**Title:** The Effect of Aerobic Training With the Consumption of Probiotic on the Myelination of Nerve Fibers in Cuprizone-Induced Demyelination Mouse Model of Multiple Sclerosis

**Authors:** Donya Sajedi¹, Ramin Shabani²*, Alireza Elmieh³

1. Ph.D. Candidate, Department of Physical Education and Sports Sciences, Faculty of Humanities, Rasht Branch, Islamic Azad University, Rasht, Iran.
2. Professor, Department of Physical Education and Sports Sciences, Faculty of Humanities, Rasht Branch, Islamic Azad University, Rasht, Iran.
3. Associate Professor, Department of Physical Education and Sports Sciences, Faculty of Humanities, Rasht Branch, Islamic Azad University, Rasht, Iran.

*Corresponding author: Ramin Shabani, Professor, Department of Physical Education and Sports Sciences, Faculty of Humanities, Rasht Branch, Islamic Azad University, Rasht, Iran. E-mail: dr.ramin.shabani@gmail.com

To appear in: Basic and Clinical Neuroscience

**Received date:** 2021/06/05
**Revised date:** 2021/11/10
**Accepted date:** 2022/01/02
This is a “Just Accepted” manuscript, which has been examined by the peer-review process and has been accepted for publication. A “Just Accepted” manuscript is published online shortly after its acceptance, which is prior to technical editing and formatting and author proofing. Basic and Clinical Neuroscience provides “Just Accepted” as an optional and free service which allows authors to make their results available to the research community as soon as possible after acceptance. After a manuscript has been technically edited and formatted, it will be removed from the “Just Accepted” Web site and published as a published article. Please note that technical editing may introduce minor changes to the manuscript text and/or graphics which may affect the content, and all legal disclaimers that apply to the journal pertain.

Please cite this article as:

DOI: http://dx.doi.org/10.32598/bcn.2022.3104.1
ABSTRACT

Introduction: Extensive human and animal research shows that exercise has beneficial effects on several clinical outcomes in patients suffering from multiple sclerosis (MS). The study aimed to address the effect of aerobic training with the consumption of probiotic on the myelination of nerve fibers in a cuprizone-induced demyelination mouse model of MS.

Methods: Mice, which were exposed to cuprizone (CPZ) for 13 weeks, were subjected to motor and balance tests in week 5. They (n = 5 per group) were assigned to five groups of control (C), MS, MS with exercise (MS + Exe), MS with probiotic (MS + Pro), and MS with probiotic and exercise (MS + Pro + Exe) randomly. The exercise groups carried out aerobic exercises 5 days/week for 2 months. The mice received probiotic by gavage. Performance and balance tests were repeated when the eight-week protocol of exercise and probiotic consumption was finished. One day after these interventions, they were sacrificed for biochemical and molecular biology analyses.

Results: The results showed that myelin basic protein (MBP) was increased in the MS + Pro + Exe, MS + Pro, and MS + Exe compared to the MS group (P<0.05). The mRNA of nestin showed an increase in MS + Pro + Exe, MS + Exe, and MS + Pro groups compared to the MS group, but this increase in MS + Pro + Exe and MS + Exe groups was not significant compared to the control and MS groups (P>0.05).

Conclusion: According to the results, lifestyle interventions can be effective against demyelinating-inflammatory processes that happen in the brains of MS patients.

Keywords: Myelination, Multiple sclerosis, Exercise, Lactobacillus plantarum, Cyclohexanes
Highlights

- Exercise can improve demyelination in mice.
- Consumption of probiotics can be effective in improving multiple sclerosis disease.
- Exercise can modify the gut microbiota.

Plain Language Summary

Multiple sclerosis (MS) is a common inflammatory disorders of central nervous system (CNS) categorized by myelin loss and decadence of neurons in the brain and spinal cord. Feeding of cuprizone causes reversible demyelination, predominantly of the corpus callosum in C57/Bl6 mice.

The results of previous studies showed that exercise training and consumption of probiotics alone effectively reduces demyelination of the nerve fibers.

In this study considering that the elimination of cuprizone from mice food causes remyelination, its use was continued until the end of the protocol.

In this study we emphasize on exercise training and consumption of probiotics.

