
# Can resistance training impact MRI outcomes in relapsing-remitting multiple sclerosis?

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**Title Page**

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Keywords:

Multiple Sclerosis; MRI; Rehabilitation; Resistance Training; Cortical Thickness; Brain Volume

**Abstract:**

Background: Multiple sclerosis (MS) is characterised by accelerated brain atrophy, which relates to disease progression. Previous research shows that progressive resistance training (PRT) can counteract brain atrophy in other populations.

Objective: To evaluate the effects of PRT by MRI and clinical measures of disease progression in people with MS.

Methods: This study was a 24-week randomised controlled cross-over trial, including a Training (n=18, 24 weeks of PRT followed by self-guided physical activity) and Waitlist group (n=17, 24 weeks of habitual lifestyle followed by PRT). Assessments included disability measures and MRI (lesion load, global brain volume, percentage brain volume change (PBVC) and cortical thickness).

Results: While the MS functional composite score improved, expanded disability status scale, lesion load and global brain volumes did not differ between groups. PBVC tended to differ between groups and higher absolute cortical thickness values were observed in 19 of 74 investigated cortical regions after PRT. Observed changes were confirmed and reproduced when comparing relative cortical thickness changes between groups for four areas: anterior cingulate gyrus, temporal pole, orbital sulcus and inferior temporal sulcus.

Conclusions: PRT seem to induce an increase in cortical thickness, indicating that PRT have a neuroprotective or even neuroregenerative effect in relapsing-remitting MS.

**Introduction:**

Multiple Sclerosis (MS) is a chronic disease of the central nervous system (CNS). The pathology entails lymphocytic infiltration of the blood-brain barrier, which leads to demyelinating processes inducing focal lesions, which *in vivo* are detectable by magnetic resonance imaging (MRI)[1]. Annually, the total lesion load of the CNS increases on average by 0.8cm^3[2]. Additionally, the pathology of MS involves neurodegenerative processes such as accelerated whole-brain atrophy[3] and cortical thinning[4]. Untreated MS is known to induce an annual loss of 1% of total brain volume compared to an annual loss of 0.1-0.3% in healthy people[5]. Current first choice medical treatments (interferon-ß 1A) is claimed to reduce the annual loss to 0.6%[6].

Over the last decades exercise has become a fundamental part of MS rehabilitation[7, 8]. In accordance, substantial improvements in maximal oxygen uptake[9], muscle strength[10], and quality of life[7] have been observed following differentiated types of exercise training, whereas more recent work also suggest improvements in cognition, fatigue and mood[7, 11]. Moreover, reviews have even suggested that exercise might possess a disease modifying and/or neuroprotective effect for people with MS (pwMS)[12, 13], which potentially could make it an even more crucial adjunct treatment to the well-established medical regimes. This idea was initially based on one study utilising the animal experimental autoimmune encephalomyelitis model[14] and on cross-sectional human studies[15-17]. Also in support of a disease modifying effect of exercise, a review found that the relapse-rate was reduced when comparing intervention and control groups across exercise studies[18]. Two recent 12 week intervention studies in pwMS, a case (n=2) study of aerobic exercise[19], that demonstrated increased hippocampal volume and a pilot randomised controlled trial (RCT) evaluating balance training[20] that observed altered microstructure of the

superior cerebellar peduncles, further stress the need for longer term studies evaluating the effects of intensive exercise on brain structures in pwMS.

Further adding to the study rationale, it has been demonstrated that 12 months of twice weekly resistance training in healthy elderly people can improve cognitive function[21] and reduce white matter atrophy[22]. This concurs with similar results of endurance training in elderly people[23], showing increases in brain volume, in both grey and white matter regions especially when exercise programs extend to six months[24].

To our knowledge no RCT has investigated the effects of a long-term exercise intervention on different measures of disease progression in MS, including clinical and patient reported outcomes. Neither has attractive sensitive and objective surrogate MRI measures of degeneration (and regeneration), such as brain volume changes and focal atrophy[25], been applied in MS exercise studies.

In an earlier report from the present study we showed substantial improvements in neuromuscular function and functional capacity after PRT[26]. This part of the study utilises MRI-measures to investigate the hypothesis that PRT influence CNS inflammation and degeneration. Secondarily, the effects of PRT on clinical measures of disease progression were evaluated.

**Methods:**

*Subjects and design*

Thirty-five relapsing-remitting subjects all treated with interferon-ß, were recruited from the MS Clinic at Aarhus University Hospital and the MS Clinic of Southern Jutland. For inclusion and exclusion criteria see Kjolhede et al.[26]. Participants gave written informed consent and the study was approved by the ethics committee of Region Midtjylland (M-20110178), and registered at ClinicalTrials.gov (NCT01518660).

Following inclusion and baseline testing, subjects were randomised (stratified by gender) to either an immediate training or waitlist group. Allocation was concealed by the sealed envelope principle. The training group immediately underwent supervised long-term PRT for 24 weeks, while the waitlist group continued their habitual lifestyle without commencing PRT. After 24 weeks, the waitlist group underwent the same PRT intervention, while the initial training group was allowed to continue community based self-guided training without supervision of the researchers. The study design is illustrated in Figure 1 and described elsewhere[26]. Each test session was separated by at least 48 hours from the last exercise bout.

