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Running-induced Functional Mobility Improvement in the Elderly Males is Driven by Enhanced Plasma BDNF Levels and the Modulation of Global Histone H4 Acetylation Status

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Abstract

Background: Emerging evidence point out that exercise is an epigenetic modulator and is able to alter brain-derived neurotrophic factor (BDNF) levels in different populations. However, little is known about the impact of exercise on these markers in well-trained aged individuals, making this research topic particularly relevant.
Objectives: Therefore, the current study aimed at investigating the impact of the regular running practice on global histone H4 acetylation and brain–derived neurotrophic factor (BDNF) levels as well as on the functional mobility in healthy elderly males.
Methods: Fifteen male volunteers aged 60 years and older were recruited. They were allocated into 2 groups: runners (RUN, n = 8) and sedentary (SED, n = 7), taking into account the question that they were sedentary or amateur street runners. Whole blood samples (15 mL) were collected for the biochemical measurements and the functional mobility assessment was performed through the timed up and go (TUG) test. The biochemical analyses were determined using specific kits, according to the manufacturer's instructions.
Results: The RUN group showed a significant increase in plasma BDNF levels ($P = 0.007$) and H4 hypoacetylation status ($P < 0.001$) in peripheral mononuclear cells (PBMCs), compared with the SED individuals. The RUN group also presented significantly lower values in TUG, an indicative of better motor performance ($P = 0.02$).
Conclusions: Collectively, the current study data suggested that the modulation of histone H4 acetylation status might emerge as an important biomarker related to the elderly runners phenotype. The data also supported the idea that the running practice enhances peripheral BDNF levels, which could be linked to the functional mobility improvement in the elderly runners.

Keywords: Exercise, Aging, Epigenomics, Brain-Derived Neurotrophic Factor

1. Background

According to the world health organization (WHO), the elderly population increases from the current 841 million to 2 billion by the year 2050, making the chronic diseases and well-being of seniors a new challenge for global public health (1). Importantly, the age-related physiological decline depends on several factors such as genetic components and epigenetic influences (2, 3).

Epigenetics, defined as the dynamic regulation of gene expression in the absence of changes in the underlying DNA sequence, seems to plays a pivotal role in both normal aging process and age-related neurodegenerative conditions (4, 5).

DNA methylation, one of the most abundantly studied epigenetic markers, is regulated by DNA methyltransferase (DNMT) enzymes and is usually associated with transcriptional silencing (6). Histone acetylation, another important epigenetic mechanism, is widely associated with enhanced transcriptional activity, whereas deacetylation is typically associated with transcriptional repression (5). Histone acetyltransferases (HATs) and histone deacetylates (HDACs), respectively, add and remove the acetyl groups of lysine residues from amino-terminal tails of histones (7). In this context, Lovatel et al. demonstrated that lower levels of global histone H4 acetylation were found in hippocampi from aged rats (8), suggesting that this epigenetic mark might be associated with the aging process.

In addition, an imbalance of several neurotrophins linked to both cognitive function and energy metabolism is widely related to the aging process. Among these, the brain-derived neurotrophic factor (BDNF) is highlighted. BDNF is known to influence synaptic plasticity, neuronal

growth and development, and is involved with learning/memory processes and motor improvement (9-11). Evidence pointed out an age-related decrease in BDNF production and release, which is linked to the development of neurodegenerative diseases, memory impairment, and metabolic dysfunctions such as diabetes mellitus (10, 12). Therefore, strategies that modulate this neurotrophin were considered important tools for elderly people.

The BDNF is able to cross the blood-brain barrier in a bidirectional manner; therefore, the peripheral levels of this neurotrophin seem to present a strong correlation with cerebrospinal fluid levels (13). In view of these considerations, peripheral BDNF levels are used as a biomarker in several clinical studies (14, 15). Furthermore, it was previously demonstrated that BDNF levels are modulated in response to different exercise protocols both in healthy individuals and patients (16-18).

Among the modalities of physical exercise, recreational running is markedly growing as an option, even among the aged population. The beneficial impact of running practice on human physiological systems is well described (19, 20) and studies conducted on young people demonstrated that these effects are related, at least in part, to the enhancement of plasma BDNF levels (21). Despite these findings, to the authors' best knowledge, this response is not yet evaluated in the elderly individuals.

