Physical Exercise Therapy for Autoimmune Neuroinflammation: Application of Knowledge from Animal Models to Patient Care

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Highlights

- Exercise positively impacts autoimmune-related diseases, but in an undefined manner
- Specific exercise guidelines for multiple sclerosis patients are still lacking
- Training can alter disease progression by immunomodulation and/or neuroprotection
- Understanding basic mechanisms of training can contribute to patient treatment
Abstract

Physical exercise (PE) impacts various autoimmune diseases. Accordingly, clinical trials demonstrated the safety of PE in multiple sclerosis (MS) patients and indicated beneficial outcomes. There is also an increasing body of research on the beneficial effects of exercise on experimental autoimmune encephalomyelitis (EAE), the animal model of MS, and various mechanisms underlying these effects were suggested. However, despite the documented favorable impact of PE on our health, we still lack a thorough understanding of its effects on autoimmune neuroinflammation and specific guidelines of PE therapy for MS patients are lacking.

To that end, current findings on the impact of PE on autoimmune neuroinflammation, both in human MS and animal models are reviewed. The concept of personalized PE therapy for autoimmune neuroinflammation is discussed, and future research for providing biological rationale for clinical trials to pave the road for precise PE therapy in MS patients is described.

Key words: Physical exercise; Autoimmunity; Neuroinflammation; Multiple Sclerosis; Experimental Autoimmune Encephalomyelitis
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1. Introduction

Physical exercise (PE) has long been acknowledged as beneficial for health, whereas physical inactivity is one of the most prevalent risk factors for various acquired diseases worldwide, and the fourth leading risk factor for global mortality [1]. Autoimmune diseases are a heterogeneous group of chronic diseases that develop when the immune system fails to differentiate between self from non-self causing an immunologic response damaging its own tissues [2, 3]. Accordingly, in recent decades published findings indicate a beneficial role of PE in various autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis (MS) and others [4-6]. PE appears to improve mobility, increase quality of life, enhance mood and cognitive abilities in patients and reduce the incidence of autoimmune diseases. Furthermore, there is growing evidence linking between PE and the immune system [7-10]. PE reduces the risk of chronic inflammatory diseases, partly owing to the anti-inflammatory effects of exercise [8, 11, 12]. PE leads to elevations in regulatory T cells, decreased immunoglobulin secretion, and produces a shift in the Th1/Th2 balance to a decreased Th1 cell production. Moreover, PE promotes the release of IL-6 from muscles. IL-6 released from muscles functions as a myokine and has been shown to induce an anti-inflammatory response through enhanced IL-10 secretion and diminished release of IL-1β.

Multiple sclerosis (MS) is an immune-mediated disease of the central nervous system (CNS), leading to CNS demyelination and axonal damage [13, 14]. During the course of MS, autoreactive T cells are activated in the peripheral lymphoid organs and migrate across the blood-brain barrier (BBB) to induce inflammatory lesions within the CNS [15]. MS causes chronic irreversible functional impairments. Therefore, there is a requirement for therapeutic strategies that can reduce the deleterious impact of the disease on the quality of life of MS patients. Various clinical trials demonstrated that PE attenuates symptoms, induce various
physiological and functional beneficial effects, and delays progression of disability in MS patients [16-21], while low levels of physical fitness have been suggested as a risk factor for developing MS [22]. Anti-inflammatory effects of PE are also documented in MS patients [23]. These studies prompted additional research of PE for MS and investigation of the beneficial effects and mechanisms of action of PE on animal models of MS, including toxin-induced demyelinating models (e.g. cuprizone and lysolecithin) [24-28] and particularly experimental autoimmune encephalomyelitis (EAE) [29-48]. EAE is an autoimmune demyelinating disease of the CNS and serves as the most common animal model to study MS pathogenesis and potential therapies [49]. The auto-inflammatory process results in demyelination, axonal damage and, subsequently, progressive hind limb paralysis [50, 51].

However, although the favorable effects of PE on general health are well accepted, a thorough understanding of the effects of PE on autoimmune diseases in general and on MS in particular is still lacking. The optimal training protocol and elucidation of the underlying mechanisms whereby PE affects systemic immune responses and/or the CNS remain undefined. Thus, concrete guidelines (indications, amount, intensity and type of exercise) for MS patients are still lacking.

In this review we will summarize the current knowledge of the impact of PE on MS and experimental models of autoimmune neuroinflammation, discuss the key elements involved in the effects PE on EAE, and pinpoint the limitations of studies so far. Thereafter, we will address the general applicability of PE and focus on three main questions regarding PE and autoimmune neuroinflammation: (a) Whether PE ameliorates EAE by modulating the systemic immune system or exerting direct neuroprotective effects on the CNS; (b) Whether different training programs exert similar or distinct effects on the immune and nervous system; and (c) Whether PE modulates autoimmunity selectively or causes general immune
suppression. Finally, we will critically highlight possible future directions for paving the road for clinical recommendations for MS patients.

2. Physical Exercise and Multiple Sclerosis

2.1. Multiple Sclerosis

MS is an immune-mediated chronic disease of the CNS, leading to multifocal-progressive CNS demyelination and axonal damage [13, 14]. MS is the most common neurodegenerative disease in young adults between 20 and 50 years old with a two-to-one ratio between women and men [52]. The risk of developing MS includes a combination of genetic and environmental factors [53, 54]. Clinical manifestations in MS vary in accordance with the spatiotemporal spreading of lesions within the CNS. The CNS tissue damage in MS may lead to disabilities of coordination and balance skills, reduction in muscle strength, sensory and visual disturbances, increasing fatigue, pain, cognitive and affective deficits. The disease outcomes may lead to severe limitations in function of daily life. Based on progression and disease course, MS is divided into different clinical forms, including relapsing-remitting, relapsing-progressive, secondary progressive, and primary progressive MS. At 25 years after diagnosis approximately 50% of patients require permanent use of a wheelchair. The leading theory suggests that MS etiology involves a dysregulation of the peripheral immune system, leading to local CNS inflammation against myelin autoantigens [27].

2.2. Inflammation and Multiple Sclerosis

MS lesions are induced by progressive infiltration of activated leukocytes into the CNS after crossing the BBB. Acute or chronic disruption of the BBB may result in the entry of immune cells into the CNS, followed by the induction of neuroinflammatory processes that are suggested to be crucial for the pathogenesis of MS [55]. These inflammatory processes cause demyelination, neuroaxonal degeneration and gliosis resulting in the disruption of neuronal
signaling and MS related symptoms [56, 57]. Whereas the precise mechanisms of acute inflammatory lesion formation remain unknown, it is suggested that T lymphocytes, which appear early in lesion formation, induce autoimmune reaction against CNS myelin autoantigens. These autoreactive CD4+ T cells produce soluble cytokines that amplify inflammation and neurodegeneration. Macrophages were shown to dominate the CNS infiltrates, as well as CD8+ T cells. Lower numbers of B cells and plasma cells can also be found [58].

