ORIGINAL COMMUNICATION

The insula modulates the effects of aerobic training on cardiovascular function and ambulation in multiple sclerosis

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Abstract

Background Impairment of cardiovascular control is common in multiple sclerosis (MS), possibly due to damage of strategic brain regions such as the insula. Aerobic training (AT) targets cardiopulmonary system and may represent a neuroprotective strategy.

Purpose To investigate whether insular damage (T2-hyperintense lesions and volume) is associated with cardiovascular fitness (CF) and influences AT effects in MS.

Methods Sixty-one MS patients were randomized to an AT intervention group (MS-AT) and a motor training control group (MS-C). At baseline and after training (24 sessions over 2–3 months), peak of oxygen consumption (VO2max), heart rate reserve (HRR), 6-min walk test (6MWT) and whole brain and insula MRI data were collected. Two healthy control (HC) groups were enrolled for CF and MRI data analysis.

Results At baseline, MS patients vs HC showed impaired VO2max, HRR and 6MWT ($p < 0.001$) and widespread gray matter atrophy, including bilateral insula. In MS patients, left insula T2-lesion volume correlated with HRR ($r = 0.27$, $p = 0.042$). After training, MS-AT, especially those without insular T2-hyperintense lesions, showed 6MWT improvement ($p < 0.05$) and a stable insular volume, whereas MS-C showed left insular volume loss ($p < 0.001$).

Conclusions By increasing 6MWT performance, our results suggest that AT may improve walking capacity and submaximal measure of CF in MS patients. Such beneficial effect may be modulated by insula integrity.

Keywords Multiple sclerosis · Exercise · Insula · Magnetic resonance imaging · Aerobic training

Introduction

Multiple sclerosis (MS) is one of the most common causes of neurological disability in young adults and its incidence and socioeconomic impact are increasing worldwide [1]. Typical clinical manifestations include locomotor, sensory,

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visual and cognitive impairment; however many other symptoms and signs can occur [2]. Dysfunction of the autonomic system with impairment of cardiovascular function regulation, including attenuated increases in hearth rate (HR) and systolic blood pressure during exercise, has been described in up to 60% of MS patients [3].

Among the brain regions potentially implicated in modulating autonomic system function, the insula represents a key area involved in the regulation of cardiac functions, most likely through its direct projections to the lateral hypothalamic area, the parabrachial nucleus, and the nucleus of the solitary tract [4]. The insula is anatomically and functionally divided into a larger anterior and a smaller posterior lobule by the central sulcus [5]. However, the role of insular sub-regions involved in this autonomic control shows large variations across studies [5–8]. Moreover, some evidence suggests a certain degree of insular lateralization in cardiac representation [7, 8]. Cardiac acceleration has been

correlated with the right insula function, while cardiac deceleration with the left one [6].

Aerobic training (AT) directly targets cardiovascular and pulmonary systems [9]. In healthy controls (HC), AT promotes improvements in cardiopulmonary functions, including lowering HR at rest and increasing maximal oxygen consumption (VO2max) [9]. In MS patients, AT may ameliorate aerobic capacity, fatigue, quality of life and emotional aspects [10]. Some studies have suggested that AT may exert neuroprotective effects in aging [11] and several neurological diseases [12], including MS [13], possibly due to the creation of a better environment for CNS-resident cells [14].

In MS, focal demyelinating lesions and irreversible tissue loss frequently affect the insula and are clinically relevant [15, 16]. A higher T2-hyperintense WM lesion volume (LV) in the left insula was associated with a shift of sympathetic–parasympathetic cardiac modulation toward sympathetic predominance, with increased sympathetic blood pressure modulation [15] and prolongation of QT interval duration [16]. Since the insula modulates the function of the autonomic system, it is tempting to speculate that the accumulation of structural damage in this region in these patients may have a detrimental impact on the regulation of cardiovascular functions and on the beneficial effects promoted by AT.

By evaluating a quite large cohort of MS patients who were randomized to perform AT or a motor training not influencing the cardiopulmonary system, we explored whether focal WM lesions and volume of the insula were associated with cardiovascular functions and whether insula involvement modulated improvements on cardiovascular fitness (CF) and walking performances promoted by AT.

