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No immediate change in systemic cytokines following an eccentric muscle training session in people with multiple sclerosis

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Abstract

Background: Eccentric muscle contractions elicit distinct physiological responses, including modulation of the cytokine profile. Although relevant for rehabilitation, the effect of eccentric muscle training on the immune system has never been investigated in multiple sclerosis (MS).

Objectives: Examine the immediate cytokine response of interleukin-4 (IL-4), IL-6, IL-10, IL-17a, interferon-gamma, and tumor necrosis factor-alpha after a moderate eccentric training session in individuals with MS. Additionally, further investigate the association between systemic cytokine levels at rest and clinical measures of mobility and lower limb functional strength.

Design: Observational study.

Methods: The first session included blood sampling for baseline cytokine measures. Subsequently, the participant completed a battery of clinical assessments related to mobility and lower limb strength, that is, the Timed-Up-and-Go Test, Five-Repetition-Sit-to-Stand-Test (5STS), Four-Square-Step-Test, and Two-Minute-Walk-Test. The second session included the eccentric exercise training session, followed by a second blood sampling to assess the acute cytokine response to the eccentric training bout. This session comprised 10 exercises concentrating on the strength of the trunk and lower extremities.

Results: Twenty-seven people with MS (pwMS), with a mean age of 40.1 years, participated in the study. No difference was demonstrated in the cytokine concentration values between baseline and immediately after the eccentric training session. The 5STS explained 30.3% of the variance associated with interferon-gamma, 14.8% with IL-4, and 13.8% with IL-10.

Conclusion: An eccentric training bout does not impact cytokine concentration in the blood and, consequently, does not boost a pro-inflammatory response, thus, it can be performed on pwMS in a rehabilitation setting.

Plain language summary

A strength-lengthening exercise session doesn't affect inflammation markers in people with multiple sclerosis

The article explores how a specific type of exercise, called eccentric muscle training, affects people with multiple sclerosis (MS). Eccentric muscle training involves exercises where the muscle lengthens under tension, like when you slowly lower a heavy object. This type of exercise is known for causing unique physical responses, including changes in certain proteins in the blood that help control the immune system and inflammation. The main goal of the study was to see if a session of eccentric muscle training would change the levels of these proteins, called cytokines, in the blood of people with

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MS immediately after exercise. The cytokines studied included IL-4, IL-6, IL-10, IL-17a, INF-γ, and TNF-α. These proteins are important because they help regulate inflammation and immune responses. The researchers also wanted to know if there was any connection between the levels of these proteins at rest and measures of mobility and leg strength. Twenty-seven people with MS took part in the study. Their average age was 40.1 years. In the first session, blood samples were taken to measure the baseline levels of these proteins, and various tests were conducted to assess mobility and leg strength. In the second session, participants completed an eccentric training session, and another blood sample was taken immediately after to see if there were any immediate changes in the protein levels. The results showed no significant differences in the protein levels before and after the exercise session. This suggests that a single session of eccentric muscle training does not cause an immediate inflammatory response in the blood. Therefore, this type of exercise can be safely included in rehabilitation programs for people with MS without the risk of causing harmful inflammation. Overall, the study supports the safety of eccentric muscle training for people with MS and provides valuable insights into its immediate effects on the immune system.

Keywords: cytokine response, eccentric training, multiple sclerosis, muscle strength

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Introduction

Multiple sclerosis (MS) is a chronic neurological disease characterized by widespread demyelination of the white matter in the central nervous system (CNS).[1] This immune system-induced attack on the myelin sheath surrounding the CNS axons and generates the formation of plaques or lesions, which may occur anywhere in the CNS. MS presents with diverse symptoms, including limb function or sensation loss, compromised bowel or bladder control, sexual dysfunction, fatigue, optic neuritis-induced blindness, loss of balance, walking difficulties, cognitive decline, and emotional fluctuations.[2,3]

