

Association between objectively measured physical activity, chronic stress and leukocyte telomere length

Short title: Physical activity, stress and telomeres

Roland von Känel^{1,2,3*}

Erna J. Bruwer⁴

Mark Hamer⁵

J. Hans de Ridder⁴

Leoné Malan¹

¹Hypertension in Africa Research Team (HART, North-West University, South Africa)

²Department of Neurology, University Hospital Bern, Inselspital, and University of Bern, Switzerland

³Department of Psychosomatic Medicine, Clinic Barmelweid (Barmelweid, Switzerland)

⁴Physical Activity, Sport and Recreation research group (PhASRec, North-West University, South Africa)

⁵National Centre of Sport & Medicine, School of Sport, Exercise and Health Sciences (Loughborough University, UK)

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***Corresponding author:**

Prof Roland von Känel, MD
Department of Psychosomatic Medicine
Clinic Barmelweid
CH-5017 Barmelweid, Switzerland.
Tel: (+41) 62 857 2251
Fax: (+41) 62 857 2741
E-mail: roland.vonkaenel@barmelweid.ch

ABSTRACT

BACKGROUND: Physical activity (PA) attenuates chronic stress and age-related and cardiovascular disease risks, whereby potentially slowing telomere shortening. We aimed to study the association between seven-day objectively measured habitual PA, chronic stress and leukocyte telomere length.

METHODS: Study participants were African (n=96) and Caucasian (n=107) school teachers of the Sympathetic activity and Ambulatory Blood Pressure in Africans study. All lifestyle characteristics (including PA) were objectively measured. The general health questionnaire and serum cortisol were assessed as psychological and physical measures of chronic stress. Leukocyte telomere length was measured using the quantitative real-time polymerase chain reaction.

RESULTS: Africans had significantly shorter telomeres ($p < .001$) and greater psychological distress ($p = 0.001$) than Caucasians, whereas no group difference was seen for cortisol levels. Higher age [$\beta = -0.28$ (-0.40, -0.16), $p \leq 0.000$], higher alcohol consumption [$\beta = -0.21$ (-0.36, -0.08), $p = 0.003$] and increased central obesity [$\beta = -0.17$ (-0.30, -0.03), $p = 0.017$] were all significantly associated with shorter telomeres. Habitual PA of different intensity was not significantly associated with markers of chronic stress or telomere length. However, more time spent with light intensity PA time was significantly and independently correlated with lower waist circumference ($r = -0.21$, $p = 0.004$); in turn, greater waist circumference was significantly associated shorter telomeres [$\beta = -0.17$ (-0.30, -0.03), $p = 0.017$].

CONCLUSION: Habitual PA of different intensity was not directly associated with markers of chronic stress and leukocyte telomere length in this biethnic cohort. However, our findings suggest that light intensity PA could contribute to lowered age-related disease risk and healthy ageing by facilitating maintenance of a normal waist circumference.

Key words: Accelerometry; Ageing; Cardiovascular disease; Obesity; Lifestyle; Stress

INTRODUCTION

There is much evidence to demonstrate that lifestyle modifications, such as sufficient amounts of physical activity (PA) and a healthy diet, enhance quality of life, contribute to cardiometabolic disease prevention and longevity.^{1,2} Unhealthy behaviours, such as physical inactivity, poor dietary intake, alcohol abuse and smoking contribute to a number of disease processes, such as oxidative stress and low-grade inflammation which increase the risk of morbidity and mortality.³ During long-term psychological stress the increased metabolic energy supply normally used to mount “fight-or-flight” responses is conserved in the body, leading to glucocorticoid and catecholamine excess. As a consequence, wear and tear on the body called allostatic load occurs in chronic stress; for instance, as the hypothalamic-pituitary-adrenal (HPA) axis responses are not “turned off”, the secretory end product of the HPA-axis, cortisol, remains high.⁴ Heightened stress hormone secretion increase oxidative stress than can be assessed by reactive oxygen species (ROS), further downstream, thereby contributing to ageing and age-related diseases.³ Increased oxidative stress interferes for instance with the protective role of nitric oxide (NO) which functions as an antioxidant, vasodilator, as well as antiplatelet and anti-adhesive component if produced in vascular endothelial cells.⁵ An increasingly used biomarker for measuring the impact of ageing and determining cardiovascular health is telomere length.⁶ Short telomeres and low leukocyte telomerase activity were shown to be related to elevated levels of cortisol, epinephrine and norepinephrine; and that low telomerase activity was also associated with several cardiovascular disease (CVD) risk factors.⁷

