


## Original Article

# Global DNA methylation and physical fitness of elderly athletes with lifelong endurance activity

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## ABSTRACT

**Background:** Level of Global DNA methylation is associated with many diseases and the influence of physical activity is being investigated by several research groups. The aim of our study was to assess the effect of lifetime endurance physical activity on global DNA methylation, physical fitness and body composition. **Methods:** A total of 31 elderly males were involved in the study, divided into two groups based on differences in physical activity. The first group consisted of 18 volunteers with lifetime endurance activity (mean age: 65.1 ± 3.3 yr.; height: 174.8 ± 4.9; weight: 81.5 ± 6,1 kg; BMI: 24.2 ± 1.1). The control group consisted of thirteen elderly individuals with a sedentary lifestyle (mean age: 64.8 ± 3.1 yr.; height: 176.5 ± 5.5; weight: 87.9 ± 10.1 kg; BMI: 27.8 ± 2.9). Quantification of global DNA methylation was performed in DNA isolated from peripheral blood mononucleated cells by LINE-1 pyrosequencing. **Results:** Elderly individuals with lifetime endurance activity had a better level of physical fitness  $VO_{2max}$  on average 30 % (35.7 ± 10.6 vs. 31.9 ± 7,7 ml.kg<sup>-1</sup>.min<sup>-1</sup>, p <.01) and lower mean body fat content (17.46 ± 2.52 vs. 27.8 ± 2.9 %, p < .01). Global DNA methylation did not differ between studied groups (81.1 ± 2.1 vs. 80.5 ± 1.6 %). **Conclusion:** Better level of physical fitness does not influence the level of the global DNA methylation in peripheral blood mononuclear cells significantly. For future research we recommended to observe DNA methylation changes in specific tissues (e.g., skeletal muscle fibres).

**Keywords:** Global DNA methylation; Physical fitness; Ageing; Endurance.

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## INTRODUCTION

Life expectancy at birth increased very fast in the last few years. Worldwide, the average life expectancy is 76.72 years (WHO 2014). The question is: Is life quality increasing too? Growing disparities between quality and quantity of life may be one of the most significant health challenges of our time (Walsch et al. 2019). Physical inactivity that caused more than 5.3 million of the 57 million deaths that occurred worldwide in 2008 (WHO 2014) has been identified as one of the four leading risk factors for global mortality. Moreover, the lack of exercise is the sixth most important factor of diseases in the European Union (Börnhorst et al. 2016), estimated to be the main cause for approximately 21-25 % of breast and colon cancers, 27 % of diabetes and 30 % of ischemic heart disease burden (WHO 2014).

A large number of studies show that physical activity improves not only physical fitness but it reduces the risk of hypertension, diabetes, cardiovascular diseases, various types of cancers and many others diseases (Vita et al. 1998, Hurley et al. 2000, Haskell et al. 2007, Lee et al. 2012, La Vecchia et al. 2012, Wen et al. 2012, Waxman et al. 2004, Joyner et al. 2009, Kell et al. 2009, Ballard-Barbash et al. 2012).

Environmental factors have been shown to influence the functional output of the cell's genome and many complex human diseases are believed to have a strong environmental component. Gene-environment interactions are mediated by epigenetic modifications which are, in contrast to genetic changes, more dynamic and often reversible (Anderson et al., 2012).

The term 'epigenetics', first time used by Conrad Waddington in 1942, explain the complex, dynamic interactions between the developmental environment and the genome leading to the production of the phenotype (Tronick et al. 2016). In molecular epigenetics, the term 'epi' is interpreted as 'over' - sitting over and operating on the genes (Avery et al., 1944). Epigenetic mechanisms are often involved in the control of gene expression. Until recently DNA methylation was one of the most studied epigenetic mechanisms. However, recent discoveries uncovered others, e.g., histone modifications, leading to changes in chromatin conformation and subsequently in gene expression (Bannister et al. 2011). Furthermore, gene regulations through non-coding RNA-mediated pathways, having a crucial role in the number of biological processes, including diseases development and progression (Matzke et al. 2005). Epigenetics is closing the gap in our gene-environment interaction explanatory abilities and should be implemented to broaden the field of rehabilitation sciences, promote mechanism-based clinical reasoning, and develop new treatments (Poli et al. 2019).