Cytokines, a significant component of MS pathophysiology, are protein hormones regulating communication between the immune system's innate and adaptive branches.[4,5] A sense of balance exists between the T helper cytokines in a healthy population; however, in people with MS (pwMS), this balance shifts and exhibits increased pro-inflammatory cytokines [i.e. interleukin (IL)-1, IL-6] and decreased anti-inflammatory cytokines (i.e. IL-10) which can accelerate the destruction of myelin in the CNS of pwMS.[6]

Numerous studies have established that exercise as an effective symptomatic treatment in the MS population.[7–9] Moreover, previous studies have suggested that exercise produces a disease-modifying effect, reduces the annual relapse rate, and limits disease progression.[9] One of the mechanisms of exercise-mediated disease modification is the acute impact on the cytokine system.[10,11] The underlying rationale is that neuroplasticity can be induced by reducing several pro-inflammatory cytokines, thus preventing neurodegeneration and ultimately reducing disease progression.[11,12] Nevertheless, despite the logical rationale, the evidence demonstrating the effects of exercise on the cytokine response in pwMS is inconsistent.[13]

Eccentric muscle training is a potential exercise training strategy to induce a cytokine response. Eccentric muscle contractions have been shown to elicit distinct physiological responses, including modulation of the cytokine profile.[14] During eccentric contractions, muscle tissue is subjected to mechanical stress, leading to microstructural damage and subsequent inflammatory processes, eventually releasing cytokines as part of the

immune response.[15] Animal studies suggest that eccentric exercise generates a unique cytokine response compared to concentric or isometric exercise.[16–18] Eccentric training creates an upregulation of pro-inflammatory cytokines such as IL-6[16] and tumor necrosis factor-alpha (TNF-α).[17] Moreover, the cytokine upregulation following the eccentric exercise is greater than the concentric exercise.[18] Simultaneously, anti-inflammatory cytokines, that is, IL-10, might also become elevated.[19] This type of exercise strategy appears to significantly impact the immune system as opposed to other muscle-strengthening strategies; hence, it might be particularly relevant to pwMS. According to our literature search, the effect of eccentric muscle training on the immune system has never been investigated in the MS population.

Therefore, as an initial step in this investigation, our primary aim was to examine the acute cytokine response of IL-4, IL-6, IL-10, IL-17a, interferon-gamma (INF-γ), and TNF-α following an eccentric training session with pwMS. Furthermore, we explored the relationship between the selected cytokine values at rest and the clinical mobility and lower-limb functional strength measures in the MS sample.

Methods

Study design and participants

The observational study was performed at the Multiple Sclerosis Center, Sheba Medical Center, Tel-HaShomer, Israel. Participants were recruited through direct contact with the study's staff or local advertising. Inclusion criteria included (1) a diagnosis of MS according to the revised McDonald Criteria 2017,[20] (2) an age range of 18–65 years, and (3) scores <4.5 on the Expanded Disability Status Scale (EDSS).[21] Exclusion criteria included (1) orthopedic disorders that could negatively affect mobility, (2) major depression or cognitive decline limiting the ability to understand the study's instructions or complete the study protocol, (3) pregnancy, (4) MS clinical relapse or treatment with corticosteroid therapy within 6 months prior to the examination, (5) cardiovascular disorders and respiratory diseases, confirmed *via* the patient's medical records, (6) body-mass index $\geqslant 30$, and (7) use of steroids or fampridine or other conditions contraindicating physical activities. The study was approved by the Sheba Institutional Review Board (Ref# SMC-6288-19), consistent with the Declaration of Helsinki. All subjects signed an informed consent form prior to participation.

Study protocol

A physical therapist experienced in neurological rehabilitation performed and collected all outcome measures during two sessions with a 3–7-day interval between sessions. The first session included the recording of the participant's demographic and clinical information (age, gender, height, weight, disease duration, type of MS, and level of disability), patient-reported outcome measures [perceived fatigue *via* the Modified Fatigue Impact Scale (MFIS)], perceived mobility *via* the 12-item MS Walking Scale (MSWS-12), and blood sampling for baseline cytokine measures. Subsequently, the participant completed a battery of clinical assessments and patient-reported outcome measures related to mobility and lower-limb strength. The second session included eccentric exercise training and a second blood sampling to assess the acute cytokine response to the eccentric training bout.