The peripheral immune dysregulation during MS closely interacts with immune alterations inside the CNS. Studies suggest that elevation of various cytokines is an essential part of MS pathogenesis. In MS patients increased Th1 pro-inflammatory cytokines have been found, whereas levels of Th2 anti-inflammatory cytokines are decreased. Higher concentrations of peripheral pro-inflammatory cytokines were associated with CNS inflammatory activity and demyelinating processes in the CNS. Innate immune cells, mainly microglia, were also suggested to be involved in demyelinated foci [59]. Their numbers seem to correlate with tissue damage suggesting an important role for innate immune cells. The induction of peripheral inflammatory milieu was suggested to be linked to several lifestyle factors, such as a lack of PE combined with malnutrition [60].

2.3. Therapies for Multiple Sclerosis

Peripheral immune dysregulation is an important target of current therapies for MS. A broad spectrum of immunomodulatory and immunosuppressive drugs to reduce immune cell activity and entry into the CNS have been developed. Drug therapies can control disease progression, but they do not cure the disease. Moreover, current treatments are often associated with significant side effects, such as flu-like symptoms, susceptibility to develop other autoimmune disorders, malignancies or fatal opportunistic infections, and, with some exceptions, their applications are limited to relapsing-remitting MS [61]. Therefore,
multimodal management approaches should be employed to identify, develop and optimize other therapeutic strategies that diminish side effects, including lifestyle modifications to maintain CNS function, such as PE [62].

2.4. Physical Exercise and Inflammation

PE reduces inflammatory events in patients and animal models of inflammatory diseases [8, 9]. Contracting skeletal muscles produce and secrete various anti-inflammatory myokines, such as IL-6, followed by an increase in systemic levels of the anti-inflammatory cytokines such as IL-10 and IL-1RA [63]. Additionally, exercise-induced systemic elevation of hormones, such as cortisol and epinephrine, inhibits the secretion of pro-inflammatory tumor necrosis factor (TNF)-α by monocytes. Even after an acute bout of prolonged exercise, reduced expression of toll-like receptors (TLR) on monocytes can be observed, which results in subsequent inhibition of pro-inflammatory cytokines [8]. Chronic exercise also affects Th1/Th2 balance and increases the number of circulating regulatory T cells [64]. These observations indicate that a well-controlled PE program may also affect the pathogenesis of MS to reduce relapses and inflammatory progression.

2.5. Physical Exercise and MS

PE was suggested as complementary therapeutic method in MS patients [17]. PE is associated with functional recovery of several disease outcomes and quality of life [18, 20, 65]. A large body of evidence suggests that MS patients undergoing supervised exercise programs exhibit: reduced spasticity, improvements of physical fitness, balance and walking cadence, decreased levels of fatigue, and increased daily activities [66-70]. Resistance training in MS patients results in a significant increase in muscle strength [71]. The beneficial effects of PE on muscle strength were demonstrated with different exercise interventions including aquatic fitness, aerobic activity, and treadmill training [67, 72, 73]. Other symptoms, such as fatigue, depression and cognition, can also be improved by exercise [74-76]. Research on the role of
PE in MS demonstrates benefits regardless of disability level secondary to MS [77]. While a higher proportion of MS cases was demonstrated in a group of women reporting lower PE [78], others found PE unrelated to the risk of MS occurrence [79]. Taken together, it is currently well accepted that PE should be recommended in patients with MS.

Despite accumulating recognition of the beneficial effects of exercise in MS, engagement of patients in rehabilitation programs is still low [80]. In addition to the common barriers to engage in exercise, MS patients have other disease-specific perceived hurdles, such as impaired mobility, heat sensitivity, fatigue and pain [81, 82]. Additionally, implementation of PE in patients with primary progressive MS was shown to be significantly lower than in relapsing remitting MS [83]. This decrease in PE likely derives from advanced disease progression. (I suggest the sentence in yellow rather than the following one.) A probable explanation for this is their more advanced state in the course of disease, and the higher likelihood for disability.

Despite accumulating clinical data, little is known about the basic mechanisms whereby PE affects MS pathogenesis. It is unclear how PE affects immune cell activation and regulation of inflammatory signaling pathways, and how it intervenes in the invasion (and eventually transformation) of peripheral immunity into CNS inflammation. Thus, it is unknown whether PE may act as a disease-modifying therapy in MS. Furthermore, in the progressive phase of MS there is a switch in the immune pathogenesis of disease, where systemic autoimmunity declines and there is partial closure of the BBB. Instead, there are slowly expanding demyelinating lesions with low inflammatory activity, and accumulation of meningeal based tertiary lymphoid tissue, that is thought to drive cortical and subpial demyelination, as well as chronic axonal degeneration in distal tracts [84]. Whether PE may affect this CNS-compartmentalized immune process is unknown.
Moreover, the optimal training protocol and the underlying mechanisms whereby PE affects systemic immune responses and/or the CNS in MS remain undefined. Accordingly, concrete guidelines (indications, amount, type, and intensity of exercise) for MS patients are still lacking. Additionally, no specific PE-induced substances were identified as disease-modifying biomarkers in MS. One possibility to explore these issues is to test exercise in preclinical models of MS, which with some limitations thereafter addressed, enables in-depth analysis of the impact of exercise on a single pathological process.

2.6. Animal Models for Multiple Sclerosis

Rodent models have been generated to study the various aspects of human MS. The three major categories of animal models of MS include: (1) viral induced chronic demyelinating disease models, with the leading model of Theiler’s Murine Encephalomyelitis Virus (TMEV) infection; (2) toxin-induced models of demyelination, including the cuprizone model and focal demyelination induced by lysolecithin, and (3) various EAE models.

2.6.1. Virus-induced demyelination

It has been hypothesized that in the presence of a specific genetic background, a viral infection early in life, may result in immune-mediated attack against CNS tissue [85, 86]. To date there is no specific virus that has been identified as a potential cause of MS, although EBV infection has been linked to MS as a critical environmental susceptibility factor [87, 88]. To assess the contribution of viruses in human MS, virus-induced demyelination models can be utilized. Generally, pathological features of virus-induced demyelinating disease are mediated by the immune system and not by direct viral cytopathy, and the clinical presentation is very similar to that observed in chronic progressive MS [89, 90].

TMEV is a mouse enteric pathogen that belongs to the single-stranded RNA picornaviruses. In susceptible mouse strains, demyelinating disease is induced by intracerebral infection with TMEV. Several mechanisms leading to demyelination in the TMEV model were proposed,
such as direct viral cytopathic effects on oligodendrocytes, autoimmune destruction of infected oligodendrocytes, bystander demyelination due to toxic metabolites from activated macrophages, epitope spreading and molecular mimicry [91]. Various combinations of these mechanisms could be responsible for myelin damage throughout the course of the disease. Use of the TMEV-induced model, including its pathology, is relevant for human MS since viral infections are associated with clinical exacerbations of MS. This approach also impacts the autoimmune response triggered by viral infection in the CNS, its chronic nature that lasts for the entire lifespan, and its pathological abnormalities are limited to the CNS.