Materials and methods

Subjects

In this secondary analysis of an interventional ongoing study, 61 MS patients (20 relapsing–remitting and 41 progressive) enrolled between 2017 and 2021 were retrospectively analyzed. To be included they had to be between 18 and 65 years old, right-handed, without additional neurologic, psychiatric, orthopedic or rheumatologic diseases and not engaged in structured physical activity for more than 3 times a week. MS patients had to have an Expanded Disability Status Scale (EDSS) < 7.0, be relapse- and steroid free for at least 3 months and on a stable treatment for at least 1 month. Data from two groups of HC without any neurologic diseases or systemic disorders potentially affecting the CNS recruited at our Unit for ongoing studies were also evaluated. The first group ("HC-clinic") ($n = 20$) was selected to analyze baseline cardiopulmonary data, whereas the second one ("HC-MRI") ($n = 60$) was selected for MRI analysis. Two different HC groups were selected since subjects included in the HC-clinic group did not perform the MRI acquisition, while subjects of the HC-MRI group did not have data on CF.

Clinical evaluation

At the beginning and immediately after the training period (see below for details), MS patients underwent clinical evaluation with rating of Expanded Disability Status Scale (EDSS) score, disease duration, clinical phenotype, body mass index (BMI), and cardiopulmonary exercise testing to assess peak of VO2max, maximal peak of HR (HRmax), heart rate reserve (HRR), 6-min walk test (6MWT) and timed 25-foot walk (T25FW). Relapses and adverse events were recorded during the whole training period. At baseline, HC-clinic group underwent the same cardiopulmonary evaluation of patients.

Intervention

MS patients were randomly allocated to two groups: intervention group (MS-AT, $n = 31$) and control group (MS-C, $n = 30$). Both groups performed 24 training sessions of 30–40 min for 2–3 times a week with constant monitoring of HR over a period of 2–3 months. MS-AT performed moderate AT with HR between 55 and 75% of HRmax, while MS-C performed a non-specific motor training in which subjects performed lower limb stretching, passive–active mobilization and balance exercises without a direct involvement of cardiopulmonary system established as no increase of HR during physical effort.

MRI acquisition

Using a 3.0 Tesla Philips Ingenia CX scanner (Philips Medical Systems) and standardized procedures for subjects positioning and repositioning, the following brain MRI sequences were acquired at baseline and immediately after the training for all MS subjects and at baseline for HC-MRI group: (a) sagittal 3D fluid attenuation inversion recovery (FLAIR), FOV = 256 × 256 mm, pixel size = 1 × 1 mm, 192 slices, 1 mm thick, matrix = 256 × 256, TR = 4800 ms, TE = 270 ms, inversion time (TI) = 1650 ms, echo train length (ETL) = 167, acquisition time (TA) = 6.15 min; (b) sagittal 3D T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE), FOV = 256 × 256, pixel size = 1 × 1 mm, 204 slices, 1 mm thick, matrix = 256 × 256, TR = 7 ms, TE = 3.2 ms, TI = 1000 ms, flip angle = 8°, TA = 8.53 min.

MRI analysis

Focal T2-hyperintense WM lesions were identified by a fully automated approach using co-registered 3D FLAIR and 3D T1-weighted images as inputs [17]. T2-hyperintense WM LVs were obtained for each patient from their lesion masks, after a careful visual check of the results provided by the automatic segmentation from expert physicians.

At baseline, normalized brain volume (NBV), normalized GM volume (NGMV) and normalized WM volume (NWMV) were measured on 3D T1-weighted images, after lesion-filling, using the FSL-SIENAx software.

Three masks of bilateral, right and left insula were created merging the six bilateral sub-regions defined by the Human Brainnetome Atlas (http://atlas.brainnetome.org) [18]. T2-hyperintense WM LVs and volumes of right and left insula were extracted by non-linearly registering the insular masks on both FLAIR and 3D T1-weighted images of each subject, respectively.

At follow-up, new T2-hyperintense WM lesions were identified by estimating the difference of lesion mask between follow-up and baseline (after co-registration between the two time-points). Longitudinal percentage brain volume change (PBVC) was also obtained from 3D T1-weighted images using SIENA toolbox (FSL version 5.0.9).

Voxel-based morphometry (VBM) was applied on T1-weighted images to obtain a voxel-based distribution of regional GM atrophy. VBM was performed using SPM12 (Matlab version 2017) and the Diffeomorphic Anatomic Registration using Exponentiated Lie algebra (DARTEL) toolbox [19]. First, T1-weighted images were segmented into GM, WM and cerebrospinal fluid. An initial template was obtained by aligning GM and WM images for all subjects, then refined by non-linear registration including the DARTEL toolbox. Finally, images were spatially normalized to the MNI space, modulated and smoothed with an 8-mm FWHM Gaussian kernel.

Tensor-based morphometry (TBM), as implemented in SPM12, was used to map longitudinal regional volume changes within and between patient groups [20]. The method produces a mid-point average template image after group-wise alignment of each subject's scans [21]. The rate of volume change was quantified from images of the Jacobian determinants as the ratio between volume differences and the volume in the mid-point average template, which was used as reference: negative values indicate tissue volume loss, positive values volume increase.