Eccentric exercise training session

The eccentric exercise session comprised 10 exercises concentrating on the strength of the trunk and lower extremities, five exercises each for each body region (Supplemental Material 1). During the execution of the exercises, patients were instructed to emphasize the eccentric phase by performing the movement slowly and in a controlled manner against gravity. The exercises focused on body weight resistance, complemented by therapeutic elastic bands and small weights. A physical therapist supervised the training session. Each eccentric exercise was performed for 2–3 sets with 10 repetitions. Intensity was controlled by a self-reported perceived exertion (RPE) rating *via* the Borg Scale.[22] Adequate training intensity was set by an RPE score between 13 (somewhat hard) and 17 (very hard). Additional modifications to exercise complexity and intensity were made individually by controlling the range of motion, lever arms, motion velocity, and further resistance.

Blood samples and cytokine measures

Venous blood samples were obtained twice by venipuncture: (1) during the morning after an

overnight fast (baseline) and (2) within 30 min after the eccentric training session. The participants refrained from strenuous exercise for at least 2 days before baseline blood sampling. For the pre-menopausal females, blood sampling was conducted in the mid-follicular phase of their menstrual cycle (between days 6–9). Moreover, the blood sample was collected for at least 48 h after any MS-related drug administration to control for the possible impact of the drug on cytokine regulation.[23] Blood samples were drawn from the antecubital vein in a seated position. This blood collection protocol was chosen due to the variation in cytokine dynamics.[24,25] Blood samples remained at room temperature for 30 min. Once the blood had clotted, it was centrifuged at 3000 rpm for 15 min. Serum was then quickly removed, frozen at −20°C, and preserved for assessment. Two weeks later, the sample was moved to a deep freeze at −80°C, where it was kept until needed. Before running the analysis, samples were defrosted on ice. Multiple cytokine expression was measured using Luminex technology. Based on the literature, primary cytokine measures were interferon-gamma (IFN-γ) and IL-6.[13,26–28] Secondary measures were TNF-α, IL-4, IL-10, and IL-17A cytokine levels.

Postacquisition cytokine data processing

Quantitative, multiplexed protein measurements from the serum samples to detect specific cytokines were performed using the ProcartaPlex High Sensitivity Immunoassay Mix and the Max panel kit from the eBioscience division of Affymetrix. ProcartaPlex immunoassays employ magnetic microsphere technology, allowing for the capture and detection of specific analysts from a serum sample. By using the Luminex MAGPIX®, Luminex coorperation, Austin, Texas, USA compact analyzer, simultaneous detection from a single sample of up to 50 protein targets was possible. Analysis was carried out per the manufacturer's protocol. The concentration of samples was calculated by plotting the expected concentration of the standards against the median fluorescent intensity generated by each standard. A 5PL algorithm was used for the best curve fit. Assayed samples were analyzed in accordance with the operation manual of the Luminex MAGPIX instrument. Procedures align with our previous experimental study of the MS population.[29]

Mobility and lower-limb strength tests

Timed Up and Go Test. The Timed Up and Go Test (TUG), a clinical test assessing a person's mobility, requires static and dynamic balance. The study participants wore their usual footwear and any foot assistive device. The starting point was determined after the participant sat in a standard-height chair with their back flush against the chair and arms resting on the armrests. They were then instructed to stand up, walk 3 m, turn around, return to the chair, and sit down. Timing begins when the individuals start to rise and end when they return to the chair and sit back down. Two trials were performed; the average time was considered the final score. The TUG validly measures functional mobility in pwMS.[30]