Changes in global DNA methylation levels are associated with different diseases such as cancer (Cheung et al. 2010, Kanai et al. 2010, Weber et al. 2005, Spence et al. 2011, Woo et al. 2012, Brennan et al. 2012), type 2 diabetes mellitus, (Volkmar et al. 2012, Weng et al. 2018), multiple sclerosis (Brooks et al. 2010), asthma (Ho SM et al. 2010) and Alzheimer's disease, schizophrenia or myelodysplastic syndrome (Chouliaras et al. 2010, Romermann et al. 2008), inflammation, neurodegenerative diseases and ageing process (Ferioli et al. 2019). Loss of CpG methylation at repetitive sequences which are normally highly methylated is associated with increased genomic instability (Sharma et al. 2010). Global DNA hypomethylation and gene-specific hypermethylation were the initial epigenetic abnormalities recognized in cancers (Dawson et al. 2012).

In addition to the role of epigenetic changes in diseases pathogenesis, there can be epigenetic variability among individuals due to ageing, which is also, with a few exceptions, associated with CpG hypomethylation (Tra et al. 2002, Bollati et al. 2009, Bormann et al. 2016).

Our study focused on the assessment of the effect of chronic aerobic exercise on DNA methylation. Although the association between physical activity and DNA methylation changes was the subject of several studies in recent years, only a few of them were focused on the potential relationship between the long-lasting physical activity and the level of global DNA methylation (Luttrop et al. 2013). Aim of our study was to assess the influence of lifetime physical activity on global DNA methylation, physical activity and body composition.

## **METHODS**

### ***Subjects***

Thirty-one elderly males from Slovak republic were divided into two groups according to the validated questionnaire focused on physical activity (Lagerros et al. 2006). The first group consisted of eighteen elderly athletes (EA) who were lifetime physically active (age:  $65.1 \pm 3.3$  yr.; height:  $174.8 \pm 4.9$  cm; weight:  $81.5 \pm 1.4$  kg; body mass index (BMI):  $24.2 \pm 1.1$ ) skinfold fat:  $17.46 \pm 2.52$  %). In this group were active distance runners, cyclist and triathlons. Athletes had different training structure during professional carrier in various clubs but the main ingredient of training was endurance training and performance on the competition. The inclusion condition was to have at least four-time endurance training per week during all life. The second group consisted of thirteen elderly male individuals without experience of regular sport or organized physical activity (SG) (age:  $64.8 \pm 3.1$  yr.; height:  $176.5 \pm 5.5$  cm; weight:  $87.9 \pm 10.1$  kg; BMI:  $27.8 \pm 2.9$ ); skinfold fat:  $27.8 \pm 2.9$  %). Written informed consent was obtained from all individual participants included in the study. The study was submitted to and approved by the ethics committee of Faculty of Physical Education and Sports of the Comenius University in Bratislava, Slovakia.

### ***Data collection***

Subjects were examined and tested during the years 2018 and 2019 in the Diagnostic Center on Faculty of Physical Education, Bratislava. Global DNA methylation was analysed in the Cancer Research Institute, Biomedical Research Center, Slovak Academy of Sciences. The blood sampling and physical fitness tests were organized on separate days. The blood sampling was carried out in the morning when participants didn't eat and conditioning tests were realized two days later. The participants were instructed to keep usual diet behaviour and the exercise testing were done after an overnight fast and breakfast prior to examination.

### ***Aerobic capacity testing***

#### ***Power at $VO_{2max}$ during cycling ergometer***

All participants were familiar with cycling on a bicycle ergometer (Cosmed Metabolic Company, Italy). Participants completed a maximal incremental cycling test. During all tests environmental conditions were standardized, the temperature was kept at  $20^{\circ}\text{C}$ , and relative humidity varied between 50% and 60%. After short warm-up (5 minutes, 50 W) the subjects starting at 40 W. The power was set to increase by 10 W every 1 minute for elderly individuals who did not practice any physical exercise and 20 W every minute for elderly athletes with lifetime physically activity until volitional exhaustion. Last power step was held for an entire minute.

### ***Knee extension isometric strength***

The knee extension isometric strength was performed in a seated position on a backward-inclined ( $15^{\circ}$ ) chair by a dynamometer (S2.0 science to practice, Slovenia). The hips and the shoulders were stabilized with safety belts. The rotational axis of the dynamometer was aligned with the transverse knee-joint axis and connected to the distal end of the tibia using a length-adjustable rigid lever arm. The subjects performed a maximal voluntary isometric contraction of the knee extensors twice. The knee-joint angle was  $130^{\circ}$ . The

isometric contractions lasted for 3 seconds each and were separated by a 2-minute rest interval. The highest torque (Nm) was recorded as isometric strength performance.

