalls M, Cummings SR, Hsueh WC. Shorter telomeres are associated with obesity and weight gain in the elderly. *Int J Obes*. 2012;**36**(9):1176–1179.
28. Friedman GD, Cutter GR, Donahue RP, Hughes GH, Hulley SB, Jacobs DR, Jr, Liu K, Savage PJ. CARDIA: study design, recruitment, and some characteristics of the examined subjects. *J Clin Epidemiol*. 1988;**41**(11):1105–1116.
29. Cawthon RM. Telomere measurement by quantitative PCR. *Nucleic Acids Res*. 2002;**30**(10):e47.
30. Krishnan KJ, Bender A, Taylor RW, Turnbull DM. A multiplex real-time PCR method to detect and quantify mitochondrial DNA deletions in individual cells. *Anal Biochem*. 2007;**370**(1):127–129.
31. American Diabetes Association. Standards of medical care in diabetes–2013. *Diabetes Care*. 2013;**36**(suppl 1):S11–S66.
32. Mancia G, Parati G. Office compared with ambulatory blood pressure in assessing response to antihypertensive treatment: a meta-analysis. *J Hypertens*. 2004;**22**(3):435–445.
33. National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation*; 2002;**106**(25):3143–3421.
34. Schmitz KH, Jacobs DRJ, Jr, Leon AS, Schreiner PJ, Sternfeld B. Physical activity and body weight: associations over ten years in the CARDIA study. Coronary Artery Risk Development in Young Adults. *Int J Obes Relat Metab Disord*. 2000;**24**(11):1475–1487.
35. Twisk JWR. Longitudinal data analysis. A comparison between generalized estimating equations and random coefficient analysis. *Eur J Epidemiol*. 2004;**19**(8):769–776.
36. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Series B Stat Methodol*. 1995;**57**(1):289–300.
37. Révész D, Milaneschi Y, Terpstra EM, Penninx BW. Baseline biopsychosocial determinants of telomere length and 6-year attrition rate. *Psychoneuroendocrinology*. 2016;**67**:153–162.
38. Weischer M, Bojesen SE, Nordestgaard BG. Telomere shortening unrelated to smoking, body weight, physical activity, and alcohol intake: 4,576 general population individuals with repeat measurements 10 years apart. *PLoS Genet*. 2014;**10**(3):e1004191.
39. McCracken J, Baccarelli A, Hoxha M, Dioni L, Melly S, Coull B, Suh H, Vokonas P, Schwartz J. Annual ambient black carbon associated with shorter telomeres in elderly men: Veterans Affairs Normative Aging Study. *Environ Health Perspect*. 2010;**118**(11):1564–1570.
40. Svenson U, Nordfjäll K, Baird D, Roger L, Osterman P, Hellenius ML, Roos G. Blood cell telomere length is a dynamic feature. *PLoS One*. 2011;**6**(6):e21485.
41. Verhoeven JE, Lin J, Révész D, Wolkowitz OM, Penninx BW. Unresolved issues in longitudinal telomere length research: response to Susser et al. *Am J Psychiatry*. 2016;**173**(11):1147–1149.
42. Kim JH, Kim HK, Ko JH, Bang H, Lee DC. The relationship between leukocyte mitochondrial DNA copy number and telomere length in community-dwelling elderly women. *PLoS One*. 2013;**8**(6):e67227.
43. Cree LM, Patel SK, Pyle A, Lynn S, Turnbull DM, Chinnery PF, Walker M. Age-related decline in mitochondrial DNA copy number in isolated human pancreatic islets. *Diabetologia*. 2008;**51**(8):1440–1443.
44. Laderman KA, Penny JR, Mazzucchelli F, Bresolin N, Scarlato G, Attardi G. Aging-dependent functional alterations of mitochondrial DNA (mtDNA) from human fibroblasts transferred into mtDNA-less cells. *J Biol Chem*. 1996;**271**(27):15891–15897.

45. Short KR, Bigelow ML, Kahl J, Singh R, Coenen-Schimke J, Raghavakaimal S, Nair KS. Decline in skeletal muscle mitochondrial function with aging in humans. *Proc Natl Acad Sci USA*. 2005; **102**(15):5618–5623.