Five-Repetition Sit-to-Stand Test. The sit-to-stand test (STS) quantifies functional lower-extremity strength. The score was calculated according to the time the participant took to stand from a seated position and return to a sitting position five times. The participants sat with arms folded across their chest with their back against the chair and were instructed as follows: 'I want you to stand up and sit down five times in succession, as quickly as you can, when I say "Go." Be sure to stand up straight and not allow your back to touch the back of the chair between repetitions. Do allow the back of your legs to touch the chair'. The amount of time expended after the fifth repetition when the participant's body touched the chair was documented. Two trials were performed, and the average time was considered the final score. The 10-repetition sit-to-stand test (10STS) was previously validated in PwMS.[31]

Four Square Step Test. The Four Square Step Test (FSST) measures dynamic balance and clinically assesses the person's ability to step over objects when walking forward, sideways, and backward. A square is formed by four canes resting flat on the floor. The participants were instructed to try and complete the sequence as fast as possible without touching the canes, with both feet touching the floor in each square. One practice trial was allowed before administering the test. Two trials were performed, and the better time was used as the preferred score. The FSST has been established as a valid measure of dynamic balance in pwMS.[32]

Two-Minute Walk Test. The Two-Minute Walk Test (2MWT) is a test in which the participants are instructed to walk as far as possible in 2 min. Participants were instructed to walk at their fastest speed and cover as much distance as possible along a 30-m hallway, circling the cones at each end. They were allowed to use their habitual assistive device at each testing session. Psychometric properties of the 2MWT have been established in pwMS.[33]

Perceived mobility. Perceived mobility was measured by the MSWS-12, a valid self-reported questionnaire assessing walking ability in pwMS. This test is the most widely used patient-reported measure of perceived limitation in walking attributable to MS.[34] The MSWS-12 has been used in numerous clinical trials of pwMS due to its excellent psychometric properties.[35,36]

Statistical analysis

Descriptive statistics determined the study sample's demographics, clinical characteristics, mobility metrics, and cytokine values. The Kolmogorov–Smirnov test verified normal distribution. Cytokine data were examined with boxplots, and the data were removed in the event of outlier identification. To test whether the eccentric training session significantly affected the cytokine values, we chose the repeated measure Analysis of Variance (ANOVA) (within-subject factor at 2) controlling for age, gender, and body mass index (BMI). The relationship between cytokine values and clinical mobility measures at baseline was examined by linear regression analysis (stepwise method), performed separately for each cytokine, and defined as the dependent value. Clinical mobility measures (TUG, STS, FSST, 2MWT, MSWS-12), age, gender, and BMI were incorporated into the analysis as independent variables. Correlation coefficient scores between all outcome measures (cytokine level and clinical mobility scores) were analyzed *via* Pearson's correlation coefficient. All analyses were performed using the SPSS software (IBM SPSS Statistics for Windows, Version 28.0; IBM Corp., Armonk, NY, USA). p Values were two-tailed. The level of significance was set at $p < 0.05$.

Results

Twenty-seven pwMS participated in the study, with a mean age of 40.1 (SD = 8.8) years, 13 women and 14 men, a mean disease duration of 8.5 (SD = 8.1) years, and a median EDSS score of 2.5 (1.5–4.5), indicating mild disability. The demographics and clinical characteristics (including patient-reported outcome measures) of the study sample are presented in Table 1. Serum cytokine levels at baseline and following the eccentric exercise session are presented in Table 2 and illustrated in Figure 1. No significant difference was demonstrated in the cytokine concentration values between baseline and immediately after the eccentric training session. This finding was confirmed in all six cytokines included in the study (IFN-γ, IL-6, IL-10, IL-17a, IL-4, TNF-α).

