

No Change in Brain-Derived Neurotrophic Factor Levels Following a Single Session of Light to-Moderate Intensity Walk in Chronic Stroke Patients

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Abstract

Background: Many studies show the beneficial effects of overground gait training in stroke patients. However, it is not known whether it is able to induce changes in brain-derived neurotrophic factor (BDNF) levels, which could positively influence neurophysiological mechanisms.

Objective: To evaluate the effect of a single session of light-to-moderate intensity walk on BDNF serum levels and its precursors (proBDNF) in chronic ischemic stroke patients.

Methods: Patients were asked to walk for 30 minutes in the target heart rate training zone (30-60% of maximal heart rate). Blood samples were collected immediately before and after a single session.

Results: Fifteen individuals with 60.8 (7.7) years old participated of this study. There was no significant difference in proBDNF ($p=0.573$) and BDNF ($p=0.563$) serum levels between pre- and post-session.

Conclusion: This study used light-to-moderate intensity because the goal is to approach the gait training in clinical setting. The lack of increase in BDNF levels can be explained by two reasons: the intensity zone of the heart rate selected during the session and not having a gradual increase in this intensity.

Keywords: Stroke; Brain-derived neurotrophic factor; Exercise; Overground gait training

Introduction

Stroke is a disease that can cause considerable disability or lead to death [1]. According to the World Health Organization, 15 million people suffer from stroke worldwide, with 5 million becoming dependent on family members and 5 million at risk of death [2]. The increasing number of stroke events leads to costs for the individual, family, and government health care [3]. After a stroke, patients commonly show muscle weakness of the hemibody contralateral to the brain injury (hemiparesis), sensory deficits, balance, and gait disturbances [4,5]. Conventional physiotherapy aims at evaluating and treating these impairments and disabilities, but restoring the ability to walk is one of the most important goals of stroke rehabilitation [6-8]. Gait impairment is a significant contributor to long-term disability and decreased walking resistance is the most prominent functional limitation after stroke. Approximately two-thirds of patients have difficulties in walking soon after a stroke and almost a third are still unable to walk without assistance in the chronic phase i.e., after six months of injury [5].

Many studies show the beneficial effects of over ground gait training in such individuals in different phases after stroke [9,10]. Even during single sessions, over ground gait training is effective in promoting improved performance in the timed up and go test [11]. However, it is not known whether the conventional gait training widely used in clinical physical therapy practice is able to induce changes in serum levels of brain-derived neurotrophic factor (BDNF), which could positively influence neurophysiological mechanisms. Studies have shown that acute and chronic exercises increase peripheral levels of BDNF, mainly after performing aerobic exercise [12-16]. Additionally,

BDNF levels in the central nervous system are positively associated with peripheral BDNF levels, particularly serum and plasma, and therefore, circulating peripheral BDNF level has been suggested as a good biomarker for concentration brain this neurotrophin [17].

BDNF is a member of the neurotrophin family. It is a protein with widespread distribution in the central and peripheral nervous system [18]. Physiological responses to neurotrophins are mediated by the activation of two distinct classes of transmembrane receptors, the tropomyosin-related kinase (Trk) family of receptors and the neurotrophin receptor (p75NTR) [18,19]. BDNF may be released in the mature form, which preferentially activates TrkB receptors, or as proBDNF, which is coupled to the stimulation of p75NTR.

The phosphorylation of the TrkB receptor activates three signaling pathways: protein kinase cascade activated by mitogens, signaling pathway phosphatidylinositol-3 kinase, and through phospholipase C- γ [20-22]. This allows BDNF to exert its effects on neuronal survival, among several other functions such as neuronal excitability,

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transmission and synaptic plasticity, including the motor cortex and subcortical motor structures [20-22]. Its receptors are abundantly expressed in neuron related motor functions. Such BDNF functions can contribute to the modulation of motor behavior in physiological and pathological situations such as stroke [20-22]. Therefore, the objective of this study was to evaluate the effect of a single session of light-to-moderate intensity walk on serum levels of proBDNF and BDNF in chronic stroke patients with reduced lower leg muscle force and who performed poorly in the 6-minute walk test (6MWT).

