Original Article

DOI: 10.4274/cjms.2022.2021-247

Preliminary Study of the Effect of Neuroeducational Methods on the 20th Percentile Telomere Length Dynamics

Serapinas et al. Neuroeducation Effect on Telomere Length

Danielius Serapinas[1,2], Akvilė Staškūnaitė[2], Gabija Pajedaitė[2], Antanas Valantinas[1], Rasa Pilkauskaite Valickiene[1], Marija Mendele Leliugiene[3]

[1]Institute of Psychology, Mykolas Romeris University, 08303 Vilnius, Lithuania
[2]Department of Family Medicine, Lithuanian University of Health Sciences, 44307 Kaunas, Lithuania
[3]Institute for Personality Development 'Rafaelis', 14188 Vilnius, Lithuania

Received: 23.01.2022
Accepted: 30.04.2022

Correspondence: Gabija Pajedaitė
E-mail: gabija.pajedaite@stud.lsmu.lt

ORCID ID of authors:
D.S. 0000-0002-2976-0682; A.V. 0000-0002-7266-7870; R.P.V. 0000-0003-2356-7715

Cite this article as: Serapinas D, Staškūnaitė A, Pajedaitė G, Valantinas A, Valickiene RP, Leliugiene MM. Preliminary Study of the Effect of Neuroeducational Methods on the 20th Percentile Telomere Length Dynamics. Cyprus J Med Sci. 2022. DOI: 10.4274/2022.2021-247. [Online Ahead of Print].

Abstract

Background/Aims: Telomeres are nucleotide repeats that cap the end of each chromosome arm and ensure the stability of the genome. The telomere length is amongst the most dependable markers of senescence at the cellular level. It is known that telomeres become shorter with each cell division; accordingly, telomere loss correlates with the process of aging in vivo. This research aimed to investigate the effect of neuroeducational methods on 20th telomere length shortening.

Material and Methods: Twenty healthy women participated in this study, and ten of them attended regular neuroeducational sessions. We were searching for the impact of lifestyle on cellular aging by measuring the value of the 20th percentile of telomeres before and after the neuroeducational sessions.

Results: The median of the 20th percentile value in the experimental group was 5.8 kb before the study and 5.6 kb after the study ($p < 0.05$). The median of the 20th percentile value in the control group was 6.4 kb before the study and 5.7 kb after the study ($p < 0.001$).

Conclusion: The findings show that neuroeducational methods relieve stress and make telomere shortening slower, as we were expecting.
Keywords: telomere shortening; neuroeducation; 20th percentile

Introduction

Aging is a physiological process that involves finite changes at the genetic and epigenetic levels [1]. On the genetic level, the most important drivers tend to be epigenetic dysregulation, DNA damage, mitochondrial dysfunction, and telomere damage, which can cause stable, irreversible growth of cells [2]. In recent studies, telomeres have been intensively studied, and their reliability in indicating biological aging on the cellular level is significant [3]. Telomeres are caps (highly conserved tandems of nucleotide repeats) that include proximal double-stranded and distal single-stranded regions that, in complex with shelterin proteins, afford protection at the chromosomal ends to maintain genomic solidity [4]. If there is no compensatory elongating mechanism, telomeres get shorter with every cell division. Telomerase can elongate telomeres de novo during cell severance, but after birth, most telomerases are silenced in somatic cells, and telomeres then undergo oxidative stress and age-dependent incremental attrition [5]. Short or dysfunctional telomeres are recognized as DNA double-stranded breaks, triggering cells to undergo replicative senescence [4].
The telomere 20th percentile is the shortest quintile of telomeres, indicating a telomere length below 20%, while usually, the median telomere length represents the 50th percentile in the distribution of cell telomere lengths. The telomere 20th percentile more precisely represents the impact of telomeres on the general health state. This is important because the latest research shows that short telomeres cause the aging processes and collateral effects of aging [6]. It is known that short telomeres trigger permanent and harmful damage to the cell, in this way provoking precocious biological aging, unless they are repaired by telomerase [7]. Furthermore, there are telomere-based therapies, for example, TA-65 (natural telomerase activator product), that have provided apparent positive immune remodeling and beneficial effects on metabolic, bone, and cardiovascular health, although they have failed to prolong life [8]. In this context, not only pharmaceutical preparations but also lifestyle and emotional state are being intensively examined, and some studies have contributed to demonstrating that this can prolong telomere lengths. A theory was put forward and some clinical trials were conducted to prove the impact of lifestyle on telomere length [9]. Oxidative stress is one of the most important triggers that interfere with the normal functioning of the body. It can arise due to the accumulation of ROS, a phenomenon that increases with age and is accompanied by a reduction in protective mechanisms; this eventually causes a wide range of DNA lesions, leading to mutations and disruption in the epigenetic state of the cell [10].
As suggested, telomeres can be restored by physical activity, which is one of the main ways to relieve stress [2]. One of the first studies that explored the correlation between regular exercise and telomere length in humans was directed by Cherkas et al. In a cross-sectional survey of 2401 white men and women, they showed that lymphocyte telomere length (LTL) was positively associated with higher physical activity levels [1]. However, an inactive lifestyle, particularly stress, can potentially lead to oxidative stress through chronic activation of the autonomic and neuroendocrine stress responses; also, it was proved that oxidative stress shortens telomeres in cells cultured in vitro [11]. Recently, the emerging field of "neuroeducation", also frequently referred to as "mind, brain, and education" or "educational neuroscience", has been developing. It is known that on a psychological level some art and mindfulness, and stress management techniques can help people to maintain their emotional stability. But it is still a question if those techniques can protect against telomere shortening or if they can be useful on a biological level. This field is also considered the scientifically

