

The effect of neuroeducational methods on telomere length dynamics

Wpływ metod neuroedukacyjnych na dynamikę długości telomerów

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Słowa kluczowe: neuroedukacja, odporność psychiczna, telomery, styl życia.

Abstract

Introduction: Telomere length is one of the most reliable indicators of biological ageing at the cellular level, and shortening of the telomere is an indicator of oxidative stress. Telomere length is associated with the capacity of the immune system responsible for protection against infectious and non-infectious diseases.

Aim of the research: The evaluation of the effect of neuroeducational methods on individuals' biological age, as indicated by telomere shortening, compared to a control group.

Material and methods: The study was conducted on 20 relatively healthy subjects aged 23–59 years. The data summarise the findings on the length of telomeres in the neuroeducational and control groups before and after the 6-month intervention. The experimental group had regular (20 h/month) neuroeducational sessions. HT-Q-FISH (LifeLength, Spain) was used to measure the median telomere length (TL).

Results: The main finding of this study was that while telomere shortening within 6 months was significant in the control group (median telomere length before the survey was 11.05 kb (min. 9.5 kb; max. 12 kb) and after the study 10.50 kb (min. 9.1 kb; max. 11.4 kb) – $p < 0.05$), there was no significant change in telomere length in the experimental group – 10.40 kb (min. 9.4 kb; max. 11.6 kb) before the study and 10.45 kb (min. 9 kb; max. 11.5 kb) after the survey – $p > 0.05$.

Conclusions: Slower telomere shortening is positively associated with neuroeducational sessions and may affect some biochemical pathways associated with stress-induced mechanisms, and also may contribute to the “decreased aging” phenotype.

Streszczenie

Wprowadzenie: Długość telomerów jest jednym z najbardziej wiarygodnych wskaźników biologicznego starzenia się na poziomie komórkowym, a skrócenie telomerów jest wyznacznikiem stresu oksydacyjnego. Długość telomerów wiąże się z wydolnością układu odpornościowego, który odpowiada za ochronę organizmu przed chorobami zakaźnymi i niezakaźnymi.

Cel pracy: Ocena wpływu metod neuroedukacyjnych na wiek biologiczny osób badanych w porównaniu z grupą kontrolną na podstawie skrócenia długości telomerów.

Materiał i metody: Badanie przeprowadzono w grupie 20 ogólnie zdrowych osób w wieku 23–59 lat. Przedstawione dane opisują wyniki pomiarów długości telomerów w grupie, w której stosowano metody neuroedukacyjne, oraz w grupie kontrolnej, przed i po 6-miesięcznej interwencji. W grupie eksperymentalnej realizowano regularne (20 h/miesiąc) sesje neuroedukacyjne. Do pomiaru mediany długości telomerów (TL) zastosowano test HT-Q-FISH (LifeLength, Hiszpania).

Wyniki: Głównym ustaleniem badania było istotne skrócenie długości telomerów w okresie 6 miesięcy w grupie kontrolnej (mediana długości telomerów przed badaniem wynosiła 11,05 kpz (min. 9,5 kpz; maks. 12 kpz), a po badaniu 10,50 kpz (min. 9,1 kpz; maks. 11,4 kpz) – $p < 0,05$). Nie stwierdzono natomiast istotnej zmiany w długości telomerów w grupie badanej – 10,40 kpz (min. 9,4 kpz; maks. 11,6 kpz) przed badaniem i 10,45 kpz (min. 9 kpz; maks. 11,5 kpz) po badaniu – $p > 0,05$.

Wnioski: Wolniejsze skracanie długości telomerów wykazuje dodatnią zależność z sesjami neuroedukacyjnymi i może oddziaływać na niektóre szlaki biochemiczne związane z mechanizmami indukowanymi stresem, co przyczynia się do tzw. fenotypu „spowolnionego starzenia”.