2.6.2. Toxin- induced demyelination

The two most widely used toxins to induce demyelination in animal models of MS are lysolecithin and cuprizone. Lysolecithin is an activator of phospholipase A2 and cuprizone is a copper chelator. Compared to EAE and virus-induced demyelinating syndrome, toxin-induced demyelination models do not attempt to mimic MS but are used mainly to study the process of focal demyelination and remyelination [92].

Lysolecithin injections into the spinal cord have been described in several mammals, including cat, rabbit, rat and a mouse [93]. Usually, the dorsal or ventrolateral funiculi of the spinal cord at the thoracolumbar level are used as an injection site of lysolecithin. Demyelination is not immune-mediated and is evident even in immune deficient mice. In the acute phase, immediately following the lysolecithin injection, lesion sites are often infiltrated with T-cells, B cells and macrophages. This short-lived infiltration is proposed to have a beneficial role in CNS repair [94]. However, chronic inflammation in lesions is minimal and complete remyelination occurs in 5–6 weeks.

Cuprizone is a toxin that induces myelin damage and features of the model vary depending on the utilized animal species. Addition of cuprizone to the diet in young adult mice results in demyelination in several white matter structures in the brain, including cerebellar
peduncles, corpus callosum, internal capsule, anterior commissure and thalamic white matter [92]. Specific targets are mature oligodendrocytes, which fail to fulfil the extensive metabolic demand and eventually undergo apoptosis. Other cell types in the CNS are less affected. The main reason for metabolic failure is copper deficiency due to the copper chelating properties of cuprizone. While it is considered as a toxic-mediated oligodendrocytopathy, a central role for the brain’s innate immune system (eg. microglia) has been shown in mediating demyelination [95]. After cuprizone is removed from the diet, extensive remyelination is evident within 3–4 weeks. If dietary cuprizone is continued, oligodendrocytes are completely depleted, and subsequent demyelination becomes persistent. The cuprizone model is reproducible and appears to be suitable for therapeutic trials designed to repress demyelination or accelerate remyelination in MS.

2.6.3. Experimental Autoimmune Encephalomyelitis

EAE is the most extensively studied animal model of CNS autoimmune disease [49, 96]. EAE can be induced in a variety of mammal species, including mice, rats, guinea pigs, rabbits, goats, sheep, marmosets and primates. There are two methods to induce EAE: In the classic form, named active EAE, synthetic peptides of myelin proteins, such as myelin oligodendrocyte glycoprotein (MOG), myelin basic protein (MBP) or proteolipid protein (PLP), or a spinal cord homogenate are injected subcutaneously in the form of an emulsion that contains an adjuvant. Within 10-12 days clinical symptoms of paralysis develop. In the adoptive-transfer model, EAE is induced by immune-cell transfer from autoantigen-immunized donors to naive recipients, which develop paralytic disease. Transgenic mice that develop spontaneous EAE have also been reported.

In the active EAE model, immunization with myelin auto-antigens leads to auto-activation and expansion of peripheral antigen-specific T-cells. Following activation, these cells, along with monocytes, cross the BBB, enter the CNS, encounter the specific myelin
antigen and induce local CNS inflammation. The local CNS inflammation also involves local immune cells, such as resident microglia. CNS inflammation results in demyelination, axonal damage and subsequently hind limb paralysis. Myelin protein-specific CD4+ T-cells are generally considered necessary for EAE induction, as adoptive transfer of these cells from immunized donors into naïve recipient mice elicits EAE in 100% of animals [97]. It is generally considered that CD4+ Th1 cells and Th17 cells are the primary mediators in acute EAE development [98]. Additionally, a role for autoimmune MOG specific CD8+ T-cell responses has also been discussed in development of EAE [99].

The extent of EAE relevance to MS is somewhat controversial. In most EAE models, CD4+ T-cells predominate in the perivascular CNS infiltrates. However, MS lesions can be very diverse, from those that show extensive inflammation to others that feature oligodendrogiopathy with minimal inflammation [100]. Moreover, analysis of human MS lesions revealed that the predominant immune cell types are CD8+ T-cells and macrophages with CD4+ T-cells being much less frequent [101, 102]. In addition to inflammation and demyelination, both acute and chronic axonal injuries can be observed in some EAE lesions [103]. The mechanism of axonal damage in EAE remains to be more firmly defined, since no specific effector cell type has been identified that elicits axonal injury, although macrophages have been proposed to play this role [104, 105].

Studies involving EAE have had an important role in identifying and delineating several aspects of MS pathogenesis: inflammation, immune surveillance and immune-mediated tissue injury. Moreover, this experimental model has directly led to the development of medications approved for MS, such as glatiramer acetate, mitoxantrone and natalizumab [106]. Thus, while caution is advised in the applicability of its findings to human MS, the EAE model has been used as the most frequently studied model leading to a major, important expansion of our knowledge about neuroinflammation and CNS autoimmunity.
None of the three main animal models for MS described here can be considered superior; rather, they are best viewed as complementary to one another. Despite their limitations, they are currently the most useful tools to study human demyelinating diseases. However, the rational, application and utilization of each of these models for studying PE effects and mechanisms of action should carefully be considered.

3. Physical Exercise in Animal Models of Multiple Sclerosis

3.1. Exercise Paradigms in Rodents

Different paradigms of PE interventions can be applied to rodents. The main two categories are forced or voluntary exercise training [107]. In forced exercise, animals are subjected to defined programs provided by means of dedicated apparatuses. For endurance training the main devices are motorized treadmills and swimming pools. For strength training a vertical ladder is usually utilized [108]. Voluntary exercise consists of housing animals in cages endowed with a wheel to which they have either free or timed access. The main difference between forced and voluntary exercise relies on the amount of stress caused to animals by forced exercise [109]. Another difference relates to the amount of exercise, since in voluntary exercise the activity is based on the animals’ willingness to run. Another type of life-style intervention is environmental enrichment. In this case rodents are housed in extra-size cages containing toys, running wheels, tunnels, and other devices [110, 111]. Another consideration in designing experimental PE is whether the regimen is preventive (applied prior to disease onset) or therapeutic (applied after disease onset).

The impact of PE has been studied in various animal models of MS, using different PE regimens and protocols (Figure 1). Most of the studies addressing the effects of exercise in experimental MS, have been conducted in EAE and to a lesser extent in cuprizone- and lysolecithin-induced demyelinating models. Currently, none has been performed in TMEV-induced demyelination. Most studies employ preventive regimens, while some utilize
therapeutic regimens. The most common forced exercise training model is treadmill running and swimming, followed by voluntary running wheel. Few studies employ strength training or environmental enrichment.

