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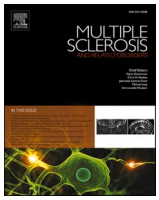

Original article

# Brain-derived neurotrophic factor, neurofilament light and glial fibrillary acidic protein do not change in response to aerobic training in people with MS-related fatigue – a secondary analysis of a randomized controlled trial

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ABSTRACT

*Background:* Neuroinflammation and neurodegeneration are pathological hallmarks of multiple sclerosis (MS). Brain-derived neurotrophic factor (BDNF), neurofilament light (NfL), and glial fibrillary acidic protein (GFAP) are blood-based biomarkers for neurogenesis, axonal damage and astrocyte reactivity, respectively. We hypothesize that exercise has a neuroprotective effect on MS reflected by normalization of BDNF, NfL and GFAP levels.

*Objectives:* To investigate the neuroprotective effect of aerobic training (AT) compared to a control intervention on blood-based biomarkers (i.e. BDNF, NfL, GFAP) in people with MS (pwMS).

*Methods:* In the TREFAMS-AT (Treating Fatigue in Multiple Sclerosis - Aerobic Training) study, 89 pwMS were randomly allocated to either a 16-week AT intervention or a control intervention (3 visits to a MS nurse). In this secondary analysis, blood-based biomarker concentrations were measured in 55 patients using Simoa technology. Changes in pre- and post-intervention concentrations were compared and between-group differences were assessed using analysis of covariance (ANCOVA). Confounding effects of age, sex, MS-related disability assessed using the Expanded Disability Status Scale (EDSS), MS duration, use of disease-modifying medication, and Body Mass Index were considered.

*Results:* Blood samples were available for 30 AT and 25 control group participants (mean age 45.6 years, 71% female, median disease duration 8 years, median EDSS score 2.5). Within-group changes in both study groups were small and non-significant, with the exception of BDNF in the control group (median (interquartile range) -2.1 (-4.7; 0)). No between-group differences were found for any biomarker: BDNF ($\beta$ = 0.11, 95%CI (-3.78 to 4.00)), NfL ($\beta$ = -0.04, 95%CI (-0.26 to 0.18)), and GFAP ($\beta$ = -0.01, 95%CI (-0.16 to 0.15)), adjusted for confounders.

*Conclusion:* Aerobic exercise therapy did not result in statistically significant changes in the tested neuro-specific blood-based biomarkers in people with MS.

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*Abbreviations*

| | |
|---|---|
| ANCOVA | analysis of covariance |
| AT | aerobic training |
| BDNF | brain-derived neurotrophic factor |
| BMI | body mass index |
| CI | confidence interval |
| CNS | central nervous system |
| DMD | disease-modifying drugs |
| DMT | disease-modifying treatment |
| EDSS | Expanded Disability Status Scale |
| GFAP | glial fibrillary acidic protein |
| IQR | interquartile ranges |
| LN | natural logarithm |
| MS | multiple sclerosis |
| NfL | neurofilament light |
| PMS | progressive multiple sclerosis |
| pwMS | people with multiple sclerosis |
| RCT | randomized controlled trial |
| RRMS | relapse remitting multiple sclerosis |
| SD | standard deviation |
| sBDNF | serum brain-derived neurotrophic factor |
| sGFAP | serum glial fibrillary acidic protein |
| sNfL | serum neurofilament light |
| TREFAMS | Treating Fatigue in Multiple Sclerosis research program |
| VEGF | vascular endothelial growth factor |
| VIF | variance inflation factor |

## 1. Introduction

Multiple sclerosis (MS) is an inflammatory, neurodegenerative disease of the central nervous system. The primary pathological characteristics of MS are demyelination and axonal loss (Thompson et al., 2018). As people with MS (pwMS) often experience a disabling impact on their daily functioning and quality of life (Thompson et al., 2018), there is a need for pharmacological and non-pharmacological disease-modifying treatments (DMTs) that modulate both clinical progression and the underlying pathophysiological hallmarks of MS. Exercise interventions are known to be effective in symptom management, improve daily functioning and quality of life (Motl and Pilutti, 2012), while preliminary data suggest that exercise therapy may potentially have disease-modifying effects in MS (Dalgas et al., 2019; Proschinger et al., 2022).

Several theories have been proposed on how exercise therapy might induce disease-modifying effects and attenuate neurodegeneration (Guo et al., 2020; Mahalakshmi et al., 2020). Studies in animal models of MS suggest that exercise interventions may slow demyelinating processes in MS (Guo et al., 2020; Mahalakshmi et al., 2020). Exercise seems to promote an anti-inflammatory environment in the central nervous system via upregulation of anti-inflammatory cytokines and anti-inflammatory M2 microglia, together with downregulation of pro-inflammatory cytokines and pro-inflammatory M1 microglia (Guo et al., 2020; Mahalakshmi et al., 2020). Furthermore, growth factors such as brain-derived neurotrophic factor (BDNF), which is associated with neuroplasticity, neuronal survival and neuronal growth, and vascular endothelial growth factor (VEGF), which has been linked to improved angiogenesis, are upregulated after exercise in animal models of MS (Cotman et al., 2007; De Almodovar et al., 2009; Guo et al., 2020; Mahalakshmi et al., 2020). In addition, exercise therapy might also result in improved mitochondrial function and upregulation of mitochondrial biogenesis, thereby reducing oxidative stress (Mahalakshmi et al., 2020).

