Physical Activity in Multiple Sclerosis: A Comparative Study of Vitamin D, Brain-Derived Neurotrophic Factor and Regulatory T Cell Populations

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Abstract

Background: Previous studies suggest beneficial effects of exercise in multiple sclerosis (MS). However, knowledge on the effects of physical activity on the immune system is limited. Objective: To assess potential relationships between cardiorespiratory fitness, cognitive function, and immune parameters in physically active and inactive MS patients. Methods: We identified 83 patients with relapsing-remitting disease, an unrestricted walking range, and stable interferon-β treatment from our data base. Based on the subjective report of physical activity, the lower/inactive (n = 21) and upper/active quartiles (n = 21) of patients were selected. We assessed the frequency of T cells, B cells, NK cells, monocytes and regulatory T cell populations by flow cytometry, measured brain-derived neurotrophic factor and vitamin D serum levels by ELISA, and conducted spiroergometry and transcranial sonography. Results: Physical activity and cardiorespiratory fitness were not associated with brain-derived neurotrophic factor, frequency of T regulatory cells or any other immune cell subpopulation. However, we found a positive correlation of vitamin D serum levels with cardiorespiratory fitness. Conclusion: Overall, we found no negative effect of physical activity on the immune system. The association between vitamin D and cardiorespiratory fitness most likely reflects longer hours of sunlight exposure in active patients, suggesting a desirable ‘side-effect’ of physical activity.

Key Words
Multiple sclerosis · Exercise · Sport · Cardiorespiratory fitness · HLA-G · Foxp3 · Brain-derived neurotrophic factor · Vitamin D

Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system that might lead to considerable physical disability due to motor weakness and spasticity. In addition, fatigue, heat sensitivity and mental depression are common problems. Although not evidence based, in the past patients were advised to avoid physical activity in order to stabilize the immune-mediated disease. Today, however, an increasing number of studies have shown beneficial effects of exercise in MS. It has been clearly shown that exercise in patients with MS provides similar physical and psychological benefits as in healthy controls. Exercise has shown promising effects on quality of life [1], depression [2] and fatigue [3], and may improve cognitive functions [4]. However, physical inac-
tivity is still prevalent in MS patients [5, 6] and knowledge on the effects of exercise on the immune system of patients with neurological diseases is limited.

So far, no systematic analysis of the immunoregulatory effects of physical activity in MS patients has been performed. It is not clear if physical activity has such an effect at all. In addition, there is no answer to the question whether physical activity above a certain level might even be harmful due to immunological alterations. There is evidence from the literature that physical activity impacts the human immune system and cytokine homeostasis. It has been suggested that exercise significantly induces brain-derived neurotrophic factor (BDNF) production in MS patients and controls [7]. In athletes, a profound immunosuppressive effect of exercise with decreased numbers of granulocytes, macrophage oxidative burst activity and a decrease in cytotoxic T cells was demonstrated [8].

Studies evaluating similar effects in MS patients are lacking. This is why we evaluated potential relationships between levels of physical activity and serum levels of BDNF, vitamin D and cellular immunity in relapsing-remitting MS patients. Transcranial sonography and the third ventricle width as well as functional cognitive tests (MUSIC [9] and PASAT [10]) were employed to identify relationships between physical activity and cognitive function in MS patients. In order to obtain a reliable marker for physical fitness and physical activity, we assessed cardiorespiratory fitness.

Since exercise and sport are inherent parts of our modern society, we believe that the relation of physical activity and immunity is of paramount importance to the care of MS patients.

**Patients and Methods**

**Patient Selection and Clinical Samples**

The study was approved by the local ethics committee and written informed consent was obtained from all patients. Questionnaires on demographics, disease course, treatment and comorbidities were collected from 632 patients with MS as previously described [6]. Self-reported data on disease course, walking range and immunomodulatory therapy was used to identify 83 subjects with relapsing-remitting MS who had no significant comorbidities, an unrestricted walking range (EDSS ≤3.5) and were treated with interferons at high frequency, i.e. interferon-β-1a 3×/week (Rebif®) or interferon-β-1b (Betaseron®).