Telomeres are DNA-protein complexes that protect chromosomal ends and shorten with each cell division, triggering cell senescence at crucial lengths.^{6,8} Possible mechanisms for telomere shortening are multifactorial and include current perceived stress, chronic psychological stress (in other words, the pathways involving stress-related hormones),

oxidative stress, estrogen deficiency, metabolic disturbances (i.e., hypertension, diabetes, and obesity), and inflammation.⁸⁻¹⁰ The National Institute of Environmental Health Sister Study in the USA indicated that the effects of perceived stress on telomere length are dependent on neuroendocrine responsiveness and exposure to environmental stressors.¹¹ Research suggests that a physically active lifestyle can buffer the detrimental effects of chronic stress.^{12,13} Prescription of exercise has been recommended to individuals reporting high levels of psychological stress for the sake of preserving telomere length.¹⁴ The notion is that PA improves cellular conditions and, therefore, has the potential to reduce age-related disease risk via telomere biology.¹⁰

A limitation of previous research is the assessment of PA, which has often been self-reported and thus fails to accurately estimate PA in all metabolic equivalent of task (MET) categories. Therefore, we hypothesize that 7-day objectively measured habitual PA will be associated with psychological and physical measures of chronic stress and leukocyte telomere length in a cohort of Africans and Caucasians.

MATERIALS AND METHODS

Design and participants

The follow-up data of the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) prospective cohort study, collected from February to May 2011 and 2012, respectively, was used for the purpose of this sub-study. Permission for the study was obtained from the North West Department of Education, as well as the South African Democratic Teachers Union and all participants signed informed consent. The SABPA study conforms to the principles outlined in the Declaration of Helsinki (revised 2004) and abided by the institutional guidelines and was approved by the Ethics Committee of the North-West

University, South Africa (0003607A6). The cohort profile of the SABPA study has been described elsewhere.¹⁵

In brief, a cohort of urban African (n=173) and Caucasian (n=186) primary and secondary school teachers, grades 1-7, ensuring socioeconomic equality, were recruited from the Dr Kenneth Kaunda Education District in the North-West Province of South Africa.¹⁵ Pregnant or lactating women, individuals who donated blood or had been vaccinated in the three months prior the commencement of testing and those with a tympanum temperature greater than 37.5°C were excluded prior to commencement of testing. Only 216 of the participants complied with wearing the Actiheart for a full seven days and with less than 40 minutes of total daily interruption periods at the follow-up investigation. Allowable interruption periods (lost- and non-wear time) when using accelerometry to measure PA, are inconsistent in the literature.¹⁶ After close inspection of the current study's raw Actiheart data, a total interruption period of no more than 40 minutes awake time per day was chosen to ensure habitual PA data as close to a full period of seven days as possible. Further, all HIV-positive participants (n=13) were excluded because the average telomere lengths of these individuals differed significantly from the group average, thereby leaving a final sample of 203 participants (Africans, n=96 and Caucasians, n=107) for the analysis.

Measurements and equipment

Blood pressure. The Cardiotens apparatus (Meditech CE0120[®], Meditend, Hungary), a validated British Hypertension Society device, was used to obtain the 24-hour ambulatory blood pressure measurement (ABPM) from the non-dominant arm using suitable cuff sizes. Measures were obtained every 30 minutes during the day and set at 60-min intervals at night. Successful mean inflation rates for the ABPM period were 85.29% ($\pm 9.35\%$) in Africans and 93.62% ($\pm 6.34\%$) in Caucasians. Participants were asked to continue with normal daily

activities and record any abnormalities such as visual disturbances, headache, nausea, fainting, palpitations, PA and emotional stress on their ambulatory diary cards. The data were analysed using the CardioVisions 1.15.2 Personal Edition software (Meditech[®]). We defined hypertension status according to the European Hypertension Society guidelines for ambulatory BP: SBP \geq 130 and / or DBP \geq 80.¹⁷