Figure 2 illustrates the correlation scores (rho) between all study outcome measures (cytokine values at rest and clinical mobility scores) collected at baseline. According to the linear regression analysis, the Five-Repetition Sit-to-Stand Test (5STS) test was significantly associated with three (out of six) cytokine values. The 5STS explained 30.3% of the variance associated with IFN-γ ($R^2 = 0.304$, $p = 0.002$), 14.8% of the variance associated with IL-4 ($R^2 = 0.148$, $p = 0.030$), and 13.8% of the variance associated with IL-10 ($R^2 = 0.138$, $p = 0.035$). The TUG was significantly associated with the TNF-α, explaining 23.3% of the variance ($R^2 = 0.233$, $p = 0.007$) and associated with IL-10 (in addition to the 5STS). No significant correlations were found between the clinical mobility measures and IL-17 and IL-6. The results of the linear regression analysis are presented in Table 3.

Discussion

The primary aim of the current study was to examine the acute effect of eccentric training on selected cytokines in pwMS. We found no immediate change occurred in the IL-4, IL-6, IL-10, IL-17a, TNF-α, and IFN-γ levels following an eccentric training session. Nevertheless, a functional measure of lower-limb muscle strength (5STS) correlated with the resting-state values of several cytokines (IL-4, IL-10, TNF-α, and IFN-γ).

Our primary result is in general agreement with the existing literature. Two recent systematic reviews in 2018 and 2022 investigated the effect of exercise on the immune system in pwMS, focusing mainly on aerobic training.[13,37] According to these reviews, three studies

Table 1. Demographics and clinical characteristics of the study sample ($n = 27$).

Variable	Mean (SD)	Range
Age, years	40.1 (8.8)	20.5–51.0
Gender, *n* (%)		
Male	14 (52%)	–
Female	13 (48%)	–
Height (cm)	173.2 (8.5)	156.0–190.0
Weight (kg)	76.0 (18.7)	45.0–120.0
BMI (kg/m^2)	25.2 (5.2)	16.9–39.7
Disease duration (years)	8.5 (8.1)	0.8–26.7
Median EDSS (score)	2.5	1.5–4.5
Clinical tests and patient-reported outcome measures		
Perceived fatigue (MFIS, score)	28.1 (10.7)	4.0–77.0
Perceived mobility (MSWS-12, score)	34.3 (12.8)	12.0–60.0
Timed-Up and Go test (s)	7.2 (2.4)	4.7–15.9
5-Sit-to-Stand test (s)	13.3 (3.3)	6.2–20.5
Four Square Step Test (s)	11.4 (7.8)	5.2–39.5
2-Minute Walk Test (m)	168.0 (39.2)	90.0–240.0

BMI, body mass index; EDSS, Expanded Disability Status Scale; MFIS, Modified Fatigue Impact Scale; MSWS-12, 12-item MS Walking Scale; SD, standard deviation.

Table 2. Comparison of cytokine levels prior to and immediately after the eccentric training session.

Cytokine (pg/ml)	Baseline	Acute response (following the eccentric exercise session)	*p* Value
INF-γ	91.98 (47.2)	92.6 (50.7)	0.441
IL-10	65.5 (84.6)	62.7 (92.1)	0.653
IL-17a	40.8 (18.8)	40.9 (20.9)	0.222
IL-4	613.4 (1215)	556.8 (117.7)	0.728
IL-6	27.2 (43.3)	24.6 (47.0)	0.139
TNF-α	20.27 (12.1)	28.12 (28.6)	0.285

IL, interleukin; INF-γ, interferon-gamma; TNF-α, tumor necrosis factor-alpha.

explicitly focused on resistance training.[38–40] Although these three studies differed in design from the current study (e.g. long-term exercise programs and different time points of cytokine measurements), we find them relevant and present their main findings for comparison. White

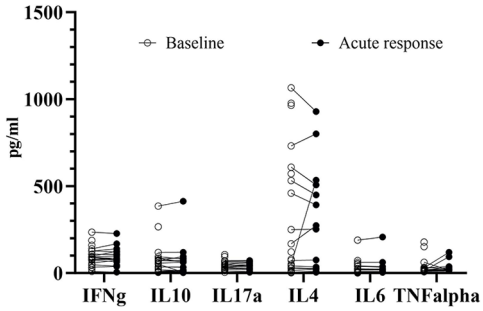

Figure 1. Individual changes in cytokine values following the eccentric training session.