substantiated art of teaching that embraces many overlapping tenets of brain-based teaching and learning and cognitive neuroscience [12]. Alberto Olivero wrote that “education has the task to shape the brain”, and neuroeducation is centered on the neural plasticity feature of the brain that produces neurons and countless connections under the influence of experience [13]. Different methods can be used in neuroeducation activity programs — meditation, art therapy, visualization, language codes, yoga, and mindfulness therapy — all of which have the same purpose: to help the human being to handle stress and protect them from harmful effects. Several study-based articles suggest mechanisms by which various therapies reduce the cortisol level, which is an indicator of stress level [13,14].

Materials and Methods

Study Design

To assess the neuroeducational methods’ influence on telomere shortening, we used a paired-sample, prospective, randomized study. The Institute for Personality Development 'Rafaelis' designed and managed the study; participants' medical histories were gathered, blood samples were taken at the InMedica Clinic (Vilnius), and genetic tests were done at the Life Length laboratory (Madrid). This study is a continuation of a previous one in which we analyzed the length of telomeres [15].

The research lasted for six months and involved 20 healthy women aged 20 to 59 years. We included only women in the study for the cohort would be more homogenous because studies show that there are differences in telomere length between genders [16].

For four continuous months, 50% of participants (10 women) attended regular neuroeducational sessions (20-hour-per-month) in which various methods were applied (theoretical and neuroeducational). The neuroeducational methods we used in our research were copyrighted works created by Marija Mendele-Leliugienė (Institute for Personality Development ‘Rafaelis’). Altogether, 57 different neuroeducational methods and their varied algorithms were used in the research. Participants who were in the study group participated in intense (20 h/month; 80 h in total) neuroeducational classes, including art therapy, stress management techniques, visualization, and meditation, as described in the methodological book [17]. The main neuroeducational methods were divided into groups:

1) exercises / tests. These are additional components of education that are specifically designed to achieve the desired educational goal. Exercises are designed for relaxation, concentration, emotional recognition, control, and release.

2) visualizations. The seven visualizations were meant to be the main motivational keys that could help a person go through his thinking process, sometimes even resulting in mindset/attitude permanent changes, helping to understand, comprehend, assimilate and realize the importance of emotions management, and enabling a person to take full responsibility for their decisions.

3) Verbal codes. Verbal codes are phrases used for self-awareness / self-perception, self-integration, perception of reality, mindset, and behavioral correction. Applying verbal codes enables a person to forgive himself and others and motivates a person to choose how to live.

4) art therapy methods, create unique conditions for the participant to remember the feelings, emotions, and inner states which then are used as the main source of self-regulation.

Another 10 women participated as a control group.

Measurement of Telomere Length

The median telomere length was measured by the Life Length laboratory in Spain. In this study, we used high-throughput (HT) quantitative fluorescence in situ hybridization (Q-FISH). We chose this technique because of its ability to analyze cells, unlike other techniques such as TRF and PCR-substantiated assays where the substrate is DNA. This Q-FISH method is adapted for cells fixed in interphase. The HT technique is automatized and not labor-intensive, which makes it suitable for use in large studies.