Our results showed that in addition to the effect of exercise training and consumption of probiotics on remyelination of nerve fibers, both of these interventions have a greater effect on it.
1. Introduction

Multiple sclerosis (MS) is the most widespread demyelinating disease of the central nervous system (CNS) causing neurological disability, mostly in young adults (Eckstein et al., 2012). Women suffer from MS 2-3 times as many as men (Leray et al., 2016). MS patients show various physiological and psychological symptoms, e.g., cognitive and emotional problems, visual impairments, fatigue, general muscle weakness, and motor disturbances (Huang et al., 2017). Myelin sheath is a lipid-protein substance, which wraps nerve fibers (axons). The myelin cells, or the so-called oligodendrocytes, are the main ground for disease initiation. The term MS refers to the formation of scar tissues or plaques or lesions in certain parts of the brain, especially in the white matter (Rahmanzadeh et al., 2018). Infiltrating T cells and monocytes/macrophages are not only responsible for the death of the oligodendrocyte cells but they also employ brain resident immune cells, i.e. astroglia and microglia, which, in turn, both amplify and resolve inflammation (Popescu & Lucchinetti, 2012).

Inflammatory infiltration into the CNS destroys major myelin proteins, such as myelin basic protein (MBP) (Popescu & Lucchinetti, 2012). This inflammatory process causes demyelination, axonal damage, and subsequently progressive hind limb paralysis (Robinson et al., 2014).

In MS, where lesions throughout the CNS are demyelinated by the damage to oligodendrocytes and their myelin sheaths, spontaneous myelin repair was identified years ago. More recent studies have revealed that remyelination happens earlier and more extensively than what the earlier studies have reported (Brück et al., 2003). The expression of the intermediate filament protein nestin has been utilized as a marker for neural stem and progenitor cells in the ventricular and subventricular zones (Hockfield & McKay, 1985). Radial glia (Hockfield & McKay, 1985), which are substrates for migration and can give rise to neurons (Noctor et al., 2001), also express nestin. The second intronic enhancer of nestin signifies the expression of the gene to neural tissues (Zimmerman et al., 1994).

Most clinical studies have reported the benefits of exercise training for molecular, histopathological, and behavioral abnormalities in MS patients and animal models of MS (Motl & Pilutti, 2012). Anti-inflammatory effects of exercise training are also documented in MS patients (Florindo, 2014). Exercise-based protocols for animal models are composed of voluntary wheel running, forced treadmill running, or swimming. Although both voluntary and forced exercise
paradigms are effective preconditioning tools for the CNS (Zhang et al., 2011), forced exercise has been found to outperform voluntary exercise in promoting neuroprotection by altering brain metabolism (Kinni et al., 2011). Moreover, forced exercise protocols allow modifying the intensity, duration, and frequency of the program and may motivate a human’s schedule of gym exercises (Zhang et al., 2011). Also, it is suggested that the improvement of adaptation to stress responses and the promotion of neuroprotection requires the exercise periods to be at least three weeks (Adlard et al., 2005).

The gut microbiome is claimed to cause several autoimmune disorders including inflammatory bowel disease, rheumatoid arthritis, and MS (Bhargava & Mowry, 2014). Recent research has revealed a correlation of high frequency of intestinal T helper 17 (Th17) cells with changes in gut microbiota composition and increased disease activity in MS (Cosorich et al., 2017). Thus, it can be said that MS patients can potentially benefit from, for instance, manipulating the gut microbiome by probiotics. Probiotics are live and non-pathogenic microorganisms that can be found in certain foods. Some of these microorganisms belong to selected bacterial strains, Lactobacillus (Goudarzvand, 2016).

Some evidence suggests that exercise may modify the microbiota (Queipo-Ortuño et al., 2013). Accordingly, a study on elite rugby players reported that exercise increased gut microbiota richness and diversity (Clarke et al., 2014). Also, recent studies on animals have shown that controlled training also exerted some beneficial effects on the gut microbiome of obese and hypertensive rats (Petriz et al., 2014) and in obese mice with a phenotype induced by high-fat diet (Kang et al., 2014).

The cuprizone (CPZ) model provides a reproducible method to investigate primary demyelination, inflammation, axonal damage, and myelin repair/remyelination processes, in the absence of peripheral immune system activation. As a mitochondrial agent that chelates copper, CPZ selectively targets mature oligodendrocytes of the CNS, especially those of the corpus callosum. Demyelination completes after five weeks of CPZ intoxication, along with massive microgliosis, astrocytosis, and axonal damage (Hibbits et al., 2009). Furthermore, the removal of CPZ from the diet of animals enhances remyelination (Heckers, 2018).

