The effect of 12-weeks resistance and endurance training on the serum levels NGF, BDNF, and VDBP in women with multiple sclerosis

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Abstract:

Recent studies suggest that patients with multiple sclerosis (MS) have low levels of neurotrophic factors such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and the vitamin D-binding protein (VDBP). Moreover, the results of recent studies show that exercise training may have potential effects on these factors in people with MS. The purpose of the present study was to investigate the effect of 12-week resistance and endurance training program on the serum levels of neurotrophic factors in women with MS. A randomized controlled clinical trial was conducted in 24 volunteer MS patients (Expanded Disability Status Scale range of 1–5). The resting serum levels of BDNF, NGF, VDBP, body composition variables, and disability scale values were determined before and after the intervention. The intervention consisted of three sessions of combined training a week for 12 weeks (two sessions of aerobic and one session of resistance training, progressively). The results showed a significant decrease in the percentage of body fat (p=0.003), and disability scale value (p=0.007) in the experimental group. However no significant changes were observed in other variables. The results obtained in the present study indicated that although the resistance and endurance training for three non-consecutive days per week for 12 weeks has no significant effect on neurotrophics and VDBP, it triggered positive effects on the percentage of fat in the body, and the disability scale of women with MS. So, such a training seems a useful physical practice for people with MS.

Key words: Resistance and endurance training, Multiple Sclerosis, Neurotrophic
1. Introduction

Multiple sclerosis is often described as a chronic, immune-mediated disease of the CNS, although neurodegenerative processes are increasingly being recognised in its pathogenesis[1]. MS is often associated with some symptoms (eg, fatigue and depression) and dysfunction (eg, mobility and cognitive impairment), which often affects the quality of life and participation in everyday life activities negatively [2]. Despite much attempt made by researchers, there is no definitive medical treatment for MS, with the currently available medications and treatments being only effective in relieving its side effects, and in preventing its recurrent attacks [3,4].

Neurotrophic factors, that is, the growth factors (such as neurotrophics) necessary for the survival of nerve cells, promote the survival rate of the nerve cells through inhibiting the nerve cells involved in the initiation of the programmed death of cells. They are also important in the differentiation of stem cells from neural cells[5]. Moreover, Nerve growth factor (NGF), the first recognized member of neurotrophics, is of great importance for the growth and phenotypic maintenance of neurons in the peripheral nervous system, and also for the functional accuracy of the cholinergic neurons in the central nervous system[6].

Another member of the neartrophin family, Brain-derived neurotrophic factor (BDNF), serves to encourage the creation, growth, and survival of neurons and synapses [5]. Moreover, the results of the studies done so far indicate that vitamin D level is one of the important factors to be considered in patients with MS [7]. More specifically, the results of previous studies revealed a significant positive association between higher levels of 25-hydroxy vitamin D and a reduced risk of MS. Moreover, MS Patients had a lower level of serum 25-hydroxy vitamin D, especially before the recurrent stage [8-10]. The main plasma protein carrying vitamin D and its metabolites is Vitamin D-binding protein (VDBP). It is responsible for transferring vitamin D₃ to the liver and 25-hydroxy vitamin D to the kidneys. It also serves the function of transferring 25 and 1-hydroxy vitamin D to the targeted organs[11]. The results of recent studies have revealed that, during the acute phase of demyelination, VDBP level is low in the cerebrospinal fluid in patients with MS[12,13].

The results of numerous studies on animals have confirmed that exercise can have neuro-protective and -regenerative potential in that it induces neurogenesis in the hippocampus, an important brain structure for learning and memory[14,15]. These findings are in line with clinical observations in neurodegenerative diseases such as Parkinson's disease[16] or Alzheimer's disease[17], in which aerobic exercise has been found to be effective in reducing symptoms and delaying the disease progression. However, there is still controversy over the possible effect of physical activities in controlling MS.

Partly due to the fact that there has not been conclusive information on the most appropriate physical activities effective in controlling MS, it is widely believed that patients with MS should avoid intense physical activities in that such activities increase the body temperature, thus worsening the existing symptoms. However, over the last few years, our understanding of health and the type of physical activities (training) suitable for patients with MS has greatly improved[3,4].