Materials and Methods

Study design and subjects

The cross-sectional study was approved by the Human Research Ethics Committee of Universidade Federal de Minas Gerais. All volunteers signed an informed consent form. This study was conducted using stroke patients enrolled in Centro Metropolitano de Especialidades Médicas da Santa Casa, Belo Horizonte, Minas Gerais, Brazil.

The eligibility criteria for participation in this study were: clinical diagnosis of ischemic stroke of at least six months, patient age \geq 45 years, the ability to understand verbal commands, and walk even using a gait-assistance device. Participants were excluded if they had a history of neurological and psychiatric diseases, hearing or visual impairment, cancer or infectious diseases, heart disease limiting physical activity, having fallen due to dizziness or fainting, and not having performed physical activity in the previous 2 months. Physical activity was defined as strength training and/or more than 20 minutes of aerobic activity twice per week.

Assessment

The participants completed a demographic and health status questionnaire. Body mass index (BMI) was calculated using the standard formula and the patients were classified according to American College of Sports Medicine [23]. All subjects responded to the Mini-Mental State Examination (MMSE), and the Brazilian version was used to assess cognitive abilities [24]. The cutoff points used for the MMSE were: 13 for the uneducated, 18 for individuals with low to medium level of education (low - 1 to 4 incomplete and medium - 4 to 8 incomplete years), and 26 for individuals with high education (8 or more years) [24].

Isometric hip flexors, knee extensors, and plantar flexors muscle force production for each leg were measured with a MicroFET 2 handheld dynamometer (Hoggan-Health industries) [25]. The subject was instructed to remain in a supine position and the dynamometer was placed in standardized regions, according to the muscle group being tested. Participants were asked to push as hard as possible for 5 seconds while the investigator matched the resistance. Participants performed 3 maximal-effort trials with a 1-minute rest period between the trials, considering the average of these measures [25]. The values obtained in the muscle strength testing were expressed in pounds (lbs).

Walking resistance was assessed using the 6MWT [26]. It is an easy-to-perform and practical test that measures the distance that a patient can walk from one end of a 30-meter long corridor to the other within 6 minutes. The result reflects the integrated exercise response of complex physiology involving the pulmonary and cardiovascular systems and neuromuscular function [26,27]. Subsequently, the expected distance for each subject was calculated according to the following equation:

$D6MWT = 622.461 - (1.846 \times \text{age years old}) + (61.503 \times \text{gender } 1 - \text{men or } 0 - \text{women});$ where D6MWT is the distance covered in a 6-minute walk test [28].

Single session of low-intensity walk

Maximal heart rate (HRmax) was estimated using the formula "220 - age". The target heart rate (THR) training zone was determined using: $THR = [(HR_{max} - HR_{resting}) \times \%Intensity] + HR_{resting}$. This formula uses maximum and resting heart rate (HRresting) with the desired training intensity to get a target heart rate. The THR range was determined by finding the heart rate that corresponded to light (30%) and moderate (60%) intensity which may be beneficial to deconditioned patients [23].

Subsequently, the participants were asked to walk for 40 minutes, with the first five minutes for warming up and the last five minutes for cooling down. The participants were instructed to stop walking if they experienced chest pain, intolerable dyspnea, leg cramps, staggering, or other symptoms. An HR monitor was used during all the sessions.

Measurements of BDNF serum levels

Blood samples were collected immediately before and after a single session of aerobic exercise, between 07:00 and 10:00 hours. The patients were instructed to fast and avoid caffeine. Tubes were centrifuged to remove cells and debris, and stored in serum aliquots at -80°C until analysis. BDNF and proBDNF levels were measured using conventional sandwich enzyme-linked immunosorbent assay kits (DuoSet, R&D Systems, Minneapolis, MN, USA), according to the manufacturer's instructions. The detection limits were 10.0 pg/mL.

Statistical analysis

The sample size of this study was based on the results of BDNF serum level mean and standard deviation in stroke subjects submitted to a physiotherapy program followed by aerobic exercise [29]. In this study BDNF levels were $19,180 \pm 3710$ pg/ml and $23,830 \pm 5180$ pg/ml before and after intervention ($p < 0.001$). For this effect size with a power of 0.80 at alpha 0.05 (two-sided), we calculated a sample size of 15 participants.