Following the methodology of Serapinas and colleagues (2020), the counts of defrosted cells at 37 °C were checked, and after that, control and sample lymphocyte lines were cultured in black-walled clear-bottom 384 plates. Methanol/acetic acid (3/1, vol/vol) was used to complete cell fixation. Telomeres were hybridized in situ with a fluorescent peptide nucleic acid (PNA) probe (binds to sequence Alexa488-OOCCCTAACCCTAACCCTAA, Panagene). Later on, the cells were washed, and a fluorescent stain (DAPI) was added to improve the contrast of DNA. To accomplish the imaging of cells and telomeres, a 40 × 0.95 NA water immersion objective was used. Signals from DAPI were distinguished by UV wavelength, and those from Alexa488 were distinguished by 488 nm wavelength. Images were analyzed using the High Content Screening Opera System (Perkin Elmer, USA) on Acapella software, Version 1.8 (Perkin Elmer). Further interpretation of telomere length was carried out using Life Length's proprietary program.

Statistical analysis

Statistical analysis was conducted on all gathered data using the SPSS (Statistical Package for the Social Sciences) software (version 27.0. Armonk, NY, IBM Corp). To compare 20th percentile value changes between first and second measurements in both groups, a non-parametric paired Wilcoxon test was performed. The Mann-Whitney test was used to compare the variation in the 20th percentile of telomeres between the control and experimental groups. Spearman's correlation test was used to assess the correlation between two variables.

The study was reviewed and approved by the bioethics center of the Lithuanian University of Health Sciences; the approval number is BEC-MF-863.3.

Results

This study included an analysis of women of different ages. Quantitative fluorescence in situ hybridization (Q-FISH) was performed on all participants before and after the study. Figure 1 shows the Q-FISH of our randomly selected participants.

The participants' average biological age was 42.6 years (min. 21.6 years, max. 59.8 years). 40.05 years was the average chronological age (min. 20.7 years, max. 59.3 years). There was a statistically significant difference between biological and chronological ages ($p < 0.05$). Before the study, the median telomere length was 10.8 kb (min. 9.4 kb, max. 12 kb), and the population percentage was 40.5% (min. 1%, max. 81%). However, there was no statistically significant difference between the experimental and control groups in the demographic data (Table 1).

Changes in the Value of the 20th Percentile

The experimental group's median 20th percentile value was 5.8 kb (min. 4.9 kb, max. 7.1 kb) prior to the research and 5.6 kb (min. 4.6 kb, max. 6.5 kb) after the research ($p < 0.05$). The control group's median 20th percentile value was 6.4 kb (min. 4.9 kb, max. 7.4 kb) prior to the research and 5.7 kb (min. 4.6 kb, max. 6.3 kb) after the research. The change in the control group's 20th percentile value was statistically significant ($p < 0.001$) (Figure 2).

The mean difference of the 20th percentile of telomeres between the first and second measurements in both groups was 0.52kb (min. -0.50kb, max. 1.20kb).

Measurement of the 20th percentile value in both groups showed that in the experimental group, the 20th percentile decreased in 8 of the 10 participants. However, in the control group, the value of the 20th percentile decreased in all 10 participants.

The Influence of Daily Habits on the 20th Percentile

Every participant ($n = 20$) was asked about their lifestyle daily and habits. We have analyzed three aspects of lifestyle: stress from daily life, physical activity, and smoking. The surveys were created based on self-reports from participants: If the person was suffering extreme stress daily, life was described as stressful. Respondents were classified as physically active

if they participated in sports or other forms of physical activity for a minimum of 60 minutes each day. We observed that for participants whose lives were more stressful (n = 13), the median 20th percentile value at the initial blood sampling was 6.2 kb (min. 4.9 kb, max. 7.4 kb), while for those whose lives were less stressful (n = 7) it was 6.5 kb (min. 4.9 kb, max. 7.1 kb). There was no statistically significant difference between these groups ($p > 0.05$). Smoking women (n = 6) had a median 20th percentile value of 6.35 kb (min. 5.6 kb, max. 7.4 kb), while non-smokers (n = 14) had a value of 6 kb (min. 4.9 kb, max. 7.3 kb). The difference, however, was not statistically significant ($p>0.05$). Physically active women (n = 9) had a median 20th percentile value of 5.8 kb (min. 4.9 kb, max. 7.4 kb), while physically inactive women (n = 11) had a median 20th percentile value of 6.3 kb (min. 4.9 kb, max. 7.3 kb). Also, there was no statistically significant difference between the two groups ($p > 0.05$) (Table 2).