Anthropometric measurements. Participants' waist circumference was measured to the nearest 0.1 cm using the standardized methods of the International Society for Advancement of Kinanthropometry (ISAK).¹⁸

Psychological distress. Psychological distress was derived from the 28-item General Health Questionnaire (GHQ) that was developed as a measure of common mental health problems of depression, anxiety, somatic symptoms and social withdrawal over the past few weeks.¹⁹ We applied the binary scoring method, where any score exceeding the threshold value of 4 is classified as "psychiatric caseness", meaning that these individuals qualify for further clinical attention.²⁰

Biochemical measurements. A 65 ml blood sample was obtained in standard 10 ml EDTA-treated vacutainer tubes by a registered nurse from the brachial vein branches of the dominant arm using a winged infusion set and was immediately sent to the laboratory for storage. Fasting serum samples for gamma glutamyl transferase (γ -GT) were analysed using the sequential multiple analyser computer (Konelab 20i; Thermo Scientific, Vantaa, Finland). Serum cotinine levels were determined with a homogeneous immunoassay (Automated Modular, Roche, Basel, Switzerland). HIV-status was measured using the First Response kit (Premier Medical Corporation, India) as well as the confirmatory Pareekshak test (Bhat Biotech, India). ROS (1 Unit = 1.0 mg/L H₂O₂) was determined with the Bio-Tek FL600 Microplate Fluorescence Reader. Serum samples were analysed for cortisol and estradiol/E₂, using an electro chemiluminescence immunoassay with the Elecsys 2010 apparatus (Roche,

Basel Switzerland). Telomere length was determined from peripheral blood mononuclear cells (PBMC) that were isolated by density gradient centrifugation from each blood sample. DNA was extracted from the PBMC using the PureGene DNA isolation system. The exact protocol for measuring telomere length in SABPA is described elsewhere.²¹ Measurement of relative telomere length (Telomere PCR to single-copy gene PCR or T/S ratio) was determined by quantitative real-time polymerase chain reaction (RT-PCR).²²

Physical activity measurement. The weekly habitual PA of participants was measured over a period of seven consecutive days with an Actiheart (GB0/67703[®], CamNtech Ltd., Cambridgeshire, UK). All the raw 24-hour recordings (seven-days) of each participant were visually inspected to distinguish between time awake (including sedentary hours), and time asleep. To differentiate sleeping time from sedentary time, heart rate (HR) was considered along with the Metabolic Equivalent of Task (MET, 1 MET regarded as being asleep) and activity level. Where the HR in the evenings gradually dropped (over a period of 15 or more epochs) to less than the average HR in a selected sedentary sample period and activity level was equal to zero, the participant was considered to be sleeping. The end of sleeping was identified by an immediate increase in the HR of more than 10 to 20 beats per minute relative to preceding sleeping HR, as well as a corresponding increased MET- and activity-level. Habitual PA during the daily awake minutes was categorised according to daily awake sedentary time (≤ 1.5 METs), daily awake light activity time (> 1.5 & < 3 METs), daily awake moderate activity time (≥ 3 & < 6 METs) and daily awake vigorous activity time (≥ 6 METs).²³

Data collection procedure

Clinical assessments were performed over a 2-day period per person and the participants stayed overnight in a well-controlled environment at the Metabolic Unit

Research Facility of the North-West University. On day 1 at 0700, the Cardiotens apparatus (24-h ambulatory BP) was fitted to four participants at their selected schools. Participants then resumed their normal daily activities and were transported to the university at approximately 1500 for further clinical assessments. They were introduced to the experimental set-up to lessen anticipation stress.²⁴ The participants enjoyed a standardized dinner at around 1800, completed psychological questionnaires under supervision of a clinical psychologist and were asked to refrain from taking any beverages after 2200.

