OPEN ACCESS

UNIVERSITY OF THE WEST of SCOTLAND
UWS

UWS Academic Portal

Effects of exercise training on multiple sclerosis biomarkers of central nervous system and disease status

Negaresh, R.; Motl, R.W.; Zimmer, P.; Mokhtarzade, M.; Baker, J.S.

Published in:
European Journal of Neurology

DOI:
10.1111/ene.13929

Published: 31/05/2019

Document Version
Peer reviewed version

Link to publication on the UWS Academic Portal

Citation for published version (APA):
Negaresh, R., Motl, R. W., Zimmer, P., Mokhtarzade, M., & Baker, J. S. (2019). Effects of exercise training on multiple sclerosis biomarkers of central nervous system and disease status: a systematic review of intervention studies. *European Journal of Neurology*, *26*(5), 711-721. https://doi.org/10.1111/ene.13929

General rights
Copyright and moral rights for the publications made accessible in the UWS Academic Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
If you believe that this document breaches copyright please contact pure@uws.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 04 Oct 2022

"This is the peer reviewed version of the following article: Negaresh, R., Motl, R. W., Zimmer, P., Mokhtarzade, M., & Baker, J. S. (2019). Exercise training effects on multiple sclerosis biomarkers of central nervous system and disease status: a systematic review of intervention studies. *European Journal of Neurology*. Vol. X, pp.xx, which has been published in final form at https://doi.org/10.1111/ene.13929. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions."

https://authorservices.wiley.com/author-resources/Journal-Authors/licensing/self-archiving.html

Info to be completed on publication.

Exercise Training Effects on Multiple Sclerosis Biomarkers of Central Nervous System and Disease Status: A Systematic Review of Intervention Studies

Raoof Negaresh[1], Robert W Motl[2], Philipp Zimmer[3,4], Motahare Mokhtarzade[1], Julien S Baker[5]

1. Department of Physical Education & Sport Sciences, Faculty of Humanities, Tarbiat Modares University, Tehran, Iran
2. Department of Physical Therapy, University of Alabama at Birmingham, Birmingham, AL, USA
3. Department for Molecular and Cellular Sports Medicine, German Sport University Cologne, Cologne, Germany
4. Division of Physical Activity, Prevention and Cancer, German Cancer Research Center, Heidelberg, Germany
5. Applied Physiology Research Laboratory, School of Health and Life Sciences, University of the West of Scotland, Lanarkshire Campus, Scotland, UK

Corresponding author: Raoof Negaresh, Department of Exercise physiology, Tarbiat Modares University, Tehran, Iran,

Phone: +989164262029,

E-mail: Raoof.negaresh@modares.ac.ir

Running head: Exercise and multiple sclerosis biomarkers

Conflicts of interest: The authors revealed no conflicted of interest.

Finding: This study not received any funding.

Exercise Training Effects on Multiple Sclerosis Biomarkers of Central Nervous System and Disease Status: A Systematic Review of Intervention Studies

ABSTRACT

BACKGROUND: Multiple sclerosis is a demyelinating and neurodegenerative disease of the central nervous system that can be tracked through biomarkers of disease status. We investigated the effects of exercise on MS biomarkers associated with central nervous system status including imaging, blood-brain barrier function and neurotrophic factors.

METHODS: We conducted open-dated searches of Scopus, Medline, EMBASE and the Cochrane Library. We included studies written in English describing interventions of exercise that measured one or more of the biomarkers associated with multiple sclerosis published until October 2018.

RESULTS: We located a total of 3012 citations through searches in electronic databases. Of these, 16 studies were eligible for review; six studies focused on magnetic resonance imaging markers, nine studies focused on neurotrophic factors, and three studies focused on blood-brain barrier function markers. Of note, two studies included both neurotrophic factor and blood-brain barrier function markers and are therefore included across categories of biomarkers in this review. The existing evidence from magnetic resonance imaging studies confirmed that exercise training can improve central nervous system integrity and function. There is evidence of a positive effect of exercise training on modulation of blood-brain barrier permeability markers and brain-derived neurotrophic factor.

CONCLUSIONS: Exercise successfully improves magnetic resonance imaging outcomes and peripheral biomarkers (i.e., brain-derived neurotrophic factor) in people with multiple sclerosis.

This suggests that exercise can be recommended as an adjuvant therapy for multiple sclerosis treatment. This conclusion is tempered by some methodological limitations including small sample sizes and high drop-out rates in the reviewed studies.
Key Words: Multiple sclerosis, Biomarkers, MRI, Neurotrophic factor, Blood-brain barriers

Introduction

Multiple sclerosis (MS) is a common neurological disease [1] of the central nervous system (CNS) that occurs when lymphocytes cross the blood–brain barrier (BBB) and initiate axonal demyelinating processes [2]. MS is further characterized by the presence of plaques in the brain that are detectable through magnetic resonance imaging (MRI), disturbances in neurotrophic factors, BBB dysfunction and accelerated whole-brain atrophy [3]. These CNS indictors, including MRI outcomes (i.e., lesion status and brain connectivity), neurotrophins, immune function and BBB function markers, are often considered biomarkers of disease status, and are typically included in experimental trials and during the clinical monitoring of MS progression [4 5]. Such biomarkers may further become mechanistic targets of therapeutic approaches in the management of MS.

There are multiple systematic reviews and meta-analyses that support exercise training for symptomatic management, and perhaps disease-modification, in MS [6 7]. Regarding exercise training and MS biomarkers, we located a single systematic review that included both animals and humans [8], and the study only included 2 studies that assessed neurotrophic factors [9 10]. We are unaware of other reviews regarding exercise training effects on biomarkers of

disease status in MS, yet such a review is important for the further understanding of exercise training effects on biomarkers of disease status and progression. This may identify plausible mechanisms as targets of exercise training in MS, and such an understanding of biological mechanisms has been identified as a rate limiting step in exercise promotion among persons with MS via healthcare providers [11].

The current study involved a systematic literature review and examined the possible effects of exercise training on MS biomarkers of CNS integrity and disease status. This was necessary as much of the literature suffers from small sample sizes with low power for detecting statistically significant effects, and the studies have further included various disease biomarkers with a variety of intervention protocols and this needed to be addressed using a systematic review. The presence of heterogeneity among participants (sex, age etc.), types of the disease (i.e., RRMS), and the interventions (intensity, duration etc.) suggested against a meta-analysis based on the Cochrane Library Guidelines [12]. Accordingly, we performed a systematic review of interventional studies that investigated the effects of exercise training on validated and established pathophysiological biomarkers in people with MS (pwMS).

Methods

The current study reviewed research that investigated the effects of exercise training on the CNS and disease status biomarkers of MS. The study was undertaken using the guidelines and principles outlined by the PRISMA statement and checklist [13]. We did not register the review using PROSPERO.