Experimental studies highlighted that the BDNF upregulation following exercise occurs, at least in part, through epigenetic modulation (22, 23). Likewise, a significant increase on global histone H4 acetylation levels was observed in the hippocampus of aged rats submitted to a chronic protocol running on a treadmill (8). Emerging clinical evidence also showed that exercise is an epigenetic modulator in a lot of tissue, including peripheral blood (19, 24). However, there is a relative lack of literature concerning the impact of exercise on epigenetic modulation in aging individuals, making this research topic particularly relevant.

Therefore, the current study aimed at investigating the impact of the regular practice of running on the levels of global histone H4 acetylation, BDNF, and functional mobility in the elderly males.

2. Methods

2.1. Participants

The study was approved by the ethics committee of Methodist University Center IPA (protocol no. 1190948) and all participants provided informed written consent prior to participation.

Participants of both groups (RUN and SED) were recruited through an advertisement on the social network sites. They were allocated into 2 groups: runners (RUN, n = 8) and sedentary (SED, n = 7), taking into account the question that they were sedentary or amateur street runners. As the inclusion criteria to compose RUN group, volunteers had to be regular amateur runners, practicing running 3 times a week for at least 2 years. The amount of physical activity in the RUN group was assessed by a self-report questionnaire of training characteristics. To be included in SED group, individuals should not have been engaged in regular practice of physical exercise in the last 6 months prior to data collection.

Exclusion criteria for both groups were the presence of chronic diseases, the use of drug-containing HDAC inhibitors and smoking. The participants were oriented not to use anti-inflammatory and antibiotic medicines 72 hours before all blood collections. All volunteers had to be physically independent and not to have cognitive limitations that could prevent the understanding of tests.

2.2. Experimental Design

In the current comparative, controlled, cross sectional study, volunteers went to the laboratory of exercise physiology at the Centro Universitário Metodista-IPA after a 12-hour fasting period and blood samples (15 mL) were obtained from an antecubital vein (basal period) to measure BDNF and global histone H4 acetylation levels.

The body composition and the body mass index (BMI) were assessed (25, 26). The waist to hip ratio (WHR) in both groups was also measured (27). Functional mobility was evaluated by the timed up and go test (28). This test requires that individuals start from a sitting position, get up from a standard chair, walk 3 meters, turn around, return to the chair, and sit again. If the task is performed for a period longer than 20 seconds, this is an indication of increased risk for falls and functional dependence. The time spent to do the task was recorded and used as a comparison parameter between the groups.

2.3. Blood Processing

After the blood collection, the PBMCs were extracted by Ficoll gradient Histopaque® (Sigma-Aldrich 1077) and added into the collected sample in a 1:1 proportion in a conical tube and, then, centrifuged at 1,500 rpm for 30 minutes at room temperature. After that, the buffer coat was removed from the portion between plasma and Histopaque®. The buffer coat was washed 5 times with phosphate buffered saline solution (PBS - pH 7.4) and, then, centrifuged at 1800 rpm for 10 minutes at room temperature. The formed pellet was collected and used to evaluate acetylation levels of histone H4. The remaining plasma was stored in conical tubes (1.5 mL) at -80°C for later determination of BDNF levels.

2.4. Determination of Plasma BDNF Levels

BDNF levels in plasma were determined by the enzyme-linked immunosorbent assay (ELISA) technique, using Sigma-Aldrich commercial kit (catalog number RAB0026) according to manufacturer's instructions. Briefly, the sample and BDNF specific standards were added to ELISA microplate and incubated for 2.5 hours at room temperature. Subsequently, the solutions were discarded and the same plate was washed 4 times with wash buffer (phosphate-buffered saline, 0.01% Tween 20). After washing, the secondary antibody bound to biotin was added and incubated for 1 hour at room temperature with gentle agitation. The plate was again washed with wash buffer, and streptavidin solution was added and incubated at room temperature for 45 minutes with gentle agitation. The solution was discarded and the plate went through the washing process. Tetramethylbenzidine (TMB) was added and, then, it was incubated for 30 minutes at room temperature, light deprivation and gentle agitation. The stop solution was added and the plate was read in a spectrophotometer at a wavelength of 450 nm. The plasma BDNF levels were expressed as ng/mL.