The present study aimed to investigate the impact of exercise with Lactobacillus plantarum (L. plantarum) probiotic on motor deficits associated with the CPZ model and the myelination. To address this issue, the research adopted a model of exercise training that can mimic endurance
training in humans and is associated with the enhancement of wellness in both healthy individuals and subjects suffering from neurodegenerative disorders.

2. Methods

2.1. Animals

Female C57BL/6 mice (n = 25, 8 weeks old, 20 ± 4 gram), procured from Pasteur Institute of Iran, were taken the Histogenotech Lab of Tehran, Iran. They were kept in communal cages at 22 ± 1°C under a 12-h light/dark cycle (lights on at 07:00) with free access to food and water. All procedures, approved by the Islamic Azad University (Rasht, Iran) and Institutional Animal Care and Use Committee (Ethical code: IR.IAU.RASHT.REC.1399.028), complied with the National Institute of Health (NIH) guidelines. Behavioral experiments were conducted from 08:00 am to 04:00 pm.

2.1.1. Experimental design and animal group

The mice (n = 5 per group) were randomly assigned to five groups: control (C) that did not receive any intervention, MS that was only exposed to cuprizone, MS with exercise (MS + Exe), MS with probiotic (MS + Pro), and MS with probiotic and exercise (MS + Pro + Exe).

2.2. Cuprizone (CPZ) model

Mice were exposed to cuprizone for 13 weeks. To induce demyelination, the mice were fed on a diet composed of 0.2% CPZ (bis cyclohexylidenehydrazide; Sigma–Aldrich Inc., St. Louis, MO, USA) mixed with rodent food triturated pellets (Gudi et al., 2014b). Since remyelination is increased with there is no CPZ in the diet of animals (Heckers, 2018), its use was kept on until the end of the protocol. So, the daily food of the animals contained it during the exercise period too.
2.3. Beam test

The mice were subjected to the mobility and balance tests by the beam test in week 5 of CPZ feeding and at the end of the eight-week protocol of exercise and probiotic consumption. The animals were trained to travel from the suspended end of a narrow beam (120 cm length, 7 cm width, elevated 100 cm above a thick foam cushion) into a goal box (24.5 * 20 * 18). Two observers recorded the number of footslips to start a movement. A footslip is defined as one single paw descending 1.5 cm or more beneath the surface of the beam (Mu et al., 2011).

2.4. Probiotic supplement

*L. plantarum* strain PTCC (Persian Type Culture Collection) 1058 was chosen by the Iranian Research Organization for Science and Technology, Tehran, Iran for testing. After the bacteria were cultured and their purity was confirmed, they were grown anaerobically in de Man, Rogosa and Sharpe (MRS) broth at 37°C for 48 h at a volume of 500 ml. After 48 hours, they were centrifuged, washed with PBS, and re-centrifuged to be isolated from the growth media. Each animal was treated with 1.5 mL of resuspended bacteria in saline with $10^8$ CFU concentration/kg. The probiotic was gavaged 5 times a week 1 hour after exercise training.

2.5. Treadmill exercise training

The mice were subject to physical training and performance tests on a specifically designed 8-lane treadmill (Tajhiz Gostar Omid Iranian, Iran). Before the exercise performance tests and the training program were initiated, the mice were gotten acquainted with treadmill running for 10 min on three consecutive days (8 cm/s). The performance was tested before training and at the end of the 8-week training protocol. These two performance test types were conducted 72 hours apart.

2.5.1. Exhaustion speed performance test

The exhaustion speed performance test was employed to measure the maximal running speed. For this test, the mice were run for 8 cm/s and then the speed was increased at a rate of 2 cm/s per minute until exhaustion [modified from (Qi et al., 2011)]. Exhaustion was recorded when the mouse could not or refused to continue when encouraged with a bottle brush or a small puff of air.
2.5.2. Exercise tolerance performance test

To evaluate the exercise tolerance, the exercise tolerance performance test was employed for which each mouse was individually run to exhaustion at 30 cm/s on a rodent-specific treadmill [modified from (Ritchie et al., 2014)]. Exhaustion was defined as above.