3.2. Physical Exercise and toxin-induced demyelinating models

Preventive PE has been tested in the cuprizone-induced demyelination model. Two treadmill running protocols, the high-intensity interval training (HIIT) and the low-intensity continuous training (LICT), started 4 weeks before cuprizone feeding, prevented the abnormal neurological movements induced by cuprizone and increased mRNA levels of the neurotrophic factors BDNF, GDNF and NGF in the hippocampus, as well as the numbers of oligodendrocyte and microglia cells [28]. The HIIT program was more effective than the LICT program.

Therapeutic PE interventions were also tested in the cuprizone-induced demyelination model. Forced treadmill running and swimming performed during and after a 12 week-cuprizone feeding significantly improved locomotor activity [24]. This study highlights possible links between PE and modulation of cholesterol/oxysterol homeostasis.

Moreover, voluntary running initiated in parallel with cuprizone feeding improved motor dysfunction associated with toxicant treatment [112]. PE induced early protection against myelin damage and loss of myelin basic protein (MBP) and 2',3'-Cyclic-nucleotide 3'-phosphodiesterase (CNPase), together with reduced microglia activation in the corpus callosum. At later stages, astrogliosis was significantly attenuated as well. The authors proposed that wheel running could exert direct protection on myelin that, in turn, might limit microglia proliferation and activation in the damaged white matter area, with the consequence of a reduced recruitment of new oligodendrocytes and increased myelin content during the late phase of cuprizone feeding.

Voluntary running wheels have beneficial effects in lysolecithin-induced demyelination [25]. Voluntary running wheel training initiated soon after lysolecithin injection
into the spinal cord induced an early shift from a pro-inflammatory to an anti-inflammatory phenotype of microglia/macrophages, which, by removing myelin debris, allowed the proliferation of oligodendrocyte progenitor cells (OPCs). The combination of training and clemastine, an anti-muscarinic drug with OPC pro-differentiating properties, resulted in an additive increase of new oligodendrocytes, suggesting that exercise created a permissive environment for remyelination. Moreover, using proteomics analysis, a later study demonstrated that voluntary wheel running, performed for 4 days post lesion induction, induced upregulation of 86 and downregulation of 85 proteins in the spinal cord and upregulation of 14 and downregulation of 11 proteins in serum. The changes in protein expression correlated with altered pathways of oxidative stress response, metabolism and transmission across chemical synapses [26].

The first paper employing environmental enrichment intervention, initiated one week before lysolecithin induction, reported reduced functional impairment and increased recruitment of subventricular zone progenitor cells into demyelinated lesions possessing oligodendrocyte commitment [113]. The latter effect was also confirmed in an EAE model when enriched environment was initiated on the day of EAE induction [113]. These finding raises the question whether voluntary training itself has disease-modifying effects, or if such effects simply reflect environmental enrichment.

3.3. Physical Exercise and Experimental Autoimmune Encephalomyelitis

Considerable research has examined the impact of PE on the development and progression of EAE, as well as its underlying mechanisms of action. These studies investigated the therapeutic as well as the preventive role of PE, using various EAE models (active and passive-transfer EAE, different strains of rodents), and various training modes (forced swimming, treadmill running, voluntary running wheel and environmental enrichment) (Figure 1).

3.3.1. Therapeutic Physical Exercise Regimens in EAE
An early study utilized the transfer EAE model in Lewis rats and assessed whether severe endurance treadmill running performed before and after EAE induction, affects the development of EAE [39]. Overall, this study showed that PE did not alter the symptoms of EAE.

Later studies examined the therapeutic effect of PE on EAE, focusing mainly on immunomodulatory properties and neurotrophic factors in the brain as underlying putative mechanisms [29, 37, 38, 41-44](Figure 1).

Voluntary running wheel performed following EAE induction has been associated with attenuated disease; however, contrasting results were provided regarding its immunomodulatory action [29, 40, 42, 43]. Three studies have shown that ad libitum access to wheel running was able to decrease immune cell infiltration in the spinal cord of EAE mice [29, 40, 42]. Two of these studies demonstrated that 1 hour per day of free access to a running wheel reduced T cell infiltration and microglia activation in the spinal cord [29]. Benson et al. showed that voluntary running wheel was also able to reduce the abnormal neuronal activity in the spinal cord and pain hypersensitivity in EAE mice [29]. Interestingly, Mifflin et al. demonstrated that voluntary running wheel was effective in attenuating clinical signs and CNS inflammation in males but had little effect on female EAE mice [40]. Conversely, another group did not highlight any alterations in immune cell infiltrates in the CNS of EAE mice following running wheel training initiated on the day of EAE induction, despite the improved disease course [43]. Based on their findings that running wheel training rescued the downregulation in the sensitivity of striatal GABA synapses to the stimulation of cannabinoid CB1 receptors and reduced dendritic spine loss in striatal neurons, the authors argued that therapeutic running wheel exerted a neuroprotective effect [43].

Forced treadmill running as a therapeutic intervention has also been tested and yielded conflicting results. Treadmill running which started at 20 days post EAE induction for 4 weeks
was shown to be protective against memory loss, which has generally been attributed to hippocampal demyelination [37]. Trained EAE mice demonstrated better performance on step-down avoidance tasks which are employed to assess memory ability. Furthermore, a decline in demyelination with concomitant increase in brain derived neurotropic factors was noted in the trained group. Additionally, the level of apoptosis in the hippocampus of the trained mice was shown to be decreased. This was reflected by the decrease in the apoptotic signal (bax) and increase of anti-apoptotic proteins (bcl-2) in the trained EAE group [37]. However, other studies failed to find significant differences in clinical disability following forced treadmill running [114]. Patel et al. could not detect any changes in brain mass, nor in TNFα and BDNF levels, but found a significant increase in NGF in trained EAE brains [114]. Similarly, forced treadmill running, as well as voluntary running wheel, performed during the remission period after disease onset, did not induce significant effects on disease course nor the levels of hippocampal BDNF [38].

The impact of endurance training by treadmill running was compared to that of strength training by climbing ladder [44]. Both modes of training, initiated 2 weeks before EAE induction and for a total of 4 weeks, attenuated disease course and reduced brain levels of pro-inflammatory cytokines. Strength training induced reduction in IL-6, MCP-1 and TNFα production from spleen cells suggesting that different modes of training induce distinct immunomodulatory activities. Additionally, this altered cytokine profile was associated with a decreased expression of adhesion molecules, as well as increased expression of tight junctions such as occludins which decreases BBB permeability and inflammatory immune cells diapedesis [44].