The data were analyzed using the SPSS statistical package, version 15.0 (Inc., USA) and GraphPad Prism, version 5.0 (Inc., USA). Descriptive statistics were determined for all demographic and health-related variables. Shapiro-Wilk was applied to evaluate the normality of results. Log transformations were used to normalize proBDNF and BDNF serum levels. The Paired Student's t-test was performed to compare these levels before and after aerobic exercise. The significance level was 5% ($\alpha < 0.05$).

Results

Twenty-one patients were recruited but only 15 (71.4%) were able to perform the walk protocol or undergo a measurement of their proBDNF and BDNF serum levels before and after the session. Demographic and clinical features are shown in Table 1.

There was a significant difference in lower leg muscle force between the paretic and non-paretic side (Table 2). The distance walked in the 6MWT was less in relation to the lower limit of the normal range provided by reference equation in study Gold et al. (2011) ($p = 0.004$).

There was no significant difference in proBDNF (Figure 1) and BDNF (Figure 2) serum levels between pre- and post-session of the light-to-moderate intensity walk. Mean and standard deviation

were: proBDNF - 535.8 ± 655.3 pg/ml and 504.1 ± 653.9 pg/ml; BDNF - $12,317.9 \pm 4304.9$ pg/ml and $12,536.9 \pm 4234.9$ pg/ml, before and after session, respectively (Table 3). The ratio of pro BDNF: BDNF before and after testing were same (0.04).

Variables	Stroke patients
Gender (male/female)	9/6
Age in years	60.8 ± 7.7
Duration of illness in years	7.1 ± 8.4
BMI (kg/m ²)	27.0 ± 3.2
Normal (18.5-24.9 kg/m ²)	3
Overweight (25.0-29.9kg/m ²)	10
Obesity (Class I: 30.0-34.9 kg/m ²)	2
MMSE	22.2 ± 3.9
6MWT (m)	332.0 ± 147.7

Values represent in mean and standard deviation or number of participants. BMI: Body Mass Index; 6MWT: Six-Minute Walk Test.

Table 1: Demographic and clinical features of chronic stroke patients (n=15).

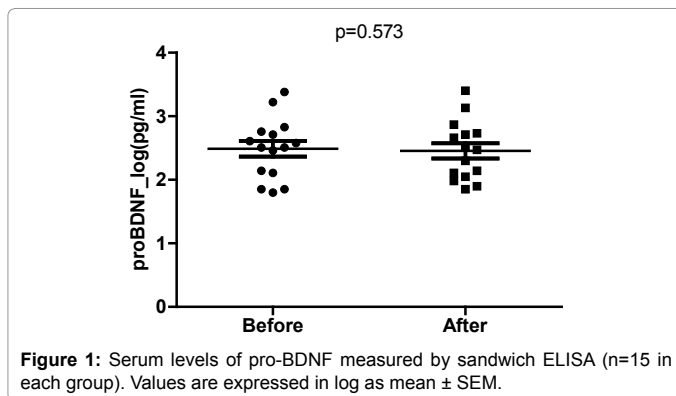


Figure 1: Serum levels of pro-BDNF measured by sandwich ELISA (n=15 in each group). Values are expressed in log as mean \pm SEM.

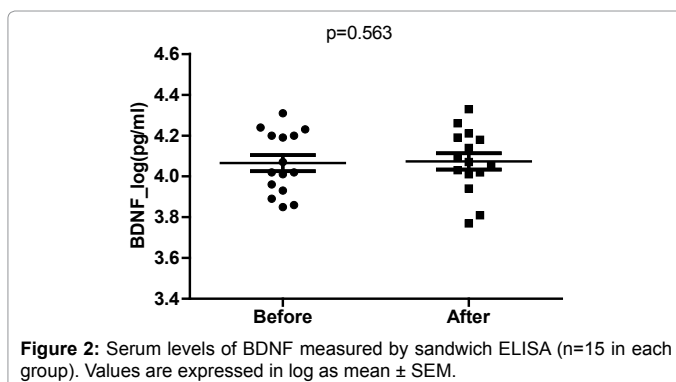


Figure 2: Serum levels of BDNF measured by sandwich ELISA (n=15 in each group). Values are expressed in log as mean \pm SEM.