2.5.3. Exercise training protocol

The mice were subjected to an 8-week treadmill running, 5 days per week, 1 session per day at a rate of 23 cm/s. This training speed is equivalent to an exercise intensity of 55–60% of maximal speed as per the baseline exercise speed performance tests. An incremental exercise training protocol was applied to the trained mice. Each training session was composed of a 5-min warm-up at 8 cm/s. The warm-up was followed by 10 min of training in week 1 and by 20 min of training in week 2. In the subsequent 6 weeks, the warm-up was followed by 30 min of training at 23 s/min. The potential intervening factors, such as differences in stress, sound, and light exposure, were minimized by leaving the sedentary control mice on the treadmill without running for the same duration as the exercise groups. All animals were included in the experiments (Figure 1).
Figure 1. Experimental protocol for exploring the effects of exercise training. The mice were initially exposed to cuprizone for 5 weeks. The performance tests and beam test were carried out before the training. Then, the eight-week training protocol was initiated. The training protocol was composed of 10 minutes of training for the first week, 20 minutes of training for the second week and 30 minutes of training with an intensity of 23 cm/s during the next 6 weeks. During the eight-week training period, the mice were given probiotics by gavage 5 days a week. There was an hour break between activity and probiotics. The performance tests and beam tests were repeated when the 8th week was over.

2. 6. Tissue preparation

One day after the eight weeks of the exercise training protocol and taking probiotics were finished, the mice were sacrificed under CO₂ gas. The whole brain was exposed and divided anteriorly-posteriorly in bregma place using a coronal section. The anterior part was used for western blot and real-time PCR and the posterior part was used for histological assessment.

2.7. Histological evaluation and luxol fast blue (LFB) staining

To assess myelination, the corpus callosum was removed after the mice were sacrificed and post fixed overnight. Then, the samples were dehydrated in ascending alcohol series, rinsed with xylene, and infiltrated with paraffin. Afterward, all the paraffin-embedded specimens were coronally sectioned at a thickness of 5 μm. The samples were stained with luxol fast blue (Sigma-Aldrich) according to the manufacturer’s instructions. Briefly, a graded serial of ethanol to 1% solution of LFB in 0.05% acetic acid and 96% ethanol was used to deparaffinized and transform
corpus callosum sections. Sections were put in LFB solution at 56°C overnight, then rinsed in 95% ethyl alcohol and distilled water prior to differentiating in 70% ethyl alcohol. After differentiation in the lithium carbonate solution, they were placed in distilled water again and counterstained. The samples were investigated under a light microscope (Labomed, USA). Image J software was used to evaluate the amount of Myelin. For this reason, the amount of dark blue to whole tissue in each section was assessed.

2.8. Striatal total protein extracts preparation and western blot (WB)

The protein concentration of each sample was measured with Lowry assay to make sure that equal amounts have been loaded during western blotting. First in the running the gel stage, mix the protein sample with the sample buffer and boil for 5-10 minutes and then run on polyacrylamide gel for 2-3 hours at 100 volts. After running, the gel was prepared for the transfer stage. In the transfer step, we first cut the nitrocellulose paper as needed and put it inside the transfer buffer along with the device pads and sponges. To cut the nitrocellulose paper, we used a clean scalpel and avoided touching paper with our hands. Then, the nitrocellulose paper was inserted with gel and sponges and the device pads inside a tank and it was exposed to 350 amps electrophoresis for 1 hour. After transfer, the nitrocellulose paper was washed with a TBS buffer for 5-10 minutes. At this stage, the nitrocellulose paper was immersed in the TBS buffer (or blocking) for 1 hour at room temperature, and it was shaken gently. Then, the nitrocellulose paper was washed several times using a TBS buffer. Then, the nitrocellulose paper was incubated for 1-2 hours at room temperature with the primary antibody diluted in the TBS buffer (antibody accuracy was 1000/1). The nitrocellulose paper was rinsed several times using a TBS buffer. Then, it was incubated for 1-2 hours at room temperature with a secondary antibody diluted in the TBS buffer (antibody accuracy was 3000/1). The nitrocellulose paper was rinsed several times using a TBS buffer. This step of nitrocellulose paper using the emergence and fixation solution using ECL appears in a dark room on the photographic film. Finally, after the band appeared, the paper could be washed with distilled water.
2.9. mRNA gene expression analysis