Physical activity was evaluated using the German version [11] of the Baeecke Questionnaire [12], which quantifies structured exercise (sport index), physical activity acquired in leisure time (leisure time index) and at work (occupational index). Each index adopts values from 1 to 5, with 5 indicating the highest possible physical activity. A sport index value of 3 can be achieved by, for example, 2–3 h of moderate intensity exercise per week. The sport index was used to define quartiles of patients according to their level of physical activity. Patients that belonged to the lower (n = 21) and upper quartile (n = 21) were recruited for further testing. EDSS performance and relapse rates were assessed by two neurologists blinded to the sport index. Peripheral blood was obtained by venipuncture prior to spirometry in all patients. All samples were obtained during a period of 6 weeks between June and September 2008.

**Spiroergometry**

Spiroergometry was conducted on a bicycle ergometer (Kardiomed Diagnostic Cycle, Proxomed, Germany) using the Oxycon® Mobile System (Viasys Healthcare, Germany). A ramp protocol was used to assess maximum aerobic capacity (VO_{2max}) and the respiratory exchange ratio. Following a 3-min warm-up (25 W), the intensity was increased by 11 W/min in female and 15 W/min in male participants. Ergometry was conducted until subjective physical exertion and included a 3-min cool-down period.

**Flow Cytometry**

Peripheral blood mononuclear cells were isolated via Ficoll density centrifugation (Lymphoprep™; Axis-Shield, Oslo, Norway). Cells were washed twice in phosphate-buffered saline containing 0.1% sodium azide and 1% bovine serum albumin, followed by Fc receptor blocking with human IgG (Sigma-Aldrich, Munich, Germany). Afterwards, cells were incubated for 30 min with specific, fluorescence-conjugated monoclonal antibodies. The following anti-human monoclonal antibodies were used: anti-CD3 (HIT3a), anti-CD4 (SK3), anti-CD8 (SK1), anti-CD14 (MOP9), anti-CD19 (HB19), anti-CD16 (3G8), anti-CD25 (M-A251) (all from BD Biosciences, Heidelberg, Germany) and anti-HLA-G (MEM-G/9) (Exbio, Prague, Czech Republic). Intracellular stainings using anti-Foxp3 (PCH101) antibody were performed using the Foxp3 staining kit (eBioscience, San Diego, Calif., USA) according to the manufacturer’s protocol. The respective isotype controls were purchased from BD Biosciences or eBioscience. All antibodies were titrated for optimal concentrations. Samples were measured on a FACSCalibur (surface stainings) or FACSCount II (intracellular staining) flow cytometer (BD Biosciences). Data was analyzed using FlowJo Software, Version 8.8.6 (Tree Star, Ashland, Oreg., USA).

**ELISA**

Vitamin D serum levels were analyzed using a 25-OH-vitamin D enzyme immunoassay (Immunodiagnostic Systems, Frankfurt, Germany) according to the manufacturer’s instructions. For the analysis of BDNF concentrations in serum samples the Quantikine® Human BDNF Immunoassay (R&D Systems, Wiesbaden, Germany) was used.

**Transcranial Sonography**

The diameter of the third ventricle was supervised by M.M. The third ventricle was measured using a standardized transcranial sonography protocol as previously described [13]. All patients were assessed by one independent, experienced examiner who was unaware of the degree of physical activity or clinical details of the patient. Transcranial sonography of the third ventricle was supervised by M.M.
Cognitive Function Tests

The MUSIC (Multiple Sclerosis Inventarium Cognition [9]) and PASAT (Paced Auditory Serial Addition Test [10]) were performed according to the protocol.

Statistical Analysis

All data was analyzed using GraphPad Prism® Software. A Shapiro-Wilk normality test was employed and group differences were assessed by a two-tailed Student’s t or a Mann-Whitney U test. Where applicable, Spearman or Pearson coefficients were calculated to assess bivariate correlations.

Results

Clinical Details and Physical Performance of the Study Population

There were no significant differences concerning basic demographic and disease-specific parameters between physically active and inactive patients (table 1). Self-reported physical activity as assessed by the Baecke Questionnaire correlated well with physical performance parameters (relative VO2peak (percentage of age and gender-specific normative values of healthy subjects), r = 0.61, p < 0.0001; relative maximum Watt (percentage of normative values), r = 0.60, p < 0.0001). The absolute VO2peak values were within the range of normative values for healthy adults [14] but differed significantly between the group of active (34.69 ± 8.36) and inactive patients (27.31 ± 6.09). The duration of the test was higher for the active group (14:25 ± 3:09 min) compared to the inactive group (11:07 ± 03:20 min). Of note, the peak respiratory exchange rate did not differ between the two groups (active: 1.16 ± 0.06; inactive: 1.17 ± 0.07), indicating adequate and comparable physical exertion in both groups.