Only some of these proposed exercise-induced neuroprotective pathways have been studied in pwMS thus far. Cross-sectional studies in pwMS found that being more physically active or having a better physical fitness resulted in better preserved brain volumes and an improved integrity of the nervous system (Motl et al., 2015; Prakash et al., 2010). Moreover, a longitudinal study demonstrated a trend towards a better preserved total brain volume after a resistance training intervention compared to a waitlist control group (Kjølhede et al., 2018). Results regarding the effect of exercise therapy on the inflammatory status of the CNS are still inconclusive (Negaresh et al., 2018). Growth factors have received more attention in relation to MS and an improved growth factor profile following exercise interventions has been reported (Diechmann et al., 2021; Shobeiri et al., 2022).

Possible pathophysiological responses to treatment interventions in pwMS, such as exercise-induced neuroplastic changes, reduced axonal damage and reduced astrogliosis, can be objectively monitored using blood-based biomarkers (Ziemssen et al., 2019). An extensively studied neuro-specific biomarker in the context of exercise in MS is BDNF, a neurotrophin strongly linked to neuroplasticity. BDNF plays an important role in the survival and growth of neurons and is also associated with synaptic plasticity (Zuccato and Cattaneo, 2009). Another blood-based biomarker of interest is serum neurofilament light chain (sNfL). Neurofilaments are the major component of the axonal cytoskeleton (Teunissen and Khalil, 2012). Existing literature shows that elevated concentrations of sNfL are associated with a MS diagnosis, disease activity, disease progression, and neurodegeneration in MS (Barro et al., 2018; Benkert et al., 2022; Bridel et al., 2019; Kapoor et al., 2020; Khalil et al., 2018). Glial fibrillary acidic protein (GFAP) is a cytoskeletal protein of astrocytes and a biomarker of astrocyte activation (Escartin et al., 2021). Compared to healthy controls, concentrations of GFAP are elevated in pwMS, especially in the progressive subtypes of MS (Axelsson et al., 2011; Ayrignac et al., 2020; Högel et al., 2020). In pwMS, higher concentrations of GFAP were associated with worse disease severity scores, disease progression and brain volume loss (Axelsson et al., 2011; Ayrignac et al., 2020; Högel et al., 2020), suggesting that GFAP might be a potential biomarker of disease progression in MS (Högel et al., 2020).

Blood-based biomarkers are gaining increasing attention in the study of the neuroprotective effects of exercise therapy in rehabilitation patients (D'Ambrosio et al., 2015), and should preferably be assayed in all MS exercise studies (Dalgas et al., 2020). A number of studies have examined the effect of exercise interventions on neuro-specific blood--based biomarkers in pwMS, but the few results available are inconclusive, especially regarding NfL and GFAP (Diechmann et al., 2021; Ercan et al., 2021; Joisten et al., 2021; Shobeiri et al., 2022). Therefore, the aim of this study was to examine the effects of an aerobic training (AT) intervention on blood-based biomarkers (i.e. serum BDNF (sBDNF), sNfL, serum GFAP (sGFAP)) in pwMS. We hypothesize that aerobic exercise training has a neuroprotective effect reflected by increased BDNF and decreased NfL and GFAP levels.

## 2. Material and methods

### 2.1. Design

This is a secondary analysis of the 'Treating Fatigue in Multiple Sclerosis - Aerobic Training' (TREFAMS-AT) study including blood-based biomarkers. The TREFAMS-AT study is a multicenter, single-

blind, randomized controlled trial (Heine et al., 2017). In the current study, changes in sBDNF, sNfL and sGFAP concentrations were compared between an AT and a control intervention. The study was approved by the medical ethical review board of the VU University Medical Center, Amsterdam and conducted in accordance with the declaration of Helsinki and good clinical practice.

### 2.2. Participants

In total, 89 people participated in the TREFAMS-AT study. Inclusion criteria were a definite diagnosis of MS regardless of subtype, age between 18-70 years, Expanded Disability Status Scale (EDSS) $< 6.5$, with severe fatigue (Checklist Individual Strength $\geq 35$) but without severe comorbidity or depression (Heine et al., 2017). All participants gave written informed consent prior to participation. In the current study we analyzed data from a subset of 55 trial participants for whom serum samples were available.

### 2.3. Interventions

Participants were randomized to either high intensity AT or MS nurse consultations. Participants allocated to AT performed training sessions on a cycle ergometer three times a week, consisting of 6 intervals of 3 minutes at 40% of peak power, 1 minute at 60% of peak power, and 1 minute at 80% of peak power, during a period of 16 weeks. In total, 12 sessions were conducted in an outpatient clinic under supervision of an experienced physiotherapist, whereas the remaining 36 sessions were home-based using identical equipment as provided by the study team for the duration of the intervention (Heine et al., 2017) (for details see supplementary: Tidier checklist).