The corpus callosum tissue, which was prepared by Qiazol (Qiazol lysis reagent, USA), was prepared in completely sterile conditions on the ice. The concentration and purity of RNA were determined by the ratio of the absorbance at 260 nm over that at 280 nm (A260/A280) using a NanoDrop ND-100 spectrophotometer (Thermo Scientific, Waltham, MA, USA). The RevertAid cDNA synthesis kit (Thermo Scientific, USA) was used at a volume of 10 μL following the manufacturer’s recommendations to convert RNA into cDNA. The extracted RNA samples, which were already infected with genomic DNA, were treated using DNase I. PCR was amplified by 2 μL of the cDNA synthesis reaction, 12.5 μL of RealQ Plus Master Mix Green high ROX™ (Amplicon, Denmark), 0.2 μL of each forward and reverse primers (at the concentration of 10 pico-molar), and 10.1 μL of distilled water. The primers were designed by the Primer 3 software package and were verified by the NCBI BLAST Tool. Table 1 presents the primers used here.

Table 1. The characteristics of the primers used in the present study

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>NES-F</td>
<td>5’ CCCCTTTCTTCTGTGTCTCACC 3’</td>
</tr>
<tr>
<td>NES-R</td>
<td>5’ TCACTCATCATGTGCTCCTCT 3’</td>
</tr>
<tr>
<td>β-Actin-F</td>
<td>5’ TCAGAGGAAGGAGCATCC 3’</td>
</tr>
<tr>
<td>β-Actin-R</td>
<td>5’ GGTCATCTTCTACGGTTGG 3’</td>
</tr>
</tbody>
</table>

Thermocycling conditions were 95°C for 15 min, which was followed by 40 cycles of denaturation at 95°C for 15 sec and 60°C for 1 min. The ΔCT method was employed to determine the relative expressions for the nestin gene. A comparison was made between CT samples in the target gene and CT gene internal control. The real-time PCR was conducted on the ABI Stepone (Applied Biosystems, USA) detection system. The specificity of the real-time PCR reaction was double-checked by electrophoresis and melting curve analysis.
2.10. Statistical analysis

The differences of means among groups on all variables were compared using one-way analysis of variance (ANOVA). The between-group differences of means were distinguished by Tukey’s multiple comparison test. All values are expressed as mean ± standard deviation (SD). The statistical significance level was set at \( P < 0.05 \) for all tests.

3. Results

3.1. The effect of aerobic training with the consumption of probiotic in the beam test

The results showed that the error level in the beam test was significantly decreased in the MS + Pro + Exe and MS + Exe compared to the MS group (\( P < 0.05 \)). Also, the error level in MS + Pro group decreased compared to the MS group but the difference was not significant (\( P > 0.05 \)) (Figure 2).

![Beam test results in different study groups](image)

**Figure 2.** The beam test results in different study groups. The values are displayed as the means ± SD. The difference signs represent statistically significant differences between the mean values (\( P < 0.05 \)) and the same signs are not significant.

One-way ANOVA analysis and Tukey post-hoc in all groups were significantly different from the control group (* \( P < 0.05 \) vs. control group), and MS+Exe and MS+Pro+Exe groups were significantly different from the MS and MS+Pro groups (& \( P < 0.05 \) vs. MS group and # \( P < 0.05 \) vs. MS+Pro group).

MS+Pro: MS plus probiotic, MS+Exe: MS plus exercise, MS+Pro+Exe: MS plus probiotic plus exercise.
3.2. The effect of aerobic training with the consumption of probiotic in the performance tests

To examine the efficacy of the training protocol, exhaustion speed, and exercise tolerance performance tests were performed. The results of both tests in the MS + Pro + Exe, MS + Exe, and MS + Pro groups showed a significant increase in the exhaustion speed, and exercise tolerance compared to the MS group (P < 0.05) (Figure 3a,b).

![Figure 3a](image-url)  
*Figure 3a. The exhaustion speed performance test results in different study groups. The values are displayed as the means ± SD.*

One-way ANOVA analysis and Tukey post-hoc in all groups were significantly different from the MS group (* P < 0.05 vs. MS group).