2.5. Global histone H4 Acetylation Levels in PBMCs Measurement

The global histone H4 acetylation levels in PBMCs were determined using the global histone H4 acetylation assay kit (Colorimetric Detection, catalog number P-4009, EpiQuik USA) according to the manufacturer's instructions. The samples were incubated with the capture antibody followed by incubation with detection antibody. Afterwards, they were incubated with the developing solution, followed by the addition of the stop solution. The absorbance was measured on a spectrophotometer at a wavelength of 450 nm. The global histone H4 acetylation levels in PBMCs were expressed as ng/mg protein. The protein concentration of each sample was measured by the Coomassie blue method using bovine serum albumin as the standard (29).

2.6. Statistical Analysis

Data normality was verified by the Shapiro-Wilk test. Then, to compare body composition parameters, TUG, global histone H4 acetylation and plasma BDNF levels between the RUN and SED groups, an unpaired t test was utilized. Pearson correlation was used to identify the evaluated correlations. Data were expressed as means ± standard deviations (SD). Statistical significance was accepted at $P < 0.05$. Statistical analyses were performed using PASW Statistics 20.0 for Windows (SPSS).

3. Results

The sample consisted of 15 participants, 7 in the SED group and 8 in the RUN group. The participants' characteristics are described in Table 1. The characteristics of RUN group regarding training aspects are highlighted in Table 2.

Table 1. Samples Characteristics[a]

Variable	RUN (n = 8)	SED (n = 7)
Age, y	64.2 ± 3.9	64.8 ± 4.2
Weight, kg	71.1 ± 5.79	73.1 ± 5.78
Stature, m	174 ± 0.044	172 ± 0.033
BMI, kg/m²	23.3 ± 1.34	24.5 ± 2.30
WHR, cm	0.765 ± 0.041	0.810 ± 0.102
% Fat[b]	20.62 ± 1.92	23.00 ± 2.00

Abbreviations: BMI, body index mass; WHR, waist to hip ratio.
[a]Values are expressed as mean ± SD.
[b]Statistically significant difference between RUN and SED groups (t test, P = 0.05)

Table 2. Training Characteristics of the Running Group[a]

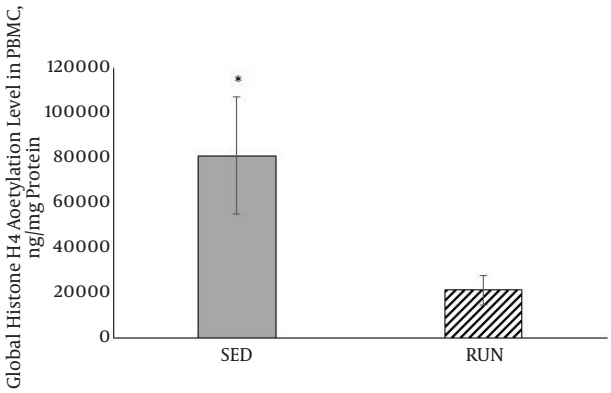
Variable	RUN Group, %
Weekly training, time	
2	25
3	50
4	12.5
6	12.5
Training session duration, min	
60	75
> 60	25
Time exposed to running training, y	
2	37.5
4	12.5
10	12.5
20	12.5
30	12.5
43	12.5

[a]Values are expressed as absolute No. (%).

The SED group presented higher levels of global histone H4 acetylation (81299.46 ± 26222.87 ng /mg protein) ($P < 0.001$), compared with the runners (21498.17 ± 6404.68 ng/mg protein), as illustrated in Figure 1. Regarding BDNF levels, the RUN individuals showed higher val-

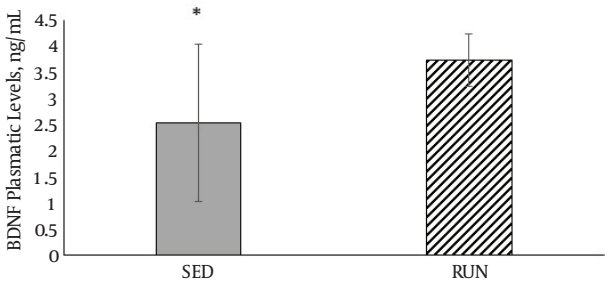
ues of this neurotrophin (4.1 ± 1.1 ng/mL), compared with SED group (2.5 ± 0.5 ng/mL) (P = 0.007; Figure 2). The RUN group had significantly lower values on TUG test, compared with the SED group (P = 0.02) (Figure 3).