Participants allocated to the MS-nurse control intervention had three 45-minute sessions with an experienced MS nurse over the course of the 16-week intervention period. During these sessions the MS nurse informed participants about MS-related fatigue and patient concerns were discussed. During the intervention period patients were not referred to any other facility for the treatment of their fatigue (Heine et al., 2017) (for details see supplementary: Tidier checklist).

### 2.4. Outcome measures

#### 2.4.1. Clinical scores

Clinical scores including age, sex, body height and weight, duration of MS, EDSS (Kurtzke, 1983), and use of disease-modifying drugs (DMDs) were determined at baseline.

#### 2.4.2. Blood draw and analysis

Blood was collected at two participating study centers in The Netherlands. Blood was drawn via vena puncture pre- and post-intervention between 09.00-17.00 using a BD Vacutainer plastic serum tube (BD, New Jersey, USA). Samples were centrifuged within 1 hour at 2000g for 10 minutes and subsequently stored at -80 $^{o}$ C in polypropylene tubes (Sarstedt, Germany) until analysis.

Before analysis, samples were thawed at room temperature and centrifuged at 10,000g. Subsequently, sBDNF, sNfL and sGFAP concentrations were determined in accordance with manufacturer guidelines using the Simoa BDNF discovery kit (Quanterix, USA), the Simoa NF-light advantage kit (Quanterix, USA) and the Simoa GFAP discovery kit (Quanterix, USA), respectively.

### 2.5. Statistical analysis

Statistical analysis was performed using STATA 14 statistical software (College Station, TX: StataCorp LP). Participant characteristics are presented as means (standard deviations (SD)) or medians (interquartile ranges (IQR)) in case of normal or non-normal distributed data, respectively. Frequencies are presented as number and percentages. Baseline group differences were tested using either an independent samples t-test, chi-square test or Wilcoxon rank sum test. To check selectivity of missing serum from trial participants, differences between the analyzed group and drop-out group were examined using either an independent samples t-test, chi-square test or Wilcoxon rank sum test.

Analysis of covariance was used to determine possible treatment effects of AT versus the MS-nurse control intervention on sBDNF, sNfL, and sGFAP concentrations. To adjust for regression to the mean, the biomarker measured at the post-treatment measurement was adjusted for the baseline value (Twisk et al., 2018). The applied method is based on regression and therefore a normal distribution is assumed. This was verified by visual inspection of the histograms, probability distribution (p-p plot) and by the Shapiro-Wilk test. If assumptions for normality were not met, a natural logarithm transformation (LN) was applied. To adjust for possible confounding effects of age, sex, EDSS, disease duration, use of DMDs and body mass index (BMI), these were added to the model if there was a minimal confounding effect on the regression coefficient of treatment group of 10% using a forward selection procedure. Correlation matrix and variance inflation factor (VIF) were checked for multicollinearity between possible confounders. Assumptions concerning the absence of multicollinearity were met with a correlation of less than 0.7 and/or VIF less than 5.

## 3. Results

### 3.1. Participant characteristics

Between 2011 and 2014, 207 pwMS were assessed for eligibility, of whom 89 participants were enrolled in the TREFAMS-AT study (Heine et al., 2017) (see Fig. 1 with flow chart). Blood samples from 55 participants were available for analysis (Fig. 1). Demographic and baseline characteristics are presented in Table 1. The AT group had a mean age of 43.5 years, with a median MS duration of 7 years, while the MS-nurse control group had a mean age of 48.1 years, with a median MS duration of 12 years. There were no statistically significant differences in demographics or disease characteristics between the AT group and MS nurse group.

Thirty-four participants were not included in the serum analysis. The analyzed and non-analyzed group did not differ significantly for possible confounders (i.e. age, sex, disease duration, EDSS, use of DMDs), with only a trend ($p = 0.06$) found for differences in BMI (i.e. higher BMI in the non-analyzed group compared to the analyzed group). (supplementary material)

### 3.2. Treatment effects on blood-based biomarkers

Serum concentrations of BDNF, NfL, and GFAP at baseline and at week 16 post-treatment, as well as within-group changes and between-group differences in blood-based biomarkers, are shown in Table 2. No statistically significant differences were found in baseline concentrations of sBDNF, sNfL and sGFAP between the AT group and MS-nurse control group.

The AT group showed no statistically significant within-group changes. On average, sBDNF showed a decrease of 1.6 ng/mL (p-value 0.43), sNfL an increase of 0.3 pg/mL (p-value 0.23) and sGFAP an increase of 1.0 pg/mL (p-value 0.65). In the MS nurse group, within-group changes revealed a statistically significant decrease in sBDNF concentrations of 2.1 ng/mL (p-value 0.04), while sNfL and sGFAP showed no statistically significant changes (p-values: 0.77 and 0.17, respectively) (Table 2).

With regards to possible between-group differences, no significant differences were found between the AT and MS-nurse control groups, either in the unadjusted or adjusted analyses. In addition to the small average differences expressed by the regression coefficients, the 95% CI's underlined the large variation in differences between both study groups (Table 3 and Fig. 2).