### **Handgrip force**

Maximal isometric handgrip force was recorded over 10 s using a handheld hand-grip ergometer (CAMRY EH101 electronic hand dynamometer, China). During measurements, the upper and lower arm were supported in such a way that 90° position of the elbow joint was achieved without additional effort.

### **Body composition**

Body composition was measured on Inbody 270 (BioSpace Seoul, Korea) is a segmental impedance device measuring the voltage drop in the upper and lower body. The participant stood on the device while it measured body weight, and age, height and sex were entered on the touch screen. The InBody uses eight points of tactile electrodes (contact at the hands and feet).

### **DNA methylation assessment**

#### *DNA extraction and sodium bisulfite modification*

Blood samples were collected in EDTA tubes and centrifuged at 1000 g for 10 min at room temperature. The supernatants were collected and centrifuged again at the same condition. Plasma samples were stored at -70°C. Peripheral white blood cell DNA was extracted using a FlexiGene DNA kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA concentrations were measured by NanoDrop1000 spectrophotometer (Thermo Fisher Scientific, Bremen, Germany). A sodium bisulfite modification was performed at 1 µg of peripheral blood cells DNA using EpiTect Bisulfite Kit (Qiagen, Hilden, Germany). The sodium bisulfite modification is based on the conversion of unmethylated cytosines to uracils, while 5-methylcytosines remain unaltered. The bisulfite modified DNA was stored at -18°C until use.

#### *Global DNA methylation analysis*

Global DNA methylation was analysed by pyrosequencing using the PyroMark Q24 CpG LINE-1 kit (Qiagen, Hilden, Germany). This assay uses real-time, sequence-based Pyrosequencing® technology to quantify the methylation levels of three CpG sites in positions 331 to 318 of the LINE-1 repetitive sequence (GenBank accession number X58075). The first step was the PCR amplification of a 146 bp fragment using bisulfite-treated DNA. Pyrosequencing runs were carried out using a PyroMark Q24 system and the PyroMark Gold Q24 Reagents (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The results of the analyses were evaluated using the PyroMark Q24 2.0.6. software (Qiagen, Hilden, Germany). Methylation data are presented as the percentage of average methylation in three CpG sites.

### **Data Analysis**

Descriptive statistic calculations are presented as means and standard deviations. The statistical analyses of data were performed using the Jamovi (Version 1.0.0), (Computer Software). The normality of data we tested by the Shapiro-Wilk normality test. The Wilcoxon Rank-Sum Test was applied to determine differences between seniors athletes and seniors with sedentary lifestyles. Pearson's product-moment correlation was applied to find relationships between variables. Significance for all analyses was set at  $p < .05$  and  $p < .01$ .

## **RESULTS**

Baseline characteristics and comparison of the physical fitness between EA and SG are presented in Table 1. Age did not differ significantly between studied groups. Among anthropometric characteristics we observed significant differences in BMI ( $24.2 \pm 1.1$  vs.  $27.8 \pm 2.9$ ;  $p < .05$ ), body fat content ( $17.5 \pm 2.5$  vs.  $23.5 \pm 5.3$

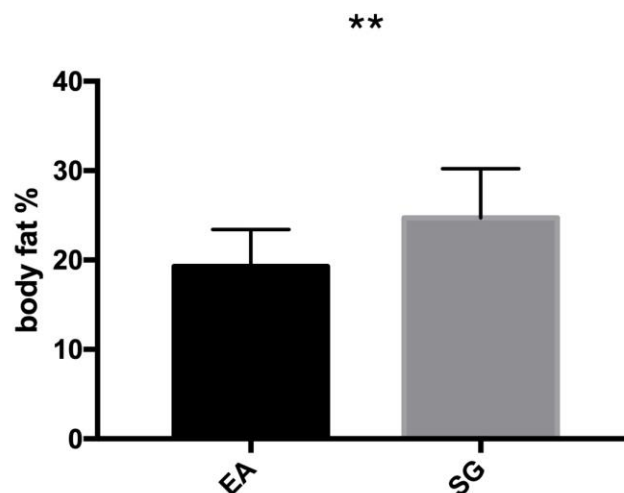
%;  $p < .01$ ) (Figure 1) and visceral fat ( $9.8 \pm 2.6$  vs.  $13.5 \pm 4.0$  level;  $p < .01$ ). Also, we found a significant difference in maximal strength parameters of the upper and lower body. Maximal strength of upper body and maximal strength of lower body ( $0.65 \pm 0.07$  vs.  $0.52 \pm 0.09$   $\text{kg.kg}^{-1}$ ). EA had also better values in  $\text{VO}_{2\text{max}}$  ( $35.7 \pm 10.6$  vs.  $31.9 \pm 7.7$   $\text{ml.kg}^{-1}.\text{min}^{-1}$ ,  $p < .01$  (Figure 2) with 30 % difference. In power at  $\text{VO}_{2\text{max}}$  had EA significant higher values ( $3.27 \pm 0.43$  vs.  $2.21 \pm 0.56$   $\text{W.kg}^{-1}$ ) (Figure 3) with 12.5 % differences.