All the participants were asked about their sleep routine, and their answers regarding their average sleep duration were divided into three groups: 6, 7, and 8 hours of sleep duration. The median 20th percentile value for those whose sleep 6 h (n = 6) was 5.65 kb (min. 4.9 kb, max. 7.1 kb); for women who sleep 7 h (n = 10), it was 6.4 kb (min.4.9 kb, max. 7.4 kb); and for participants who sleep 8 h (n = 4), it was 6.4 kb (min. 5.5 kb, max. 7 kb). However, we did not find a statistically significant correlation between the average sleep duration and the 20th percentile value ($p > 0.05$).
No statistically significant correlation between the 20th percentile value and BMI or alcohol consumption was found (Figure 3).

Discussion

Our findings were unique because for the first time in our preliminary study, we analyzed 20th telomere length shortening concerning psychological factors. 20th telomere analysis is more accurate than general mean length analysis because it more precisely represents body aging and physiological events [18].
With the outcomes of this study, we aim to contribute to the discussion on the importance of neuroeducational methods to longevity regarding the mechanism of telomere shortening. The main finding of this study was that the value of the 20th percentile of telomere shortening in the control group was statistically significant ($p <0.001$). Furthermore, in the experimental group, the value of the 20th percentile telomere shortening was also statistically significant ($p < 0.05$). These findings revealed that telomere shortening in the experimental group was less prevalent than in the control group. Nevertheless, because of the low number of participants and the very short period between measurements, these pilot study results should be considered with caution.
Other studies provided similar findings on the 20th percentile of telomere shortening in a control group, and their results echoed those in our study. In research conducted in 2016 in Spain, the impact of dietary supplement TA-65 on 20th percentile telomere length was analyzed. The difference in the value of the 20th percentile of telomere length between the low-dose TA-65 group and the placebo group was statistically significant [19]. It is important to maintain a balance in telomerase activity because excessive telomere elongation can also disrupt chromosomal stability [20].
There are many genetic and environmental factors related to telomere shortening, and one of the most mentioned in the literature is oxidative stress [21]. The latter is a consequence of an imbalance in the production of reactive oxygen species (ROS) and the defense of cellular antioxidants. Reactive oxygen species levels are known to be higher in areas of chronic inflammation and are common in chronic inflammatory illnesses [22]. Several studies have demonstrated that oxidative stress is linked to increased telomere shortening in the majority of degenerative and inflammatory illnesses [23]. That is why it is important to find ways to

relieve stress. Studies show that meditation through stress reduction mechanisms can reduce levels of the stress hormone cortisol and reduce levels of reactive oxygen species (ROS), as well as stimulate anti-inflammatory cytokines, endorphins, and neurotrophins [24]. Mindfulness meditation practices were examined in a study with medical students by measuring the serum cortisol level. The study population consisted of 30 medical students whose cortisol level before the mindfulness meditation was higher than that after the meditation practice [25].

Environmental stresses, smoking, heavy alcohol consumption, and air pollution can cause metabolic changes on the cellular level and can lead to oxidative stress and telomere shortening [26,27]. Neuroeducational methods are often mentioned in other studies as an opportunity to decelerate chronic disease development [28]. FINGER (The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability) is the biggest clinical trial on this topic, focusing on the impact of a multidomain lifestyle intervention on changes in LTL; unfortunately, over the course of two years, there was no significant difference in LTL between the intervention and control groups. However, LTL maintenance was directly associated with an improvement in lifestyle among FINGER participants [9]. The same results were visible in our research: participants who attended neuroeducational activities were monitored with fewer changes in the 20th percentile of telomere length, while in the control group, telomeres were more shortened. In another research, LTL was significantly longer in middle-aged U.S. women who were healthy, practiced a healthy lifestyle, and conformed to five low-risk factors for a healthier lifestyle (non-smoking, engaging in regular moderate to intense physical activity, maintaining optimal body weight, eating a healthy diet, and consuming alcohol in moderation) [29]. Even though these factors are usually referred to as risky for telomere shortening, in our study, alcohol, smoking, BMI, and physical activity had no statistically significant effect on the value of the 20th percentile of telomere length. It can be hypothesized that neuroeducation activities could protect from telomeres shortening even when high-risk factors are experienced. However, it could not be tested in this study because of the small number of participants. So it is a preliminary study and because of so small sample size, there is a need for further larger studies to confirm the findings.

Conclusions

The study findings showed that shortening of the 20th percentile of telomere length, which is most important to somatic health, was slower in those participating in the neuroeducational methods program. The mechanism for this is still not clear, but neuroeducation may influence some pathways involved in stress-related mechanisms. However, more studies have to be conducted to confirm the advantages of a neuroeducational methods program concerning telomere length.