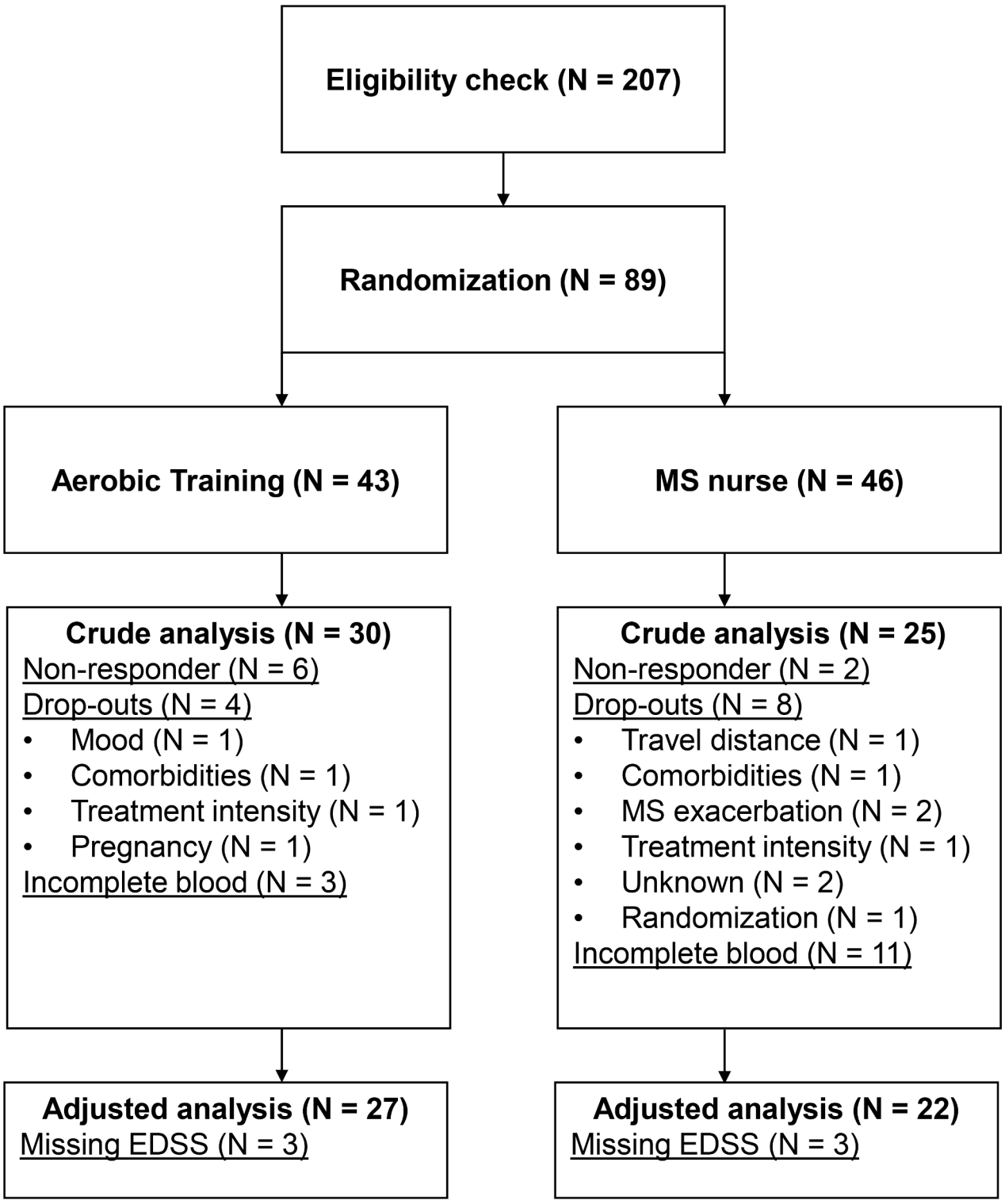


**Fig. 1.** Flowchart for the TREFAMS-AT trial (Heine et al., 2017), adapted to the blood biomarker analyses in this paper.

The adjusted model (with disease duration, DMD use, age, BMI, sex and EDSS as confounders) did not show significant improvements in sBDNF due to AT (0.111 ng/mL (95%CI: -3.782 to 4.004)). When corrected for the confounding effect of disease duration, DMD use, age and sex, sNfL concentrations also showed no significant differences between study groups. After back transformation following LN transformation, the sNfL concentration was 0.964 higher (95%CI: 0.772 to 1.202) in the AT group relative to the MS nurse group, possibly suggesting a decrease of sNfL due to AT, as theoretically expected. sGFAP levels also showed no significant between-group treatment effects. When corrected for confounders (i.e. disease duration, EDSS, DMD use, sex, age and BMI) the concentration of sGFAP in the AT group was 0.995 higher (95%CI: 0.855 to 1.160) relative to the MS nurse group after back transformation. This may suggest an average, albeit non-significant, decrease of GFAP due to AT, in the expected direction.