Introduction

The development rate of observable signs of ageing and the development and progression rate of age-associated diseases vary across individuals in a manner that is not strictly parallel to their chronological age. Such variation is influenced by environmental factors (e.g. quality of nutrition, physical activity levels, work-life balance, unhealthy habits, quality of the living environment) and internal factors (oxidative stress, genetic code). Therefore, the individual ageing process should be seen as a function of biological age rather than of chronological age. Telomere length and DNA methylation levels are the strongest indicators of the ageing process at the cellular level. Thus, they are the two most commonly used markers of biological age [1]. Telomere shortening also occurs when cells are subjected to a damaging of such factors as a viral or bacterial infection. Human telomeres are protective nucleoprotein structures of DNA found at the ends of chromosomes; they consist of the single-stranded TTAGGG repeats of DNA. Telomerase is a cellular reverse transcriptase, which adds new DNA onto the telomeres [2]. However, because of the end replication problem TTAGGG repeats shorten with every cell division, when Okazaki fragment has no place to put on and activate polymerase, bases are often lost from the end of DNA, and this results in telomere shortening during the lifetime [3, 4]. This process is associated with an increased risk of cardiovascular and degenerative diseases (osteoporosis, osteoarthritis), atherosclerosis, diabetes mellitus, vascular dementia [3, 5–8], and reduced immunity to infections [9]. According to Blackburn *et al.* and several other studies, emotional and psychological factors (for example, socioeconomic status, stress, depression) and their management have an impact on changes in telomere length [3, 10]. According to the Stress and Coping Theory, successful termination of a stressful factor or restoration of an individual's emotional wellbeing can alleviate chronic stress, thereby protecting against faster biological ageing. Recently a new field of education that connects neurosciences, psychology, and education has been developing, and the results show the impact of the psychological wellbeing of a person on the physiological and even genetic processes in the cells [11]. Neuroeducation is a field of neuroscience based on the theory of neurobiologist Giacomo Rizzolatti [12], which explores human capabilities and ways to change attitudes to life and stressful situations, as well as to develop emotionally resilient thinking and rational solutions based on problem-solving. It can be defined as a multidimensional discipline that consists of cognitive neuroscience, developmental cognitive neuroscience, educational psychology, educational technology, and other methods, which connect the mind, brain, and education [13, 14]. The neuroeducational program 'I am the Creator' is applied using

different methods – visualisations, language codes, special awareness exercises and tasks, meditations, art therapy methods, and mindfulness therapy – all of which have the overall aim of helping the person to manage stressors and protect them from further progression [14]. For example, Regev *et al.*, in their literature review, concluded that art therapy helps psychologically healthy individuals to better alleviate stress levels and manage burnout at work [15]. Also, Ghawadra *et al.*, in a literature review of nine studies, evaluated the effect of mindfulness on the emotional state of nurses and found that mindfulness can significantly reduce their psychological distress, anxiety, burnout, and depression [16].

Aim of the research

The study aim was to evaluate the effect of neuroeducational methods on individuals' biological age, as indicated by telomere shortening, compared to control groups.

Material and methods

Study design

A randomised (paired-sample), prospective study evaluating the impact of neuroeducational methods on telomere shortening was performed. The study was designed and organised by the Institute for Personality Development 'Rafaelis'; the medical history of participants was collected, and blood sampling was performed at the InMedica Clinic (Vilnius); and genetic tests were performed at the Life Length laboratory (Madrid).

The study included 20 healthy women between the ages of 20 and 59 years, all of whom participated in the survey for 6 months (from May to December 2017). Half of the participants (10 persons) attended regular (20 h/month) neuroeducational sessions for 4 consecutive months, during which different theoretical and neuroeducational methods were applied. The neuroeducational methods used were copyrighted works created by Marija Mendele-Leliugiene (Institute for Personality Development 'Rafaelis'). Fifty-seven neuroeducation methods (NEM) and their various algorithms were used in the study. Women who were in the study group participated in intensive (20 h/month; 80 h in total) neuroeducational classes. There were total of 8 days of classes during the study (split into 4 weekends and 2 days a month). NEM is based on the premise that every living person has a vertical dimension – spirit as a will to how live, has natural born powers for self-healing and creativity, human values are naturally oriented towards good, the human being has free will and the ability to choose to live and to heal, and has free will, to change one's mindset and attitude, and to delve into personal growth and exploration. NEM is based

on the fact that the human brain has plasticity and is able to change and create new neural networks. The total number of different NEMs that were created was 111. The most important methods are described in the methodological book [14]. All NEMs were divided into five 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, emotions recognition, control, and release. The tests are for self-examination, “how and where I am”, “I am here and now”. NEM tests and exercises help to perceive, assimilate, and become aware of knowledge about emotional and spiritual hygiene, to see reality, to internalise humanistic values, to change attitude and mindset.
- 2) Visualisations. The seven visualisations were meant to be the main motivational keys that could help a person to go through his/her thinking process, sometimes even resulting in mindset/attitude permanent changes, helps to understand, comprehend, assimilate, and realise the importance of emotions management, and enables a person to take full responsibility for their decisions.
- 3) Meditation. Meditation helps in stress and negative emotion management and allows for a deeper, more subtle self-exploration.
- 4) Verbal codes. Verbal codes are phrases used for self-awareness/self-perception, self-integration, perception of reality, mindset and behavioural correction. Applying verbal codes enables a person to forgive him/herself and others, and motivates a person to choose how to live.
- 5) Art therapy methods for personality development. Art therapy methods were developed by taking into account the goals of psycho-emotional and spiritual health, emphasising the person’s own decision to change his/her attitude, mindset, and way of living, taking into account the person’s emotional memory, which creates unique conditions for the participant to remember the feelings, emotions, and inner states that are then used as the main source of self-regulation, allowing one to improve, to know oneself, to develop one’s powers of consciousness and natural powers for self-healing and creativity.