Search strategy

This systematic review was conducted based on a literature search of 4 electronic databases, including Scopus, Medline, EMBASE and the Cochrane Library. The current review focused on studies that investigated the effect of exercise training on MS biomarkers including imaging, BBB and neurotrophic markers (supplemental methodology table 1).

Methodological quality

We described the methodological quality of eligible studies using the 11-item Physiotherapy Evidence Database (PEDro) scale (see supplemental methodology for more information).

Results

Results of searches

We located a total of 3012 citations through searches in four electronic databases (supplemental methodology table 1). Of these, 16 studies were eligible for review after examining inclusion and exclusion criteria. However, one of the eligible studies was included via backwards citation or forward searching (See the PRISMA flow-chart in Figure 1).

Methodology characteristics of included studies

The methodology characteristics of the included studies are represented in supplemental files. Methodological quality ranged between 3 and 9 on a 10-point scale, and the mean PEDro quality score was 6.4±1.4. This indicated fair methodological quality (Table 1). Ten studies (62.5%) reflected high methodological quality (score ≥7).

Results of reviewed studies

Six studies focused on the imaging markers of CNS in pwMS (Table 3). Reviewed studies focused on brain structure, connectivity and viscoelastic properties. Overall, results emphasized on positive effect of exercise. Three studies focused on BBB function markers in pwMS (Table 3). Studies used peripheral circulation markers including matrix metallopeptidase (MMP)-9 [14 15], MMP-2 [15], s100 calcium-binding protein B (s100b) and neuron-specific enolase (NSE) [16]. Results of BBB markers studies revealed a positive effect of exercise, particularly where s100b investigated. Nine studies focused on neurotrophic factors (Table 3) including BDNF [9 10 15-20], nerve growth factor (NGF) [10 16 19 20], neurotrophin (NT)-4 [21] (1 studies), platelet-derived growth factor (PDGF) and ciliary neurotrophic factor (CNTF) [16] in persons with MS. Among 8 studies that investigated the effects of exercise training on BDNF, 4 studies [16-18 20] reported a significant increase (see supplemental methodology for more information).

Discussion

We performed a systematic review of intervention studies investigating the influence of exercise training on MS biomarkers related to the CNS – such an analysis is important for discovering

possible mechanisms of exercise training effects on disease modification and informing clinical practice for promotion of exercise behavior [11].

Imaging markers: MRI scanning is the first line of diagnosis and monitoring of disease status and progression in MS. Imaging markers studies, with the exception of one study [22], considered ≥12 weeks of exercise training as an intervention. It is reasonable to speculate that improvement in brain structure and function needed exposure and stimulation by longer duration interventions when compared to blood biomarkers. The longest period of exercise intervention was observed by an MRI study [23], which was a conducted supervised progressive resistance training program, performed twice weekly for 24 weeks, and comprised of community-based self-guided training without supervision for 24 weeks. In this study [23], exercise training conditions demonstrated a slower progression of lesion load compared to previous evidence [24]; however, no significant differences were noted pre- to post-test between groups. Moreover, brain volume showed a trend, but was not significant, towards a reduced rate of brain atrophy during supervised exercise training. Our recent systematic review [25] concluded that exercise training (i.e., resistance training) can reduce pro-inflammatory cytokine levels which can lead to reduced brain atrophy and lesion development. Since physical activity needs to repeat co-activation of the brain areas [26-28], and continuous changes of multisensorial environmental demands during movement [28], especially movements consisting of complex skills; it is reasonable to assume that exercise is able to improve brain region communication capability. To test this hypothesis, certain studies [22 28-30] have investigated brain connectivity responses to exercise training. Exercise training can improve brain connectivity by increasing muscle strength and strengthened synapse efficiency between neurons through co-activation of brain areas associated with fatigue (i.e., caudate) in pwMS [26 29]. Furthermore exercise training can reinforce the thalamus-cortex

network, which results in improvements of thalamocortical RSFC as indicated in a previous study previously [28]. Changes in brain connectivity was associated with improvements in clinical outcomes that included cognitive fatigue and cognitive process speed (table 3) which could be due to selective neuroplasticity changes in functional connectivity in the caudate and thalamocortical areas [29 31]. Finally, the last MR scan study [31] that included elastography methodology as a non-invasive imaging technique for measuring the viscoelastic properties of the hippocampus reported a large effect of walking training ability (stiffening increase 8.4% and viscosity decreased 11.5%). Further, they concluded that improvement in hippocampal viscoelastic properties response to exercise training can be considered as a mechanism for exercise training-related changes in learning and memory that was observed in this study [31].

One of the major issues of imagining studies is that the drop-out rate of participation exceeded 15% (range between 17-38%). As a result of this, all imaging studies did not meet the eighth criteria of the PEDro scale (Outcomes were obtained from more than 85%). The high drop-out rate in addition to small sample sizes in some studies [28-31] tempers the conclusions possible from these studies. Therefore, future studies should recruit a large cohort of pwMS and include strategies for maximizing participation retention [32]. Ultimately, one study [29] did not meet the second and third criteria of the PEDro scale (random and concealed allocation) and this further weakens the results of that study.

BBB function biomarkers: S100b and NSE have been investigated in one study previously [16]. In this randomized controlled trial, only s100b demonstrated significant change in the normal weight group which exercised. Since exercise training is able to reduce reactive oxygen species and pro-inflammatory cytokines [16 25] , physical activity can be considered as a strategy to reduce BBB permeability and to improve integrity. A study [15] that examined MMP-

2 and MMP-9 as BBB indicator showed significant reductions in the serum level of MMP-2, not MMP-9 after HIIT. Based on the PEDro scale, this study [15] had the highest methodological quality (9/10). However, this study [15] did not satisfy the fifth PEDro criterion (Blinding all subjects); this criterion is not applicable for exercise training intervention studies [25]. Other research [14] confirms that resting serum levels of MMP-9 remain stable after combined training; however, this study had more than a 15% drop-out of participants that undermined the results. Since previous evidence described the role of MMP-2 in the inflammatory processes, current results can reinforce this hypothesis that regular physical activity has anti-inflammatory properties [33]. In addition, improving MMP-2 level is consistent with the improvement of cognitive function in pwMS [15]. Since that MMP-2 have emerged as important pathogenic molecules that disrupt the BBB [14 15], significant reduction of MMP-2 levels can be indicate the protective effect of exercise while improving cognitive function. Although the results indicate a positive effect of exercise training on BBB function, the studies all included indirect indicators of BBB function, and none of the studies have used the dynamic contrast enhanced-MRI as a gold-standard method. Therefore, future studies should include this methodology when investigating exercise training effects on BBB function in MS.