**Table 1**
Demographics and baseline characteristics.

| | Aerobic Training(N = 30) | MS nurse(N = 25) | p-value |
|---|---|---|---|
| Age (yr) (mean (sd)) | 43.5 (10.1) | 48.1 (10.6) | 0.11[†] |
| Sex (n (%)) | | | |
| - Male | 8 (27) | 8 (32) | 0.67[‡] |
| - Female | 22 (73) | 17 (68) | |
| MS subtype (n (%)) | | | |
| - RRMS | 17 (57) | 16 (64) | 0.32[‡] |
| - PMS | 8 (27) | 8 (32) | |
| - Unknown | 5 (17) | 1 (4) | |
| Disease duration (yr) (median (IQR)) | 7 (4; 9) | 12 (3; 19) | 0.09[§] |
| EDSS[*] (median (IQR)) | 2.5 (2.0; 3.0) | 3 (2.0; 3.5) | 0.35[§] |
| BMI ($kg/m^{2)}$ (median (IQR)) | 23.6 (22.1; 26.7) | 23.3 (21.3; 27.6) | 0.28[§] |
| DMD use (n (%)) | | | |
| - No | 17 (57) | 11 (44) | 0.35[‡] |
| - Yes | 13 (43) | 14 (56) | |

[*] number of missing EDSS values: 3 in AT group and 3 in MS nurse group
[†] independent samples t-test.
[‡] Chi-square test.
[§] Wilcoxon rank sum test
Abbreviations: yr (year), sd (standard deviation), n (number), RRMS (relapse-remitting multiple sclerosis), PMS (progressive multiple sclerosis), EDSS (expanded disability status scale), IQRs (interquartile ranges), BMI (body mass index), DMD (disease-modifying drug).

## 4. Discussion

In this secondary analysis of the TREFAMS-AT trial we examined the effect of a 16-week high intensity aerobic exercise training program on neuro-specific blood-based biomarkers and compared this to the effects of a MS-nurse control intervention in pwMS. Contrary to our hypothesis, we did not find any effects on sBDNF, sNfL and sGFAP of the AT intervention. The average between-group differences of these biomarkers were very small and do not seem of clinical relevance.

### *4.1. Concentrations of blood-based biomarkers*

Existing literature contains little information concerning normative values for these biomarkers, a problem that hampers clinical interpretation of our results, i.e. we do not know whether or not the scores of the patients are within normal range.

Median baseline concentrations of sBDNF in our study were 20.1 ng/mL in the AT group and 21.4 ng/mL in the control group. Previous studies of blood-based BDNF concentrations in people with MS reported values ranging from 1.7 ng/mL to 10678.9 ng/mL, so comparing our results to earlier studies is difficult (Shobeiri et al., 2022).

By contrast, our sNfL data can be compared to our center's in-house data. Baseline sNfL concentrations (8.8 pg/mL in the AT group and 9.8 pg/mL in the MS nurse group) are between the $75^{th}$ and $90^{th}$ percentile as compared to age-matched healthy individuals and between the $50^{th}$ and $75^{th}$ percentile as compared to age-matched MS patients. Compared to recently published reference values in pwMS, the baseline sNfL concentrations in our study fall between the $50^{th}$ and $84^{th}$ percentile for both groups (Benkert et al., 2022).

No reference values are available for GFAP. Median baseline concentrations of sGFAP in our study were 97.2 pg/mL in the AT group and 98.3 pg/mL in the MS nurse group, compared to concentrations of 78.2 pg/mL and 142.0 pg/mL in previous MS studies (Abdelhak et al., 2019; Ayrignac et al., 2020; Högel et al., 2020). Our sGFAP concentrations

**Table 3**
Unadjusted and adjusted ANCOVA outcomes of the effect of aerobic training on blood-based biomarkers.

| | $\beta_1$ coefficient | Standard error | p-values | 95% CI lower | upper |
|---|---|---|---|---|---|
| sBDNF crude model | 0.007 | 1.747 | 0.997 | -3.498 | 3.513 |
| sBDNF corrected model[*] | 0.111 | 1.926 | 0.954 | -3.782 | 4.004 |
| sNfL crude model[†] | 0.040 | 0.104 | 0.705 | -0.169 | 0.249 |
| sNfL corrected model[† ‡] | -0.037 | 0.110 | 0.734 | -0.259 | 0.184 |
| sGFAP crude model[†] | 0.007 | 0.059 | 0.911 | -0.112 | 0.125 |
| sGFAP corrected model[† §] | -0.005 | 0.076 | 0.951 | -0.157 | 0.148 |

The crude models are based on 55 participants; the adjusted models are based on 49 participants. $Y_{post} = \beta_0 + \beta_1 X + \beta_2 Y_{pre}$, with $Y_{post}$ = biomarker measured post-treatment, X = treatment group (0=MS nurse consultation, 1=aerobic training) $\beta_1$ = regression coefficient expressing the overall treatment effect and $Y_{pre}$ = biomarker measured at baseline).
[*] BDNF model corrected for disease duration, DMD use, age, BMI, sex and EDSS.
[†] natural logarithm transformation.
[‡] NfL model corrected for disease duration, DMD use, age and sex.
[§] GFAP model corrected for disease duration, EDSS, DMD use, sex, age and BMI.