Art therapy methods for personality development allow a person to enter the creative process without any restrictions, to draw what he/she wants and how he/she wants it (Free drawing, Drawing with hands (palms), My inner compass, My life tree, Secret, Heart icon. Combining these art methods with other NEMs helps a person to be in the “here and now” state for 60 to 180 min. NEM algorithms create a space where a person can experience subtle emotions, feelings, and states and thus strengthen their mental and psychoemotional health.

The remaining 10 participants (control group) did not make any changes to their lifestyle during the study period. Individuals in the control group were identified by their demographic, professional (all had higher education), familial, and medical aspects. Harmful habits (smoking, alcohol consumption) and other signs (Tables 1, 2) were also taken into account. Criteria for inclusion in the study were as follows: age between 20 and 59 years, urban resident, and female gender. Exclusion criteria were cancer or other chronic diseases, and age younger than 20 or older than 60 years. Only females were included in the study to reduce the impact of potentially distorting factors because the group of research was small and telomere length may vary among genders [17]. The study was reviewed and approved by the bioethics centre of the Lithuanian University of Health Sciences; the approval number is BEC-MF-863.

Table 1. Comparison of neuroeducational session participants and the control group according to quantitative indicators

Parameter		Experimental group	Control group
Telomere length before the experiment [kb]	Min.	9.4	9.5
	Max.	11.6	12
	Median	10.4	11.05
Chronological age	Min.	31.2	20.7
	Max.	57.2	59.3
	Median	39	41.85
Biological age	Min.	36.7	21.6
	Max.	56.7	59.8
	Median	41.35	43.85
Percentage of the population (%)	Min.	1	8
	Max.	66	81
	Median	19.5	44
Antitrypsin [g/l]	Min.	1.28	1.21
	Max.	1.71	1.55
	Median	1.41	1.45
Homocysteine [μmol]	Min.	7.23	8.05
	Max.	18.39	46.66
	Median	11.88	11.17
BMI [kg/m^2]	Min.	18.93	17.65
	Max.	28.33	29.41
	Median	22.40	21.17
Hours of sleep	Min.	6	6
	Max.	8	8
	Median	7	7

Table 2. Comparison of neuroeducational session participants and the control group according to quantitative indicators (specified number in group)

Groups	Smokers	Physical activity (> 150 min/week)	Tense (stressful) life	Consume alcohol
Experimental group	2	4	5	6
Control group	4	5	8	6

Data collection

Blood for determination of telomere length was taken twice: at the start of the project and after 6 months (i.e. at the end of the study). Study participants completed particular psychological state evaluation questionnaires both at the beginning of the study and at the end – Hospital Anxiety and Depression Scale (HADS) [18] – which were used to evaluate the patient's mental state and determine whether there was a tendency for depression or anxiety disorders. The results were calculated according to the number of points collected separately for both depression and anxiety: 0–7 normal, 8–10 borderline abnormal, and 11–21 abnormal, in both subgroups. However, for more detailed evaluation, not groups of normal/borderline abnormal/abnormal, but instead scores were used further in the study. Also, participants identified their anthropometric data, described their daily habits (stress, smoking, consumption of alcohol, physical activity, work, and rest regime), which allowed them to be divided into analogous groups and the impact of lifestyle on telomere shortening to be evaluated.

Measurement of telomere length

The life length of participants was measured in Spain by measuring the median telomere length. A method of high throughput (HT) quantitative fluorescence in situ hybridisation (Q-FISH) was used. The Q-FISH analyses cells, unlike other methods such as TRF- and PCR-based assays, where the substrate is DNA [19].