MS+Pro: MS plus probiotic, MS+Exe: MS plus exercise, MS+Pro+Exe: MS plus probiotic plus exercise.
b)

**Figure 3b.** The exercise tolerance performance test results in different study groups. The values are displayed as the means ± SD. The difference signs represent statistically significant differences between the mean values (P < 0.05) and the same signs are not significant.

One-way ANOVA analysis and Tukey post-hoc in MS+Pro and MS+Exe groups were significantly different from the MS group (* P < 0.05 vs. MS group), MS+Exe and MS+Pro+Exe groups were significantly different from the control group (# P < 0.05 vs. control group), MS+Pro+Exe group was significantly different from the MS group (µ P < 0.05 vs. MS group), and MS+Pro+Exe group was significantly different from the MS+Pro group (& P < 0.05 vs. MS+Pro group).

MS+Pro: MS plus probiotic, MS+Exe: MS plus exercise, MS+Pro+Exe: MS plus probiotic plus exercise.

### 3.3. Induction of demyelination in cuprizone-fed mice

The histological changes in this study were performed using LFB staining method. Figure 4a,b shows the myelination changes in different groups. As shown in the figure, the myelination of the nerve fibers was significantly (P < 0.05) higher in the MS + Pro + Exe, MS + Pro, and MS + Exe groups than in the MS group.
Figure 4a. Histological changes in myelination in different treatment groups. The above images show the longitudinal cross-section of nerve fibers stained with LFB staining. Arrows indicate areas of myelination. Bright areas indicate a decrease in myelin density in the MS group (D, E and F) and the myelination of the nerve fibers is lower in this group than in the other groups. In the intervention groups (MS + Exe, MS + Pro and MS + Pro + Exe), an increase in the amount of myelin is observed in the images, respectively. The MS + Pro + Exe group (M, N and O), has the highest rate of myelination compared to the control group. Also in this group, the myelination of the nerve fibers is arranged in a regular, coherent structure. The diameter of the nerve fibers is also higher in this group than in the other groups.

MS+Pro+Exe: MS plus probiotic plus exercise, MS+Pro: MS plus probiotic, MS+Exe: MS plus exercise.
Figure 4b. The myelination of the nerve fibers in different study groups. Observed percentages of myelination - Blue fibers in different groups. The values are displayed as the means ± SD. The difference signs represent statistically significant differences between the mean values (P < 0.05) and the same signs are not significant.

One-way ANOVA analysis and Tukey post-hoc in MS+Pro+Exe, MS+Pro and MS+Exe groups were significantly different from the control and MS groups (* P < 0.05 vs. control group and # P < 0.05 vs. MS group), and MS group was significantly different from the control group (& P < 0.05 vs. control group).

MS+Pro+Exe: MS plus probiotic plus exercise, MS+Pro: MS plus probiotic, MS+Exe: MS plus exercise.

3.4. Western blot analysis

The results showed that MBP expression was significantly increased in the MS + Pro + Exe, MS + Pro, and MS + Exe compared to the MS group (P < 0.05). However, a combination of probiotic with exercise training protocols showed the highest increase in MBP (Figure 5).
Figure 5. The expression of MBP in the corpus callosum area of different study groups. β-Actin was used as loading control. The values are displayed as the means ± SD. The difference signs represent statistically significant differences between the mean values (P < 0.05) and the same signs are not significant.

One-way ANOVA analysis and Tukey post-hoc in MS+Pro+Exe, MS+Pro and MS+Exe groups were significantly different from the control and MS groups (* P < 0.05 vs. control group and # P < 0.05 vs. MS group), and MS group was significantly different from the control group (& P < 0.05 vs. control group).

MS+Pro+Exe: MS plus probiotic plus exercise, MS+Pro: MS plus probiotic, MS+Exe: MS plus exercise.
3.5. Expression of Nestin

The results showed that the gene expression of nestin was higher in MS + Pro + Exe, MS + Exe, and MS + Pro groups than in the MS group, but the difference was not significant (P > 0.05) (Figure 6).

Figure 6. The mRNA expression of nestin in the corpus callosum area of different study groups. The values are displayed as the means ± SD.

One-way ANOVA analysis and Tukey post-hoc in MS and MS+Pro groups were significantly different from the control group (* P < 0.05 vs. control group).

MS+Pro: MS plus probiotic, MS+Exe: MS plus exercise, MS+Pro+Exe: MS plus probiotic plus exercise.