3.3.2. Preventive Physical Exercise Regimens in EAE

The preventive impact of PE on EAE has also been assessed [30, 32, 33, 46, 47]. Forced swimming yielded more consistent data [30, 32, 47]. Forced swim training initiated 4 weeks
before EAE induction and maintained until 10 days post-immunization attenuated EAE severity. Overall, swimming increased BDNF levels and decreased pro-inflammatory cytokines in the CNS, with no change in the number of infiltrating cells [32]. These effects were observed at 14 days post EAE induction, soon after disease onset. In a subsequent study, analysis was performed at 42 days post EAE induction to examine long term effects of the preventive swimming protocol [30]. At that time point preventive swimming significantly reduced the number of infiltrating CD4+ and CD8+ T cells and B cells in the spinal cord and increased regulatory T cells, resulting in an enhanced release of IL-10 [30]. The beneficial effect of preventive high-intensity swimming on EAE severity was confirmed by others [47]. High-intensity swimming increased the number of regulatory T cells in both spinal cord and lymph nodes, reduced the levels of the pro-inflammatory cytokines IFN-γ and IL-17 and increased the levels of anti-inflammatory TGF-β and IL-10 in the spinal cord. Interestingly, preventive high-intensity swimming increased BDNF levels in the brain but not in the blood, suggesting that the neuroprotective effects may be independent of immunomodulation.

However, the value of forced swimming to examine the effects of exercise training may be problematic, as it may cause considerable stress in the animals, which may alter disease expression. Thus, the beneficial effect of preventive PE using a voluntary regimen on EAE was also studied by utilizing environmental enrichment [33, 46]. Mice reared in environmental enrichment conditions before EAE induction exhibited attenuated EAE [33]. The attenuation was associated with a reduction in inflammatory infiltrates and demyelination in the spinal cord, without changes in IL1β levels in spinal cord [33]. Additionally, environmental enrichment preserved glutamate exocytosis from cortical terminals and increased the expression of MUNC-18 and SNAP-25 in the cortex. No effects were observed on cAMP production from cortical synaptosomes. Recently, Xiao et al. demonstrated an immune-modulatory effect of preventive environmental enrichment [46]. Environmental enrichment
significantly alleviated EAE symptoms and inhibited spinal cord inflammation through regulation of Th1 cells. The authors suggested this was mediated by glucocorticoid receptor signaling. Additionally, adoptive transfer of thymocytes from treated mice into donor sedentary mice induced an attenuated disease, highlighting the role of thymic population in environmental enrichment-mediated immunomodulation. Finally, EAE induction in mice lacking the corticosteroid receptor in thymocytes, prevented the environmental enrichment-induced beneficial effect, suggesting the involvement of the pituitary-adrenal axis.

The impact of preventive forced treadmill running on EAE has been studied by several groups. We found that 6 weeks training prior to EAE induction attenuates EAE clinical course by immune-modulation of encephalitogenic T cells [34-36]. We also demonstrated intensity-dependent neuroprotective effects of training on EAE [34, 48]. These studies will be further discussed in the next sections. The effect of forced treadmill running on the cross-sectional areas of limb muscles of EAE rats was examined [45]. Training was performed for two weeks before and 10 days after EAE induction. Training postponed the peak of hindlimb paralysis, but did not prevent muscle fiber atrophy and did not affect the cross-sectional areas of tibialis anterior, extensor digitorum longus and soleus muscles. Another study investigated the preconditioning role of forced treadmill running on the beneficial effects of glatiramer acetate and dimethyl fumarate [31]. It was shown that training before disease induction followed by drug administration at the acute phase of the disease reinforced the beneficial effects of these drugs. However, training alone did not ameliorate the severity nor the histopathological hallmarks of the disease, suggesting that training regimens can induce variable responses in EAE.

In summary, many studies indicate beneficial effects of PE on the clinical symptoms associated with EAE. These effects include preservation of axons and motor neurons, reduction in immune-cell infiltration, reduction in demyelination and axonal injury, increased synaptic
plasticity, upregulation of neurotrophins and induction of hippocampal neurogenesis, inhibition of pro-inflammatory cytokine production, antioxidant effects and restoration of tight junction expression in the CNS. It appears that the beneficial effect of PE on EAE severity depends more on exercise duration, rather than voluntary versus forced protocols. Apparently, PE over 3 weeks is necessary to promote adaptation to stress responses and neuroprotection. PE following EAE induction probably minimizes potential beneficial effects, as training occurs only for a short period after disease induction.

4. Does One Size Fit all?

4.1. Systemic Immune-modulatory versus Direct Neuroprotective Effects of PE in EAE

A key issue is whether the suggested therapeutic effects of PE on autoimmune neuroinflammation are mediated via modulation of the systemic immune system and/or by inducing direct CNS neuroprotective effects. Both induction of immune responses and modulation of T-cell reactivity to brain-derived antigens take place in peripheral lymphoid organs [115, 116]. Therefore, it can be hypothesized that PE inhibits the generation of effector T cells in the peripheral lymphoid system upon immunization with an autoantigen. On the other hand, health benefits of PE may extend to the CNS as well. PE is associated with enhanced neurogenesis and neuroplasticity in various brain pathological conditions, as well as an increased expression of various neurotrophic factors [110, 117, 118]. Recent studies suggest that exercise can modify CNS inflammation and induce an anti-neuroinflammatory phenotype similar to its effects on the peripheral immune system [119].

Most studies on the effects of PE on EAE used active EAE models (Figure 1). With this model, systemic immune and central CNS processes occur in the same immunized mouse. Therefore, effects of PE mediated via the systemic immune system to reduce encephalitogenicity cannot be distinguished from direct protective effects on the CNS. To address this issue, we employed a unique experimental set-up using the PLP peptide-induced
transfer EAE model in SJL mice that enabled us to distinguish between the potential systemic and central effects of PE on EAE [34-36, 48]. Additionally, in this model, injected T cells from donor mice attack the CNS of recipient mice, leading to a relapsing remitting disease, thus mimicking the common clinical course of human MS.