After defrosting at 37°C, cell counts were checked. Control and sample lymphocyte lines were then cultured in black-walled clear-bottom 384 plates. Cell fixation was completed with methanol/acetic acid (3/1, vol/vol). Telomeres were hybridised in situ with a fluorescent peptide nucleic acid (PNA) probe (binds to sequence Alexa488-OO-CCCTAACCCCTAACCCCTAA, Panagene). This was followed by washing of the cells, a fluorescent stain (DAPI) was added to enhance the contrast of DNA. To carry out 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 from Alexa488 – by 488 nm wavelength. Images were analysed with the High Content Screening Opera System (Perkin Elmer) on Acapella software, Version 1.8 (Perkin Elmer). Further interpretation of telomere length was carried out using Life Length's proprietary program.

The median telomere length of all the participants ($n = 20$) from the first blood sample (before the study) was compared with the same age and gender telomere length statistical rates (from the LifeLength database). This comparison allows us to assess (as a percentage) how many people of the same age and gender in the general population have shorter telomeres than our participants. The answer is presented in the results as the size called the percentage of the population. Also, the biological age was measured according to telomere length by the proprietary program of the company.

Statistical analysis

The data obtained were analysed by the IBM SPSS statistics program 25. Quantitative study data (chronological and biological age, telomere length, change in their length, percentage, percentile kilobases (kb), body mass index (BMI), hours of sleep) were converted into grades and calculated using non-parametric criteria due to the small scope of the study ($n < 30$). The values of quantitative characteristics that did not meet the conditions of a standard section in two independent groups were compared with the non-parametric Mann-Whitney U test, and in the two dependent groups the non-parametric Wilcoxon signed-rank test was used. The Spearman correlation coefficient was applied to assess the strength of the relationship between two quantitative characteristics that did not meet the conditions of a standard section. Observed differences and dependency between attributes were considered statistically significant when the calculated significance level (p -value) was lower than the selected significance level ($\alpha = 0.05$).

Results

The median biological age of the participants in the study was 42.6 years (min. 21.6 years, max. 59.8 years), and the chronological age – 40.05 years (min. 20.7 years, max. 59.3 years); the difference was considered statistically significant ($p < 0.05$). The median telomere length before the study was 10.8 kb (min. 9.4 kb, max. 12 kb), and the percentage of the population was 40.5% (min. 1%, max. 81%).

Changes in telomere length

The median length of telomeres in the experimental group was 10.40 kb (min. 9.4 kb; max. 11.6 kb) before the study and 10.45 kb (min. 9 kb; max. 11.5 kb)

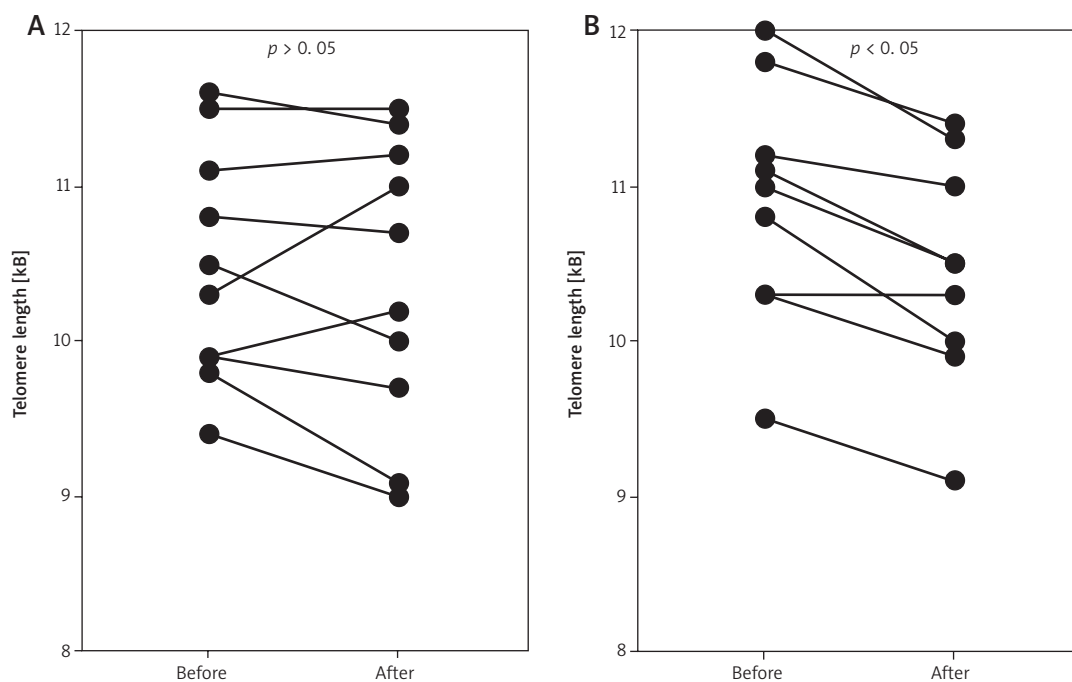


Figure 1. Comparison of telomere length before and after the study for experimental group (A) and control group (B) with the Wilcoxon matched-pairs test

after the study – telomere length changes over the 6 months were not statistically significant ($p > 0.05$). The median telomere length of the control group before the study was 11.05 kb (min. 9.5 kb; max. 12 kb), and after the study 10.50 kb (min. 9.1 kb; max. 11.4 kb) – telomere shortening over the 6 months was statistically significant ($p < 0.05$) (Figure 1).