We did not identify a difference in DNA methylation between studied groups ( $81.1 \% \pm 2.1 \%$  vs.  $80.5 \% \pm 1.8 \%$ ). Similarly, no significant differences were found in the level of DNA methylation at individual CpG dinucleotides ( $84.5 \pm 3.2$  vs.  $83.6 \pm 3.0 \%$ ;  $78.2 \pm 1.5$  vs.  $77.3 \pm 1.8 \%$ ;  $80.5 \pm 3.1$  vs.  $80.6 \pm 3.1 \%$ ) between studied groups (Table 2). Between global DNA methylation and physical fitness, we didn't find any relationship.

Table 1. Baseline characteristics and comparison of the physical fitness between elderly athletes ( $n = 18$ ) and seniors with a sedentary lifestyle ( $n = 13$ ).

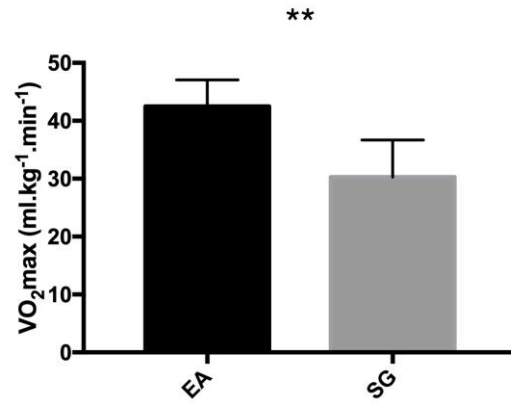
	EA	SG	p-value
Age	$65.1 \pm 3.3$	$64.8 \pm 3.1$	n.s.
Height (cm)	$174.8 \pm 4.9$	$176.5 \pm 5.5$	n.s.
Weight (kg)	$81.5 \pm 1.4$	$87.9 \pm 10.1$	$p < .05$
BMI	$24.2 \pm 1.1$	$27.8 \pm 2.9$	$p < .05$
Body fat (%)	$17.5 \pm 2.5$	$23.5 \pm 5.3$	$p < .01$
Hand grip [ $\text{kg.kg}^{-1}$ ]	$0.65 \pm 0.07$	$0.52 \pm 0.09$	$p < .05$
Isometric strength of knee extensors [ $\text{nm.kg}^{-1}$ ]	$4.79 \pm 1.1$	$4.46 \pm 0.8$	n.s.
$\text{VO}_{2\text{max}}$ [ $\text{ml.kg}^{-1}.\text{min}^{-1}$ ]	$41.8 \pm 8.1$	$30.1 \pm 6.5$	$p < .01$
$\text{VO}_{2\text{max}}$ [ $\text{l.min}^{-1}$ ]	$3.0 \pm 0.9$	$2.7 \pm 0.6$	$p < .01$
Power max [ $\text{W.kg}^{-1}$ ]	$3.27 \pm 0.43$	$2.21 \pm 0.56$	$p < .01$

Note: (n.s.) represents nonsignificant differences.



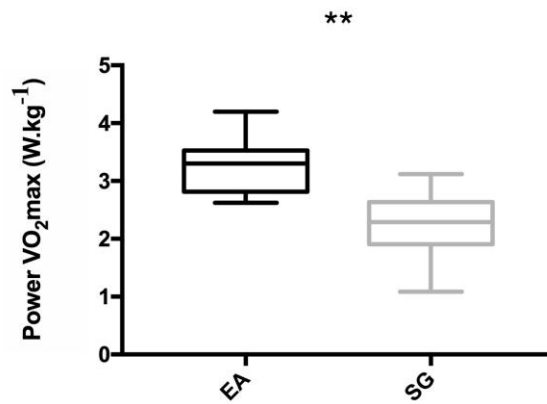
Note: (\*\*) represents significantly differences at  $p < .01$ .