On day 2, participants were woken at 0700, the Cardiotens apparatus was disconnected and anthropometric measurements were taken. A resting 12-lead ECG was performed after each participant had rested in the semi-recumbent position for half an hour and fasting blood sampling commenced one hour later to avoid the cortisol awakening response.²⁵ The participants then showered and the Actiheart device for the seven-day PA measurement was fitted. Each participant was given four extra electrodes, as well as plaster to immediately secure the Actiheart back onto the chest if it became disconnected during the course of the seven days. Participants were instructed to carry on with their habitual daily activities and to wear the monitor at all times whilst awake and asleep. The Actiheart was collected from each participant at the various schools on the eighth day and downloaded onto a computer for storage, viewing and analysis.

Statistical analyses

Statistical analyses were performed with the Statistica 12 (StatSoft Inc., 2014) programme. Data normality was evaluated using both the Shapiro-Wilk test and Quantile-Quantile plots. γ -GT was normalized by log transformation and serum cotinine was categorized. Moderate and vigorous PA time was not log-transformed as all residual plots of the multivariate regression analyses that included these two measures indicated normal

distribution. One-way analyses of covariance (ANCOVA) were used to determine significant ethnic differences in physiological, anthropometric and PA measures adjusting for covariates age and log γ -GT. Multivariate regression analyses were computed. Firstly, a forward stepwise regression analysis evaluated the associations with serum cortisol (dependent marker), adjusting for ethnicity sex, age, cardiovascular- and/or kidney disease, hypertension- and/or diabetes drug use, log γ -GT, smoking, waist circumference, ROS, MAP, estradiol, GHQ total score and time spent in different MET-categories (each entered separately into the model). Partial correlations, adjusted for age and log γ -GT were used to assess if any relationship between time spent in the different MET-categories and psychological distress (GHQ total score) exist. A second forward stepwise regression analysis determined associations with telomere length (dependent marker), adjusting for ethnicity, sex, age, cardiovascular and/or kidney disease, hypertension and/or diabetes drug use, γ -GT, smoking, waist circumference, cortisol, GHQ total score, ROS, mean arterial pressure (MAP) and time spent in different MET-categories (each entered separately into the model. Despite the ethnicity interaction observed with telomere length, the separate groups did not comply with sample size requirements as calculated by the formula of Tabachnick and Fidell as quoted by Pallant: $N > 50 + 8m$ (where m = the number of independent variables).²⁶ The regressions were therefore performed for the group as a whole (Africans and Caucasians combined), entering ethnicity as an independent variable. Lastly, a sensitivity analysis (partial correlations adjusted for age, log γ -GT and cortisol) assessed whether any of the different MET-categories were associated with waist circumference (a variable associated with both cortisol and telomere length) in this study population. Data were considered statistically significant for all the analyses at $p \leq 0.05$.

RESULTS

Participant characteristics

The study population consisted of African men (n=45), African women (n=51), Caucasian men (n=52) and Caucasian women (n=55). The average GHQ total score for this study population was above the clinical threshold of 4,²⁰ indicating high levels of psychological distress – less than half of the participants scored below 4 (n=99, 49%) (Table I). More than half of the participants could be classified as hypertensive according to the European guidelines for ambulatory blood pressure;¹⁷ however, not all hypertensive participants used anti-hypertensive drugs. The average daily awake time of the group was slightly over 17 hours per a 24-hour cycle. Although the habitual PA time spent in light and moderate activity may seem high, it accumulated during an average uninterrupted wearing period of 17.1 awake hours in which activity was recorded every minute (one-minute epoch intervals). Almost half of this awake time (45.66%) was spent sedentarily.

Ethnic differences

Significant ethnicity interactions were observed with telomere length (F(1, 176); 25.31, $p \leq 0.000$). Independent T-tests revealed significant ethnic differences in mean age ($p=0.024$) and serum γ -GT ($p < 0.001$) – with the Africans being slightly younger (Africans = 48.33 years; Caucasians = 51.06 years) but with a much higher alcohol consumption (γ -GT in Africans = 59.84 U/L; γ -GT in Caucasians = 27.02 U/L). When adjusting for age and log γ -GT, the Africans had significantly shorter telomeres, as well as significantly higher psychological distress (GHQ total scores) and ambulatory BP (24-h SBP, DBP and MAP) than the Caucasians (Table II). Both groups presented with a GHQ score above 4, however, only the African population presented with elevated 24-h SBD and DBP levels.