Main points

- The shortening of the 20th percentile of telomere length was slower in the neuroeducational group than in the control group.
- Neuroeducational exercises might help to slow the 20th percentile of telomere length shortening due to unknown mechanisms and need further studies.
- There was a statistically significant difference between the biological and chronological ages of participants.

Author Contributions

D.S.: Conceptualization, Methodology, Investigation, Data gathering, Data analysis, Writing, Original draft preparation, Reviewing and Editing; A.S. and G.P.: Methodology, Writing, Data gathering, Data analysis; M.M.L.: Supervision, Funding acquisition, Methodology, Validation; R.P.V. and A.V.: Data gathering, Visualization, Validation. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Ethics Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Ethics Committee: Bioethics center of the Lithuanian University of Health Sciences; the approval number is BEC-MF-863.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Semeraro, M.D.; Smith, C.; Kaiser, M.; Levinger, I.; Duque, G.; Gruber, H.-J.; Herrmann, M. Physical activity, a modulator of aging through effects on telomere biology. *Aging* **2020**, *12*, 13803–13823, doi:10.18632/aging.103504.
2. Lulkiewicz, M.; Bajsert, J.; Kopczynski, P.; Barczak, W.; Rubis, B. Telomere length: How the length makes a difference. *Mol. Biol. Rep.* **2020**, *47*, 7181–7188, doi:10.1007/s11033-020-05551-y.
3. Terman, A.; Kurz, T.; Navratil, M.; Arriaga, E.A.; Brunk, U.T. Mitochondrial Turnover and Aging of Long-Lived Postmitotic Cells: The Mitochondrial–Lysosomal Axis Theory of Aging. *Antioxid. Redox Signal.* **2010**, *12*, 503–535, doi:10.1089/ars.2009.2598.
4. Srinivas, N.; Rachakonda, S.; Kumar, R. Telomeres and Telomere Length: A General Overview. *Cancers* **2020**, *12*, 558, doi:10.3390/cancers12030558.
5. Vera, E.; Blasco, M.A. Beyond average: Potential for measurement of short telomeres. *Aging* **2012**, *4*, 379–392, doi:10.18632/aging.100462.
6. Alter, B.P.; Baerlocher, G.M.; Savage, S.; Chanock, S.J.; Weksler, B.B.; Willner, J.P.; Peters, J.A.; Giri, N.; Lansdorp, P.M. Very short telomere length by flow fluorescence in situ hybridization identifies patients with dyskeratosis congenital. *Blood* **2007**, *110*, 1439–1447, doi:10.1182/blood-2007-02-075598.
7. Nalobin, D.; Alipkina, S.; Gaidamaka, A.; Glukhov, A.; Khuchua, Z. Telomeres, and Telomerase in Heart Ontogenesis, Aging, and Regeneration. *Cells* **2020**, *9*, 503, doi:10.3390/cells9020503.
8. Da Costa, J.P.; Vitorino, R.; Silva, G.M.; Vogel, C.; Duarte, A.C.; Rocha-Santos, T. A synopsis on aging—Theories, mechanisms, and future prospects. *Ageing Res. Rev.* **2016**, *29*, 90.
9. Sindi, S.; Solomon, A.; Kåreholt, I.; Hovatta, I.; Antikainen, R.; Hänninen, T.; Levälahti, E.; Laatikainen, T.; Lehtisalo, J.; Lindström, J.; et al. Telomere Length Change in a Multidomain Lifestyle Intervention to Prevent Cognitive Decline: A Randomized Clinical Trial. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* **2021**, *76*, 491–498, doi:10.1093/gerona/glaa279.
10. Guillaumet-Adkins, A.; Yañez, Y.; Peris-Diaz, M.D.; Calabria, I.; Palanca-Ballester, C.; Sandoval, J. Epigenetics and Oxidative Stress in Aging. *Oxid. Med. Cell. Longev.* **2017**, *2017*, 9175806, doi:10.1155/2017/9175806.