Figure 1. Comparison of the body fat content between elderly athletes ( $n = 18$ ) and sedentary group ( $n = 13$ ).



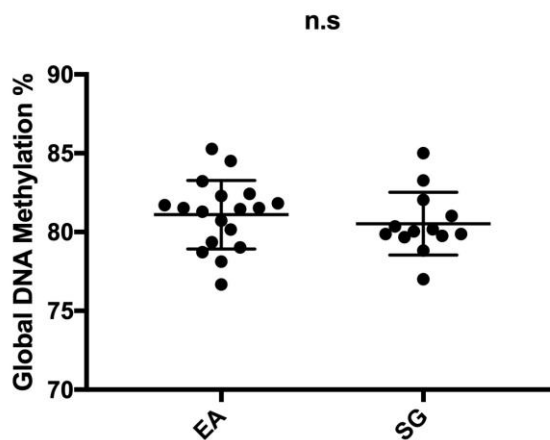
Note: (\*\*) represents significantly differences at  $p < .01$ .

Figure 2. Comparison of the VO<sub>2</sub>max [ml.kg<sup>-1</sup>.min<sup>-1</sup>] between elderly athletes (n = 18) and sedentary group (n = 13).



Note: (\*\*) represents significantly differences at  $p < .01$ .

Figure 3. Comparison of the Power max [W] between elderly athletes (n = 18) and sedentary group (n = 13).



Note: (n.s.) no significant difference.

Figure 4. Comparison of the levels of global DNA methylation (%) between elderly athletes (n = 18) and sedentary group (n = 13).

Table 2. Comparison level of the global DNA methylation between elderly athletes (n = 18) and seniors with a sedentary lifestyle (n = 13).

	EA	SG	Significant difference p-value
Global methylation (%)	81.1 ± 2.1	80.5 ± 1.8	n.s.
Pos.1 Meth. (%)	84.5 ± 3.2	83.6 ± 3.0	n.s.
Pos.2 Meth (%)	78.2 ± 1.5	77.3 ± 1.8	n.s.
Pos.3 Meth (%)	80.5 ± 3.1	80.6 ± 3.1	n.s.

Note: (n.s.) represents nonsignificant differences.

## DISCUSSION

The study examined thirty-one healthy elderly males. Our data showed that EA had 30 % better physical fitness. Better level of physical fitness was a result of lifetime physical activity. This outcome is in accordance with the older study, focusing on well-trained master endurance athletes and elderly sedentary control subjects. The authors found 59 % higher  $VO_{2max}$  in endurance athletes compared to sedentary controls (54.0 vs. 33.9 ml.kg<sup>-1</sup>.min<sup>-1</sup>) (Rogers et al. 1990). In our study we identified 28 % higher  $VO_{2max}$  for EA (41.8 vs 30.1 ml.kg<sup>-1</sup>.min<sup>-1</sup>) than in SG. The widely accepted theory states that the threshold cardiorespiratory fitness needed to independently perform typical activities of daily living is 20 ml.kg<sup>-1</sup>.min<sup>-1</sup> for older adults (Arnett et al. 2008) while another study by Herdy et al. 2011 defined 24.5 ml.kg<sup>-1</sup>.min<sup>-1</sup> as a mean value for healthy older adult males.

In the present study, EA had on average 30 % lower body fat content, BMI and visceral fat than SG. The healthy weight range for older adults over 65 years of age was defined as BMI between 22 and 27 kg/m<sup>2</sup> (Park et al. 2011). Average weight values for older males from our study was 24.2 (EA) and 27.8 kg/m<sup>2</sup> (SG).

Although we assumed association between global DNA methylation and physical fitness, we did not find a significant difference between studied groups in the level of global DNA methylation. This result corresponds with meta-analyses performed by Boyne and collaborators, who assessed the association between global DNA methylation and physical activity (Boyne et al. 2018). They found that higher levels of physical activity had a trend toward higher levels of DNA methylation, but the difference was not significant.

The other cross-sectional studies have also investigated the correlation between physical activity and blood DNA methylation level. The main aim was to identify the type of exercise and duration of physical activity which can elicit specific epigenetic changes favouring health benefits and chronic diseases prevention. However, similarly to our findings, these cross-sectional studies didn't find correlation between global DNA methylation and physical activity (Zhang et al. 2012, Gomes et al. 2012, Morabia et al. 2012).