[Insert Figure1]

*Training protocol*

Supervised PRT sessions were conducted twice weekly for 24 weeks. Each session consisted of four lower and two upper body exercises. Further details of the training protocol can be found elsewhere[26].

*MRI*

For each patient, MR scans were performed at baseline (T0), after 24 (T24) and after 48 (T48) weeks. All MR measurements were performed on a 1.5-Tesla MRI- scanner (MAGNETOM Avanto, Siemens Medical Systems, Erlangen, Germany). The MR protocol included a 3D Magnetization Prepared Rapid Acquisition Gradient-Echo (MPRAGE) T1 weighted sequence with scan-matrix= 256x192x192, voxel size=0.94x0.94x0.90mm$^3$, field-of-view (FOV)=240x180mm, slice thickness=0.9mm, TR=1900ms, TE=3.4ms, TI=1100ms and total scan time of 5m55s. Next, a transversal dual echo fast spin-echo sequence (T2- and proton density weighted) with the following parameters was performed: scan-matrix=256x204 pixels, voxel size=1.0x1.0 mm$^2$, FOV=256x204mm, number of slices=55, slice thickness=3.0mm, TR=9590ms, TE(proton density)=12ms, TE(T2)=107ms, echo train length=7 and total scan time of 4m59s.

Standard atrophy measures included global volumes, lesion load and PBVC. An assessor blinded to group allocation marked T2 lesions on T2w images, and lesion number and volumes were determined for each patient and timepoint using the software Analyze 10.0 (AnalyzeDirect Inc., KS, USA).

Longitudinal atrophy was assessed by SIENA[27] producing values of PBVC. Brain masks were manually corrected to minimize false tissue assignment by the FSL (Fluid Science Laboratory)-segmentation, if needed.

Freesurfer software (Version 5.2.0) for cortical reconstruction and volumetric segmentation (http://surfer.nmr.mgh.harvard.edu/) was used for the determination of regional cortical thickness. To extract reliable and comparable thickness estimates from all timepoints, images where automatically processed by the Freesurfer longitudinal stream[28]. Specifically an unbiased within-subject template space and image is created using robust, inverse consistent

registration[29]. Several processing steps, such as skull stripping, Talairach transforms, atlas registration as well as spherical surface maps and parcellations are then initialized with common information from the within-subject template, significantly increasing reliability and statistical power[28].

Volumes of global grey and white matter as well as thickness of regional GM were determined. Again, brain masks and white or grey matter segmentations were manually corrected if needed.

*Measures of disease severity*

Expanded Disability Status Scale (EDSS) was at all test-sessions assessed by the same trained neurologist blinded to group allocation. The MS Funtional Composite (MSFC), consisting of a timed 25ft walk test (T25FWT), the paced auditory serial addition test (PASAT) and the 9-hole peg test (9HPT), was performed and analysed in accordance with the administration and scoring manual[30]. Additionally, the MS Impact Scale (MSIS)-29[31] physical and psychological subscales was scored by all subjects.

*Muscle strength*

Isometric muscle strength of the knee extensors and flexors for both legs was assessed in an isokinetic dynamometer (Humac Norm, CSMi, Stoughton, MA, USA), to document the effectiveness of PRT as described elsewhere[26]. Briefly, after standardized instructions (to contract as strongly and fast as possible), two familiarisation trials preceded three to five maximal voluntary contractions (MVC) for both knee extensors and flexors and the contraction showing the

highest MVC for each leg was used for further analysis. A combined muscle strength score ($MVC_{com}$) was calculated from the sum of the knee flexor and extensor MVC.

Functional capacity

As reported earlier[26], the 2 Minute Walk Test (2MWT) and the 5x Sit-To-Stand test (5STS) was also measured. In this paper, correlational analysis with MRI data described below will be provided.

*Statistics*

The power calculations for this study has been described elsewhere[32]. Baseline demographic measures were checked for normality by visual inspection of histograms and subsequently analysed using Student's t-test or non-parametric rank sum tests. Muscle strength, EDSS, MSFC, T2 lesion number and volume) were analysed with a mixed-effects ANOVA for repeated measures and used to detect any group (Training vs Waitlist) x time (T0, T24, T48) interaction. Normality was checked by visual inspection of residuals derived from the ANOVA. If a significant interaction was found, unadjusted post hoc linear pairwise comparisons were performed to test within and between group changes. As the PBVC are change-scores, these data were analysed by paired and unpaired Student's t-test for within and between group changes, respectively. As numerous cortical segments exist, an exploration and validation approach was used here to account for multiple testing. *Exploration*: To assess longitudinal changes in cortical thickness, absolute thickness of each cortical segment was first compared pairwise before and after PRT for all participants (i.e. T0-T24 for the training group, and T24-T48 for the waitlist group)

using paired t-tests. *Validation of findings*: To increase reliability, we compared the changes of thickness in significant regions from waiting and intervention group (baseline to Week 24). *Finally, we investigated if* cortical segments that showed significant changes in exploration and validation were correlated with corresponding relative changes of clinical parameters, muscle strength and functional capacity using linear regression analysis.