11. Epel, E.S.; Blackburn, E.H.; Lin, J.; Dhabhar, F.S.; Adler, N.E.; Morrow, J.D.; Cawthon, R.H. Accelerated telomere shortening in response to life stress. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 17312.
12. Tokuhama-Espinosa, T.N.; Kays, E.; Bruce, C. The Scientifically Substantiated Art of Teaching. Ph.D. Thesis, Capella University, Minneapolis, MN, USA, 2008.
13. Debora Di, J. Synaptic Plasticity and Learning Processes: A Neuroeducation Perspective. *OBM Neurobiol.* **2020**, *4*, 1.
14. Visnola, D.; Sprudša, D.; Arija Baķe, M.; Piķe, A. Mākslas terapijas ietekme uz stresu un trauksmi darbiniekiem. *Proc. Latv. Acad. Sci. Sect. B Nat. Exact. Appl. Sci*. **2010**, *64*, 85–91.
15. Serapinas, D.; Serapiniene, A.; Simaityte, P.; Daugirdaite, I.; Valantinas, A.; Leliugiene, M. The effect of neuroeducational methods on telomere length dynamics. *Med. Stud.* **2020**, *36*, 73–82, doi:10.5114/ms.2020.96784.
16. Gardner M, Bann D, Wiley L, et al. Gender and telomere length: systematic review and meta-analysis. Exp Gerontol. **2014**;51:15-27
17. Mendele-Leliugiene, M. Development of social responsibility in education a socially responsible personality. *Soc. Educ.* **2013**, *34*, 26–39.
18. Gutierrez-Rodrigues F, Alves-Paiva RM, Scatena NF, Martinez EZ, Scheucher PS, Calado RT. Association between leukocyte telomere length and sex by quantile regression analysis. Hematol Transfus Cell Ther. **2021** Feb 6:S2531-1379(21)00005-5.
19. Salvador, L.; Singaravelu, G.; Harley, C.B.; Flom, P.; Suram, A.; Raffaele, J.M. A Natural Product Telomerase Activator Lengthens Telomeres in Humans: A Randomized, Double-Blind, and Placebo-Controlled Study. *Rejuvenation Res.* **2016**, *19*, 478–484, doi:10.1089/rej.2015.1793.
20. Rivera, T.; Haggblom, C.; Cosconati, S.; Karlseder, J. A balance between elongation and trimming regulates telomere stability in stem cells. *Nat. Struct. Mol. Biol.* **2017**, *24*, 30–39, doi:10.1038/nsmb.3335.
21. Boonekamp, J.J.; Bauch, C.; Mulder, E.; Verhulst, S. Does oxidative stress shorten telomeres? *Biol. Lett.* **2017**, *13*, 20170164.
22. Barnes, R.; Fouquerel, E.; Opresko, P.L. The impact of oxidative DNA damage and stress on telomere homeostasis. *Mech. Ageing Dev.* **2019**, *177*, 37–45, doi:10.1016/j.mad.2018.03.013.
23. Zhang, J.; Rane, G.; Dai, X.; Shanmugam, M.K.; Arfuso, F.; Samy, R.P.; Lai, M.K.P.; Kappei, D.; Kumar, A.P.; Sethi, G. Ageing and the telomere connection: An intimate relationship with inflammation. *Ageing Res. Rev.* **2016**, *25*, 55–69.
24. Venditti, S.; Verdone, L.; Reale, A.; Vetriani, V.; Caserta, M.; Zampieri, M. Molecules of Silence: Effects of Meditation on Gene Expression and Epigenetics. *Front. Psychol.* **2020**, *11*, 1767, doi:10.3389/fpsyg.2020.01767.
25. Mekseepralard, C.; Busarakumtragul, P. Effects of mindfulness meditation on serum cortisol of medical students Effects of Barakol on Vascular Functions in Rat View project. *J. Med. Assoc. Thail.* **2013**, *96*, 90–95.
26. Aseervatham, G.S.B.; Sivasudha, T.; Jeyadevi, R.; Ananth, D.A. Environmental factors and unhealthy lifestyle influence oxidative stress in humans—An overview. *Environ. Sci. Pollut. Res.* **2013**, *20*, 4356–4369, doi:10.1007/s11356-013-1748-0.
27. Saenen, N.D.; Martens, D.S.; Neven, K.Y.; Alfano, R.; Bové, H.; Janssen, B.G.; Roles, H.A.; Plusquin, M.; Vrijens, K.; Nawrot, T.S. Air pollution-induced placental alterations: An interplay of oxidative stress, epigenetics, and the aging phenotype? *Clin. Epigenet. BioMed Cent.* **2019**, *11*, 124.
28. Rathore, M.; Abraham, J. Implication of Asana, Pranayama, and Meditation on Telomere Stability. *Int. J. Yoga* **2018**, *11*, 186.

29. Sun, Q.; Shi, L.; Prescott, J.; Chiuve, S.; Hu, F.B.; De Vivo, I.; Stampfer, M.J.; Franks, P.; Manson, J.E.; Rexrode, K. Healthy Lifestyle and Leukocyte Telomere Length in U.S. Women. *PLoS ONE* **2012**, *7*, e38374, doi:10.1371/journal.pone.0038374.