4.1.1. Systemic Immune-modulatory Effects of PE in EAE

We first investigated the effects of forced treadmill running on the systemic immune system. Donor mice were trained and the encephalitogenicity of their lymph node- T cells following PLP immunization was examined in vivo and in vitro. We first showed that both moderate-intensity continuous training (MICT) and high-intensity training (HICT) inhibit T cell activation and proliferation in response to the PLP autoantigen in vitro [34, 35]. Transfer of PLP- reactive T cells obtained from trained donor mice resulted in reduced immune cell infiltration to the CNS, reduced demyelination and axonal pathology and milder clinical course in recipient mice. These results indicate that exercise training attenuates EAE by modulating the systemic immune system. We then showed that high-intensity continuous training induces a superior immuno-modulatory effect than moderate-intensity training [35]. We further demonstrated that the inhibitory effects of both HICT and high-intensity interval training (HIIT) on the encephalitogenicity of autoreactive T cells was exerted by distinct immune-modulatory mechanisms [36]: HICT induced a general reduction in autoreactive T cell proliferation and in macrophage fraction but did not affect T-helper cell polarization. In contrast, HIIT did not inhibit T-cell proliferation nor macrophage fraction, but rather induced specific inhibition of polarization into autoreactive Th1 and Th17 cells. In PLP-stimulated cultures of T cells obtained from the HICT group, cytokine secretion reached similar levels as in the sedentary group. Strikingly, in the HIIT group there were marked decreases in Th1 and Th17 type cytokines and to a lesser extent in Th2 cytokines (only in IL10, but not in IL4). Thus, there was a specific inhibitory effect of HIIT on polarization into Th1 and Th17 cell
populations. HIIT also induced marked reduction in IL6, whereas HICT did not affect IL6 levels. This finding suggests that the inhibitory effect of HIIT on IL6 secretion is responsible for the decrease in Th1 and Th17 populations. Additionally, similar fractions of CD4+, CD25+, FoxP3+ regulatory T cells were detected in PLP-reactive T cells obtained from HIIT, HICT and sedentary mice [36]. Distinct immune-modulatory effects of different modes of training were also shown when forced treadmill running was compared to strength training [44].

4.1.2. Direct Neuroprotective Effects of PE in EAE

We also investigated the direct effects of forced treadmill running on neuroprotection and the development of autoimmune neuroinflammation by employing the transfer EAE model, whereby we administered encephalitogenic T cells into trained and sedentary mice. Initially, we found that moderate-intensity training did not result in a direct protective effect on the CNS from encephalitogenic T cells [34]. This prompted us to investigate whether high-intensity training, in addition to its systemic immune-modulatory effect, will also induce a protective effect directly on the CNS against autoimmune neuroinflammation, as well as potential mechanisms whereby training may induce direct neuroprotection [48]. High-intensity training indeed provided direct protection to the CNS from encephalitogenic T cells, resulting in attenuated disease progression, and reduction in inflammation-driven demyelination and axonal loss. Training induced a substantial decrease in the number of iNOS+ microglia in EAE mice but did not affect the number of Arg-1+ microglia. These findings suggest that training reduces the neurotoxic profile of microglia, rather than inducing their shift to an M2 phenotype. Additionally, our results ex vivo suggested that training modulates microglia phenotype following EAE induction to attenuate neuroinflammation. A key finding was that training resulted in a reduction in PLP-mediated secretion of the pro-inflammatory cytokine IL-6 and the MCP-1 chemokine and ROS formation by microglia. Thus, the observed reduction in immune cell infiltrates in the CNS of the trained group compared to the sedentary group at the
acute phase of EAE can be attributed to reduced release of chemotactic factors (such as MCP-1) by microglia in the trained group, which is consistent with the idea that training reduces microglial-driven pathogenesis of neuroinflammation.

Taken together, the neuroprotective effect of HICT appears to be mediated, at least partly, by modulating the CNS innate immune system and reducing the microglial neurotoxic and pro-inflammatory response to T-cell mediated autoimmune neuroinflammation. In our experimental paradigm, training did not prevent the formation of encephalitogenic T cells, which were obtained from donor mice, but rather affected brain innate immune cells in trained recipient mice, and prevented neuroinflammation and tissue destruction. Further studies are required to examine other mechanisms by which high-intensity training induces inhibition of CNS autoimmunity.

4.2. Significance of Training Intensity

Previous studies on the effects of PE on EAE yielded variable results (Figure 1). Clinical outcomes ranged from worsening of symptoms, no effect on clinical severity, to disease attenuation, as indicated by delayed onset and peak of disease and even overall attenuation of the clinical course. The inconsistencies in these studies likely derived from variations in training modes (running, swimming) and protocols (intensity, speed, volume, duration) that were employed. The variations in training programs between studies make them difficult to compare and raise the question of whether program parameters affect training outcomes.

Indeed, studies indicate that training intensity plays a major role in PE effects. Numerous studies have confirmed the beneficial effects of PE on fitness and reducing cardiovascular disease risk factors in healthy adults, with higher intensities producing a greater increase in fitness [120, 121]. Moreover, PE of various intensities can differentially affect the balance of T cells and pro- versus anti-inflammatory cytokines in mice [122-126]. It was shown
that regular intense PE provides peripheral anti-inflammatory effects in mouse models of systemic inflammation [122, 123]. Additionally, in the cuprizone-induced demyelination model it was shown that high-intensity interval treadmill running was more effective than low intensity training in inducing upregulation of neurotrophins in the CNS [28].

Therefore, to provide standardization of the association between PE intensity and its effects on EAE, we developed three controlled treadmill running protocols: moderate-intensity continuous training (MICT), high-intensity continuous training (HICT) and high-intensity interval training (HIIT) [34-36, 48] (Table 1). The MICT and HICT protocols were distance matched, but differed in their intensity level, and therefore were comparable in terms of training volume (i.e. work output). Moreover, performance tests were employed to assess the effectiveness of the training protocols. The three training protocols improved physical performance, but the high-intensity-training programs achieved superior performance, as expected with training at higher intensity.

Our studies indicate that different training paradigms exert distinct immune-modulatory and neuroprotective effects on EAE (Table 2). Three training paradigms (MICT, HICT and HIIT) induced peripheral-systemic immune-modulatory effects by reducing the encephalitogenicity of autoreactive T cells, and attenuated transfer EAE [34-36]. However, when the effects of MICT and HICT on the systemic immune system were compared using the transfer EAE model, we found an intensity-dependent decrease in the proliferative response of PLP-reactive T cells [35]. Positive effects of high-intensity, but not moderate-intensity swimming was recently reported in myelin oligodendrocyte glycoprotein-induced EAE [47]. In contrast, we found that both training intensities attenuated autoimmunity in an intensity-dependent manner. We also compared the clinical and immunologic effects of HIIT and HICT on development of systemic autoimmune response that causes EAE [36]. The beneficial effect of HIIT on clinical course of EAE was less effective than that of HICT.
Thus, HICT induced superior benefits in preventing autoimmunity in EAE and provided a superior clinical outcome compared to MICT and HIIT [34-36], demonstrating that training intensity is an important factor in peripheral immunomodulation in EAE. These results underscore the importance of identifying the mechanism(s) by which training intensity impacts the systemic development of autoimmune neuroinflammation.

Furthermore, recent research highlights a favorable outcome for extensive exercise training in the treatment of various neurodegenerative diseases, such as Parkinson's Disease [127, 128] and Alzheimer Disease [129, 130], as well as in other brain diseases such as stroke [131], mood and anxiety disorders [132]. These studies suggest neuroprotective effects of PE. In our work, we utilized the PLP-transfer EAE model and showed that performing high- but not moderate-intensity training in recipient mice protected the CNS from the deleterious effects of encephalitogenic T cells, resulting in milder tissue pathology and clinical symptoms of EAE [34, 48]. Our study provided the first demonstration of direct neuroprotective effect of training on autoimmune neuroinflammation and highlights the critical role of training intensity in this process.