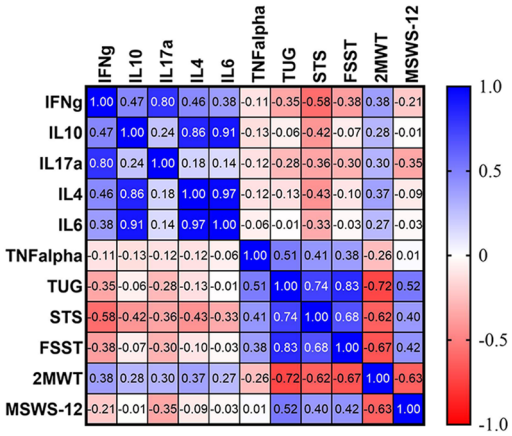

Figure 2. Correlation matrix scores at baseline between cytokine values and clinical mobility parameters.

et al. examined females with MS who participated in an 8-week (twice-weekly) progressive resistance-training program. Posttraining, they observed that IL-4, IL-10, and IFN-γ reduced resting concentrations in the blood, whereas TNF-α, IL-2, and IL-6 remained unchanged.[38] Similarly, Kierkegaard *et al.* reported that 12 weeks (twice-weekly) of high-intensity resistance training decreased the concentration level of TNF-α in pwMS, albeit no significant changes were observed in the concentration levels of IL-6 and IL-17 ($n = 17$). Finally, Kjølhede *et al.*[40] examined the acute and chronic cytokine response to resistance training in 35 pwMS. No differences were observed in the acute response for any measured cytokines (IL-1β, IL-4, IL-10, Il-17F, IL-23, IFN-γ, TNF-α) following a single bout of resistance exercise. These findings align with our current results, demonstrating no immediate significant changes in the cytokine response following an eccentric training session.

Notably, the lack of acute changes in resting cytokine levels does not exclude the possibility that cytokine responses occur during the early phase of posteccentric exercise recovery in pwMS. Although the current study included several key anti-and-proinflammatory cytokines, it was impossible to include all, that is, IL-1, IL-2,

Table 3. Linear regression analysis (stepwise method) showing associations between cytokine level at baseline (dependent value) with clinical mobility measures (independent values) adjusted on covariates.[a]

Cytokine	Variables	Adjusted R^2	*B*	95% CI for *B*	*p* Value
IFN-γ	5STS	0.304	−8.81	−14.08 to −3.54	0.002
IL-4	5STS	0.148	−134.95	−255.40 to −14.49	0.030
TNF-α	TUG	0.233	8.649	2.56 to 14.74	0.007
IL-10	5STS	0.138	−20.73	−34.24 to −7.21	0.035
	5STS and TUG	0.247	19.02	0.381 to 37.66	0.015
IL-17	No variables entered into the equation				
IL-6	No variables entered into the equation				

[a]TUG, 5STS, 2MWT, FSST, MSWS-12, age, gender, and BMI were entered into the linear regression analysis as independent variables.
BMI, body mass index; FSST, Four-Square-Step-Test; IL, interleukin; INF-γ, interferon-gamma; MSWS-12, 12-item MS Walking Scale; 2MWT, Two-Minute-Walk-Test; 5STS, Five-Repetition-Sit-to-Stand-Test; TNF-α, tumor necrosis factor-alpha; TUG, Timed-Up-and-Go Test.

IL-12, and IL-18, which was not evaluated. Furthermore, numerous interactions between cytokines might have changed but were not expressed in our results. Another research feature relates to tissues of cytokine origin. By only measuring plasma concentrations of cytokines, we could not determine from which tissue the cytokines were secreted. Moreover, we could not determine if secretion was genuinely associated with eccentric exercise-induced changes in the immune responses or in energy utilization. Finally, the cytokine values may be affected by differences in the habitual physical activity level of pwMS. To clarify this, future research should devote extra effort to minimizing the potential confounders that influence the cytokine response in pwMS.