Although no significant ethnic differences were detected for the awake time spent in different MET-categories, Africans spent nearly an hour more of the average daily awake time sedentary (54.77 minutes) than the Caucasians (Figure 1). Light PA time was more or less the same for the two groups, while the average daily awake moderate PA time was higher in the Caucasians. Both groups recorded less than 10 minutes of daily awake vigorous PA time.

Associations with serum cortisol

Including ethnicity and sex in the models, forward stepwise regressions evaluated the associations between various physiological, biochemical and lifestyle characteristics (including habitual PA) with serum cortisol (Table III, Adjusted $R^2=0.23$). All the models (MET-categories were each entered separately into the model) had the same outcome – indicating significant positive associations with log γ -GT and ROS and also a significant negative association with waist circumference. No associations were observed with ethnicity and sex. None of the PA MET-categories entered the cortisol models. Also, partial correlations (adjusted for age and log γ -GT) did not show any PA associations with the GHQ scores. Psychological distress (GHQ total scores) entered the models, but was not associated with serum cortisol levels.

Associations with telomere length

Unadjusted analyses (Pearson's correlation matrices) with the main dependent in the total group revealed that shorter telomeres were significantly associated with older age ($r=-0.25$, $p=0.001$), greater waist circumference ($r=-0.27$, $p=0.004$), greater log γ -GT ($r=-0.35$, $p\leq 0.000$), as well as higher 24-h SBP ($r=-0.22$, $p=0.007$), 24-h DBP ($r=-0.18$, $p=0.028$) and

MAP ($r=-0.20$, $p=0.012$). No significant relations were observed with time spent in the different MET-categories and telomere lengths.

Ethnicity and sex, as well as cardiovascular disease and -medication use, were included as covariates in forward stepwise regression models to assess associations with telomere length (Table IV). Significant negative associations were indicated with age, log γ -GT and waist circumference that is in accordance with the above mentioned correlations. In contrast with the Pearson correlations, the MAP now indicated a positive association, along with ROS. The four models used (entering sedentary time, light PA-, moderate PA- and vigorous PA time separately) explained between 34% and 35% of the variance in telomere length and all four models indicated similar significant covariates. Table IV also shows that ethnicity was associated with telomere length.

Association of light physical activity with waist circumference

While none of the MET-categories indicated any significant associations with either cortisol or telomere length (Tables III and IV), partial correlations (adjusting for age, log γ -GT and cortisol) revealed a significant negative relationship between waist circumference and daily awake time spent in light PA ($r=-0.21$, $p=0.004$). In other words, more light PA during the day was related to lower central obesity. This inverse relationship showed significance in all regression models and a greater waist circumference was also shown to be associated with higher 24-h MAP for the group as a whole, as well as in both ethnic groups (Total group, $r=0.40$, $p=0.000$; Africans, $r=0.28$, $p=0.008$; Caucasians, $r=0.54$, $p\leq 0.000$).

DISCUSSION

The present study aimed to investigate the associations between habitual PA, psychological distress, cortisol, and telomere length in African and Caucasian teachers. There

were no differences in PA measures between Africans and Caucasians, but Africans presented with significantly higher 24-h ambulatory BP (SBP, DBP and MAP). This more vulnerable African group had significantly shorter telomeres than the Caucasians (even after adjusting for age and log γ -GT). They also showed higher psychological distress levels with a trend towards significance for attenuated or possibly down-regulated cortisol levels. A study investigating telomere length in healthy Caucasian and African-American adolescents indicated that the African-American adolescents had longer telomeres than their Caucasian peers.²⁷ Another study, exploring ethnic effects on telomere length, found that African-Americans had longer leukocyte telomeres than Caucasians at nearly all ages.²⁸ Telomere shortening (adjusted for sex and BMI) was, however, at a steeper slope in the African-Americans.