The potential effects of exercise training on the physical fitness of MS patients have been summarized in one systematic review[18] and two meta-analyses[19,20]. The results of several studies have indicated...
that an appropriate exercise therapy can be effective in suppressing (reducing) the symptoms of the disease[21-26]. In these studies, some MS patients were given either a resistance or aerobic physical activities while other MS patients[22,24,25] received a combined exercise training[23,26]. Generally speaking, MS patients suffer several functional problems including a decreased muscle strength and endurance[2], as well as a comparatively low cardiorespiratory endurance and flexibility[3]. Therefore, patients with MS should be given a combination of aerobic and resistance training. To our knowledge, in the literature, there are only 6 projects on the effects of these important factors on the brain functioning of MS patients. However, the results obtained in these projects were contradictory[27-32]. Moreover, the majority of these studies focused on the effects of aerobic training, with none of them studying the effects of a combined exercise training program on these three factors in MS patients. Hence, the present study is aimed at examining the effect of the provision of one course of combined exercise training (aerobic and resistance) on the biochemical factors in patients with MS.

2. Method

The participants for the present randomized controlled trial (RCT) were patients with primary or secondary progressive MS (PPMS/SPMS)[33] and moderate disability (EDSS 1–5) approved by specialists through tests of MS diagnosis, including MRI or CT scan and functional tests on balance, power, ability, walking, and visual and tactile tests) selected from among the members of the MS society at Sabzevar (IRAN).

After the approval by specialists, 24 interested female MS patients, between 20 to 50 years of age, were selected using an available sampling procedure, and were then assigned to the experimental (12 subjects) and control (12 subjects) groups. However, those with medical contraindications for exercise therapy (such as cardiovascular or major orthopedic disease), severe developmental, psychiatric, or neurological disorders other than MS, or any regular exercise training were excluded from the study.

After the initial enrollment of the subjects, the participants took part in an introductory meeting to get informed of the overall plan of the study, and the nutritional and medical programs to be followed. Having passed some cardiorespiratory tests necessary for the inclusion in the study, the final participants delivered their formal written consent to participate in the study. Then, for each subject, the necessary demographic and anthropometric information including age, weight, height, and subcutaneous fat percentage were recorded. This study being in accordance with the Helsinki declaration as revised in 2013, the ethics committee of the university approved the study morally (IR.MEDSAB.REC.1394.137)(IRCT2017032633146N1). The training programs were tailored to the individual level of fitness of the participants, as determined by Rockport walk test at baseline.

Blood samples were taken from all subjects in the control and experimental group in the morning before breakfast. Then, the subjects in the experimental received exercise training while the subjects in the control group received no exercises and were asked to do their normal activities. After an interval of 12 weeks, the second blood samples were taken. However, two subjects from the experimental and 2 from the control group did not show up for the second blood sampling (post test). But the variables of weight, body fat, and disability scale were measured for these subjects.

2.1. Demographic information and disability scale:

A measuring tape fitted on a wall and a digital scale were used to measure the
height and the weight of the subjects, respectively. In addition, caliper was used to measure the fat percentage in the body (by an expert). Finally, the physical disability of the subjects in both groups was measured using the expanded disability status scale.

2.2. Training program

Under the supervision of a specialist in exercise physiology, the participant received training three sessions in a week for a total period of 12 weeks (2 sessions of resistance training and 1 session of endurance training). The sessions were conducted in the afternoon in the gym with tolerable and appropriate levels of temperature and humidity for the patients.

In each session, balance and flexibility exercises were performed along with a 15 minute warm-up and a 10-minute cool-down. Resistance training included leg press, bench press, biceps curl, knee extension and flexion, shoulders, leg dorsiflexion and plantar flexion performed with body-building devices whereas endurance training included jogging and age-appropriate rhythmic aerobic training, which were done in intervals. At the end of each session, cooling down was conducted in the form of slow running and stretching activities.

The intensity of resistance training was gradually increased in the course of training period following ACSM guidelines, with 60 to 80% of one repetition maximum, one session per week, 2 to 4 sets, 8-14 repetitions, and 3 to 4 minutes intervals between the sets. The aerobic training were done, similarly; with intervals and intensity of 40 to 55% HRR, 4-13 repetitions, and 1-2 minutes intervals between the sets[2]. Table 1 and 2 summarize these protocols.