Statistical calculations were performed in Stata version 14.1 (StataCorp LP, TX, USA) and R 3.2.0. Statistical significance was set at $p \leq 0.05$.

**Results:**

*Subjects:*

From a total of 244 pwMS, 102 were eligible. From those a total of 35 pwMS with a mean and SD age of 43±8 years, disease duration of 7±7 years, height of 171±8 cm and weight of 75±13 kg. were included in the study. Mean EDSS was 2.9 ranging from 2-4. During the study, six participants dropped out and were excluded (for full flowchart see [26]). Of the remaining 29 included subjects, 10 patients received Avonex, 6 received Extavia, 12 received Rebif and 1 patient received Betaferon. No significant differences in these demographics and clinical characteristics were observed between groups at baseline.

*Clinical outcomes*

A significant group x time interaction was only observed for $MSFC_{TOTAL}$, $MSFC_{T25FWT}$, $MSFC_{9HPT}$ and $MVC_{COM}$. Post hoc tests revealed that both the Training and Waitlist group significantly improved $MSFC_{TOTAL}$ from T0 to T24, with the change in Training superseding that of the Waitlist group ($p<0.05$). From T24 to T48 the Waitlist group (undergoing PRT) showed further improvement, while the Training group maintained their change (during self-guided physical activity). Two subscores of the MSFC, the 9HPT and the T25FWT, demonstrated similar patterns of change: both groups improved after supervised PRT, and the initial Training group was able to maintain their change at T48. The $MVC_{com}$ improved in the Training group following from T0-T24 and was maintained until T48. No interaction was observed for any other measures of disease progression or MSIS subscores. EDSS demonstrated an overall effect of time ($p<0.001$), with post hoc tests showing that all participants had lower EDSS at T48 compared to both T0 ($p<0.001$) and T24 ($p=0.001$). See Table 1.

**[INSERT TABLE1]**

*Structural and volumetric MRI findings*

For T2 lesion count, an overall effect of time was observed (p=0.01), with post hoc test showing that all participants had a mean[95% CI] increase of 0.40[0.001 ; 0.558] in the number of lesions from T0 to T48 (p<0.01). Neither interaction, group or time effect was observed for T2 lesion volume. See Table 1.

No significant PBVC was observed for either group during the PRT intervention, but a trend (p=0.08) towards a larger decrease was observed for the waitlist group (Figure 2). From T24-T48, the Waitlist group (undergoing delayed PRT) showed a similar pattern compared to the former Training group (p=0.13).

**[Insert Figure2]**

*Cortical thickness*

Exploration: When comparing absolute values of cortical thickness before and after PRT of all included participants, 19 out of 74 cortical areas showed a significant absolute increase in cortical thickness (mean 0.03 ± 0.01mm) (Table 2). The highest absolute increase in cortical thickness of 0.048mm was found in the orbital H_Shaped sulcus, while the lowest increase in cortical thickness of 0.018mm was found in the subcentral gyrus and sulcus intrapariet_and_P_trans. There was no significant absolute increase neither in global white or grey matter volume nor volumes of subcortical grey matter structures (p>0.05).

Validation: A significant relative increase in cortical thickness could be reproduced in four of the 19 cortical areas when analysing the absolute increase in trained patients from before to after treatment (Training group T0-T24, Waitlist group T24-T48), as well as the relative increase compared to the Waitlist group during control period T0-T24 (Table 2). Here, the highest relative increase in cortical thickness of 0.032% in the Waitlist group (T24-T48) was found in the temporal inferior sulcus and of 0.04 % in the Training group (T0-T24) in the anterior cingular gyrus and sulcus.

Association with disability: Correlations of changes in clinical measures with cortical thickness changes were calculated. Of the four cortical areas with significant absolute and relative increase in thickness after PRT in the Waitlist and Training group, changes in the anterior cingular sulcus and gyrus (G_and_S_cingul.Ant) showed a significant positive correlation with $MVC_{com}$ and 5STS and a negative correlation with $MSIS_{PSYCHOLOGICAL}$. Furthermore, changes in the orbital H shaped sulcus (S_orbital_H_shaped) showed a significant negative correlation with the $MSIS_{PSYCHOLOGICAL}$ and $MSIS_{PHYSICAL}$ subscales. Finally, changes in the temporal pole (Pole_temporal) showed a negative correlation with EDSS. Neither of the cortical areas were significantly correlated with the 2MWT or the MSFC. See Table 3 and Figure 3.