Measurement the telomere length changes of both groups showed that the telomere length of the experimental group decreased in six participants (of 10 recruited), and the median of telomere shortening was 0.3 kb (min. 0 kb; max. 0.7 kb), and in the control group it decreased in nine participants (of 10 studied), the median was 0.4 kb (min. 0 kb; max. 0.8 kb), but telomere shortening difference in kb between both groups was statistically insignificant ($p > 0.05$).

The influence of daily habits on telomere length

All the participants of the study ($n = 20$) completed questionnaires about their lifestyle and daily habits, and based on the responses they were divided into groups. There were three categories of groups: the stress of everyday life, smoking, and physical activity. Questionnaires were based on participants' self-report – life was considered stressful if the participant was experiencing severe strain on a daily basis, and participants were considered physically active if they engaged in physical activity for at least 60 min daily. It was determined that the median of telomere length dur-

ing the first blood sampling was 10.8 kb (min. 9.4 kb; max. 12 kb) for females whose life was more stressful ($n = 13$), and for those whose life was not stressful ($n = 8$) – 11.1 kb (min. 9.5 kb; max. 11.6 kb); however, the difference between groups was not statistically significant ($p > 0.05$). The median telomere length of participants who smoked ($n = 6$) was 10.9 kb (min. 9.9 kb; max. 12 kb), and for non-smokers ($n = 14$) – 10.55 kb (min. 9.4 kb; max. 11.8 kb); the difference between the groups was not statistically significant ($p > 0.05$). The median telomere length of those physically active ($n = 9$) was 10.3 kb (min. 9.5 kb; max. 12 kb), and of those physically inactive ($n = 11$) – 10.8 kb (min. 9.4 kb; max. 11.8 kb); the difference between the groups was also not statistically significant ($p > 0.05$) (Table 3).

No statistically significant correlation was found among telomere length and BMI, sleep duration, and alcohol consumption (Figure 2).