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Rest between stations (Minutes)

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Table 2: Endurance training protocol

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2.3. Blood sampling and biochemical analyses

From all participants, a blood sample of 5 cc was taken from the brachial vein, 48 hours after the last training session in the fasting state and half an hour after break in the sitting state. 2 cc of the blood were sent to laboratory to test for CBC. The remaining 3 cc were centrifuged and kept in three alicots, in the refrigerator at -80 °C for biochemical tests (measuring VDBP, BDNF, and NGF).

The NGF, VDBP, and BDNF levels in the blood were measured with an Eliza kit following the instruction offered by the manufacturing country, Human Nerve Growth Factor (NGF) ELISA Kit, Eastibiopharm, Torrance, USA; Human Vitamin D-Binding Protein(VDBP) ELISA Kit, Eastibiopharm, Torrance, USA and Human Brain-Derived Neurotrophic Factor(BDNF) ELISA Kit, Boster Biological Technology, Pleasanton, CA, USA and at sensitivity degree of NGF(2.48 pg/ml), VDBP(5.41 µg/ml) and BDNF(<2 pg/ml).

2.4. Statistical Methods

The descriptive statistics including mean and standard deviation were run on the collected data. The Shapiro-Wilk test was performed on the data to check the normality of the distribution. The results showed that, except for the disability scale, the remaining variables enjoyed a normal
distribution. Moreover, an analysis of covariance (ANCOVA) was performed on the data to compare the obtained means for the variables. All the descriptive and inferential statistical test were performed on the collected data using SPSS22, at a significance level of p<0.05.

However, exercise training led to a significant decrease in the percentage of the body fat, and the disability of the patients.

In addition, exercise training did not induce any significant change in the biochemical variables (BDNF, NGF, VDBP). Table 3 summarizes the results.

Table 3. Changes in anthropometric variables, subjects’ disabilities, and biochemical variables before and after training (mean± standard deviation)

<table>
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<tr>
<th>Variables</th>
<th>Experimental</th>
<th>Control</th>
<th>P value</th>
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<tr>
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<td>Pre-test</td>
<td>Post-test</td>
<td>Pre-test</td>
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<tr>
<td>Weight (kg)</td>
<td>60.8±13.3</td>
<td>60.1±12.7</td>
<td>59.7±11</td>
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<tr>
<td>Body fat percentage</td>
<td>36.3±8.6</td>
<td>34.1±6.3</td>
<td>33.6±8.1</td>
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<tr>
<td>Disability scale (EDSS)</td>
<td>3.1±0.5</td>
<td>2.5±0.6</td>
<td>3.8±1.1</td>
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<tr>
<td>BDNF (pg/ml)</td>
<td>4223±2084</td>
<td>4707±1918</td>
<td>3900.1±1658</td>
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<tr>
<td>NGF (pg/ml)</td>
<td>698.9±128</td>
<td>679.4±104</td>
<td>646.3±132.3</td>
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<tr>
<td>VDBP (ug/ml)</td>
<td>2356.5±778.9</td>
<td>2077.8±502.8</td>
<td>2227.7±413</td>
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*significant difference with the control group at significance level 0.05

4. Discussion
There is a lack of therapeutic strategies for progressive multiple sclerosis patients with a higher and average grade of disability and substantial neurodegeneration and axonal dysfunction, which are of utmost clinical need. The clinical data obtained in recent studies indicate that exercise might have positive effects on neurocognitive functions in patients with progressive multiple sclerosis; however, the underlying molecular pathways and quantifiable markers are currently missing [34].

In the present study, the potential effects of combined exercise training on previously proposed serum biomarkers as possible underlying mediators were examined in a cohort of progressive MS patients. The results obtained in the present study suggested that 12 weeks of combined exercise training (two sessions of aerobic training and one session of resistance training in a week) could lead to a significate improvement of the body fat percentage and disability scale in women with MS. However, the exercise training had no significant effects on the body weight and the biochemical variables (BDNF, NGF, VDBP).