**[Insert Table2]**

**[Insert Table3]**

**[Insert Figure3]**

**Discussion**

To our knowledge, this is the first study analysing MRI outcome parameters in a longitudinal RCT of MS patients following an intensive long-term exercise intervention. Previously, we reported improved neuromuscular function of the knee extensors and flexors, which translated to improved functional capacity following the intervention[26]. Here, we present MRI data showing a notable trend towards maintenance of total brain volume following 24 weeks of supervised PRT compared to Waitlist controls. Furthermore, PRT induced significant increases in cortical thickness of distinct cortical brain regions. However, MRI-scans also demonstrated an overall minor increase in T2 lesion number. In addition, total lesion volume did not change indicating shrinkage of lesions.

Clinically, 24 weeks of PRT improved muscle strength as well as $MSFC_{TOTAL}$ significantly, while EDSS significantly decreased from T0 to T48 for all participants by 0.4 points, which might be of clinical relevance[33]. The EDSS change could not be directly related to PRT as the statistical analysis did not show a significant interaction, thus preventing us from directly attributing this to the PRT intervention. However, the EDSS have been criticised for being insensitive to change, limiting our possibility of detecting changes in a small sample in the given time-frame. This is also in line with a previous 12-week study of PRT for relapsing-remitting MS patients having a higher mean EDSS[34]. The MSFC constitutes another approach to monitoring disease severity and progression for pwMS by combining two motor and one cognitive test. Of note, $MSFC_{TOTAL}$ improved as a result of PRT compared to the Waitlist group during control period (T0-T24), despite the Waitlist group showing a minor improvement most likely reflecting a learning effect[33]. Consequently, a clear beneficial effect of PRT on MSFC was observed. In support, the Waitlist group improved further after receiving the delayed PRT intervention (T24-T48).

Our study is also the first RCT to evaluate the effects of exercise on standard MRI

measures of brain damage, lesion load accumulation and whole brain atrophy. Whereas we observed no increase in lesion load for either group, lesion number increased on average by 0.4 for all participants throughout the 48 weeks of the research study, which collectively suggest that the existing lesions are shrinking which contrasts the expected MS pathogenesis induced increase in lesion load of 0.8cm^3 pr year[2]. PBVC showed a trend towards a reduced rate of atrophy during the periods, when both groups underwent supervised PRT (T0-T24 for training group, and T24-T48 for the waitlist group), while a normal atrophy rate was observed when participants did not exercise. Collectively, and considering our previous results on circulating cytokines[32], this suggest that PRT have negligible effect on inflammation and thus lesion accumulation, but may affect neurodegeneration, possibly by induction of neurotrophic factors such as brain derived neurothrophic factors through the PGC1-α/FDNC-5/BDNF pathway[35].

However, we acknowledge that both our sample size, highly selected population and relatively short study duration limits our conclusions in this regard. Our results gain support from recent cross-sectional studies in MS patients linking physical activity or aerobic exercise to different brain volumes. One study showed that moderate to vigorous level of physical activity, determined from 7 days accelerometry, was associated with volumes of normalized grey matter, normalized white matter, hippocampus, thalamus, caudate, putamen and pallidum after controlling for sex, age, clinical course of MS, and EDSS[36]. Another study from the same group showed that cardio-respiratory fitness was associated with volumes of the caudate, putamen, pallidum and hippocampus in pwMS[17]. Prakash et al.[15] reported that higher levels of fitness were associated with greater grey matter volume in the midline cortical structures including the medial frontal gyrus, anterior cingulate cortex and the precuneus. Furthermore, higher levels of fitness were associated with higher fractional anisotropy in the left thalamic radiation and right anterior

corona radiate indicating stronger neuronal fiber density.

A novel finding of the present study was increased thickness in specific cortical regions following PRT, when T0-T24 for the Training group and T24-T48 for the waitlist group is combined. This is in line with studies focusing on aerobic exercises in elderly or diseased populations. Using voxel-based morphometry (VBM), Colcombe et al.[37] showed that regular aerobic exercises may cause an increase in regional grey matter volume (GMV) in seniors. Other studies demonstrated cortical changes in exercising Parkinson patients[38] and in practitioners of Tai-Chi[39]. However, the areas of GMV increase included brain structures that are involved in motor function, learning and memory[19]. In our study the most prominent change could be detected in the orbital H shaped sulcus and the temporal inferior sulcus. An explanation might be that different kinds of exercise and motor skill learning are associated with site specific changes in brain morphology, in regions that are related to age and exercise type. This has been demonstrated for various activities and tasks, such as practicing golf, improving one's balancing skills and learning how to juggle *(for a critical review see Hötting & Röder[23]).*

Changes in the cingular anterior gyrus and sulcus as well as the orbital H shaped sulcus showed correlation to changes in three of the clinical measures – namely the two directly targeted by the intervention ($MVC_{com}$ and 5STS) and to the MSISpsychologcal and MSISphysical. The temporal lope showed correlation to the EDSS. However, a two-way interaction was only observed for the $MVC_{com}$.