Variables	Paretic Side	Non-paretic Side	p-value
Hip flexors	22.5 ± 10.7	30.3 ± 14.0	0.025
Knee extensors	22.6 ± 10.3	29.1 ± 12.1	0.029
Plantar flexors	13.8 ± 7.1	20.6 ± 6.6	0.009

Values represent in mean and standard deviation. Paired Student's t-test to compare paretic and non-paretic sides.

Table 2: Lower leg muscle force of chronic stroke patients (n=15).

	Before	After	p-value
proBDNF	535.8 ± 655.3	504.1 ± 653.9	0.573
BDNF	$12,317.9 \pm 4,304.9$	$12,536.9 \pm 4,234.9$	0.563

Values represent in mean and standard deviation and expressed in pg/ml.

Table 3: proBDNF and BDNF serum levels before and after session (n=15).

Discussion

Because stroke is one of the major causes of disability in adults with a negative impact on patients' lives and public health, it has been intensively studied [30]. Physical inactivity after stroke contributes to increased muscle weakness, fatigue, and cardiovascular deconditioning, resulting in functional limitation. Therefore, motor recovery involves a relearning process mediated via neuroplasticity processes, and one of the main factors involved in this relearning process is the BDNF [18-22]. This is the first study that assesses the effect of a single session of light-to-moderate intensity walk on BDNF serum levels and its precursor, proBDNF, on chronic stroke patients. The lack of increase in BDNF levels can be explained by two reasons: the intensity zone of the HR selected during the session and not having a gradual increase in this intensity. Probably, the patients selected a more comfortable intensity within the determined zone and this could have been influenced by age and weight [31]. It is noteworthy that the participants of this study had a limited functional capacity considering the results of the test assessing the muscle strength of the lower limb and the distance walked on 6MWT.

In the literature, there is some controversy concerning the effect of the exercise, acute and chronic, on BDNF peripheral concentrations. Tang et al. detected a transient increase of this neurotrophin induced by 15 minutes of step in healthy subjects, and intra-individual levels before and after the activity were relatively stable [32]. Already, Rojas Vega et al. had found that short periods (ten minutes) of walking at moderate intensity were not able to change BDNF serum levels [33]. Gold et al. observed an increase in serum BDNF levels in patients with multiple sclerosis and healthy individuals after moderate aerobic activity for thirty minutes, keeping 60% of maximum rate of oxygen consumption (VO_{2max}), although there was no difference at baseline between groups before exercise. However, this study used an ergometric test to determine VO_{2max} and exercise was carried out on a stationary bike by individuals who were not elderly [34]. Furthermore, studies generally use ergometric tests with increasing intensity until exhaustion [35] or, in some cases, shorter periods of high intensity [36] to evaluate the effects of exercise on BDNF peripheral levels. This was different from our study since our subjects remained on self-selected-intensity-to-low-intensity exercises being a conventional gait training method, and did not increase intensity over the forty-minute walk. These data show that elevated serum levels of BDNF are dependent on exercise type and intensity [37,38].

According to a systematic review by Coelho et al., no exercise type and intensity have been established to increase BDNF levels, although studies suggest that moderate-intensity exercises appear to be more effective for this purpose in the elderly [12]. However, this study used light-to-moderate intensity because the goal is to approach the gait training in clinical setting.

It is important to clarify that the levels of these neurotrophins can be influenced by various factors such as obesity and habitual physical activity [39-42]. BDNF is also expressed in adipose tissue and may be accompanied by elevated levels of cortisol, and a subliminal inflammatory process that occurs in obesity [39,40].

Although there was no control group in this study, the number of participants according to the sample calculation. In addition, patients were recruited if they had reduced lower leg muscle force and performed poorly in the 6-minute walk test. It would be revealing to carry out further longitudinal studies to clarify the effects of chronic exercises and different intensities on BDNF levels.

Conclusion

This is the first study to evaluate the effect of a single session light-to-moderate intensity exercise on proBDNF and BDNF serum levels in chronic ischemic stroke patients. Light-to-moderate intensity walk was selected considering the conventional gait training in the clinical setting. However, there was not change in this neurotrophin and its precursors. The lack of increase can be explained by two reasons: the intensity zone of the heart rate selected during the session and not having a gradual increase in this intensity. It is necessary to study which parameters including exercise type, duration, and intensity that may affect BDNF levels in chronic stroke patients.

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