There have been so far few research studies on the potential effect of long-term physical activities on regulating neurotrophics in patients with MS. In this context, it is hard to account for the biochemical significance
of changes triggered by physical activities. However, some researchers have studied the effect of aerobic and resistance training separately, with few of them investigating the effect of the combined exercise training. Moreover, few researchers have examined the biochemical factors while studying MS, and the changes resulting from training. Thus, more research studies on the effects of physical activities, especially the combined exercise training, on the biochemical variables are needed for a deeper understanding of MS and how it can be controlled. Schulz et al (2004) showed that 8 weeks of bicycling brought about no significant changes in BDNF and NGF levels in patients with MS [28]. However, Gold et al (2003) showed that bicycling for 30 minutes, an activity which consumed 60% of maximum oxygen, resulted in an increased NGF but no change in BDNF [29]. Similarly, the results of the study by Bansi et al (2013) revealed that BDNF level in patients with MS increased significantly after 3 weeks of aerobic training in that such training consumed 60% of maximum oxygen. The results of their study suggested that NGF levels tend to increase [30]. However, Castellano and White (2008) claimed that, after 4 weeks of exercise training with 65% of aerobic capacity, MS patients showed an increase in BDNF level, however, after 8 weeks, it returned to the initial level [31]. Wens et al (2016) observed an increased concentration of BDNF after 24 weeks of aerobic-resistance training [27]. Briken et al (2016) showed that bicycling for 30 minutes resulted in a significant increase of BDNF level in patients with progressive MS, but after 22 sessions, there were observed no changes in BDNF levels [32]. The results of the present study showed that a course of combined exercise training triggered no significant changes in neurotrophic factors and VDBP, a finding which is in line with the results obtained by Schulz et al (2004), Briken et al (2016), and to some extent, with those reported by Gold et al (2003). However, the results of the present study are congruent with those obtained by Castellano and White (2008), Bansi et al (2012), and Wens et al (2016). This lack of congruence can be partly accounted for by the difference in subjects' disability scales in pre-test, the difference in initial levels of BDNF and NGF, and the difference in the type and the duration of training. In fact, training in and by itself cannot trigger changes in the serum levels in neurotrophics; it is the intensity of training that plays an important role [35]. Olliff et al (1998) reported that exercise training had no effect on gene expressions of BDNF and NGF as long as they did not have enough threshold intensity [36]. Therefore, it seems that the exercise training in this study did not have enough load. Although, in the present study, the BDNF level induced, to some extent, a significant increase, the load of training was not high enough to produce significant effects on the variables.

On the other hand, the basic levels of BDNF and NGF in patients with MS showed an increase at the escalation and recovery stages rather than at the stable stage [37]. Some of the patients in this study were at recovery stage and had high basic levels of BDNF and NGF. This can account for why the variables experienced no changes. These factors response better to short-term training [31,32,38] seemingly due to the fact that the brain picks BDNF and NGF quickly, preventing any considerable increase in their levels observable in the blood [31,39]. In this study, the blood sampling was taken 24 hours after the last training session, which can partly account for the fact that no significant increase in these variables was observed. Similar to the study conducted by Briken et al (2016) who reported the small sample size as one of the reasons why no significant changes were observed in concentration of BDNF after 22 weeks, this study was limited by a small size of the sample, a fact which can explain why no significant changes were observed in these variables. In the present
study, no changes were observed in VDBP level, a finding which is not in line with the results obtained by Waschbisch et al (2012) who reported a positive relationship between fitness level and serum level of vitamin D [40]. However, there is no training program in this study and the level of vitamin D is estimated rather than VDBP level, but apparently this is the only study focusing on this variable. Generally, brain is a complex system that may be affected by various environmental factors or drug treatment [31]. Alternatively, the fact that no significant changes in these variables were observed in this study can be partly the result of the medications taken by the patients.

5. Conclusion
In summary, the provision of exercise training including aerobic and resistance training for three non-consecutive days in a week can improve the disability and the percentage of body fat in women with MS. Therefore, it seems advisable for doctors and specialists to encourage their patients with MS to receive such training sessions in order to improve their recovery chance. However, it was shown that such exercise training for 12 weeks had no significant changes in the levels of biochemical factors BDNF, NGF and VDBP).

Limitations of this research are composed of the lack of control of genetic status, sleep, the nutrition of participants, and their motivation to participate in exercises. In general, differences in the results from the scientific background and the present study may be due to such differences including the type of MS, the initial level of EDSS, the number of participants, the length of the training duration, the different kind of training program, the volume and intensity of exercise. It seems that according to the types of MS and the use of training groups based on the type of disease can be obtained for these patients in more accurate and practical results.

Acknowledgements
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Reference
Resistance and endurance training, multiple sclerosis

7 (1)