Although cellular structures such as neurons or glial cells themselves may be highly stable and non-plastic, they are integrated into highly dynamic and plastic neural networks that adapt to environmental and intrinsic changes[40]. To our knowledge, no studies have so far analysed the effect of PRT on cortical thickness. In humans, Pereira et al. demonstrated in vivo correlates of

exercise-induced neurogenesis in the dentate gyrus by measurements of cerebral blood volume confirming the theoretical possibility of angiogenesis underlying plasticity processes[41]. Similarly, one study utilising the EAE animal model of MS has demonstrated that exercise might protect against dendritic spine loss[14] offering another avenue for future studies.

Study limitations include, that despite being a long-term exercise study, the duration and sample size merely classifies our study as a pilot/explorative study (in particularly with regard the MRI outcomes). Especially, the small sample size inhibits the reliable quantification of exercise effect on cortical thickness. Additionally, a highly selected MS population using IFN-based medication and a narrow EDSS-range from 2-4 was investigated.

*Conclusion*

These results suggest a possible restorative effect of PRT on brain structures, but the interpretation should be cautious due to the study duration and sample size. Consequently, longer (years) and larger exercise studies are warranted, to confirm the observed trends, as these findings implicate that exercise might provide an adjunct therapy to the medical treatments.

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**Conflict of interest:**

The study was supported by The Augustinus Foundation, Hestehandler Ole Jacobsens Mindelegat and Biogen Idec. All authors declare no conflict of interest.

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| | Absolute values Mean±SE | | | Interaction (p-level) | Comparison of changes between groups Mean[95%-CI] | |
|---|---|---|---|---|---|---|
| | T0 | T24 | T48 | | Δ(T24 – T0) | Δ(T48 – T24) |
| **EDSS [a.u.]** | | | | | | |
| ***Training (n=17)*** | 2.9±0.2 | 2.9±0.2 | 2.5±0.2 | *0.75* | Non-significant | |
| ***Waitlist (n=12)*** | 2.9±0.2 | 2.8±0.2 | 2.5±0.2 | | | |
| **$MSFC_{total}$ [a.u.]** | | | | | | |
| ***Training (n=17)*** | 0.02±0.18 | 0.43±0.18*** | 0.49±0.19††† | ***0.05*** | 0.26 [0.05; 0.47] | (reference) |
| ***Waitlist (n=12)*** | -0.02±0.18 | 0.14±0.19* | 0.37±0.19**, ††† | | (reference) | 0.18 [-0.05; 0.41] |
| **$MSFC_{9HPT}$ [a.u.]** | | | | | | |
| ***Training (n=17)*** | -0.03±0.23 | 0.46±0.24*** | 0.46±0.24††† | ***<0.05*** | 0.49 [0.09; 0.90] | (reference) |
| ***Waitlist (n=12)*** | 0.03±0.24 | 0.03±0.25 | 0.46±0.25**, †† | | (reference) | 0.43 [0.01; 0.86] |
| **$MSFC_{T25FWT}$ [a.u.]** | | | | | | |
| ***Training (n=17)*** | -0.09±0.24 | 0.36±0.24*** | 0.21±0.24†† | ***<0.01*** | 0.48 [0.20; 0.78] | (reference) |
| ***Waitlist (n=12)*** | 0.09±0.24 | 0.05±0.25 | 0.31±0.25* | | (reference) | 0.41 [0.10; 0.72] |
| **$MSFC_{PASAT}$ [a.u.]** | | | | | | |
| ***Training (n=17)*** | 0.17±0.23 | 0.47±0.24 | 0.28±0.23 | *0.11* | Non-significant | |
| ***Waitlist (n=12)*** | -0.18±0.23 | 0.33±0.23 | 0.77±0.24 | | | |
| **T2 lesion count [#]** | | | | | | |
| ***Training (n=17)*** | 21.5±4.6 | 21.7±4.6 | 21.8±4.6 | *0.26* | Non-significant | |
| ***Waitlist (n=12)*** | 27.0±5.5 | 27.1±5.5 | 27.6±5.5 | | | |
| **T2 lesion load [cm^3]** | | | | | | |
| ***Training (n=17)*** | 3.64±1.08 | 3.38±1.08 | 3.37±1.08 | *0.27* | Non-significant | |
| ***Waitlist (n=12)*** | 4.56±1.29 | 4.49±1.29 | 4.66±1.29 | | | |
| **$MSIS_{PHYSICAL}$ [a.u.]** | | | | | | |
| ***Training (n=17)*** | 40.5±2.8 | 37.2±2.8 | 40.6±2.8 | *0.55* | Non-significant | |
| ***Waitlist (n=12)*** | 39.3±2.9 | 36.6±2.9 | 36.6±3.1 | | | |
| **$MSIS_{PSYCHOLOGICAL}$ [a.u.]** | | | | | | |
| ***Training (n=17)*** | 17.3±1.2 | 17.0±1.3 | 16.8±1.3 | *0.59* | Non-significant | |
| ***Waitlist (n=12)*** | 20.9±1.3 | 19.3±1.3 | 18.4±1.5 | | | |
| **$MVC_{COM}$ [Nm]** | | | | | | |
| ***Training (n=17)*** | 216.0±20.3 | 259.6±20.5*** | 247.5±20.5†† | ***<0.01*** | 34.3 [5.4; 63.2] | (reference) |
| ***Waitlist (n=12)*** | 217.5±20.9 | 226.8±21.2 | 266.8±21.7***,††† | | (reference) | 52.2 [21.8; 82.5] |