Cognitive Function, Third Ventricle Diameter and BDNF in Active and Inactive MS Patients

Sedentary and active MS patients performed comparably in cognitive function tests (PASAT inactive: 0.08 ± 1.67, active: −0.51 ± 1.57, p = 0.11); MUSIC inactive: 26.4 ± 4.6, active: 23.7 ± 4.7, p = 0.074). There were no significant differences concerning the diameter of the 3rd ventricle between the group of inactive and active MS patients (fig. 1a). Basal BDNF serum levels were within the range of previously published data on healthy controls [15] and did not differ between active and sedentary MS patients (fig. 1b). In line with that, there were no correlations of cognitive tests, ventricle diameter or BDNF levels with any of the physical performance parameters.

Immune Cell Subpopulations in Physically Active and Inactive Patients

Basic parameters of cellular immunity were assessed by flow cytometry. The percentages of circulating monocytes, CD4+ T cells, CD8+ T cells, B cells and NK cells were within the normal range in both groups (as exemplarily depicted for T and B lymphocytes, fig. 2). No differences were observed between inactive and active MS patients. The frequency of Foxp3+ T regulatory cells within the peripheral blood did not differ between active (5.3 ± 1.99% of CD4+ T cells) and inactive (6.1 ± 2.01% of CD4+ T cells) MS patients and did not correlate with physical performance parameters (fig. 2). In line with that we found no differences concerning the percentages of CD4+HLA-G+ T regulatory cells or CD8+ T cells and monocytes that stained positive for the immune-tolerogenic molecule HLA-G (data not shown).

Vitamin D Serum Levels in Physically Active and Inactive MS Patients

The 25-OH-vitamin D serum levels of MS patients were within the normal range (mean 91.02 ± 34.61 nmol/l). However, there was a trend towards higher 25-OH-vitamin D serum levels in active versus inactive MS patients, and 25-OH-vitamin D serum levels were positively correlated with VO2peak as an objective parameter of physical performance (r = 0.4, p < 0.01; fig. 3). 81% of active MS patients reported outdoor activities that may...

Table 1. Basic demographic and clinical characteristics of inactive and active relapsing-remitting MS patients

<table>
<thead>
<tr>
<th></th>
<th>Active patients</th>
<th>Inactive patients</th>
<th>Group difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sport index</td>
<td>3.6 ± 0.6</td>
<td>2.33 ± 0.5</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Age, years</td>
<td>39 ± 8.3</td>
<td>36 ± 8.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>Sex ratio m:f</td>
<td>1:3</td>
<td>1:5</td>
<td>n.s.</td>
</tr>
<tr>
<td>BMI</td>
<td>23.3 ± 2.9</td>
<td>23.5 ± 3.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>EDSS</td>
<td>1.44 ± 1.0</td>
<td>1.21 ± 0.9</td>
<td>n.s.</td>
</tr>
<tr>
<td>Disease duration years</td>
<td>7.8 ± 6.5</td>
<td>6.3 ± 4.7</td>
<td>n.s.</td>
</tr>
<tr>
<td>Relapses in the last 2 years</td>
<td>0.95 ± 0.97</td>
<td>1.60 ± 1.64</td>
<td>n.s.</td>
</tr>
<tr>
<td>Relative VO2peak % of normal</td>
<td>117.3 ± 15.6</td>
<td>94.9 ± 20.1</td>
<td>p = 0.0003</td>
</tr>
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</table>
Fig. 1. Ventricular width and BDNF serum levels do not correlate with physical activity in relapsing-remitting MS patients. Transcranial sonography demonstrated no differences in the 3rd ventricle diameter between active (n = 21) and inactive (n = 21) patients with relapsing-remitting MS (a). BDNF serum levels as assessed by ELISA showed no association with physical activity or parameters of cardiorespiratory fitness as exemplarily depicted for VO$_{2\text{peak}}$ (b).