Statistical analysis

Baseline demographic, clinical and MRI measures and their within-group and between-group longitudinal changes were compared using the χ^2 Pearson test for categorical variables and Mann–Whitney or t test for continuous variables. In MS patients, correlations between baseline clinical and MRI measures were assessed using Spearman's partial correlations (age- and sex adjusted). T2-hyperintense WM LVs were log-transformed before statistical analysis. Longitudinal changes of global variables were expressed as baseline normalized percentages.

Longitudinal differences of clinical variables between MS-AT and MS-C groups were assessed by a 2×2 (group by time) repeated-measures analysis of variance.

To explore the impact of insular lesions on baseline CF, MS patients were dichotomized according to the presence (MS with insular lesions [MS-WL]) or absence (MS without insular lesions [MS-WOL]) of T2-hyperintense lesions.

To understand the role of insular damage on AT effects, differences of cardiopulmonary variables over time between MS patients with and without baseline insular lesions of the two study groups were assessed by a 2×2 (group by time) repeated-measures analysis of variance. Such an analysis was limited to those cardiopulmonary variables that changed after AT or that showed a correlation at baseline with insular damage.

All previous analyses were performed using SPSS software (version 25.0) with p-value < 0.05.

Using a general linear model and the theory of Gaussian fields [22], voxel-wise between-group comparisons of baseline GM volume as well as within-group and between-group longitudinal changes of GM volumes were performed using SPM12 and an analysis of covariance (ANCOVA), including age, sex, and the intracranial volume as covariates. To limit the analysis to the GM, masks obtained from the GM DARTEL templates, transformed to standard space, smoothed and thresholded at 0.25, were used.

Using SPM12, Spearman's partial correlations (age- and sex adjusted) were performed between baseline clinical and MRI measures as well as between clinical and MRI longitudinal changes.

VBM and TBM results were assessed at a threshold of $p < 0.05$, family-wise-error (FWE) corrected for multiple comparisons and at a threshold of $p < 0.001$ uncorrected (cluster extension $kE \geq 10$).

Results

Baseline demographic, clinical and MRI findings

MS patients and HC clinic did not differ for age ($p = 0.08$) and sex ($p = 0.53$), whereas MS patients had impaired VO2max, HRR, 6MWT and T25FW ($p < 0.001$ for all comparisons) (Table 1).

Table 1 Baseline demographic, clinical and MRI variables among MS and HC groups and between MS subgroups