***Table 1:*** *Clinical measures. Result of within-group post hoc test compared to previous measurement: *p≤0.05, **p≤0.01, ***p≤0.001. T48 compared to T0 within-group: †p≤0.05, ††p≤0.01, †††p≤0.001. Abbreviations: a.u. = arbitrary units, EDSS = Expanded Disability Status*

*Scale, MSFC = MS Functional Composite, 9HPT= 9 Hole Peg Test, T25FWT= Timed 25ft Walk Test, PASAT= Paced Auditory Serial Addition Test, MSIS = MS Impact Scale. $MVC_{COM}$ = Maximal Voluntary Contraction combined for knee extensors and flexors.*

| CORTICAL REGION | ABSOLUTE CHANGE FROM PRT (N=29) [MM] | P-VALUES UNCORRECTED | MEAN RELATIVE CHANGE [%] | | |
|---|---|---|---|---|---|
| | | | Waitlist, control n=12 (T0-T24) | Waitlist, delayed PRT n=12 (T24-T48) | Training, PRT n=17 (T0-T24) |
| G_and_S_subcentral | 0.018 | *0.048* | 0.001 | 0.011 | 0.003 |
| **G_and_S_cingul.Ant *** | 0.038 | *0.044* | -0.026 | 0.025 | 0.009 |
| G_and_S_cingul.Mid.Ant | 0.026 | *0.024* | -0.011 | 0.010 | 0.011 |
| G_oc.temp_lat.fusifor | 0.022 | *0.024* | 0.000 | -0.002 | 0.016 |
| G_pariet_inf.Angular | 0.020 | *0.026* | -0.009 | 0.013 | 0.004 |
| G_temporal_inf | 0.026 | *0.038* | -0.001 | 0.006 | 0.011 |
| G_temporal_middle | 0.025 | *0.025* | -0.006 | 0.009 | 0.009 |
| Lat_Fis.post | 0.020 | *0.037* | 0.000 | 0.007 | 0.010 |
| **Pole_temporal *** | 0.025 | *0.021* | -0.016 | 0.016 | 0.003 |
| S_circular_insula_sup | 0.027 | *0.024* | -0.009 | 0.016 | 0.008 |
| S_interm_prim.Jensen | 0.028 | *0.038* | 0.000 | 0.016 | 0.013 |
| S_intrapariet_and_P_trans | 0.018 | *0.010* | 0.001 | 0.002 | 0.014 |
| S_oc_sup_and_transversal | 0.020 | *0.014* | -0.001 | 0.004 | 0.016 |
| S_oc.temp_lat | 0.032 | *0.013* | -0.004 | 0.013 | 0.016 |
| **S_orbital.H_Shaped *** | 0.048 | *0.004* | -0.021 | 0.025 | 0.017 |
| S_precentral.inf.part | 0.021 | *0.030* | -0.004 | 0.010 | 0.009 |
| S_precentral.sup.part | 0.031 | *0.001* | 0.003 | 0.010 | 0.018 |
| **S_temporal_inf *** | 0.045 | *0.003* | -0.015 | 0.019 | 0.020 |
| S_temporal_sup | 0.029 | *0.013* | -0.010 | 0.010 | 0.015 |

***Table 2:*** *Freesurfer defined cortical regions demonstrating significant change with groups collapsed (Training, PRT period T0-T24 and Waitlist, delayed PRT T24-T48). * indicate the areas that still demonstrated significant change after comparing relative changes following PRT with control intervention (Validation of findings).*

| | G_and_S_cingul.Ant | Pole_temporal | S_orbital_H_Shaped | S_temporal_inf |
|---|---|---|---|---|
| **EDSS** | *p = 0.144*<br>R = -0.204 | ***p = 0.050****<br>R = -0.311 | *p = 0.242*<br>R = -0.135 | *p = 0.469*<br>R = -0.015 |
| **MS Functional Composite** | *p = 0.528*<br>R = -0.014 | *p = 0.772*<br>R = -0.146 | *p = 0.495*<br>R = 0.003 | *p = 0.770*<br>R = -0.144 |
| **$MSIS_{PSYCHOLOGICAL}$** | ***p = 0.001****<br>R=-0.569 | *P = 0.234*<br>R=-0.141 | ***p = 0.042****<br>R=-0.325 | *p = 0.270*<br>R=-0.119 |
| **$MSIS_{PHYSICAL}$** | *p = 0.051*<br>R=-0.310 | *p = 0.541*<br>R=0.020 | ***p = 0.027****<br>R=-0.360 | *p = 0.597*<br>R=0.048 |
| **$MVC_{com}$** | ***p = 0.030****<br>R = 0.353 | *p = 0.475*<br>R = 0.012 | *p = 0.369*<br>R = 0.065 | *p = 0.538*<br>R = -0.018 |
| **5STS** | ***p = 0.037****<br>*R = 0.337* | *p = 0.278*<br>*R = 0.114* | *p = 0.274*<br>*R = 0.116* | *p = 0.382*<br>*R = 0.058* |
| **2MWT** | *p = 0.189*<br>*R = 0.173* | *p = 0.316*<br>*R = 0.095* | *p = 0.342*<br>*R = 0.081* | *p = 0.417*<br>*R = 0.042* |