**Table 2**
Pre- and post-concentrations of sBDNF, sNfL, and sGFAP, within-group changes and between-group differences.

| | Aerobic Training (N = 30) | | | | MS nurse (N = 25) | | | | AT vs. MS nurse Between-group differences | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Pre Median (IQR) | Post Median (IQR) | Within-group change | p-value | Pre Median (IQR) | Post Median (IQR) | Within-group change | p-value | Crude β (95%CI) | Adjusted β (95% CI) |
| sBDNF (ng/mL) | 20.1 (15.6; 25.0) | 18.2 (14.8; 24.4) | -1.6 (-6.4; 4) | 0.43 | 21.4 (17.4; 28.0) | 21.5 (17.0; 24.1) | **-2.1 (-4.7; 0)** | **0.04** | 0.007 (-3.498 to 3.513) | 0.111 (-3.378 to 4.004) |
| sNfL (pg/mL) | 8.8 (5.3; 14.2) | 7.9 (5.9; 12.5) | 0.3 (-0.6; 1.7) | 0.23 | 9.8 (7.6; 12.2) | 10.3 (6.9;13.7) | 0.3 (-1.7; 2) | 0.77 | 1.041 (0.845 to 1.283)[*] | 0.964 (0.772 to 1.202)[*] |
| sGFAP (pg/mL) | 97.2 (72.8; 137) | 93.3 (68.8; 140) | 1 (-10.5; 8.1) | 0.65 | 98.3 (87.5; 136) | 107 (83.4;137) | -7 (-17; 7.1) | 0.17 | 1.007 (0.894 to 1.133)[*] | 0.995 (0.855 to 1.160)[*] |

Within-group change scores (medians and IQRs) were obtained by subtracting post from pre raw biomarker concentrations. Within-group changes were tested using Wilcoxon signed rank test. Between-group differences were tested using an ANCOVA: $Y_{post} = \beta_0 + \beta_1 X + \beta_2 Y_{pre}$, with $Y_{post}$ = biomarker measured post-treatment, X = treatment group (0=MS nurse consultation, 1=aerobic training) $\beta_1$ = regression coefficient expressing the overall treatment effect and $Y_{pre}$ = biomarker measured at baseline). Significant effects are in bold.
[*] Back transformed by calculating exponential value, transformed 95% CIs that include the value 1.0 indicate non-significance, values $< 1$ indicate a decreased and values $> 1$ indicate an increased biomarker concentration due to AT.
Abbreviations: 95%CI 95% confidence intervals AT aerobic training; IQR interquartile ranges; sBDNF serum brain-derived neurotrophic factor; sNFL serum neurofilament light; sGFAP serum glial fibrillary acidic protein.