Interestingly, one group in a study by Zhang et al. 2011 was composed of obese subjects. In this category of volunteers mean level of global DNA methylation was 73.2 %, what is 10 % lower than values in our groups (SG 80.6 % and EA 81.1 %). These data suggest that health status, including obesity, has a bigger impact on global DNA methylation than physical activity. This can explain an insignificant difference between our study groups of healthy volunteers. Two studies performed by Morabia and Gomes surveyed the correlation between physical activity measured by the number of steps taken per day and global DNA methylation (Morabia et al. 2012, Gomes et al. 2012). A significant correlation was not found even after stratification by moderate and vigorous physical activity measured by accelerometer.

On the other side, literature focusing on physical activity-induced methylation changes is controversial. Some, mainly interventional studies, found an association between physical activity and global DNA methylation (White et al. 2013, Luttrupp et al. 2013, Kawakami et al. 2019, Van Roekel et al. 2019). Luttrupp and colleagues found a negative correlation between exercise activity and global DNA methylation in leucocytes from 509 elderly individuals measured by luminometric methylation assay. Surprisingly, compared to a group with higher physical activity, decreased physical activity was associated with higher global DNA methylation, which remained significant after adjusting for multiple cardiovascular risk factors (Luttrupp et al. 2013). According to Kawakami and colleagues, high level of leisure-time physical activity was associated with decreased levels of DNA damage (Kawakami et al. 2019). Their study included 501 Japanese workers in age between 20 to 65 years. Another study used linear mixed models to assess the association between physical activity, television viewing measures and DNA methylation. Changes in leisure-time physical activity between baseline and follow-up were associated with methylation changes ( $p < .05$ ) (Van Roekel et al. 2019). However, in another study of 647 women, those who reported physical activity had only 0.33 % higher level of DNA methylation in comparison to those who were not physically active. The average level of global DNA methylation was 76.2 % is what is approximately 5.9 % lower than in our men. Significant differences in global genomic DNA methylation were reported previously (Zhang et al., 2011). While in males LINE-1 methylation was  $75.0 \pm 2.5$  %, in females it was  $73.2 \pm 3.0$ ,  $p < .001$ .

Despite these controversies, emerging evidence indicates that DNA methylation can be reprogrammable through physical activity (Voisin et al. 2015). We supposed that physical activity can protect our DNA against global DNA hypomethylation. Although useful, global methylation status can be misleading, as it describes average percent methylation of LINE-1 sequences only. Gene-specific DNA methylation, on the other hand refers to the average percent methylation within a specific gene expressed in relevant tissues. Hypermethylation of tumour-suppressor genes was repeatedly associated with elevated cancer risk (Ehrlich et al. 2002, Yu W et al. 2008, Bjornsson et al. 2008, Terry et al. 2009, Yuasa et al. 2009). Inconsistencies in the conclusion of the published studies could be caused by several reasons. Among them are large interindividual variability across a human subject, relatively small sample size or methodological differences. Therefore, more research is needed to provide conclusive results regarding the hypothesis, that beneficial effect of physical activity can be mediated through the epigenome, demonstrating impact of gene-environment interactions on human health.

## CONCLUSION

Our data show that elderly athletes with lifelong endurance activity have on average 30 % better physical fitness than seniors with lifetime sedentary lifestyle, but a better level of physical fitness doesn't significantly influence the level of the global DNA methylation in peripheral blood mononuclear cells.

We are aware of the limitation of our pilot study, which is the small sample size. We recommended assessment of DNA methylation changes in relevant tissues (e.g. skeletal muscle) which have demonstrated a direct relationship with acute or chronic physical activity, including gene-specific DNA methylation changes. Despite our negative findings, exercise training was confirmed to be favourable for physical fitness. The role of exercise-induced epigenetic modifications and their potential influence on ageing and athletic performance will have to be considered.



## AUTHOR CONTRIBUTIONS

V.Bi. and B.S. designed the experiment. V.Bi. and L.L. collected the research data. B.Bu. and L.W. processed the sample isolation and preparation. B.S., B.Bu. and L.W. analysed Global DNA methylation. L.L. performed statistical analyses. B.S. and V.Bi. conceived and supervised the entire study. All of the authors read and approved the final manuscript.

## SUPPORTING AGENCIES

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## DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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