Influence of neuroeducational methods on the wellbeing of study participants

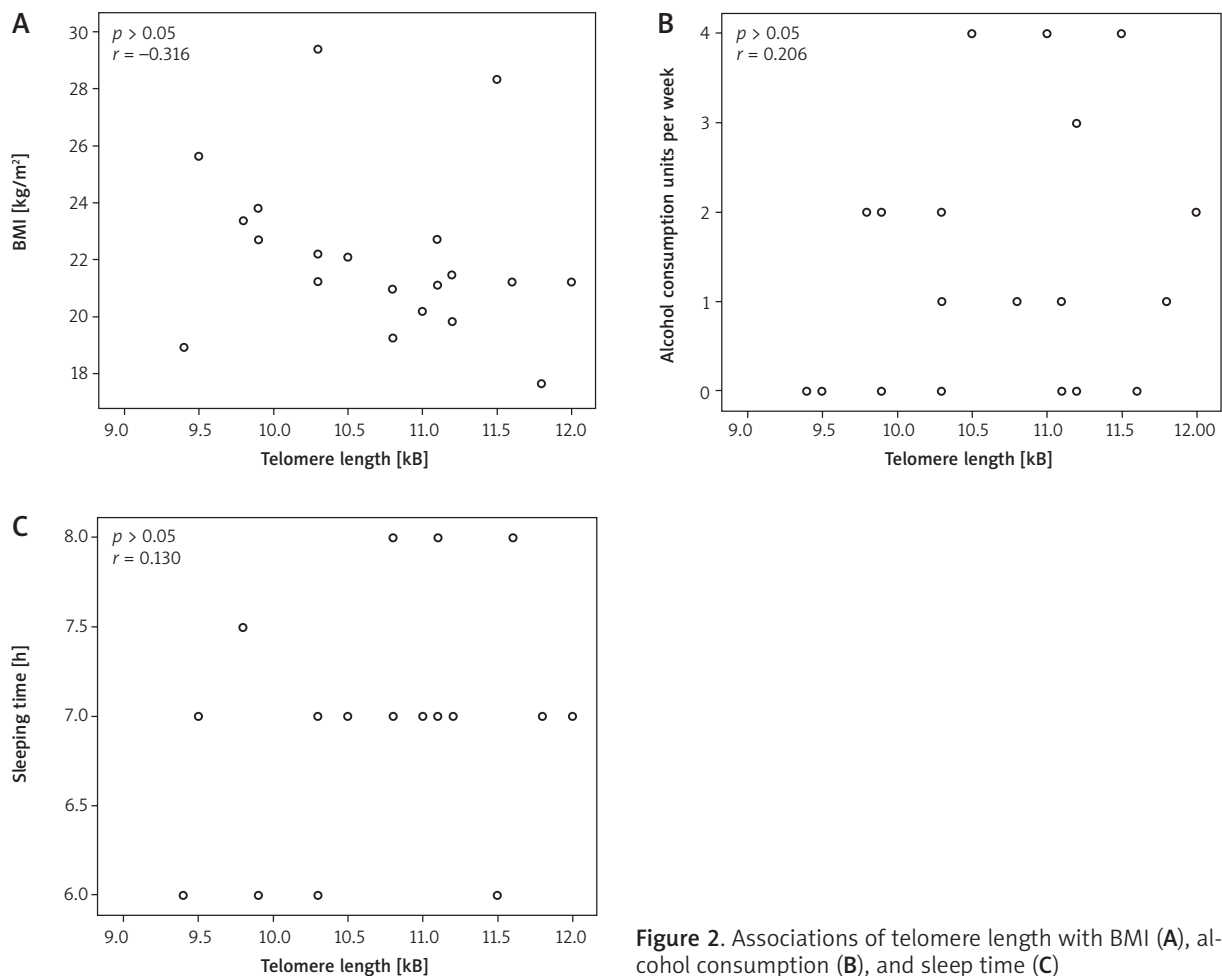
All participants in the study completed a HADS at the beginning and end of the study, based on which the risk of anxiety disorder and depression were individually evaluated. Comparing the scores of both the experimental and the control groups, it was found that the depression risk scores in the experimental group were statistically significantly reduced after the survey.

The median of the HADS anxiety scores of the experimental group was 5 (min. 0; max. 16) before

Table 3. The impact of lifestyle on telomere length

Parameter	Yes			No			P-value
	Median	Min.	Max.	Median	Min.	Max.	
Tense life	10.8*	9.4	12	11.1	9.5	11.6	> 0.05
Smoking	10.9	9.9	12	10.55	9.4	11.8	> 0.05
Physical activity	10.3	9.5	12	10.8	9.4	11.8	> 0.05

*All sizes in the table are measured in kb.

**Figure 2.** Associations of telomere length with BMI (A), alcohol consumption (B), and sleep time (C)

the study, and 3 (min. 0; max. 7) after the study. The change of the sum of scores was not statistically significant after 6 months ($p > 0.05$). The median of HADS anxiety scores of the control group before the study was 8 (min. 2; max. 15), and after the survey – 7 (min. 2; max. 7). The change of the sum of scores was not statistically significant over 6 months ($p > 0.05$) (Figure 3).

The median of the HADS depression scores of the experimental group was 4 (min. 1; max. 6) before the study and after the study 1.5 (min. 0; max. 4). The

change of the sum of scores over 6 months was statistically significant ($p < 0.05$). The median of the HADS depression scores of the control group was 3 (min. 1; max. 5) before the study, and after the study – 4 (min. 2; max. 7). The change in the sum of scores over 6 months was not statistically significant ($p > 0.05$) (Figure 4).

Discussion

The main finding of this study was that while telomere shortening was significant in the control

group, there was no significant change in telomere length in the experimental group. Hence, neuroeducational methods, which are relatively easy to access, have the potential to be used in clinical practice as a means of slowing down the process of telomere shortening. Similar findings are described in other studies; for example, as established by Lengacher *et al.*, mindfulness-based stress reduction psychotherapy slows down telomere shortening [20]. Although these scientists have not investigated and explained the benefits of telomere shortening, the results of our study revealed that deceleration of telomere shortening improves the wellbeing of individuals and reduces the incidence of depression. However, our study, in contrast to the study by Lengacher *et al.*, was conducted with healthy volunteers. Furthermore, longer telomeres were identified among people who engage in Zen meditation compared to non-practitioners [21]. In particular species the Hayflick limit (cells lost their ability to divide) is dependent on telomere length. However, not in all species higher Hayflick limit is associated with longer lifespan.