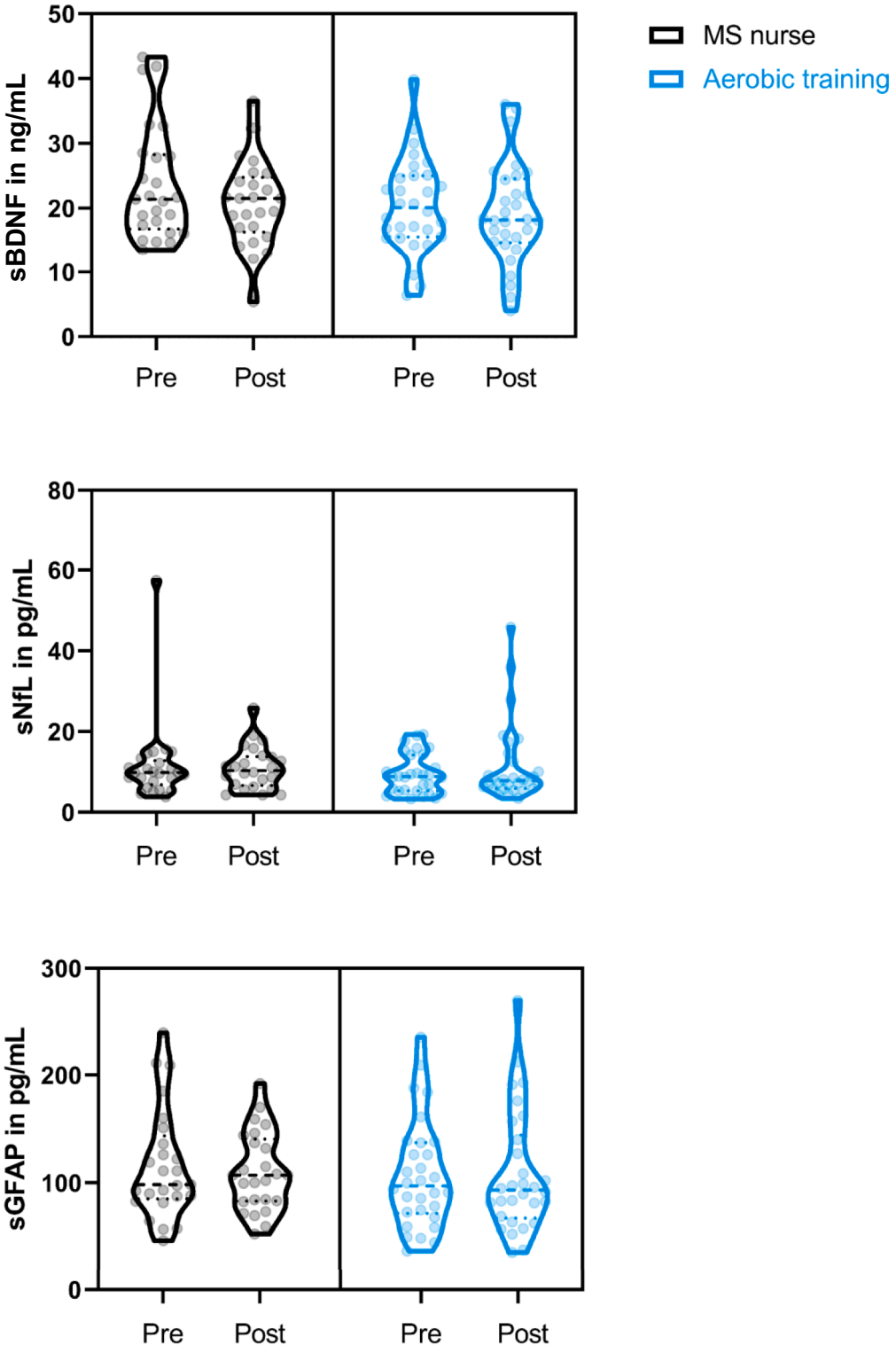


**Fig. 2.** sBDNF (upper panel), sNfL (middle panel) and sGFAP (lower panel) concentrations pre (left in each panel) and post (right in each panel) the 16-week intervention period for MS-nurse control group (black) and AT group (blue). Striped lines represent median scores and dotted lines represent interquartile ranges. (For interpretation of the references to color in the text, the reader is referred to the web version of this article.)

seem to be in line with these studies. Biomarker concentrations can be influenced by disease severity as well as disease subtype (e.g. relapsing remitting MS or progressive MS) (Abdelhak et al., 2019; Ayrignac et al., 2020; Högel et al., 2020), which should be taken into account when comparing studies. The very large differences in biomarker concentrations between studies can also be partly explained by the different measurement techniques used, making one-to-one comparison difficult.

### 4.2. *Exercise-induced changes in biomarker concentrations*

Several studies have investigated the effect of exercise interventions on BDNF in pwMS (Diechmann et al., 2021; Shobeiri et al., 2022). In our

study, we found no significant improvement in sBDNF after AT (adjusted $\beta_1 = 0.11$ ng/mL, 95%CI -3.78 to 4.00 ng/mL). In contrast, two recent meta-analyses found an overall increase in BDNF concentrations after exercise interventions (i.e. smallest mean differences of 0.78 (0.27; 1.28) (based on 9 studies) and 0.26 (0.04; 0.62) (based on 12 studies); 7 studies overlapped between meta-analyses) (Diechmann et al., 2021; Shobeiri et al., 2022), although not all included studies found improvements (Abbaspoor et al., 2020; Briken et al., 2016; Devasahayam et al., 2020; Savsek et al., 2021; Schulz et al., 2004). The differences in type of exercise interventions (e.g. resistance training, AT or combined interventions), duration and intensity have been suggested as possible reasons for these inconsistencies. Previous RCTs, with samples sizes ranging from 22 to 90, have reported significant between-group differences in BDNF following mainly combined exercise interventions (i.e. combining aerobic training, resistance training and/or Pilates) (Banitalebi et al., 2020; Khademosharie et al., 2018; Wens et al., 2016). In line with our data, two studies that focused solely on AT (sample sizes of N = 37 and N = 25) did not report any between-group differences (Briken et al., 2016; Schulz et al., 2004). All of these studies compared an exercise intervention to either a waitlist or sedentary control group (Banitalebi et al., 2020; Briken et al., 2016; Khademosharie et al., 2018; Schulz et al., 2004; Wens et al., 2016). The sample sizes of studies that did not find differences fall between the sample sizes of interventions that found significant differences, so an issue with the power of these studies is unlikely. It seems that changes in BDNF concentrations may be more sensitive to a combination of exercise modalities rather than a single type of exercise.

In contrast to BDNF, sNfL and GFAP have received less attention in relation to exercise interventions in pwMS. One study examined the effect of a 3-week high intensity interval training intervention in comparison to a moderate continuous AT intervention. No between-group differences were found after the exercise intervention period, which is in line with our results (Joisten et al., 2021). In terms of the direction of change, the authors found a 0.5 pg/mL increase in plasma NfL in both groups. As a decrease in NfL concentration is considered an improvement, these AT interventions had no beneficial effect. When considering the within-group changes of sNfL in the present study (i.e. a decrease in the AT group and an increase in the MS-nurse control group), our findings favor the AT intervention. Similarly, another study found a decrease in NfL concentrations of 1.8 ng/mL in the AT group, while the control group (home-based exercise program) showed a decrease of only 0.4 ng/mL (Ercan et al., 2021). Furthermore, this study found significant between-group differences. Overall, we can conclude that the effect of exercise on sNfL levels are still ambiguous and require further research.

To the best of our knowledge, only one study has investigated the effect of exercise on GFAP concentrations in people with MS (Ercan et al., 2021). In the AT group, the authors found a decrease in GFAP concentrations of -272 pg/mL (p-value = 0.02), while no significant change was noted in the control group (-127 pg/mL (p-value = 0.84)). In our study we also found no significant within-group changes in GFAP concentrations (median change scores 1 pg/mL (p-value = 0.65) and -7 pg/mL (p-value = 0.17) for AT and MS nurse groups, respectively). There were also no significant differences in sGFAP between groups, while in the aforementioned study the authors reported a trend (p-value = 0.05) towards a significant improvement in GFAP due to the exercise intervention (Ercan et al., 2021).