Fig. 2. Immune cell subpopulations in physically active and inactive relapsing-remitting patients. Percentages of circulating CD4+ and CD8+ T cells, CD19+ B cells and Foxp3+ T regulatory cells (Tregs) do not differ between physically active and inactive MS patients as assessed by flow cytometry.
account for longer hours of sunlight exposure such as Nordic Walking, jogging, cycling or horse riding as their primary and/or secondary leisure activity. There was no statistically significant association of vitamin D serum levels and circulating Foxp3+ T regulatory cells in our study population.

Discussion

The assessment of physical activity via questionnaire is not without pitfalls but considered appropriate in MS patients [6, 16]. The psychometric properties of the Baecke Questionnaire have been confirmed for non-diseased populations [17]. There are no validation studies with MS patients, which might be a drawback. However, the Baecke Questionnaire has shown sensitivity to change in exercise interventions with MS patients [18].

In line with previous reports we found significant correlations between self-reported physical activity and VO_{2peak} as an objective measurement of cardiorespiratory fitness [19] in MS patients, which legitimated our recruitment strategy. Interestingly, compared to our study, others have reported considerably lower absolute VO_{2peak} values in MS patients [19, 20]. Since neurological disability was reported as an independent risk factor for low cardiorespiratory fitness [19], the high VO_{2peak} values in our study might be explained by the exclusion of patients with a restricted walking range, resulting in a very low mean EDSS of 1.35 in our study population. The reason for excluding these patients was the conduction of a performance test (spiroergometry), which could possibly not have been interpreted properly in patients with early fatigability of the lower extremities, and the overall intention to study the effects of physical activity on the immune system during the early, inflammatory phase of the disease.

Cognitive dysfunction is prevalent in individuals with MS and treatment options are very limited. Exercise has consistently been reported to improve cognitive function in the elderly with beneficial effects on information processing speed, learning, memory and executive function [21, 22]. In contrast, little is known on the effects of exercise or physical fitness on cognitive function in MS patients. In a limited number of patients, significant correlations were reported between VO_{2peak} performance in the Paced Auditory Serial Addition Test and functional MRI parameters [23]. In addition VO_{2peak} and PASAT results were found to be positively correlated with MRI surrogate markers of disease such as grey matter volume and white matter integrity [24]. Instead of performing MRI we measured the width of the 3rd ventricle by transcranial sonography, which had previously been described as a good correlate of neurocognitive function in MS patients [13, 25]. We failed to detect an association of cardiorespiratory fitness or self-reported physical activity with performance in cognitive function tests (PASAT, MUSIC) or transcranial sonography in our small sample size, which is most likely due to differences in patient selection (e.g. exclusion of patients with secondary progressive disease). BDNF has been reported to play an important role in neural repair and plasticity. Physical activity may modulate BDNF serum levels and thereby contribute to the beneficial effects of exercise on cognitive functions [15]. Whether basal BDNF serum levels of MS patients are higher [26], lower [27, 28] or equal [7] to controls is a subject of ongoing debate. Nevertheless, it has been suggested that acute exercise induces circulating BDNF in individuals with MS [7]. The fact that circulating BDNF did not differ between physically active and inactive patients with MS nor correlate with cardiorespiratory fitness in our study argues against a long-term induction of BDNF in response to exercise in MS and is in line with a previous report [28]. However, based on our ex vivo analysis, we cannot preclude that physical activity modulates BDNF secretion by immune cells at the site of inflammation.

T regulatory cells play a key role in the maintenance of peripheral immune tolerance and are protective in autoimmune diseases.

![Fig. 3. Vitamin D serum levels and cardiorespiratory fitness in relapsing-remitting MS patients. 25-OH-vitamin D serum levels as assessed by ELISA are positively correlated with VO_{2peak} as a parameter of cardiorespiratory fitness (n = 42).](image)
Vitamin D has recently emerged as a protective factor against the development of MS and its therapeutic value is currently evaluated in randomized controlled trials [36]. In line with previously published findings in healthy individuals [37, 38], we found an association between physical activity and vitamin D levels which may be considered to be secondary to outdoor exercise and sunlight exposure. The finding that vitamin D serum levels correlate with VO$_{2peak}$ most likely reflects the longer hours of sunlight exposure in patients performing outdoor activities and should therefore be considered as a desirable effect of a well-tolerated, supportive treatment in MS. We hope to encourage further studies evaluating the therapeutic effect of physical activity on MS that will eventually lead to the introduction of physical exercise as another mainstay of MS therapy.

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**Disclosure Statement**

The authors declare that they have no conflicts of interest.

**References**