Neurotrophic factors: Eight studies focused on BDNF, four of which reported a significant increase in the concentration of this biomarker [16-18 20]. These results agree with previous human studies in a variety of psychiatric conditions, and can be found in the recent reviews on BDNF response to exercise training in healthy individuals and psychiatric conditions [8 34]. Importantly, it has been suggested that BDNF increases as a result of exercise training also promotes cognitive function and alleviates psychiatric symptoms [8]. However, due to different clinical measurements for psychiatric conditions compared with MS, we must be

careful when extrapolating the results. The most appropriate modality and intensity of exercise training remains controversial for altering BDNF levels in MS. Increases in BDNF levels after cycling training were demonstrated only in individuals who cycled in water (not on land) [20] which can be interpreted with anti-inflammatory beneficial effects of immersion. It has also been suggested that immersion in water results in lower adrenalin and noradrenalin levels. This indicates that immersion affects sympathetic and parasympathetic pathway activity [35].

Several important issues can influence the measurement of BDNF. For instance socio-demographic background such as body composition determines response of BDNF to exercise training [34] as demonstrated previously [16], which also supports the metabolic role of BDNF [16]. It seems that weight gain is associated with low-grade inflammation [36], which can lead to limited neurotrophic factor (i.e., BDNF, PDGF) production. Therefore, the authors [16] suggested that perhaps weight management in pwMS in overweight and obesity could modulate and enhance the effect of interventions (i.e., exercise training).

Although there is a larger corpus of research on neurotrophic factors than imaging and BBB markers, these studies are limited to peripheral blood flow, and evaluating cerebrospinal neurotrophic factors might provide more interesting results [16 20]. Accordingly, future studies focused on exercise training should include cerebrospinal neurotrophic factors. Additionally, the assessment of clinical and psychological indicators (i.e., cognitive function) alongside neurotrophics can add strengths to the results that have been neglected in most studies. However, studies with exercise training as interventions used in other medical conditions, such as stroke [37 38], further align with the current findings and indicate that exercising at moderate to high intensity increases neurotrophic factors (i.e., BDNF).

Limitation and future research

There are some observations during the preparation of this manuscript that need clarification. Firstly, we did not register the review in PROSPERO for a-priori establishing the parameters of the systematic review. The small number of studies that investigated the effects of exercise training in pwMS that satisfied our inclusion is a main limitation, particularly for imaging markers. The sample size of some studies was very low (especially imaging markers), and 4 of 6 studies on MR scan [28 29 31] included only three to seven subjects per condition. Some studies of neurotrophic factors [10 17 19 21] also had small sample sizes (<16 in each condition). These small samples increase the risk of a type II error. The current study is limited to the focus on special biomarkers; for instance, most papers focused on neurotrophic factors, and emphasized BDNF, therefore, in order to better understand the mechanisms and effect of exercise training, a more complete review of biomarkers is need.

The reviewed studies were heterogeneous in several areas, including subject characteristics (i.e., age, MS type and disease), exercise modalities (i.e., resistance and endurance), duration of interventions (3 to 48 weeks), and MS biomarkers related to CNS. Therefore, meta-analysis may not applicable according to the Cochrane Library Guidelines, and we only performed a systematic review.

Conclusion

Collectively, moderate intensity exercise training performed, 2-3 sessions per week for at least 4 weeks with moderate intensity, can improve brain function and integrity. In addition, the

improvement of BBB permeability markers and the increase of some neurotrophic factors (e.g., BDNF) can lead to improvement of disease status; however, some limitations of the reviewed studies prevent the conclusive conclusions about the effect of exercise training on MS biomarkers. Data revealed that moderate to high intensity exercise training, 2-3 sessions per week, for at least 30 min duration, can improve neurotrophic factors levels. However, results of the current systematic review generally indicate that exercise training can be considered as an important complementary therapy for MS. There is a requirement for more research studies to be completed using rigorous methodologies in large samples in order to establish the role of exercise training on MS status from a biomarkers perspective.

Declaration of Conflicting Interests

The author(s) declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Rieckmann P, Centonze D, Elovaara I, et al. Unmet needs, burden of treatment, and patient engagement in multiple sclerosis: A combined perspective from the MS in the 21st Century Steering Group. Multiple Sclerosis and Related Disorders 2018;**19**:153-60
2. Majdinasab N, Motl RW, Mokhtarzade M, et al. Acute responses of cytokines and adipokines to aerobic exercise in relapsing vs. remitting women with multiple sclerosis. Complementary Therapies in Clinical Practice 2018
3. McDonald WI, Compston A, Edan G, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. Annals of neurology 2001;**50**(1):121-27
4. Novakova L, Axelsson M, Khademi M, et al. Cerebrospinal fluid biomarkers as a measure of disease activity and treatment efficacy in relapsing-remitting multiple sclerosis. Journal of neurochemistry 2017;**141**(2):296-304
5. Bielekova B, Martin R. Development of biomarkers in multiple sclerosis. Brain 2004;**127**(7):1463-78
6. Ensari I, Motl RW, Pilutti LA. Exercise training improves depressive symptoms in people with multiple sclerosis: results of a meta-analysis. Journal of psychosomatic research 2014;**76**(6):465-71
7. Pilutti LA, Greenlee TA, Motl RW, Nickrent MS, Petruzzello SJ. Effects of exercise training on fatigue in multiple sclerosis: a meta-analysis. Psychosomatic medicine 2013;**75**(6):575-80