A novel finding of our study was the significant relationship between the 5STS test, IL-4, IL-10, and IFN-γ concentration levels. Participants who performed the test faster, indicating improved lower-limb muscle strength, demonstrated a higher resting-state concentration level of IL-4, IL-10, and IFN-γ. Moreover, participants performing better (faster) on the TUG test demonstrated lower TNF-α values. It is worth noting that the TUG includes sit-to-stand and stand-to-sit phases, similar to the 5STS test. Therefore, the inclusion of these two specific tests in the regression model seemed logical. No relationships were noted between the concentration cytokine values and the clinical walking and balance tests.

The cytokines' specific roles in the inflammation process might explain this novel finding. IL-10 is classified as an important anti-inflammatory cytokine, functioning as a critical immune regulator, decreasing excessive Th1.[41] Similarly, IL-4 is a Th2 cytokine with potent anti-inflammatory properties.[42] The primary role of IFN-γ is to potentiate a pro-inflammatory response by activating macrophages to increase phagocytosis.[43] Furthermore, TNF-α is a potent pro-inflammatory agent that regulates many facets of macrophage function.[44]

Merging these statements leads to an interesting finding: improved functional muscle strength indicates the dominance of an anti-inflammatory cytokine resting state in pwMS. Proof of this statement can be seen in a systematic review that demonstrated that resistance training reduces low-grade inflammation.[45] However, since we used a simple, functional clinical test, future research is warranted to explore this finding further *via* advanced measurement tools of muscle strength (e.g. isokinetic device).

This study provides valuable information for the MS community, especially for pwMS engaged in physical activity. However, we acknowledge several limitations of our study. First, a large variability was found in the cytokine level change from baseline to the posteccentric training session, a phenomenon common in cytokine research.[46] Cytokine measurements were taken at baseline and ~30 min following the exercise session. If further testing points had been added (e.g. 6 h later), the cytokine response would have been more distinct. Furthermore, although the present data set included the central cytokines involved in resistance exercise, investigating additional cytokines may increase our understanding of how eccentric training impacts the pro-inflammatory/anti-inflammatory balance. Second, our study was limited to patients with a relapsing-remitting form of the disease, relatively young, and with a mild-moderate disability; therefore, our conclusions cannot be confirmed in progressive pwMS, older patients, or patients with severe disabilities. Additionally, including more physiological fitness measures (e.g. cardiorespiratory endurance and body composition) could have contributed to a deeper understanding of the study findings. In a similar context, using a different measure of intensity than the BORG scale (e.g. heart rate) and/or increasing the training exercise intensity might have initiated a different cytokine response. Finally, the sample size was relatively small; however, our data should serve as sample size calculations for future trials.

Conclusion

Our findings provided preliminary data suggesting that an eccentric training bout does not impact cytokine concentration in the blood and does not boost a pro-inflammatory response; thus, it could be performed on pwMS in a rehabilitation setting. Nonetheless, we found that improved lower-limb muscle strength is related to an anti-inflammatory cytokine resting state in pwMS. Future research is warranted to examine further the impact of a long-term eccentric training program on the innate immune system in pwMS.

Declarations

Ethics approval and consent to participate

The study was approved by the Sheba Institutional Review Board (Ref# SMC-6288-19), consistent with the Declaration of Helsinki. All subjects signed an informed consent form prior to participation.

Consent for publication

MW, who is an author on this article, has provided consented to publication of her images in the Supplemental Material.

Author contributions

Yasmin Alt: Data curation; Formal analysis; Investigation; Writing – review & editing.

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Shay Menascu: Investigation; Resources; Writing – review & editing.

Frank Mayer: Conceptualization; Resources; Supervision; Writing – review & editing.

Alon Kalron: Conceptualization; Methodology; Supervision; Writing – original draft.

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Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

The data supporting this study's findings are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

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Supplemental material

Supplemental material for this article is available online.

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