4. Discussion

In this study, we investigated the efficacy of aerobic training along with the consumption of probiotic on myelination, histological changes, and the expression of the gene nestin in the corpus callosum for the animal model of demyelination using CPZ administration. The present study used the CPZ model to induce demyelination. It is a famous experimental animal model that is used in research on the mechanisms of pathophysiology of MS (Kipp et al., 2017). The results showed that aerobic training significantly improved motor deficits in animal models of MS. The MS + Exe group exhibited more improvement in motor function than the MS + Pro group. However, the highest rate of motor improvement was observed in the MS + Pro + Exe group. This is in agreement with a study in the past (Mandolesi et al., 2019) that has shown that exercise can improve clinical scores in mice with demyelination.
The study revealed an increase in the amount of MBP and myelination with exercise and consumption of probiotic. Also, the myelination rate and MBP level were increased in MS + Pro group to a greater extent than in the MS + Exe group. A study reported the significant effect of probiotic treatments on clinical improvement, which was ascribed to the retention of myelin content and the reduction of astrocytosis and CD3+ T cell infiltration (Consonni et al., 2018).

Reportedly, physical exercise influences myelination with various effects according to the analyzed brain area (Tomlinson et al., 2018). Functional recovery, which was enhanced by exercise, was associated with limited myelin destruction and the loss of myelin-associated proteins in white matter tracts of the corpus callosum and reduced axonal pathology. Moreover, exercise significantly intensified innate immune response, i.e., microgliosis and, to a lesser extent, astrogliosis. In rodents, exercise has been found to be associated with significant changes in brain anatomy in terms of weight and size, hippocampal neurogenesis and synaptogenesis (Van Praag et al., 2000), and synaptic plasticity (Patten et al., 2013). Myelination and remyelination rely on several complex mechanisms of the differentiation of oligodendrocyte precursor cells through different stages into mature myelinating oligodendrocytes (Bercury & Macklin, 2015). In the CNS, the activation of microglia and astrocytosis are important components of the lesion environment that can impact the demyelination process, and these cells have been crucially involved in demyelination/remyelination processes during CPZ (Gudi et al., 2014a). Nonetheless, it has been established that long-term exercise-based protocols improve immune function. As far as macrophages concern, these effects can be related to the higher phagocytic activity (Sugiura et al., 2001) or accelerated phenotype switching that leads to quicker wound healing (Goh & Ladiges, 2014). It has already been established that although isolated inflammation is not effective in changing the number of oligodendrocyte precursor cells, it enlarges the cell bodies and increases the number of the processes (Di Bello et al., 1999) and young macrophages recruited during remyelination, which facilitates the differential of oligodendrocyte precursor cells by removing inhibitory myelin debris (Ruckh et al., 2012). Accordingly, the modulation of immune functions plays a key role in myelin regenerative processes contributing to the remyelination course. Altogether, these data suggest that some oligodendrocyte precursor cells die between peak and chronic disease and do not remyelinate axons. Furthermore, proliferation, migration, differentiation, and remyelination are the behaviors of oligodendrocyte precursor cells that can be modulated not just by the CNS disease conditions and trauma lesions but also by changes in the
environment and the practice of physical activity. They may even sometimes act via opposite

directing forces (Ehninger et al., 2011). In fact, in demyelinating lesion models in which

remyelination was observed, a relationship was detected between the decreased number of

oligodendrocyte precursor cells and the myelin repair (Watanabe et al., 2002).

The study revealed an increase in nestin levels after aerobic training and probiotic consumption.
The amount of nestin in the MS + Exe group increased to a greater extent than that in the MS +
Pro group. Various types of CNS damage are followed by so-called reactive neurogenesis, i.e., the

formation of new neurons to replace the ones that have been lost by the damaging factor (Parent,
2003). It has been observed that nestin expression is increased in some pathological states, such as

inflammation (Cameron & Mckay, 2001), and others. Nestin expression in astrocytes is detected

as early as one day after damage and continues for one month. Astrocytes may be involved in

neuroprotection by forming a glial barrier/scar around the damage zone (Nakamura et al., 2003).