8. Devasahayam AJ, Downer MB, Ploughman M. The effects of aerobic exercise on the recovery of walking ability and neuroplasticity in people with multiple sclerosis: a systematic review of animal and clinical studies. Multiple sclerosis international 2017;**2017**
9. Briken S, Rosenkranz SC, Keminer O, et al. Effects of exercise on Irisin, BDNF and IL-6 serum levels in patients with progressive multiple sclerosis. Journal of neuroimmunology 2016;**299**:53-58
10. Schulz K-H, Gold SM, Witte J, et al. Impact of aerobic training on immune-endocrine parameters, neurotrophic factors, quality of life and coordinative function in multiple sclerosis. Journal of the neurological sciences 2004;**225**(1):11-18
11. Motl RW, Sandroff BM, Kwakkel G, et al. Exercise in patients with multiple sclerosis. The Lancet Neurology 2017;**16**(10):848-56
12. Furlan AD, Malmivaara A, Chou R, et al. 2015 updated method guideline for systematic reviews in the Cochrane Back and Neck Group. 2015;**40**(21):1660-73
13. Moher D, Shamseer L, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Systematic reviews 2015;**4**(1):1
14. Deckx N, Wens I, Nuyts AH, et al. 12 weeks of combined endurance and resistance training reduces innate markers of inflammation in a randomized controlled clinical trial in patients with multiple sclerosis. Mediators of inflammation 2016;**2016**
15. Zimmer P, Bloch W, Schenk A, et al. High-intensity interval exercise improves cognitive performance and reduces matrix metalloproteinases-2 serum levels in persons with multiple sclerosis: A randomized controlled trial. Multiple Sclerosis Journal 2017:1352458517728342
16. Mokhtarzade M, Motl R, Negaresh R, et al. Exercise-induced changes in neurotrophic factors and markers of blood-brain barrier permeability are moderated by weight status in multiple sclerosis. Neuropeptides 2018
17. Wens I, Keytsman C, Deckx N, Cools N, Dalgas U, Eijnde BO. Brain derived neurotrophic factor in multiple sclerosis: effect of 24 weeks endurance and resistance training. European journal of neurology 2016;**23**(6):1028-35
18. Ozkul C, Guclu-Gunduz A, Irkec C, et al. Effect of combined exercise training on serum brain-derived neurotrophic factor, suppressors of cytokine signaling 1 and 3 in patients with multiple sclerosis. Journal of neuroimmunology 2018;**316**:121-29
19. Khademosharie M, Tadibi V, Behpoor N, Hamedinia MR. The effect of 12-weeks concurent training on the serum levels NGF, BDNF, and VDBP in women with multiple sclerosis. International Journal of Applied Exercise Physiology 2018;**7**(1):77-86
20. Bansi J, Bloch W, Gamper U, Kesselring J. Training in MS: influence of two different endurance training protocols (aquatic versus overland) on cytokine and neurotrophin concentrations during three week randomized controlled trial. Multiple Sclerosis Journal 2013;**19**(5):613-21
21. Askari A, Rashid Lamir A, Bijeh N, Momeni Moghadam M. Effect of 8-Week Aquatic Training and Resistance Training on Plasma NT-4 Levels and NT-4 Expression in Peripheral Blood Mononuclear Cells in Women with Multiple Sclerosis. Medical Laboratory Journal 2017;**11**(6):1-6
22. Tavazzi E, Bergsland N, Cattaneo D, et al. Effects of motor rehabilitation on mobility and brain plasticity in multiple sclerosis: a structural and functional MRI study. 2018;**265**(6):1393-401
23. Kjølhede T, Siemonsen S, Wenzel D, et al. Can resistance training impact MRI outcomes in relapsing-remitting multiple sclerosis? Multiple Sclerosis Journal 2017:1352458517722645
24. Fisniku L, Brex P, Altmann D, et al. Disability and T2 MRI lesions: a 20-year follow-up of patients with relapse onset of multiple sclerosis. Brain 2008;**131**(3):808-17
25. Negaresh R, Motl RW, Mokhtarzade M, et al. Effects of Exercise Training on Cytokines and Adipokines in Multiple Sclerosis: A Systematic Review. Multiple sclerosis and related disorders 2018

26. Harmelech T, Preminger S, Wertman E, Malach R. The day-after effect: long term, Hebbian-like restructuring of resting-state fMRI patterns induced by a single epoch of cortical activation. Journal of Neuroscience 2013;**33**(22):9488-97
27. Leavitt V, Cirnigliaro C, Cohen A, et al. Aerobic exercise increases hippocampal volume and improves memory in multiple sclerosis: preliminary findings. Neurocase 2014;**20**(6):695-97
28. Sandroff BM, Wylie GR, Sutton BP, Johnson CL, DeLuca J, Motl RW. Treadmill walking exercise training and brain function in multiple sclerosis: Preliminary evidence setting the stage for a network-based approach to rehabilitation. Multiple Sclerosis Journal–Experimental, Translational and Clinical 2018;**4**(1):2055217318760641
29. Akbar N, Sandroff BM, Wylie GR, et al. Progressive resistance exercise training and changes in resting-state functional connectivity of the caudate in persons with multiple sclerosis and severe fatigue: A proof-of-concept study. Neuropsychological rehabilitation 2018:1-13
30. Leonard G, Lapierre Y, Chen J-K, et al. Noninvasive tongue stimulation combined with intensive cognitive and physical rehabilitation induces neuroplastic changes in patients with multiple sclerosis: a multimodal neuroimaging study. 2017;**3**(1):2055217317690561
31. Sandroff BM, Johnson CL, Motl RW. Exercise training effects on memory and hippocampal viscoelasticity in multiple sclerosis: a novel application of magnetic resonance elastography. Neuroradiology 2017;**59**(1):61-67
32. Motl RW, Pekmezi D, Wingo BCJMSJE, Translational, Clinical. Promotion of physical activity and exercise in multiple sclerosis: Importance of behavioral science and theory. 2018;**4**(3):2055217318786745
33. Petersen AMW, Pedersen BK. The anti-inflammatory effect of exercise. Journal of applied physiology 2005;**98**(4):1154-62
34. Dinoff A, Herrmann N, Swardfager W, et al. The effect of exercise training on resting concentrations of peripheral brain-derived neurotrophic factor (BDNF): a meta-analysis. PLoS One 2016;**11**(9):e0163037
35. Grossman E, Goldstein DS, Hoffman A, Wacks IR, Epstein M. Effects of water immersion on sympathoadrenal and dopa-dopamine systems in humans. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 1992;**262**(6):R993-R99
36. Heilbronn LK, Campbell LV. Adipose tissue macrophages, low grade inflammation and insulin resistance in human obesity. Current pharmaceutical design 2008;**14**(12):1225-30
37. Austin MW, Ploughman M, Glynn L, Corbett DJNr. Aerobic exercise effects on neuroprotection and brain repair following stroke: a systematic review and perspective. 2014;**87**:8-15
38. Ploughman M, Austin MW, Glynn L, Corbett DJTsr. The effects of poststroke aerobic exercise on neuroplasticity: a systematic review of animal and clinical studies. 2015;**6**(1):13-28

Figure 1. Search and selection of studies.