Physical activity, psychological distress and telomere length

Time spent in the four PA MET-categories did not indicate any associations with cortisol or telomere length in the current study population. This contradicts some previous studies indicating that participation in regular moderate or vigorous levels of PA is associated with attenuation in telomere erosion and buffers the detrimental effects of chronic stress on cellular longevity.^{13,14,29-32} Telomere length of individuals participating in leisure time PA for 199 minutes and more per week were found to be the same as in sedentary individuals ten years younger.¹³ These findings were supported by twin studies showing leukocyte telomere length of the more active twin to be longer than in the less active one.¹³ A major limitation of many of these studies is self-reported PA that may introduce measurement error and cannot account for 24-h time use, thereby negating the influence of sedentary time and light intensity PA.

The evidence regarding a relationship between PA and cortisol remains controversial. Although there was no significant difference in time spent in different MET-categories between the two ethnic groups, Africans on average recorded 54.77 more daily awake sedentary minutes and 45.08 minutes less moderate intensity PA time than Caucasians. Indeed, the accumulation of time spent in different MET-categories was high in both groups due to the recording during the average 17-h awake time. Although none of the PA MET-categories was associated with psychological distress, the scores of the Africans were significantly higher than those of the Caucasians. Reduced physiological (salivary cortisol levels and heart rate) and psychological (calmer mood prior to stressor and less anxiety throughout stressor) acute stress responsiveness has been shown in trained compared to untrained men.³³ This is, however, contradictory to a more recent study in which no associations were revealed between stress responses (during the stress task and recovery) and both self-report and objective PA measures.³⁴

Lifestyle risk, blood pressure and shortened telomeres

Shorter telomeres were associated with older age, alcohol abuse and increased central obesity in the current study. Despite the elevated psychological distress and MAP levels observed in both ethnic groups (especially the Africans), borderline significantly attenuated cortisol levels were observed more in Africans than Caucasians. While psychological distress did not associate with telomere length, lowered cortisol levels showed a tendency towards an association with longer telomeres. Similarly, telomere shortening was related to stress arousal (increased cortisol, epinephrine and norepinephrine), but not with negative mood in another study.⁷ It has previously been discussed that undergoing urbanization is not only associated with decreases in PA, but is also accompanied by insecurities and social relationship disruption that additionally increase the level of psychological stress.³⁵ Chronic psychosocial

stress has the potential to contribute to an increased oxidative stress burden by chronic activation of the autonomic and neuroendocrine stress response systems.⁶ One could therefore argue that cortisol levels might be down-regulated in the current African study population in an attempt to protect against chronically elevated autonomic arousal. Previous studies suggested that the adrenal gland becomes hypoactive in chronic stress-related states and due to the down-regulation of the cortisol receptors, hypocortisolism in chronic stress may occur.^{36,37} Interestingly, although hypocortisolemic subjects scored high on measures of depression, perceived stress and physical complaints, they did not show allostatic load.³⁸ This may indicate that a hypocortisolemic stress response might counteract cardiovascular and metabolic disturbances. Our data support this mechanism as attenuated cortisol levels were associated with significantly increased waist circumference, decreased ROS and also showed a tendency to be associated with longer telomeres. Adding further support to such reasoning, higher levels of MAP and ROS were also associated with longer telomeres.

Physical activity and shortened telomeres

Both the African and Caucasian participants in the current study spent more than 35% of their awake time doing PA of light intensity. This was also the only MET-category associated with smaller waist circumferences in the group as a whole and, moreover, lower central obesity was associated with longer telomeres. It is therefore possible that increased daily light PA time could be beneficial by lowering central obesity – thus having an indirect protective effect on telomere shortening. A review of PA and telomere biology indicated that exercise slows or prevents the symptoms of age-related diseases and is therefore able to indirectly alter telomere biology and reduce disease risk.¹⁰ Long-term exercise (moderate intensity treadmill walking for 45 minutes, three times per week for six months) down-regulated oxidative stress in obese women and, although not significant, the rate of telomere

shortening was slower in the exercise group than in controls. This research also stated that exercise-induced variations in antioxidant enzymes may be relevant in maintaining telomere length.³⁹ Recent research indicates lengthening of telomeres with a reduction in sitting time.⁴⁰ However, we did not observe a significant association between daily sedentary time and telomere length.