Table 1. Demographic data of experimental and control groups.

Parameter	**Experimental group (n=10)**	**Control group (n=10)**	***p - value***
Biological age in years (mean ± SD)	44.90 ± 8.17	42.93 ± 12.11	> 0.05
Chronological age in years (mean ± SD)	41.00 ± 7.88	42.02 ± 11.90	> 0.05
BMI (mean ± SD)	22.36 ± 2.65	21.97 ± 3.30	> 0.05
Physical activity (number of women)	5	4	> 0.05
Smoking status (number of women)	2	4	> 0.05
Daily life stress (number of women)	5	8	> 0.05
Value of the 20th percentile (kb) before the research (median ± SD)	5.97 ± 0.72	6.34 ± 0.74	> 0.05
Value of the 20th percentile (kb) after the research (median ± SD)	5.60 ± 0.73	5.67 ± 0.53	> 0.05

Table 2. The impact of lifestyle on the 20th percentile (kb).

Factor	**Yes**			**No**			***p-Value***
	Median	**Min**	**Max**	**Median**	**Min**	**Max**	
Smoking	6.35	5.6	7.4	6.0	4.9	7.3	>0.05
Tense life	6.4	4.9	7.4	6.5	4.9	7.1	>0.05
Physical activity	5.8	4.9	7.4	6.3	4.9	7.3	>0.05

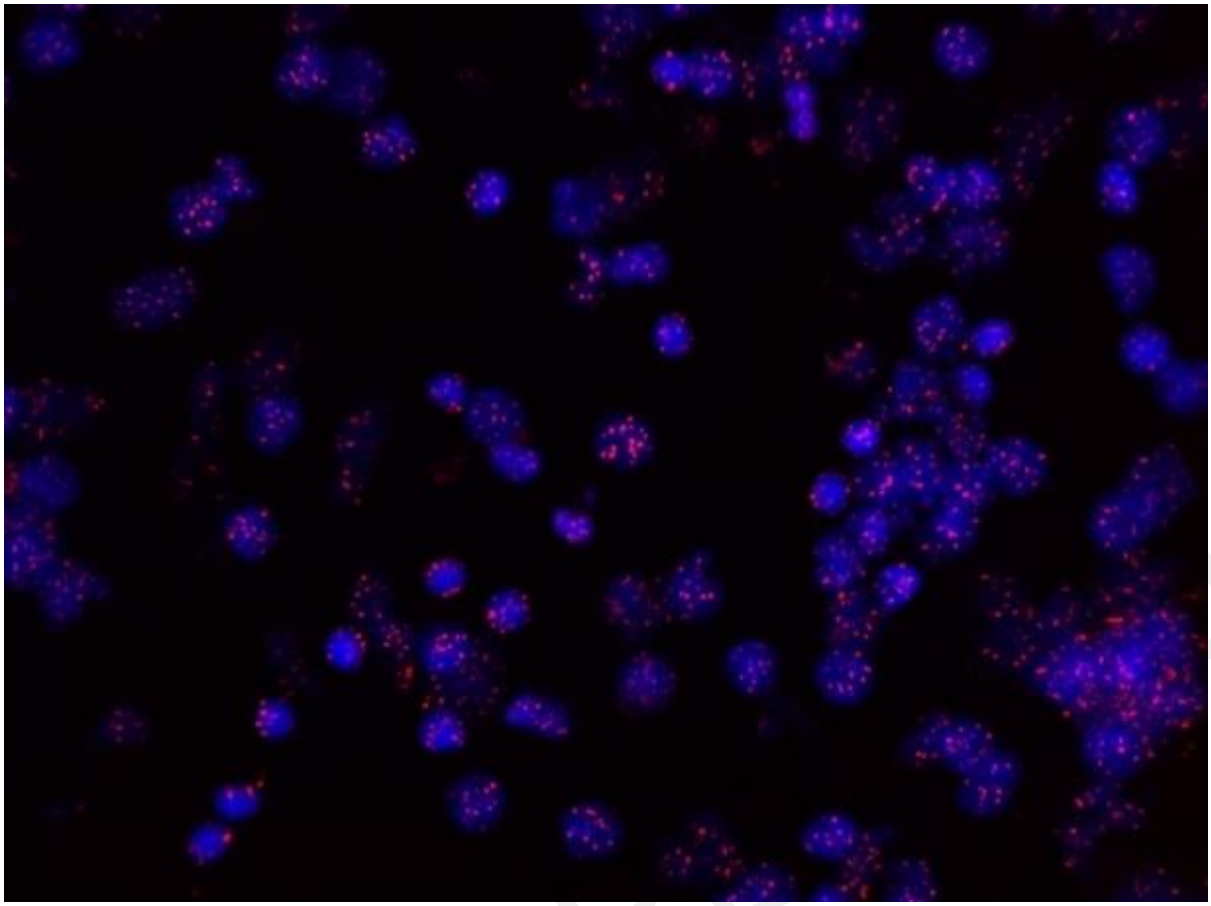

Figure 1. Telomere visualization with Quantitative fluorescence in situ hybridization (Q-FISH)