Nonetheless, the length of telomeres is directly connected with the regeneration of organs, because shortened telomeres are connected with the risk of multiple diseases such as atherosclerosis, Alzheimer's disease, and diabetes [22, 23]. These findings are important because telomere shortening has an impact on the development of ageing-related diseases such as cancer [22], atherosclerosis, and plaque instability which leads to cardiovascular diseases [23], which are

some of the most common causes of death in developed countries.

The difference between the chronological and biological age of the participants was evaluated before the study. The calculated biological age of all participants, as indicated by telomere length, was higher than their chronological age (median biological age was 42.6 years, and median chronological age was 40.05 years); the difference was statistically significant ($p < 0.05$). Based on the results, we can determine that 59.5% of our participants had shorter telomeres than matched individuals from general population. This result indicates that our study participants were more biologically obsolete before the study than the overall worldwide population average, which could be due to stress, living in the city, and other risk factors identified by the participants.

Within our study, smoking, alcohol consumption, BMI, and sleep duration had no statistically significant effect on telomere length (possibly due to the small sample size). Even though it is difficult to determine a definite link between telomere length and smoking [24] or alcohol consumption [25], these factors are often referred to as risky for telomere shortening [26]. Insufficient sleep duration and quality are also associated with shortening of telomere length [27, 28]. The Shamas study found that obesity can also lead to a reduction in telomere length [26]; this effect was not confirmed in our study because of the low and moderate BMI of the participants. Overall, in the study the relationship between daily life habits and telomere

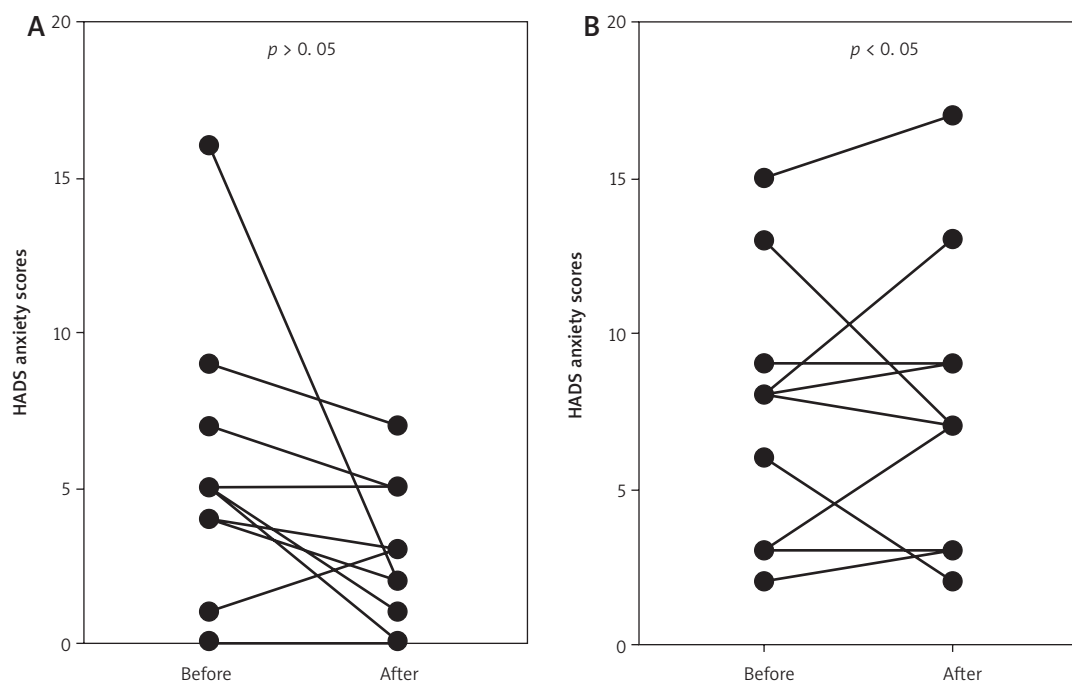


Figure 3. Comparison of HADS anxiety scores before and after the study with Wilcoxon signed-rank test (A – experimental group, B – control group)