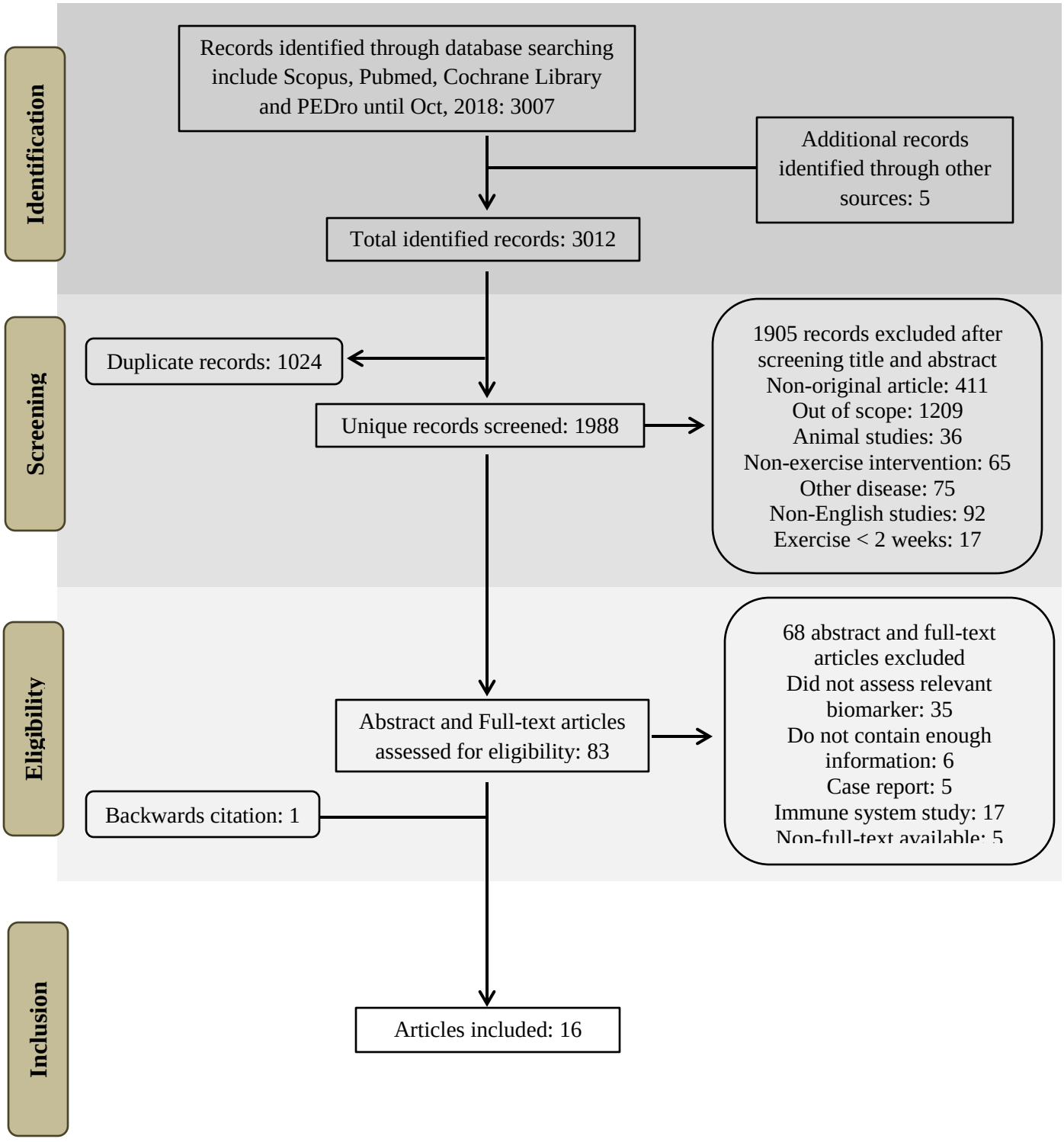
Identification
Records identified through database searching include Scopus, Pubmed, Cochrane Library and PEDro until Oct, 2018: 3007
Additional records identified through other sources: 5
Total identified records: 3012
Screening
Duplicate records: 1024
Unique records screened: 1988
1905 records excluded after screening title and abstract
Non-original article: 411
Out of scope: 1209
Animal studies: 36
Non-exercise intervention: 65
Other disease: 75
Non-English studies: 92
Exercise < 2 weeks: 17
Eligibility
Abstract and Full-text articles assessed for eligibility: 83
68 abstract and full-text articles excluded
Did not assess relevant biomarker: 35
Do not contain enough information: 6
Case report: 5
Immune system study: 17
Non-full-text available: 5
Backwards citation: 1
Inclusion
Articles included: 16

Table 1. The methodological quality of the reviewed studies using the PEDro scale.

	Scales											
First author (Year)	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**#9**	**#10**	**#11**	**Total**
Akbar (2018)	1	0	0	1	0	0	1	0	1	1	1	5/10
Kjølhede (2017)	1	1	1	1	0	0	1	0	1	1	1	7/10
Sandroff (2018)	1	1	1	1	0	0	1	0	1	1	1	7/10
Sandroff (2017)	1	1	1	1	0	0	1	0	1	1	1	7/10
Leonard (2017)	1	1	1	1	0	0	1	1	1	1	1	8/10
Tavazzi (2018)	1	1	1	1	0	0	1	0	1	1	1	7/10
Bansi (2013)	1	1	0	1	0	0	0	1	1	1	1	6/10
Zimmer (2017)	1	1	1	1	0	1	1	1	1	1	1	9/10
Mokhtarzade (2018)	1	1	0	1	0	0	1	1	1	1	1	7/10
Briken (2016)	1	1	1	1	0	0	0	1	1	1	1	7/10
Schulz (2004)	1	1	0	1	0	0	1	1	1	1	1	7/10
Wens (2016)	1	1	0	1	0	0	0	1	1	1	1	6/10
Askari (2017)	1	0	0	0	0	0	0	1	1	0	1	3/10
Ozkul (2018)	1	1	0	1	0	0	0	1	1	1	1	6/10
Khademosharie (2018)	0	0	0	1	0	0	0	0	1	1	1	4/10
Deckx (2016)	1	1	1	1	0	0	1	0	1	1	1	7/10

#1: Eligibility criteria (not included in the total score); #2: Random allocation; #3: Allocation was concealed; #4: The groups were similar on important criteria at the baseline; #5: Blind all subjects; #6: Blind therapy administration; #7: Blind all assessors; #8: Outcomes were obtained from more than 85%; #9: Intention to treat analysis; #10: Statistical comparisons between groups at least for one key factor; #11: Point estimates and variability.

Table 2. Subjects and exercise training protocol characteristics of reviewed studies.

First author (year)	Subjects (Training /Control /health)	MS type	Drop out (Training /control)	EDSS	Week	Session	Type	Intensity	Session duration (min)
Akbar (2018)	5/5/0	RRMS/ SPMS /PPMS	3/3	NA	16	3 per week	Resistance training with elastic band	NA	NA
Kjølhede (2017)	17/12/0	RRMS	1/5	2-4	48	2 per week	Resistance training	6-15 RM	NA
Sandroff (2018)	5/3/0	RRMS	0/2	1.5-4	12	3 per week	Treadmill walking	40-80 HR_{rest}	15-40
Sandroff (2017)	5/3/0	RRMS	0/2	1.5-4	12	3 per week	Treadmill walking	40-80% HR_{rest}	15-40
Leonard (2017)	7/7/0	NA	0/0	<6	14	2-3 per day 5 day per week	Intensive physical therapy and working memory training	NA	45
Tavazzi (2018)	7-9/0/0*	RRMS/ SPMS/ PPMS	9/0	4.5-6.5	4	2 per day 5 day per week	Resistance and Endurance training	10-15 RM and 16-17 Borg	30-45
Bansi (2013)	28-24/0/0*	NA	2/6	1-6.5	3	3 per week	Aerobic cycling	70% HR_{peak}	30
Zimmer (2017)	27/30/0	RRMS/ SPMS	2/1	1-6.5	3	3 per week	High intensity and continues cycling	80-90% and 70% HR_{max}	20-30