Strengths and Limitations

The average Actiheart wear time of the current study population was 6.84 days and awake and sleep times were carefully separated. Objective measurement of time spent in the different PA MET-categories captured during true awake time (17 hours per 24-h cycle) for the assessment of associations with physiological and biochemical markers for disease in two different ethnic groups with equal socio economic status are notable strengths of our study. However, the cross-sectional design with single point measurements of telomere length and serum cortisol in particular prevents us from making causal inferences. Moreover, relatively little time was spent with vigorous PA.

CONCLUSIONS

Habitual PA (expressed as time spent in different MET-categories) was not significantly associated with either cortisol or telomere length in the current study. However, shorter telomeres were associated with older age, increased alcohol consumption and higher central obesity. A tendency towards shorter telomeres with increased cortisol was also observed. Of clinical relevance is the finding of more daily PA of light intensity being associated with a smaller waist circumference, which, in turn, was associated with longer telomeres. This may suggest that habitual PA of light intensity could indirectly contribute to lessen DNA damage via constraining central obesity. Waist circumference also indicated

strong positive correlations with 24-h MAP in both ethnic groups. Therefore, increasing daily awake light PA could be recommended for lifestyle modification in programmes promoting healthy aging in this biethnic cohort. Limiting alcohol use may also be recommendable, as log γ -GT was directly associated with cortisol levels and inversely with telomere length. As an important next step translating our findings to clinical practice, our findings may inform intervention studies, incorporating lifestyle changes such as long-term PA of light intensity, to explore their effects on chronic stress responses and age-related diseases and markers such as leukocyte telomere length.

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Table I. Descriptive statistics of the study population

	Total population (N=203)
Characteristics (<i>Unadjusted Analysis</i>)	Mean \pm SD
African participants, n (%)	96 (47.3)
Caucasian participants, n (%)	107 (52.7)
Age (years)	49.77 \pm 8.67
Telomere length (ng/ μ l)	0.96 \pm 0.3
Cortisol (nmol/l)	235.14 \pm 94.7
GHQ total score	6.09 \pm 6.4
Waist circumference (cm)	96.38 \pm 15.8
γ -GT (U/l)	42.28 \pm 57.7
Smoking, n (%)	24 (11.8)
ROS (1 Unit=10 mg/l H ₂ O ₂)	79.88 \pm 22.8
24-h SBP (mmHg)	129 \pm 17.0
24-h DBP (mmHg)	79 \pm 10.5
Mean arterial pressure	96 \pm 12.2
Hypertensive (SBP \geq 130 and / or DBP \geq 80), n (%)	105 (51.7)
Anti-hypertensive and / or anti-diabetic drugs, n (%)	69 (34.0)
Kidney disease history, n (%)	9 (4.4)
CVD history, n (%)	29 (14.3)
Physical activity measurements (<i>Unadjusted Analysis</i>)	Mean \pm SD
Average Actiheart wear time (days)	6.84 \pm 0.3
Daily awake time (average minutes per 24-hour cycle)	1024.23 \pm 64.9
Sedentary time, minutes (% of awake time)	467.7 (45.7)
Light activity time, minutes (% of awake time)	375.8 (36.7)
Moderate activity time, minutes (% of awake time)	175.4 (17.1)
Vigorous activity time, minutes (% of awake time)	5.43 (0.5)

GHQ, General Health Questionnaire ; γ -GT, Gamma Glutamyl Transferase; ROS, Reactive Oxygen Species; SBP, Systolic blood pressure ; DBP, Diastolic blood pressure; CVD, Cardiovascular disease.