Figure 1. Global Histone H4 Acetylation Levels in PBMCs from SED (n = 7) and RUN (n = 8) Groups



Results are expressed as means ± standard deviations. t test (P < 0.001) *were statistically different from the RUN group. PBMC: Peripheral blood mononuclear cell; SED: Sedentary; RUN: Runner.

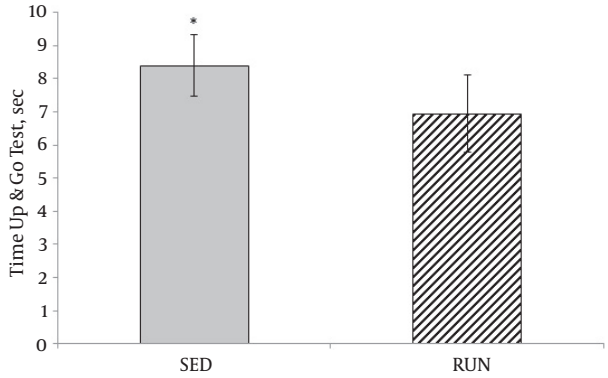
Figure 2. Plasma BDNF Levels in the SED (n = 7) and RUN (n = 8) Groups



Results are expressed as means ± standard deviations. t test (P = 0.007) *were statistically different from the RUN group. BDNF: Brain-derived neurotrophic factor; SED: Sedentary; RUN: Runner.

A positive correlation was found between the experience (in years) of running in the RUN group and plasma levels of BDNF (r = 0.75; P = 0.002). The plasma BDNF levels were also correlated negatively with the TUG (r = -0.57; P = 0.03). Likewise, the WHR was positively correlated with TUG (r = 0.64; P = 0.009). Finally, the frequency of running was negatively correlated (r = -0.78; P = 0.004) with global histone H4 acetylation levels.

Figure 3. TUG Test Performance from SED (n = 7) and RUN (n = 8) Groups



Results are expressed as means ± standard deviations. t test (P = 0.02) *were statistically different from the RUN group. TUG: Timed up and go; SED: Sedentary; RUN: Runner.

4. Discussion

The current study demonstrated that elderly males who regularly practice running presents higher levels of BDNF, hypoacetylation histone H4 status and better mobility capacity, compared with the sedentary ones.

The results of the current study showed that regular running was an important strategy to enhance BDNF levels in the elderly individuals as a significant difference was observed in neurotrophin levels between the RUN and SED groups. It was the first evidence that evaluated the impact of this particular aerobic exercise modality in the elderly population on BDNF modulation. The current study data corroborates those obtained by Zoladz et al. (21) who showed that basal plasma BDNF levels were higher in young, well-trained athletes including sprinters, jumpers, and runners, compared with untrained participants. In agreement, da Silveira et al. (30) also showed that plasma BDNF levels increased in middle-aged amateur runners compared to the sedentary group.

Coelho et al. (31), in a systematic review, described that both aerobic and resistance exercise protocols increased the peripheral concentrations of BDNF in the healthy and elderly patients. Taken together, these findings indicated that independent of the age and health status, peripheral BDNF levels enhance in response to the running practice. These data can be related to the idea previously proposed by Mattson et al. (32) that BDNF modulation exerts a pivotal role on runners' phenotype.