46. Frahm T, Mohamed SA, Bruse P, Gemünd C, Oehmichen M, Meissner C. Lack of age-related increase of mitochondrial DNA amount in brain, skeletal muscle and human heart. *Mech Ageing Dev*. 2005;**126**(11):1192–1200.
47. He Y, Tang J, Li Z, Li H, Liao Y, Tang Y, Tan L, Chen J, Xia K, Chen X. Leukocyte mitochondrial DNA copy number in blood is not associated with major depressive disorder in young adults. *PLoS One*. 2014;**9**(5):e96869.
48. Nisoli E, Clementi E, Carruba MO, Moncada S. Defective mitochondrial biogenesis: a hallmark of the high cardiovascular risk in the metabolic syndrome? *Circ Res*. 2007;**100**(6):795–806.
49. Harte AL, da Silva NF, Miller MA, et al. Telomere length attrition, a marker of biological senescence, is inversely correlated with triglycerides and cholesterol in South Asian males with type 2 diabetes mellitus. *Exp Diabetes Res*. 2012;**2012**:895185.
50. Verhulst S, Dalgård C, Labat C, Kark JD, Kimura M, Christensen K, Toupance S, Aviv A, Kyvik KO, Benetos A. A short leucocyte telomere length is associated with development of insulin resistance. *Diabetologia*. 2016;**59**(6):1258–1265.
51. Sniderman AD, Bhopal R, Prabhakaran D, Sarrafzadegan N, Tchernof A. Why might South Asians be so susceptible to central obesity and its atherogenic consequences? The adipose tissue overflow hypothesis. *Int J Epidemiol*. 2007;**36**(1):220–225.
52. Fontana L, Eagon JC, Trujillo ME, Scherer PE, Klein S. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. *Diabetes*. 2007;**56**(4):1010–1013.
53. Marseglia L, Manti S, D'Angelo G, Nicotera A, Parisi E, Di Rosa G, Gitto E, Arrigo T. Oxidative stress in obesity: a critical component in human diseases. *Int J Mol Sci*. 2014;**16**(1):378–400.
54. Picard M, Turnbull DM. Linking the metabolic state and mitochondrial DNA in chronic disease, health, and aging. *Diabetes*. 2013;**62**(3):672–678.
55. Rao Kondapally Seshasai S, Kaptoge S, Thompson A, Di Angelantonio E, Gao P, Sarwar N, Whincup PH, Mukamal KJ, Gillum RF, Holme I, Njølstad I, Fletcher A, Nilsson P, Lewington S, Collins R, Gudnason V, Thompson SG, Sattar N, Selvin E, Hu FB, Danesh J; Emerging Risk Factors Collaboration. Diabetes mellitus, fasting glucose, and risk of cause-specific death. *N Engl J Med*. 2011; **364**(9):829–841.
56. Aubert G, Hills M, Lansdorp PM. Telomere length measurement—caveats and a critical assessment of the available technologies and tools. *Mutat Res*. 2012;**730**(1–2):59–67.
57. Malik AN, Czajka A. Is mitochondrial DNA content a potential biomarker of mitochondrial dysfunction? *Mitochondrion*. 2013; **13**(5):481–492.
58. Daniali L, Benetos A, Susser E, Kark JD, Labat C, Kimura M, Desai K, Granick M, Aviv A. Telomeres shorten at equivalent rates in somatic tissues of adults. *Nat Commun*. 2013;**4**:1597.
59. Urata M, Koga-Wada Y, Kayamori Y, Kang D. Platelet contamination causes large variation as well as overestimation of mitochondrial DNA content of peripheral blood mononuclear cells. *Ann Clin Biochem*. 2008;**45**(Pt 5):513–514.
60. Liu Y, Kha H, Ungrin M, Robinson MO, Harrington L. Preferential maintenance of critically short telomeres in mammalian cells heterozygous for mTert. *Proc Natl Acad Sci USA*. 2002;**99**(6): 3597–3602.