Table II. Ethnic differences in participant characteristics

Variables	Africans	Caucasians	P-value
	(n=96)	(n=107)	
Telomere length (ng/ μ l)	0.84 (0.8, 0.9)	1.06 (1.0, 1.1)	$\leq 0.001^*$
Serum cortisol (nmol/l)	219.83 (199.9, 239.7)	248.02 (229.5, 266.5)	0.05
GHQ total score	7.80 (6.5, 9.1)	4.62 (3.4, 5.9)	0.001*
Waist circumference (cm)	97.47 (94.3, 100.6)	95.09 (92.2, 98.0)	0.30
24-h SBP (mmHg)	135 (131.9, 138.2)	123.21 (120.3, 126.1)	$\leq 0.001^*$
24-h DBP (mmHg)	82.66 (80.7, 84.6)	76.49 (74.7, 78.3)	$\leq 0.001^*$
MAP	100.12 (97.9, 102.4)	92.06 (90.0, 94.2)	$\leq 0.001^*$
Serum ROS	80.14 (75.39, 85.1)	79.91 (75.3, 84.5)	0.95

Data analysis used ANCOVAS (Mean (95% CI)) Adjusted for covariates age and log γ -GT.

* $P \leq 0.05$, statistical significance. GHQ, General Health Questionnaire; SBP, Systolic blood pressure, DBP, Diastolic blood pressure; MAP, Mean arterial pressure; ROS, Reactive Oxygen Species.

Table III. Associations of cortisol with physiological, biochemical and lifestyle biomarkers

	Total group (N=167)	
	β ($\pm 95\%$ CI)	P-value
Models statistics: $F(13, 153)=4.60$, $p<0.000$, <i>Adjusted R² = 0.23</i>		
Log γ -GT	0.23 (0.1, 0.4)	0.007*
ROS (1 Unit = 1.0 mg/l H ₂ O ₂)	0.31 (0.2, 0.5)	$\leq 0.001^*$
Waist circumference (cm)	-0.24 (-0.4, -0.1)	0.005*
MAP	-0.16 (-0.3, 0.02)	0.06
CVD and/or kidney disease	-0.10 (-0.2, 0.04)	0.18
Age (years)	0.10 (-0.04, 0.03)	0.18
GHQ total score	0.08 (-0.1, 0.2)	0.29

Data analysis used forward stepwise regression. *=Statistical significance is considered when $p \leq 0.05$. γ -GT, Gamma Glutamyl Transferase; ROS, Reactive Oxygen Species; MAP, Mean arterial pressure; CVD, cardiovascular disease; GHQ, general health questionnaire. Additional covariates considered for models: race, sex, age, hypertension- and/or diabetes drug use, smoking, Estradiol, Time spent in different MET-categories (each entered separately into the model).

Table IV. Associations of telomere length with physiological, biochemical and lifestyle biomarkers

	Total group (N=190)	
	β (±95% CI)	P-value
Models statistics $F(9, 180)=12.30, p<0.001, Adjusted R^2 = 0.34-0.35$		
Ethnicity	0.47 (0.3, 0.6)	≤0.001*
Age (years)	-0.28 (-0.4, -0.2)	≤0.001*
Log γ-GT	-0.21 (-0.4, -0.08)	0.003*
ROS (1 Unit = 1.0 mg/l H ₂ O ₂)	0.14 (0.02, 0.3)	0.02*
MAP (mmHg)	0.17 (0.01, 0.3)	0.03*
Waist circumference (cm)	-0.17 (-0.3, -0.03)	0.02*
Cortisol (nmol/l)	-0.12 (-0.2, 0.00)	0.07
Sedentary time (minutes)	0.12 (-0.06, 0.2)	0.06
Light activity time (minutes)	-0.09 (-0.2, 0.03)	0.14
Moderate activity time (minutes)	-0.08 (-0.2, 0.04)	0.18
Smoking	-0.07 (-0.2, 0.04)	0.15

Data analysis used forward stepwise regression. γ-GT, Gamma Glutamyl Transferase; ROS, Reactive Oxygen Species; MAP, Mean arterial pressure. Additional covariates considered in the four models: sex, cardiovascular- and/or kidney disease, hypertension- and/or diabetes drug use, smoking, GHQ total score, Time spent in different MET-categories (each entered separately into the model).

Figure 1. Percentage of daily awake time spent in different MET-categories per ethnic group