Mokhtarzade (2018)	34/27/0	RRMS	2/3	≤4	8	3 per week	Lower and upper limb interval cycling	60-75 W_{max}	42-66
Briken (2016)	32/10/0	PPMS/ SPMS	4/1	4-6	9	5 per two week	Incremental cycling	Increase 12.5 w/min until exhaustion	10-20 min
Schulz (2004)	15/13/0	RRMS/ SPMS/ PPMS	0/0	<5	8	2 per week	Interval cycling	75% W_{max}	30
Wens (2016)	15/7/19	RRMS	0/0	2.6 ± 0.2	24	5 per two week	Combined training	12-14 Borg	45-75
Askari (2017)	10-10/10/0*	NA	0/0	2-4	8	3 per week	Combined training (aquatic & resistance)	70% HR_{peak} & 40-70% 1RM	30-45
Ozkul (2018)	18/18/18	RRMS	3/2	<5	8	3 per week	Combined training (Pilates& aerobic)	60-80% HR_{max}	30-60
Khademoshar rie (2018)	10/10/0	PPMS/ SPMS	2/2	1-5	12	3 per week	Combined training (resistance & aerobic)	60-80% 1RM & 40-55% HR_{rest}	NA
Deckx (2016)	29/16/0	CPMS/ RRMS	9/9	≤6	12	5 per two week	Combined training (resistance & aerobic)	NA	NA

* These studies used two exercise training group. # mean (95% coefficient intervals). NA: not available, HR: heart rate, W: watt, 1RM: one-maximum repetition.

Table3. The main results of the reviewed studies.

First author (Year)	Sample size*	Sample				Main factors	Main results	Clinical results	Biochemical results**
		S	P	C	M				
Akbar (2018)	10				×	Caudate RSFC	Functional connectivity between the caudate and the left inferior parietal region, right and left inferior frontal regions, left middle frontal region, and right insula: ↑ Increase in functional connectivity between these caudate and left inferior parietal lobule was significantly associated with physical activity level.	Godin leisure-time exercise: No significant change Grip strength: No significant change MS impact scale score: No significant change	NA
Kjølhede (2017)	29				×	PBVC, Lesion load, Cortical thickness	PBVC: No significant change T2 lesion count and load: No significant change global white or grey matter volume: No significant change Cortical thickness: ↑ Increase in cortical thickness was significantly associated with increasing strength and walking ability and decreasing EDSS and MS impact scale score.	MS functional composite and strength scores: ↑	NA
Sandroff (2018)	8				×	Thalamocorical RSFC	thalamic/right superior frontal gyrus RSFC: ↑ thalamic/left medial frontal gyrus RSFC: ↑ Increasing thalamocorical functional connectivity was	Symbol digit modalities test score: ↑ Aerobic capacity: ↑	NA

							significantly assosited with increasing symbol digit modalities test and aerobic capacity.		
Sandroff (2017)	8				×	Shear stiffness, Viscosity	Hippocampal shear stiffness: ↑ Hippocampal viscosity: ↓ Increase in California verbal learning test-II score was significantly associated with increasing hippocampal shear stiffness and decreasing viscosity.	Cardiorespiratory fitness: ↑ Verbal learning and memory: ↑	NA
Leonard (2017)	14				×	Motor-task fMRI	BOLD signal in the left primary motor cortex (gait task): ↑ BOLD signal in the dorsolateral prefrontal cortex (working memory task): ↑	Sensory organization tests score: ↑ Depression and anxiety: No significant change.	NA
Tavazzi (2018)	16[#]				×	Motor-task fMRI, RSFC, DTI	Pre-central gyrus RSFC: ↑ Post-central gyrus RSFC: ↑ Fractional anisotropy, mean diffusivity, axial diffusivity, and radial diffusivity: No significant change Activation of the left precentral gyrus: ↓ Motor areas activation: ↓	Two-min walk distance: ↑ Dynamic gait index: ↑ Berg balance scale: ↑	NA
Bansi (2013)	32[#]		×			BDNF, NGF	BDNF in water group: ↑ BDNF in land group: No significant change NGF: No significant change	Fatigue: ↓ Cardiovascular fitness: ↑	TNF-α, IL-6 and sIL-6r level: No significant change
Zimmer (2017)	57	×				BDNF, MMP-9, MMP-2	BDNF: No significant change MMP-2 in HIIT group: ↓ MMP-2 in CT group: No significant change MMP-9: No significant change Decrease in MMP-2 was	Verbal learning memory: ↑ Cardiovascular fitness: ↑	Serotonin level: No significant change

							significantly associated with increasing aerobic capacity.		
Mokhtarzade (2018)	61	×				BDNF, NGF, CNTF, PDGF, s100b, NSE	BDNF in normal weight group: ↑ BDNF in overweight group: No significant change NGF: No significant change PDGF in normal weight group: ↑ PDGF in overweight group: ↑ CNTF: No significant change S100b in normal weight group: ↓ S100b in overweight group: No significant change NSE: No significant change	Cardiovascular fitness: ↑	IL-10, TNF-α and cortisol level: No significant change
Briken (2016)	42	×				BDNF	BDNF: No significant change BDNF was significantly associated with lower fatigue and upper alertness.	NA	Irisin and IL-6 level: No significant change.
Schulz (2004)	28		×			BDNF, NGF	BDNF: No significant change NGF: No significant change	Quality of life: ↑ Cardiovascular fitness: ↑ Fatigue: ↓	IL-6 and sIL-6 level: No significant change.
Wens (2016)	22	×				BDNF	BDNF: ↑	Muscle strength: ↑	NA
Askari (2017)	30		×	×		NT-4	NT-4 in plasma: ↑ NT-4 in peripheral blood mononuclear cell: ↑	NA	NA
Ozkul (2018)	36[#]	×				BDNF	BDNF: ↑	Balance: ↑ Walking ability: ↑ Fatigue: ↓	SOCS1 and SOCS3 level: No significantly change.
Khademosharie (2018)	20	×				BDNF, NGF	BDNF: No significant change NGF: No significant change	NA	NA

Deckx (2016)	45		×	×		MMP-9	MMP-9 in plasma: No significant change MMP-9 production response to lipopolysaccharides and IFN-γ stimulation: ↓	NA	IL-6, IL-10, IL-12, TNF-α and TGF-β level: No significant change TNF-α production upon lipopolysaccharides and IFN-γ stimulation: ↓ Number of plasmacytoid DC: ↓

* The number of participant with multiple sclerosis who completed the analysis (exclude drop-out). # These studies used two exercise training group. ** In these cells, biochemical results have been reported which have not been addressed in the text.