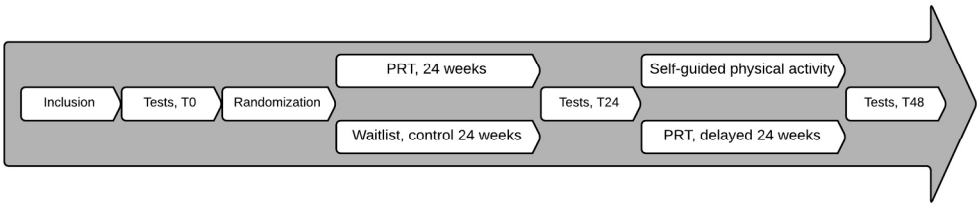
***Table 3:*** *Correlation coefficient (R) and significance level (p) of the major clinical outcomes and the four distinct areas where cortical thickness changed. * indicate significant correlations (p≤0.05). EDSS= Expanded Disability Status Scale. 5STS= 5x Sit-to-Stand. 2MWT= 2 Minute Walk Test*

**Figure Legends:**

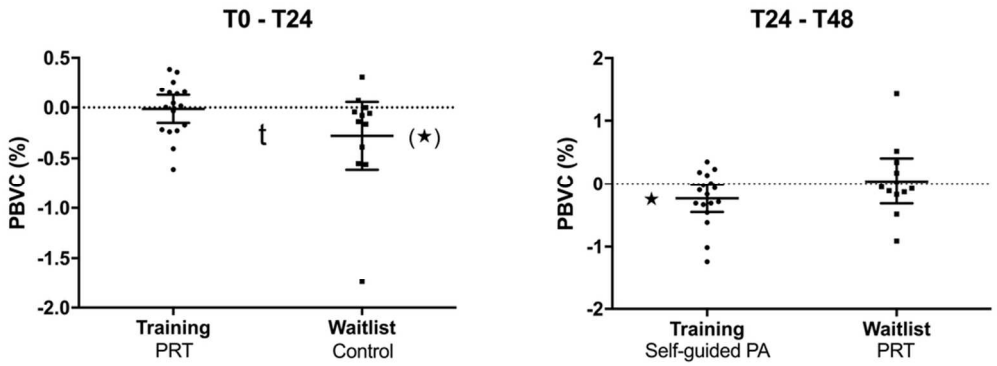
**Figure 1:** *Study design. PRT = Progressive Resistance Training*

**Figure 2:** *Percentage brain volume change (PBVC) after the first 24 weeks (T0 – T24) and the last 24 weeks (T24 – T48) for both Training and Waitlist group. Mean[95% CI] PBVC for Training was -0.01[-0.15; 0.13] following PRT (T0-T24) and -0.23[-0.45; -0.02] (p<0.1 within group, marked with (★)) following the self-guided physical activity (PA) (T24-T48). Mean[95% CI] PBVC for Waitlist was -0.28[-0.61; 0.06] (p<0.05 within group, marked with ★) during control period (T0-T24) and 0.05[-0.32; 0.41] during PRT (T24-T48). A tendency (p<0.1, marked with t) was observed between Training and Waitlist from T0 – T24.*