Taken together, our studies show that while moderate- intensity training modulates the peripheral immune system responses to the myelin antigen to attenuate EAE, it does not protect the CNS from encephalitogenic T cells and does not alter the progression of EAE. However, high-intensity training induces both superior systemic immunomodulation and direct protective effects on the CNS (Table 2). Hence, training intensity is paramount for inducing direct neuroprotection against autoimmune neuroinflammation. Nevertheless, although favorable effects of exercise on brain health are well accepted, a thorough understanding of the neuroprotective effects of exercise on autoimmune neuroinflammatory diseases is still lacking.

4.3. Safety of High-Intensity Training
While some show an anti-inflammatory effect of PE and suggest that PE improves the ability of the immune system to respond to deleterious stimuli [10, 122, 123, 133], others suggest complex effects of PE on the systemic immune system [8, 17, 134]. Variable outcomes may derive from different protocols of PE that can differentially affect the immune system [9, 122-126]. Accordingly, it was shown that PE of various intensities can differentially affect the balance of T cells and pro- versus anti-inflammatory cytokines in mice [125, 126]. Importantly, while moderate PE can ameliorate chronic neuroinflammation and its related pathologies and enhance antigen-specific immune response, intense PE may cause transient immune suppression, leading to an increased susceptibility to infection [8, 23, 125, 135-139]. Thus, a major unanswered question is whether PE modulates autoimmunity selectively or causes general effects on the immune system.

PE inhibited the proliferation of T cells in response to the PLP autoantigen in an intensity-dependent manner [35]. However, an important issue in our experimental set-up was whether the T cells of trained mice that exhibited an attenuated response to the PLP autoantigen keep their ability to respond to other non-specific stimuli. Several lines of evidence in our study demonstrate that PE modulates PLP-autoimmunity rather than inducing general immune suppression [34-36]. First, T cells from MICT-, HICT-, HIIT- and sedentary-, PLP–immunized mice proliferated similarly following concanavalin (Con)A stimulation in vitro. Also, carboxyfluorescein succinimidyl ester (CFSE) assay indicated that comparable fractions of cells entered the cell cycle when stimulated with ConA. These findings demonstrate that while PE inhibits the immune responses to the myelin autoantigen, it does not decrease the maximal capacity of the immune response to a non-specific mitogen. This important finding is in accordance with a suggested model of the "bio-regulatory effect of PE" [140], wherein PE reduces or prevents any excessive effects of selected inflammatory mediators in certain conditions, but still allows immune defenses in other conditions. Additionally, no differences
were measured in the secretion of IL2 cytokine by PLP-stimulated HIIT, HICT and sedentary-
derived T cells, providing another indication of preserved activation potential of T cells [36].
However, HICT induced significant inhibition of T cell proliferation in response to ovalbumin
immunization [35], indicating that the inhibitory effect of high-intensity PE on T cells is not
selective to an autoantigen, but is also observed in response to a non-autoantigen. One
potential consequence of high-intensity training may be a limitation in effective immunization
to deleterious immune challenges. Our observation that HICT does not increase susceptibility
of mice to E. coli-induced acute peritonitis suggests, that the innate immune response of
trained mice is not compromised [35]. However, since we investigated selected immunogenic
stimuli to test the effects of high-intensity training on the systemic immune system, this does
not allow for generalized conclusions on global adaptive and innate immune responses.

Moreover, it is well established that chronic stress results in immune-suppression and
amelioration of symptoms associated with MS/EAE [141]. Acute activation of the sympathetic
nervous system and the hypothalamic-pituitary-adrenal (HPA) axis is a necessary feature of
PE-induced stress [142, 143]. Stimulation of these systems may potentially affect some aspects
of the immune response [144]. Thus, it could be argued that stress and immunosuppression
associated with PE, rather than other adaptations, may mediate some of the positive clinical
effects of PE on EAE. At present the extent to which these factors impacted on EAE
progression cannot be quantitatively assessed. However, the finding that T-cell proliferation in
response to the non-specific mitogen ConA was similar in trained and sedentary mice speaks
against the idea of general immunosuppression as a mediator of beneficial effects of exercise
on EAE progression [34-36].

Importantly, our findings demonstrate that in healthy mice training reduces the number
of microglial cells, without affecting their ability to produce immune mediators, nor affecting
their M1/M2 phenotype. Furthermore, training does not induce a general suppressive effect on
microglia derived from mice prior to onset of EAE. Rather, training results in modulation of microglial neurotoxic phenotype, as indicated by reducing ROS, IL-6 cytokine and MCP-1 chemokine production in response to PLP and LPS stimulation. The lack of a general suppressive effect of training on microglial function is important in enabling the still activated, training-derived microglia to participate in their homeostatic and regenerative roles.

Taken together, these findings underscore the need to identify the optimal training protocol to achieve maximal immune-modulation against autoimmune neuroinflammation, without compromising the response to pathogenic challenges and increasing risk of infection.

4.4. Mechanisms of PE

An important facet of research on PE and autoimmune diseases is the identification of mechanisms of action. Understanding how PE exerts beneficial effects in terms of preventing or delaying/treating autoimmune diseases is paramount. Such understanding may prove useful in treating patients who are so severely incapacitated that PE is precluded in their condition. For example, recently it was demonstrated that HICT delayed the onset and decreased the burden of disease in a mouse model of transfer EAE [48]. In healthy mice, HICT led to a decrease in the number of resident microglia without affecting their profile. Isolated microglia from HICT mice after transfer of encephalitogenic T-cells exhibited reduced ROS formation and released less IL-6 and monocyte chemoattractant protein (MCP) in response to PLP-stimulation [48]. This likely derived from a training-mediated upregulation of antioxidant defenses in specific cell populations of the brain [145]. The observation that administration of a mitochondria-targeted antioxidant mimicked the effect of training disease progression in EAE-sedentary mice [146] supports the idea that an enhanced antioxidant capacity is an important mechanism whereby PE has beneficial effects on disease progression. Such findings illustrate how knowledge of exercise-dependent mechanisms can contribute to potential
treatment of incapacitated patients. However, exercise affects various metabolic pathways, and it is likely that other adaptations will also positively impact disease progression.

5. Summary

In this review we provide a summary of existing research on the effects of PE on autoimmune neuroinflammation. Accumulating evidence supports integration of PE programs in the management of MS patients. PE has consistently been shown to be safe and beneficial for improving motor and cognitive function, as well as patients’ quality of life. Thus, MS patients should include PE regimens as part of their daily life routines. As specific guidelines for PE in MS patients are currently lacking, several recommendations, based on biological rationale and results from animal studies, may be considered.