NA: not available; ↑: significant increase, ↓: significant decrease

SUPPLEMENTAL METHODOLOGY (ONLY ONLINE)

Search strategy

We sought to identify all intervention studies that examined the effect of exercise training on MS biomarkers of CNS integrity and disease status.

We initially identified the eligible outcomes that represent MS biomarkers based on the existing literature, and two authors listed the possible MS biomarkers. Further to this, two authors then performed a primary search of candidate biomarkers derived from the exercise studies related to MS subjects. The results indicated that exercise training studies included four different types of CNS-related biomarkers as outcome measures in MS. These biomarkers include a) imaging biomarkers (derived by MRI), b) BBB function and permeability related biomarkers, c) neurotrophic biomarkers, and d) immune system markers. We recently reviewed the effects of exercise training on immune system markers (i.e., cytokines) in a comprehensive systematic review [1], and have excluded immune markers from the current study.

Eligible criteria of studies and outcomes

The inclusion criteria for studies were: (1) intervention studies of exercise training that measured one or more of the aforementioned biomarkers of MS as an outcome, (2) English language, (3) recruited MS subjects, (4) adults above 15 years of age, (5) intervention period lasting more than 2 weeks, (6) published until October 2018. The exclusion criteria were: (1) using other interventions such as nutritional supplements in addition to exercise, (2) lack of full-text, (3)

conference paper, (4) lack of original data, (5) case reports, (6) editorials, and (7) measures of immune system markers only. The papers were screened for inclusion and exclusion criteria separately by two authors.

Methodological quality

We determined study quality using the 11-item Physiotherapy Evidence Database (PEDro) scale. The scale includes 11 items and the item scores are summed into an overall indication of study quality; the first item does not add to the score. Methodological quality scores ranged between 0 to 10, whereby 10 indicated the best and 0 indicated the poorest methodological quality. In addition, scores ≤ 4 reflect low, scores 5 and 6 reflect fair, and scores ≥ 7 reflect high methodological quality.[2]

Results of searches

Of the 16 eligible studies, six studies [3-8] focused on MRI markers, nine studies [9-17] focused on neurotrophic factors, and three [10 11 18] studies focused on BBB function markers. Of note, two studies [10 11] included both neurotrophic factors and BBB function markers and are therefore included across categories of biomarkers in this review.

Methodological quality of included studies

PEDro scores ranged between 3 and 9, and the mean PEDro scale score was 6.4 ± 1.4, reflecting fair methodological quality. Most studies met the fourth (the groups were similar on important criteria at the baseline), ninth (intention to treat analysis), tenth (statistical comparisons between groups) and eleventh (point estimates and variability) criteria. Regarding the nature of studies, blinding methodologies (blind all subjects and blind therapy administration) were not realized in most studies. Ten studies had high methodological quality.

Methodology characteristics of included studies

The 16 studies included in the current review contained a total of 525 participants; 488 of them were persons with MS (293 under exercise conditions, 195 in control conditions) and 37 of the subjects were healthy controls. Samples sizes of reviewed studies were very small (8 participants) through to moderate (61 participants). Among the 73 MS participants who dropped-out of the studies, 41 cases were control conditions (56.2%) and 32 cases were intervention conditions (43.8%). Of these, 8 and 4 cases of drop-out were caused by the occurrence of a relapse in the intervention and control conditions, respectively. Regarding biological sex of the MS subjects, 131 (31%) participants were male, and 302 participants were female (69%). Most studies recruited participants with a mean age ~45 years and ranged between 18 and 65 years. Studies recruited subjects with expanded disability status scale scores between 0 and 6.5.

Exercise program characteristics of included studies

Exercise modalities are varied across studies and included endurance [5 6 9-13], resistance [3 4 8 15], high-intensity interval training (HIIT) [10], aquatic [15], and combined training [7 14 16-18]. Most of the reviewed studies reported that the exercise programs were well tolerated by participants with MS. In five studies, the adherence to the interventional program was reported [3 5 8 10 14] and exceeded 80%. One study [10] compared the effect of HIIT and continuous training (CT). Most studies considered two to three training sessions per week. Two studies [7 8] used 2 to 3 session of exercise intervention per day. However, most studies used 8 to 12 weeks training program duration, the training protocol ranged between 3 [9 10] and 48 weeks. [4] It should be noted that one study [3] used 16 weeks training duration and was a home-based exercise methodology and another one [7] used 12 weeks home-based exercise after 2 weeks supervised exercise. Some studies did not report the duration of each session, often sessions lasted between 20 to 60 min. Exercise intensity varied from low to high and was designed based on heart rate, Borg scale, one-repetition maximum (1RM), maximum watts and maximal and peak aerobic capacity. The most severe exercise intensity was recorded by study [10] which used HIIT with an 80-90 % maximum heart rate (HR_{max}) for 3 weeks.

Results of studies

Imaging results: Six studies focused on the imaging markers of CNS in pwMS. One study (PEDro: 7/10) investigated changes in thalamocortical resting-state functional connectivity (RSFC) [5], and there was a significant increase in thalamic/right superior frontal and thalamic/left medial frontal gyrus RSFC after 12 weeks treadmill walking exercise. A further study (PEDro: 5/10) investigated the effects of 16 weeks home-based resistance exercise on caudate functional connectivity in pwMS with severe fatigue (fatigue severity scale score > 4)

[3]. The study reported increases in functional connectivity between caudate and the left inferior parietal region, right and left inferior frontal regions, left middle frontal region, and right insula. One study [4] (PEDro: 7/10) observed that 24 weeks of resistance training had no significant effect on percentage brain volume change, subcortical grey matter structure volumes, lesion load and count, but there was a significant increase in cortical thickness. An additional study (PEDro: 7/10) using magnetic resonance elastography reported that there was a large exercise effect on hippocampal shear stiffness and damping ratio [6]; the papers that reported thalamocortical RSFC [5] and magnetic resonance elastography [6] were derived from the same study. Another trial [7] included 4 weeks of endurance and resistance exercise (PEDro: 7/10) and reported an increased functional connectivity in the precentral and post-central gyrus. Finally, one study [8] applied intensive physical therapy and working memory training for 14 weeks as an intervention (PEDro: 8/10) and reported a significant increase in blood oxygen level dependent signal in the left motor cortex and dorsolateral prefrontal cortex during gait and working-memory task, respectively.