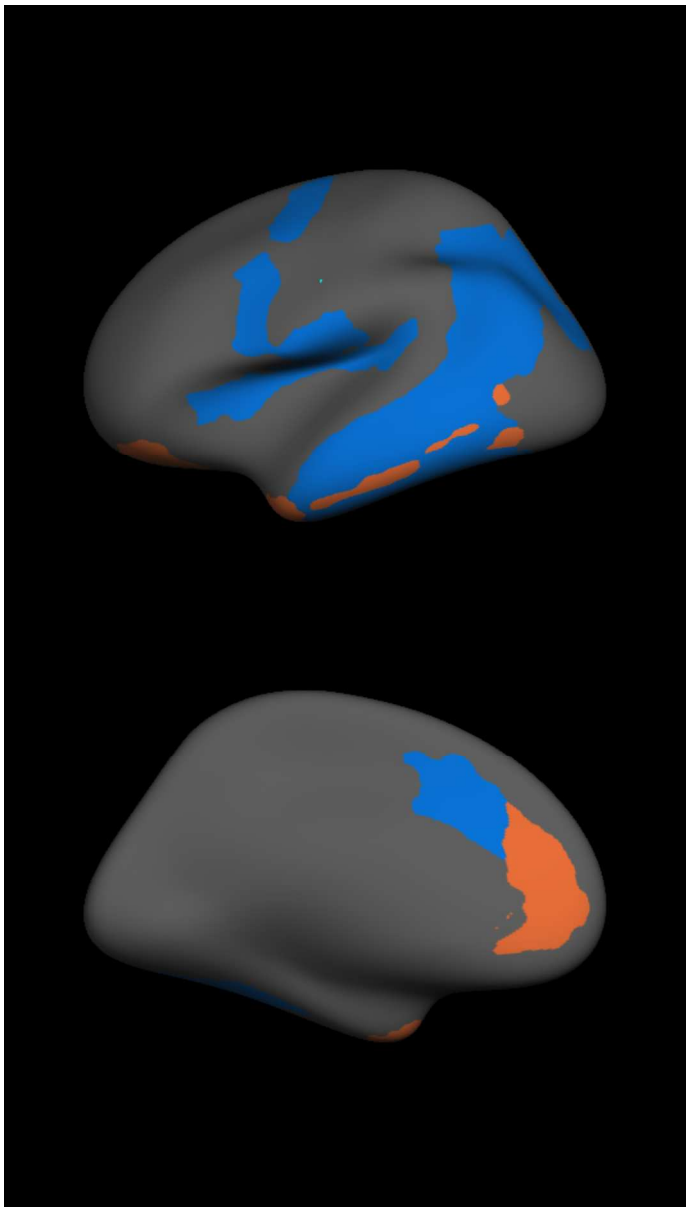
**Figure 3:** Cortical regions demonstrating significant change with groups collapsed (blue, Training, PRT period T0-T24 and Waitlist, delayed PRT T24-T48). Orange regions still demonstrated significant change after comparing relative changes following PRT with control intervention (Validation of findings). Displayed on the Freesurfer cortical surface template (left hemisphere).




189x43mm (300 x 300 DPI)




89x34mm (300 x 300 DPI)



# MS Journal Appendix for MRI methodology

| Hardware | |
|---|---|
| Field strength | 1.5T |
| Manufacturer | Siemens |
| Model | Magnetom Avanto |
| Coil type<br>(e.g. head, surface) | Head |
| Number of coil channels | 16 |

| Acquisition sequence | | |
|---|---|---|
| Type<br>(e.g. FLAIR, DIR, DTI, fMRI) | T1 (MPRAGE), T2 and PD dual-echo fast-spin echo, | |
| Acquisition time | T1: 5m55s<br>T2: 4m59s | |
| Orientation | T1: 3D<br>T2: Transversal | |
| Alignment<br>(e.g. anterior commissure/poster commissure line) | | |
| Voxel size | T1: 0.94x0.94x0.90mm^3<br>T2: 1.0x1.0mm^2 | |
| TR | T1: 1900ms<br>T2: 9590ms | |
| TE | T1: 3.4ms<br>T2: 107ms<br>T2 (PD): 12ms | |
| TI | T1: 1100ms | |
| Flip angle | | |
| NEX | | |
| Field of view | T1: 240x180mm<br>T2: 256x204mm | |
| Matrix size | T1: 256x192x192<br>T2: 256x204 | |
| Parallel imaging | Yes | No |

| Acquisition sequence | | |
|---|---|---|
| If used, parallel imaging method:<br>(e.g. SENSE, GRAPPA) | | |
| Cardiac gating | Yes | **No** |
| If used, cardiac gating method:<br>(e.g. PPU or ECG) | | |
| Contrast enhancement | Yes | **No** |
| If used, provide name of contrast agent, dose and timing of scan post-contrast administration | | |
| Other parameters: | | |

| Image analysis methods and outputs | |
|---|---|
| ***Lesions*** | |
| Type<br>(e.g. Gd-enhancing, T2-hyperintense, T1-hypointense) | T2 lesions |
| Analysis method | Manual marking of lesions |
| Analysis software | Analyze 10.0 |
| Output measure<br>(e.g. count or volume [ml]) | Lesion count and lesion load |
| ***Tissue volumes*** | |
| Type<br>(e.g. whole brain, grey matter, white matter, spinal cord) | Whole brain atrophy and cortical thickness |
| Analysis method | SIENA (on MPRAGE)<br>FreeSurfer |
| Analysis software | SIENA<br>FreeSurfer |
| Output measure<br>(e.g. absolute tissue volume in ml, tissue volume as a fraction of intracranial volume, percentage change in tissue volumes) | Percentage Brain Volume Change<br>Cortical thickness |
| ***Tissue measures (e.g. MTR, DTI, T1-RT, T2-RT, T2*, T2', $^{1}$H-MRS, perfusion, Na)*** | |
| Type<br>(e.g. whole brain, grey matter, white matter, spinal cord, normal-appearing grey matter or white matter) | |
| Analysis method | |
| Analysis software | |
| Output measure | |
| ***Other MRI measures (e.g. functional MRI)*** | |
| Type<br>(e.g. whole brain, grey matter, white matter, spinal cord, normal-appearing grey matter or white matter) | |
| Analysis method | |
| Analysis software | |
| Output measure | |

**Other analysis details:**