Cholinergic neurons, especially those that have nestin as a cofactor, do not immediately die after

a neurotoxic event, but rather they enter an atrophic quiescent state in which they do not express

the enzymes required to maintain cholinergic transmission. On the contrary, if cholinergic neurons

are exposed to nerve growth factor (NGF) (Nagahara et al., 2009) or brain-derived neurotrophic

factor (BDNF) (Morse et al., 1993) in a timely and repeated manner, a significant part of them (30-

40%) can be rescued from a pathological state. Therefore, the nestin contributes to the dynamic

remodeling of cells during development (Ruan, 2001). Evidence shows that nestin has a
cytoprotective function in the adult nervous system (Su et al., 2013). It is known that exercise
enhances neurotrophin levels – an enhancement that can last for weeks (Berchtold et al., 2010).

The present research revealed the effects of oral administration with L. plantarum. Since research

on MS has mostly addressed the differences in the gut microbiome of MS patients versus healthy

people (Jhangi et al., 2014), some have dealt with changing the composition of gut microflora by

probiotics, which modulates the immune response in an animal model of MS (Ochoa-Reparaz et

al., 2010). Nonetheless, growing evidence suggests that exercise can also alter gut microbial

composition (Bermon et al., 2015). However, the efficacy of probiotic treatment on different
diseases depends on bacterial and mice strains, host immunological state, experimental system, an

adequate dose, and its mechanisms are not well-understood yet (Isolauri et al., 2001). In the present

study, it was shown that L. plantarum can improve the myelination of the nerve fibers. The rate of
improvement in myelination was higher in the MS + Pro + Exe group. We found that oral administration of these strains delayed the development of disease and resulted in the suppression of MS progression. Probiotics inhibit the growth of other microorganisms or compete for receptors and binding sites with other intestinal microbes on the intestinal mucosa by producing antimicrobial agents or metabolic compounds (Collado et al., 2007; O'Shea et al., 2012). We know that the alteration in equilibrium between T helper 1 (Th1) and T helper 2 (Th2) responses is linked to the pathogenesis of a wide variety of autoimmune diseases such as MS (Nosratabadi, Rastin, Sankian, Haghmorad, & Mahmoudi, 2016). Although it is argued that the Th17 cells have a key role to play in MS pathogenesis, the geography of their peripheral activation and expansion in humans is still unclear. In mice, effector Th17 cells are mostly activated in the small intestine (Ivanov et al., 2009), and the pathogenicity of myelin-reactive T cells at the intestinal level and their capacity to trigger brain autoimmunity are intensified when they acquire a Th17 cell phenotype (Berer et al., 2011).

Many studies have documented that experimental autoimmune encephalomyelitis (EAE) is mediated by the Th1 and Th17 cells secreting pro-inflammatory cytokines (Kobayashi et al., 2010), while there is a relationship between disease recovery and the increased levels of Th2 cytokines (Nosratabadi, Rastin, Sankian, Haghmorad, Tabasi, et al., 2016). It seems that an up-regulation of these cytokines in probiotic-treated mice stimulates a shift towards Th2 response which may be one of the mechanisms involved in the down-regulation of the autoimmune response. Some previous studies have revealed that pro-inflammatory cytokines promote the recruitment of inflammatory cells (Haghmorad et al., 2017) while the anti-inflammatory cytokines negatively regulate the secretion of pro-inflammatory cytokines (Moore et al., 2001). In this situation, damaged axons re-generate and the healing process of myelin and neurons is better organized (Sakalidou et al., 2011).

This study as promising future therapeutics for MS provides powerful evidence for a beneficial link between the animal and human systems.
5. Conclusions

In conclusion, our study demonstrates that aerobic exercise with the consumption of probiotic influences the main pathological hallmarks in MS. In general, these results suggest lifestyle interventions as a good non-pharmacological tool for controlling disease progression against demyelinating-inflammatory processes in the brains of animals with MS. However, further research is required, especially on human subjects.

Acknowledgment

This dissertation has been registered with code 117481815632161180099 in Islamic Azad University, Rasht Branch.

Authors’ contribution

Conceptualization, Methodology, Software, Investigation, Resources and Data Curation: Donya Sajedi and Ramin Shabani, Writing – Original Draft Preparation and Funding Acquisition: Donya Sajedi, Formal Analysis, Writing – Review & Editing and Project Administration: Ramin Shabani, Visualization and Supervision: Ramin Shabani and Alireza Elmieh and Validation: All authors.
References


[KRecord #35 is using a reference type undefined in this output style.]