Studies performed in different MS models suggest that PE exerts a modulating effect on the development and/or progression of the disease. PE modifies the pathogenesis of disease mainly due to modulation of encephalitogenic T cell responses, though direct neuroprotective mechanisms mediated by PE can also be involved. Research in animal models indicates that the effects of PE depend on several factors, particularly the intensity and the training paradigm. First, studies demonstrate intensity-dependent immunomodulatory effects of PE on attenuation of EAE. These findings may have clinical relevance, where PE-mediated immunomodulation could prove useful in restraining the severity of repeated CNS attacks by systemic encephalitogenic lymphocytes in relapsing-remitting MS. Second, different training paradigms affect the immune and the nervous system as follows: continuous training attenuates EAE by decreasing T cell proliferation, and interval training by decreasing T cell polarization into deleterious Th1 and Th17 cells. These findings may also be applicable to other autoimmune diseases. In addition to its superior immunomodulatory effects, high-intensity training induces a direct neuroprotective effect in EAE, whereas direct neuroprotection is not observed following moderate-intensity training. As CNS microglia are key players in chronic
neuroinflammation, they represent an important therapeutic target, and their modulation by high-intensity PE to reduce their neurotoxic and pro-inflammatory properties may be translated clinically. Studies in toxin-induced demyelination support the notion that exercise exerts microglia-targeted effects independent of systemic immunomodulation. These findings may also apply to other neurodegenerative diseases driven by microglial neurotoxicity, such as Alzheimer’s’ and Parkinson’s’ diseases.

Various experimental designs illustrate the preventive role of PE in the development of EAE. This may be relevant for relapsing-remitting MS patients, in whom intense PE during remissions may have protective effects against development of further relapses. Thus, an active lifestyle and regular PE can modulate both the systemic and brain immune cells to optimally respond to a deleterious autoimmune challenge. Importantly, to maintain the therapeutic effect of PE, it may be necessary to constantly adjust the training program to the improvement in physical fitness, by gradually increasing its intensity.

These data, translated to clinical practice, further support the idea of personalized PE therapy for autoimmune neuroinflammation. Nevertheless, there are still some clinically relevant and interconnected questions on how to tailor a personalized optimal training and determine the PE parameters that induces the desired immunomodulatory and neuroprotective effects. Defining the optimal training programs to attenuate EAE will advance our understanding of the cellular and molecular mechanisms underlying the beneficial effects of PE.

6. Future directions

The existing research on PE in EAE is promising yet also entails limitations. The various applied models of EAE (active vs. passive models), and heterogeneity of training paradigms (forced vs. voluntary, various intensities and durations) and experimental designs (i.e. post- EAE vs. pre-post EAE combined) obfuscate conclusions. However, the current
knowledge on PE effects supports a systematic, long-term investment in this line of research. Further studies are required to elucidate the cellular and molecular mechanisms behind PE-mediated effects on autoimmune neuroinflammation. Studies should focus on potential factors that mediate the distinct immunomodulatory and neuroprotective effects of different PE paradigms on autoimmune neuroinflammation. The utilization of the transfer EAE model may reveal novel immunomodulatory and neuroprotective mechanisms of PE in EAE. While this approach is relevant mainly to relapsing MS, there is little information on how PE affects neuroinflammation in progressive MS, and no studies have been performed in clinically relevant models of chronic-progressive MS. Systematic examination of various training protocols and their differential effects on systemic autoimmunity and neuroprotection in EAE is still limited. In-depth understanding of the cellular and molecular mechanisms underlying the beneficial effects of exercise training on EAE and elucidating the training parameters that induce the optimal immunomodulation and/or neuroprotection are essential for designing effective clinical treatments in MS patients and other patients with autoimmune diseases.

7. Conclusions

A large volume of evidence supports a beneficial effect of PE in autoimmune neuroinflammation. Understanding the therapeutic potential of PE on autoimmune neuroinflammation in human and experimental MS is gaining increasing interest. Despite the apparent benefits of PE in MS and rodent MS models, engagement of MS patients to perform PE is significantly less than that of the healthy population. The lack of standardization in training protocols in human studies, as well as mechanistic understanding of how the immune and nervous system are affected by PE, limit a comprehensive analysis of the effects of PE in MS subjects and hampers efforts to motivate patients to exercise. The available evidence is still inconclusive regarding when exercise should be initiated in relation to relapses, how much is required, and what intensity or paradigm will produce the greatest benefit. The continued
investigation of PE and EAE will provide further evidence and conclusions regarding the basic science of PE. Defining the optimal training programs will enable elucidation of the cellular and molecular mechanisms underlying the beneficial effects of PE on EAE. Additionally, defining the optimal training programs will enable profiling of PE-induced serum-specific substances that can be used as biomarkers that may indicate the achievement of a disease-modifying effect by PE. These findings will provide a basic biological rationale that can be further translated to clinical trials and lay the basis for defining detailed clinical exercise recommendations (that are currently lacking) for MS patients. Translation of training programs from rodents to human patients may be challenging, yet possible.

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References


[34] O. Einstein, N. Fainstein, O. Touloumi, R. Lagoudaki, E. Hanya, N. Grigoriadis, A. Katz and T. Ben-Hur, Exercise training attenuates experimental autoimmune...
encephalomyelitis by peripheral immunomodulation rather than direct neuroprotection, Exp Neurol 299 (2018) 56-64.


[47] Y. Xie, Z. Li, Y. Wang, X. Xue, W. Ma, Y. Zhang and J. Wang, Effects of moderate- versus high- intensity swimming training on inflammatory and CD4(+) T cell subset


D. Sun, J.N. Whitaker, Z. Huang, D. Liu, C. Coleclough, H. Wekerle and C.S. Raine, Myelin antigen-specific CD8+ T cells are encephalitogenic and produce severe disease in C57BL/6 mice, J Immunol 166 (2001) 7579-87.


**Figure Legend:**

**Figure 1: Experimental Physical Exercise in Multiple Sclerosis Animal Models**

Summary of the experimental designs of studies to investigate preventive and therapeutic PE regimens in MS animal models, using different PE modes (left panels). Studies yielded variable results and suggested various mechanism of actions of PE (right panels). Numbers in circles indicate the number of published papers [24-48].
### Table 1: Training programs [34-36, 48]

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<th>1&lt;sup&gt;st&lt;/sup&gt; week</th>
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<td>Speed per session</td>
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|       | Duration per session | 10 min              | 20 min              | 30 min              | 23 min              |                     |
|       | Speed per session    | 23 cm/sec           |                     | 28 cm/sec           | 30 cm/sec           |                     |
|       | Sessions per week    | 5 d/w               |                     | 3 d/w               |                     |                     |

|       | Duration per session | 10 min              | 20 min              | 30 min              | 34 cm/sec           | 36 cm/sec           |
|       | Speed per session    | 23 cm/sec           |                     |                     | 1 min               | 2 min               |
|       | Sessions per week    | 5 d/w               |                     |                     | 5                    | 7                   |

Min - minutes, cm/sec – centimetres per second, d/w - days per week
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<td>HICT</td>
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