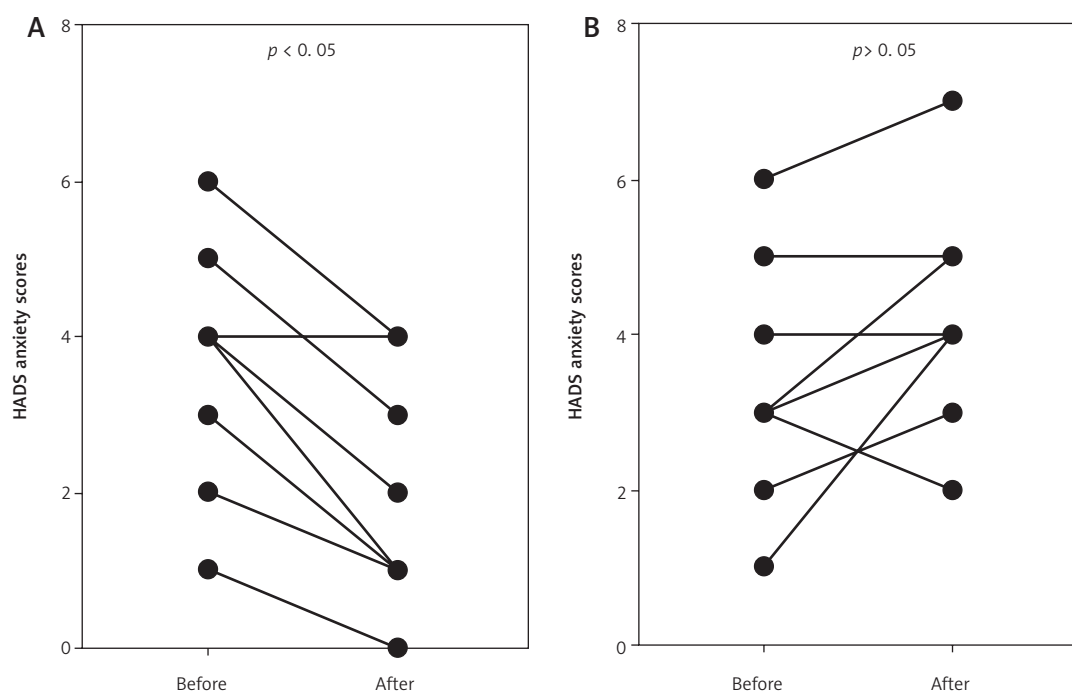


Figure 4. Comparison of HADS depression scores before and after the study with Wilcoxon matched-pairs test (**A** – experimental group, **B** – control group)

length was not determined due to the complexity of interpreting factors, the small scope of the study, and the subjective answering of the questionnaire.

Tension, anxiety, and stress are among the most commonly known telomere shortening risk factors [26, 29, 30]. After neuroeducational sessions, the HADS depression scores in the experimental group were significantly reduced, and for those in the control group, they did not change significantly. There was no statistically significant change in the HADS anxiety score. Overall, it could be said that neuroeducational methods have a positive effect on the psychological health of a person and help to reduce stress and tension.

Neuroeducation through stress reduction mechanisms can reduce the negative impact of cortisol and free radicals on telomeres and reduce the rate of telomere decline. Because several authors support the thesis that stress increases proteases and oxidative stress, which are harmful to telomeres [31, 32], these results support the theory that simple neuroeducational methods through neuronal settings and holistic mechanisms may have an impact at the cellular level. Wilson *et al.* found that neuroeducational methods reduce stress while increasing the activity of the parasympathetic nervous system [33]. Decreased activity of the sympathetic and increased activity of the parasympathetic nervous systems contribute to slowing down the shortening of telomeres [33].

The present findings, even during these times of pandemic, are of great importance because lower

decrease in telomere length is connected with better function of the immune system, which helps to fight viruses, bacteria, and other diseases [3, 10]. Telomere shortening may also explain why COVID-19 viruses affect older people to a greater degree. It was proven that some free telomeric DNA fragments in systemic circulation have anti-inflammatory action [34, 35].

The main disadvantage of this pilot study is its small scope. Because of this we could not perform multiple regression, which could have strengthened the results. We also cannot exclude possible technical or biological factors that may have influenced telomere length analysis and had an impact on the results. Although female (as was analysed in our study) telomere length tends to be higher than male [17], an age-related increase in arterial telomere uncapping and senescence is greater in females than males. At the same time, in young and middle-age groups it is similar [36]. Self-report surveys were used to assess the risk factors and experienced stress; thus, the answers could be inaccurate due to self-reporting bias. In the future, to improve the study and to assess the influence of daily habits and other factors on telomere length in a more accurate way, the scope should be extended in a greater number of participants, and men should be included as well.

Conclusions

The study findings show that neuroeducation is associated with a slower shortening of telomeres. We

hypothesise that neuroeducation can have an impact on some biochemical pathways involved in stress-related mechanisms and may contribute to a 'decreased ageing' phenotype, which is responsible for the prevention of many infectious and non-infectious diseases.

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Conflict of interest

The authors declare no conflict of interest.

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