BBB markers: Three studies focused on BBB function markers in pwMS (Table 3). Studies used peripheral circulation markers including matrix metallopeptidase (MMP)-9 [10 18], MMP-2 [10], s100 calcium-binding protein B (s100b) and neuron-specific enolase (NSE) [11]. Results of BBB markers studies revealed a positive effect of exercise, particularly where s100b investigated. In a study [10] compared high-intensity interval training (HIIT) with a continuous training program (PEDro: 9/10), and observed that MMP-2 significantly decreased in the HIIT group, whereas there was no change in MMP-9 in either group. Another study [18] (PEDro: 7/10) investigated the effects of combined training (12 weeks) on MMP-9 and reported no significant changes in this marker, however, secretion of MMP-9 in response to

lipopolysaccharides and interferon gamma stimulation decreased following the intervention. A further study [11] monitored changes of s100b and NSE in normal and overweight individuals with MS (PEDro: 7/10) with exercise training and observed that only normal weight subjects who participated in the exercise program had significant decreases in s100b, but not NSE levels.

Neurotrophic factors: Nine studies focused on neurotrophic factors. Among 8 studies that investigated the effects of exercise training on BDNF, 4 studies [9 11 14 16] reported a significant increase (PEDro: 6-9). It should be noted that in a study [9] comparing land and water based exercise (PEDro: 6/10) reported that BDNF significantly increased only during the water based condition. The reviewed studies with elevated NGF and CNTF concentrations reported non-significant effects of exercise training (PEDro: 4-7) on NGF and CNTF, expect one study (PEDro: 3/10) on NT-4 [15].

Reference

1. Negaresh R, Motl RW, Mokhtarzade M, et al. Effects of Exercise Training on Cytokines and Adipokines in Multiple Sclerosis: A Systematic Review. Multiple sclerosis and related disorders 2018
2. Foley NC, Teasell RW, Bhogal SK, Speechley MR. Stroke rehabilitation evidence-based review: methodology. Topics in stroke rehabilitation 2003;**10**(1):1-7
3. Akbar N, Sandroff BM, Wylie GR, et al. Progressive resistance exercise training and changes in resting-state functional connectivity of the caudate in persons with multiple sclerosis and severe fatigue: A proof-of-concept study. Neuropsychological rehabilitation 2018:1-13
4. Kjølhede T, Siemonsen S, Wenzel D, et al. Can resistance training impact MRI outcomes in relapsing-remitting multiple sclerosis? Multiple Sclerosis Journal 2017:1352458517722645
5. Sandroff BM, Wylie GR, Sutton BP, Johnson CL, DeLuca J, Motl RW. Treadmill walking exercise training and brain function in multiple sclerosis: Preliminary evidence setting the stage for a network-based approach to rehabilitation. Multiple Sclerosis Journal–Experimental, Translational and Clinical 2018;**4**(1):2055217318760641
6. Sandroff BM, Johnson CL, Motl RW. Exercise training effects on memory and hippocampal viscoelasticity in multiple sclerosis: a novel application of magnetic resonance elastography. Neuroradiology 2017;**59**(1):61-67
7. Leonard G, Lapierre Y, Chen J-K, et al. Noninvasive tongue stimulation combined with intensive cognitive and physical rehabilitation induces neuroplastic changes in patients with multiple sclerosis: a multimodal neuroimaging study. 2017;**3**(1):2055217317690561
8. Tavazzi E, Bergsland N, Cattaneo D, et al. Effects of motor rehabilitation on mobility and brain plasticity in multiple sclerosis: a structural and functional MRI study. 2018;**265**(6):1393-401

9. Bansi J, Bloch W, Gamper U, Kesselring J. Training in MS: influence of two different endurance training protocols (aquatic versus overland) on cytokine and neurotrophin concentrations during three week randomized controlled trial. Multiple Sclerosis Journal 2013;**19**(5):613-21
10. Zimmer P, Bloch W, Schenk A, et al. High-intensity interval exercise improves cognitive performance and reduces matrix metalloproteinases-2 serum levels in persons with multiple sclerosis: A randomized controlled trial. Multiple Sclerosis Journal 2017:1352458517728342
11. Mokhtarzade M, Motl R, Negaresh R, et al. Exercise-induced changes in neurotrophic factors and markers of blood-brain barrier permeability are moderated by weight status in multiple sclerosis. Neuropeptides 2018
12. Briken S, Rosenkranz SC, Keminer O, et al. Effects of exercise on Irisin, BDNF and IL-6 serum levels in patients with progressive multiple sclerosis. Journal of neuroimmunology 2016;**299**:53-58
13. Schulz K-H, Gold SM, Witte J, et al. Impact of aerobic training on immune-endocrine parameters, neurotrophic factors, quality of life and coordinative function in multiple sclerosis. Journal of the neurological sciences 2004;**225**(1):11-18
14. Wens I, Keytsman C, Deckx N, Cools N, Dalgas U, Eijnde BO. Brain derived neurotrophic factor in multiple sclerosis: effect of 24 weeks endurance and resistance training. European journal of neurology 2016;**23**(6):1028-35
15. Askari A, Rashid Lamir A, Bijeh N, Momeni Moghadam M. Effect of 8-Week Aquatic Training and Resistance Training on Plasma NT-4 Levels and NT-4 Expression in Peripheral Blood Mononuclear Cells in Women with Multiple Sclerosis. Medical Laboratory Journal 2017;**11**(6):1-6
16. Ozkul C, Guclu-Gunduz A, Irkec C, et al. Effect of combined exercise training on serum brain-derived neurotrophic factor, suppressors of cytokine signaling 1 and 3 in patients with multiple sclerosis. Journal of neuroimmunology 2018;**316**:121-29
17. Khademosharie M, Tadibi V, Behpoor N, Hamedinia MR. The effect of 12-weeks concurent training on the serum levels NGF, BDNF, and VDBP in women with multiple sclerosis. International Journal of Applied Exercise Physiology 2018;**7**(1):77-86
18. Deckx N, Wens I, Nuyts AH, et al. 12 weeks of combined endurance and resistance training reduces innate markers of inflammation in a randomized controlled clinical trial in patients with multiple sclerosis. Mediators of inflammation 2016;**2016**

(Only online) Table 1. Search strategy.

Search strategy	Database	Count	Fields
(‘Exercise’ OR ‘Training’ Or ‘Physical activity’ Or ‘Rehabilitation’ Or ‘Aerobic’ Or ‘Anaerobic’ Or ‘Physical therapy’ Or ‘’) AND (‘Multiple sclerosis’) AND (‘MRI’ OR ‘Imaging’ OR ‘Blood-brain barrier’ OR ‘Neurotrophic factor’ OR ‘Growth factor’ OR ‘Biomarker’ OR ‘Markers’ Or ‘Brain’ Or ‘Blood’)	Pubmed database	451	Abstract/Title
	Scopus database	2154	Abstract/Title/Keywords
	Cochrane Library database	358	Abstract/Title/Keywords
	PEDro database	43	Abstract/Title
	Other source	5	NA
	Backwards citation search	1	NA
	Total	3012	

NA: not available.