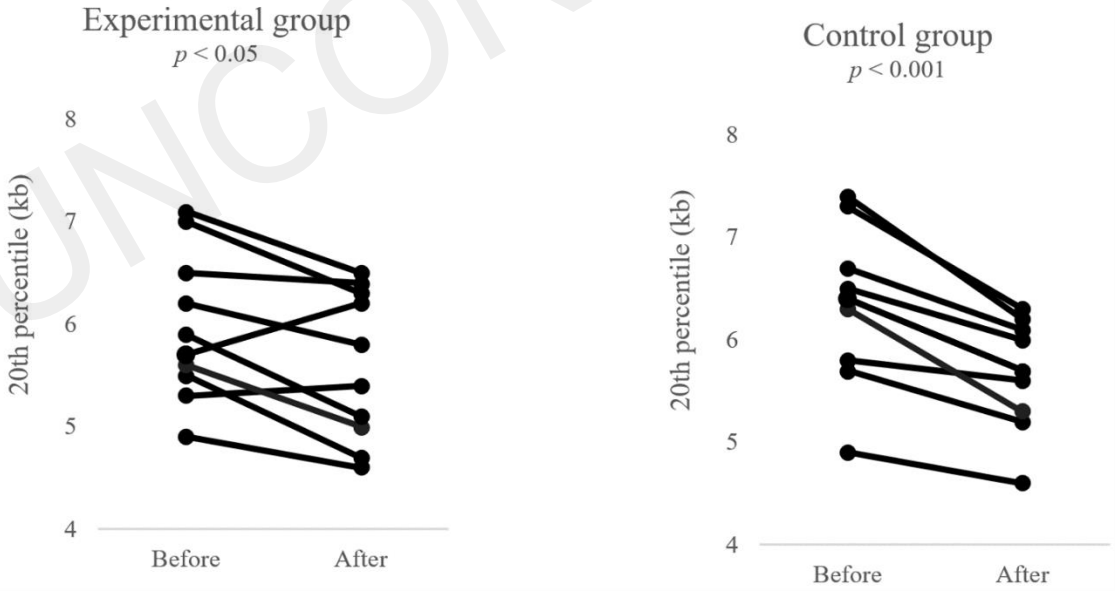

Figure 2. Comparison of 20th percentile values before and after the study using the paired t-test in the experimental group ($p < 0.05$) and control group ($p < 0.001$).

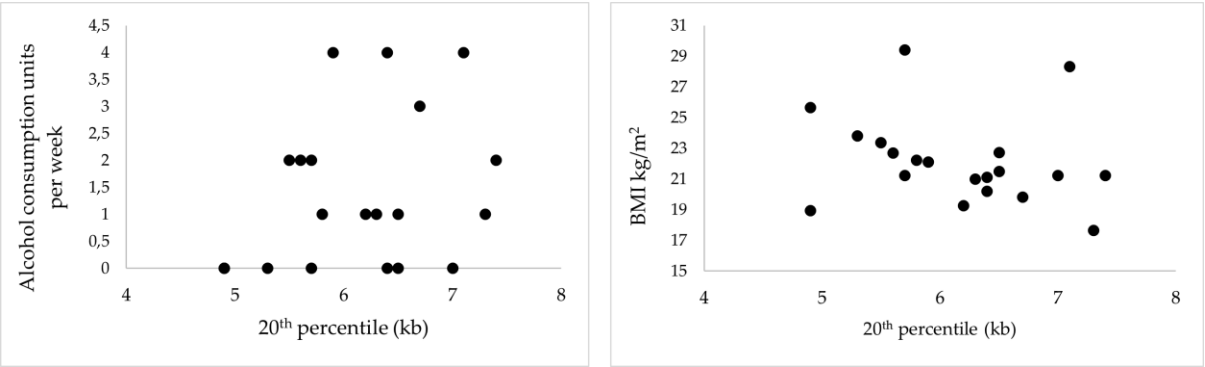

Figure 3. Associations between the 20th percentile and alcohol consumption (left) and BMI (right).