In another study, Coelho et al. (33) reported that the elderly healthy females submitted to an endurance exercise protocol during 10 weeks showed increased plasma BDNF levels after the intervention. Additionally, in accor-

dance with the current study data, the authors also identified an improvement in the mobility and balance aspects assessed by TUG test (33). Finally, they suggested that BDNF can increase the brain's resistance to degenerative damage through the ability to support the growth as neuronal survival, whereas lower BDNF levels could be associated with a marker for weakness phenotype, which affects the mobility of the elderly. Altogether, these findings led the current study to hypothesize that both aerobic and resistance exercise protocols are able to improve motor abilities in the elderly people, which is related, at least in part, to increased BDNF levels. Furthermore, this response seems not to act in a gender-dependent manner, affecting both male and female elderly.

The current study also demonstrated a positive correlation between WHR and TUG. These findings could be associated to those recently obtained by Huang et al. (34); they found that high levels of serum adiponectin in volunteers aged over 45 years were considered a predictor of falls. It is noteworthy that adiponectin is an adipocyte-derived hormone and at increased levels may be important indicative of the development of obesity and cardiovascular diseases (34). In this context, it was observed that increased adiposity was also associated with low circulating levels of BDNF (35). These considerations raised the possibility that interventions such as physical exercise and food restriction can modulate WHR and also improve executive function.

Another remarkable point to discuss was that the RUN group showed diminished levels of global histone H4 acetylation, compared with the sedentary individuals. In this sense, the current study was the first to show that exercise modulates the acetylation status in the elderly people, indicating that histone H4 acetylation can be considered as a novel biomarker that reacts epigenetically to physical activity in this population. On the other hand, a recent study (30) reported no difference in histone H4 acetylation levels between middle-aged runners and sedentary individuals. These findings were in agreement with the experimental studies reporting that histone acetylation status in response to exercise might act in an age-dependent manner (8, 36).

The histone H4 hypoacetylation status observed in RUN individuals might indicate reduced transcriptional activity and gene silencing. Similarly, Zhang et al. (37) showed that an aerobic exercise protocol during 6 months modulated DNA methylation profile in the peripheral blood of the elderly people. In fact, the NFkB2 and ASC genes, linked to the inflammatory processes in age-related diseases, were hyper-methylated after the intervention, indicating reduced transcriptional activity. As epigenetic mechanisms are not isolated events to dictate the expression of specific genes (38), it is reasonable to suppose that histone acetylation and DNA methylation signs might interact to regulate and modulate the gene transcription in response to exercise in the elderly individuals. Future studies should be conducted to elucidate this question.

An important limitation of the current study was the small sample size, which did not allow genetic analyses such as BDNF polymorphism of BDNF, an interesting topic that can be considered by future investigations. Furthermore, it measured only 1 epigenetic mark: global histone H4 acetylation levels. Thus, it is suggested that future studies with more robust sample consider the modulation of other parameters that could epigenetically respond to the running practice in the elderly such as histone H3 acetylation levels, modifications in histone methylation status, miRNA regulation as well as the expression and polymorphism of specific genes. These findings could elucidate the exact epigenetic pathways linked to the running practice in the elderly.

4.1. Conclusions

The current study reported, for the first time, that exercise, specifically the regular practice of running, significantly alters histone acetylation status in well-trained elderly individuals, suggesting that the modulation of histone H4 acetylation status might also emerge as an important biomarker related to the aged runners' phenotype. Future studies should investigate the physiological relevance of these findings. The authors believe that these preliminary data might motivate new studies to examine the impacts of running, including the appropriate frequency and intensity required, and its relationship with epigenetic mechanisms. Finally, the current study data also supported the idea that the running practice enhances peripheral BDNF levels, which could be linked to the functional mobility improvement in the elderly runners.

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Footnotes

Author Contribution: Study concept and design: Viviane Rostirola Elsner, Maristela Padilha de Souza and Anelise Ineu analysis analysis and interpretation of data: Viviane Rostirola Elsner, Maristela Padilha de Souza, Joao Jose Cunha and Anelise Ineu acquisition acquisition of data: Anelise Ineu Bard, Bard, Joao Jose Cunha, da Silva, Laura Luna Martins, Gustavo Reinaldo; drafting of the

manuscript: Viviane Rostirola Elsner, Maristela Padilha de Souza and Anelise Ineu critical critical revision of the manuscript for important intellectual content: all authors; statistical analysis: Viviane Rostirola Elsner, Maristela Padilha de Souza and Anelise Ineu

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