### 4.3. Disease-specific or generic effects of exercise

Disease-modifying effects of exercise have not only been proposed in MS but also in other (neurodegenerative) disorders (Mahalakshmi et al., 2020). For example, animal and human studies in Parkinson's disease and dementia suggest that exercise can slow progression, and there are even some indications that exercise might have a disease-modifying effect in these neurodegenerative diseases (Ahlskog, 2018; Johansson et al., 2022; Mahalakshmi et al., 2020).

Exercise-induced upregulation of neurotrophic factors, such as BDNF, has been studied extensively, with increased levels of BDNF identified in people with neurodegenerative disorders, e.g. Parkinson's disease, dementia and mild cognitive impairment, as well as in healthy people (Mackay et al., 2017; Ruiz-González et al., 2021).

In non-MS populations, only a limited number of studies have investigated the effect of exercise on NfL and GFAP. One cross-sectional study in healthy older individuals found an association between the level of physical activity and NfL concentrations (i.e. being more physically active resulted in lower NfL concentrations) (Raffin et al., 2021). Following exercise interventions in animal models of Parkinson's disease and Alzheimer's disease, GFAP concentrations decreased in several parts of the brain, indicating reduced astrogliosis (Dutra et al., 2012; Lee et al., 2014). This raises the question of whether exercise-induced neuroprotective effects are disease-specific or generic.

### 4.4. Strengths and limitations

Although based on the well-designed randomized controlled TREFAMS-AT study, some limitations of this secondary analysis of blood biomarkers have to be mentioned. Firstly, not all of the original blood samples were still available. Statistical analyses were therefore limited to 55 of the original 89 participants. The missing data on 34 participants may not be random (e.g. the non-analyzed group showed a trend towards a higher BMI compared to the analyzed group, p-value = 0.06), possibly biasing random allocation. Secondly, as the TREFAMS-AT study was specifically developed and powered to examine the effect of AT on MS-related fatigue and not on blood-based neuroprotective biomarkers, the sample size may have been too small to detect significant or clinically meaningful effects. Thirdly, the potential role of fatigue in the relationship between exercise and neuroprotective blood-based biomarkers in pwMS is still poorly understood (Aktas et al., 2020). And finally, sampling of blood was performed between 09.00 and 17.00, with fasting prior to sampling not required. This provision might have increased variability in the outcome measures. However, the small within-group changes observed in this study, together with the small between-group differences, does not support sampling effects on outcomes.

In conclusion, exercise is receiving increasing attention as a possible DMT for pwMS (Dalgas et al., 2019; Proschinger et al., 2022). Exercise has been successfully applied in the treatment of MS-related symptoms, such as mobility and balance problems, fatigue, and reduced fitness, and as such is an effective intervention with little or no side effects (Dalgas et al., 2019; Motl and Pilutti, 2012). Furthermore, neuroprotective effects of exercise have been demonstrated in various neurodegenerative disorders such as MS, Parkinson's disease and dementia (Dalgas et al., 2019; Guo et al., 2020; Mahalakshmi et al., 2020).

Nevertheless, we found no exercise-induced changes in sBDNF, sNfL and sGFAP in the current study. Despite this outcome, this research field is still in its infancy and preliminary results to date have been inconclusive (Dalgas et al., 2019). Further research on the possible disease-modifying effects of exercise interventions is needed and possible mechanisms explaining any effects should also be considered. Our goal now should be to advance this field of research by conducting well-designed randomized controlled trials that overcome methodological issues (Dalgas et al., 2020).

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## Availability of data and materials

Datasets are available from the corresponding author on reasonable request.

## CRediT authorship contribution statement

**Arianne S Gravesteijn:** Methodology, Validation, Formal analysis, Data curation, Writing – original draft, Visualization. **Heleen Beckerman:** Conceptualization, Methodology, Data curation, Writing – review & editing, Supervision, Funding acquisition, Project administration. **Eline AJ Willemse:** Validation, Writing – review & editing. **Hanneke E Hulst:** Writing – review & editing. **Brigit A de Jong:** Writing – review & editing, Supervision. **Charlotte E Teunissen:** Resources, Conceptualization, Writing – review & editing, Supervision. **Vincent de Groot:** Resources, Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition.

## Declaration of Competing Interest

HH receives research support from the ZonMW, NWO, ATARA, Biogen, Celgene/BMS, Merck and MedDay and serves as a consultant for Sanofi Genzyme, Merck BV, Biogen Idec, Roche and Novartis, and received honorary from these parties paid to her institution. She serves on the editorial board of Multiple Sclerosis Journal. CT receives research support from the National MS Society (Progressive MS alliance) and Innovative Medicines Initiatives 3TR, has a research contract with Celgene. She serves on editorial boards of Medidact Neurologie/Springer, Neurology: Neuroimmunology & Neuroinflammation. She is editor of a Neuromethods book Springer. VG, HB, AG, EW, BJ declare to have no competing interests.

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## Supplementary materials

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