	MS (n=61)	HC-clinic (n=20)	HC-MRI (n=60)	p*	p°	MS-AT (n=31)	MS-C (n=30)	p	MS-WL (n=27)	MS-WOL (n=34)	p
Age Mean (SD) [years]	49.72 (7.57)	45.67 (11.61)	48.57 (8.97)	0.081	0.448	49.06 (7.78)	50.40 (7.42)	0.901	51.78 (5.97)	48.09 (8.36)	0.058
Sex: male/female	23/38	6/14	20/40	0.532	0.615	11/20	12/18	0.716	11/23	12/15	0.427
BMI Median (IQR) [Kg/m²]	22.9 (19.4;15.5)	22.2 (20.5;24.8)	–	0.954	–	22.7 (19.5;25.9)	20.1 (19.36;25.9)	0.241	22.89 (20.2;24.9)	22.74 (19.3;27.1)	0.959
EDSS Median (IQR)	4.5 (3.0;6.5)	–	–	–	–	4.3 (3.0;6.5)	4.0 (3.0;6.5)	0.611	5.5 (4.0;6.5)	4.0 (2.5;6.0)	**0.044**
Disease duration Median (IQR) [years]	18.0 (10.9;25.8)	–	–	–	–	15.9 (10.9;25.6)	19.0 (11.0;25.8)	0.682	23.0 (13.5;27.0)	15.8 (9.5;21.0)	0.053
Phenotype: RR/P	20/41	–	–	–	–	8/23	12/18	0.238	21/6	20/14	0.171
Disease-modifying treatments: no/ first line/second line [n]	7/21/33	–	–	–	–	4/8/19	3/13/14	0.354	1/8/18	6/13/15	0.117
VO2max* Median (IQR) [mL/Kg/min]	15.4 (11.9;18.5)	28.3 (25.6;31.0)	–	**<0.001**	–	15.7 (11.8;18.7)	16.1 (11.8;18.6)	0.831	13.1 (11.6;17.9)	16.45 (11.9;18.9)	0.066
HRR* Median (IQR) [bpm]	51 (32;63)	14 (9;23)	–	**<0.001**	–	53 (34;64)	46 (34;63)	0.171	54 (34;65)	47 (32;63)	0.257
6MWT* Median (IQR) [meters]	324 (185;420)	602 (564;659)	–	**<0.001**	–	314 (175;420)	367 (180;420)	0.163	225 (165;414)	351 (188;420)	0.050
T25FW* Median (IQR) [sec]	6.67 (5.32;10.2)	4.30 (3.85;4.57)	–	**<0.001**	–	7.44 (5.31;10.45)	6.16 (5.31;10.34)	0.262	7.45 (5.48;10.77)	6.15 (5.25;9.92)	0.080
T2-hyperintense WM LV* Mean (SD) [mL]	6.30 (2.82;12.1)	–	0.02 (0.00;0.05)	–	**<0.001**	5.69 (2.76;12.07)	6.45 (2.79;12.12)	0.885	11.81 (6.70;20.80)	2.91 (1.20;6.40)	**<0.001**
NBV* Mean (SD)	1474 (60)	–	1550 (42)	–	**<0.001**	1481 (1433;1518)	1467 (1431;1517)	0.369	1448 (47)	1494 (62)	**0.002**
NGMV* Mean (SD) [mL]	821 (46)	–	859 (35)	–	**<0.001**	826 (801;850)	816 (800;848)	0.404	800 (47)	837 (38)	**0.002**
NWMV* Mean (SD) [mL]	653 (34)	–	691 (28)	–	**<0.001**	655 (631;674)	651 (631;673)	0.643	648 (31)	657 (36)	0.283
Left Insula T2-hyperintense LV* Median (IQR) [mL]	0.00 (0.00;1.00)	–	0.00 (0.00;0.01)	–	**<0.001**	0.00 (0.00;1.50)	0.00 (0.00;1.25)	0.499	2.00 (0.00;18.50)	0.00 (0.00;0.00)	**<0.001**
Right Insula T2-hyperintense LV* Median (IQR) [mL]	0.00 (0.00;3.00)	–	0.00 (0.00;0.01)	–	**<0.001**	0.00 (0.00;2.00)	0.00 (0.00;3.25)	0.089	5.00 (0.00;11.50)	0.00 (0.00;0.00)	**<0.001**
Left Insula volume* Mean (SD) [mL]	6.07 (0.96)	–	7.06 (0.75)	–	**<0.001**	6.64 (5.53;6.74)	6.45 (5.50;6.69)	0.604	5.70 (0.90)	6.37 (0.96)	**0.007**
Right Insula volume* Mean (SD) [mL]	5.54 (0.87)	–	6.38 (0.70)	–	**<0.001**	6.07 (4.87;6.16)	5.81 (4.87;6.05)	0.387	5.22 (0.83)	5.79 (0.82)	**0.009**

Statistically significant comparisons are shown in bold

BMI body mass index, *bpm* beats per minute, *EDSS* Expanded Disability Status Scale, *HC-clinic* healthy control group for baseline cardiopulmonary and clinical comparison, *HC-MRI* healthy control group for baseline magnetic resonance imaging comparison, *HRR* heart rate reserve, *IQR* interquartile range, *Kg* kilograms, *min* minutes, *LV* lesion volume, *mL* milliliters, *MRI* magnetic resonance imaging, *MS* multiple sclerosis, *MS-A* MS group that performed aerobic training, *MS-C* MS group that performed motor control training without direct involvement of cardiopulmonary system, *MS-WL* MS patients with baseline insula lesions, *MS-WOL* MS patients without baseline insula lesions, *NBV* normalized brain volume, *NGMV* normalized gray matter volume, *NWMV* normalized white matter volume, *P* progressive, *p* p-value, *p* p-value, *RR* relapsing–remitting, *SD* standard deviation, *T25FW* timed 25-foot walk test, *VO2max* maximal oxygen consumption, *6MWT* six-minute walk test

Compared to HC-MRI, MS patients were comparable for age ($p = 0.45$) and sex ($p = 0.62$), whereas they showed significant higher brain and insular T2-hyperintense WM LV as well as lower NBV, NGMV, NWMV and bilateral insular volume ($p < 0.001$ for all comparisons) (Table 1).

At baseline, demographic, clinical, cardiopulmonary and MRI measures did not differ between MS-AT and MS-C. In addition, the type of disease-modifying treatments was equally distributed between the two groups of patients ($p \geq 0.163$) (Table 1).

Compared to MS-WOL, MS-WL showed higher EDSS score ($p = 0.044$), lower NBV ($p = 0.002$), lower NGMV ($p = 0.002$), and lower bilateral insular volumes ($p \leq 0.009$). They also showed a trend for older age ($p = 0.058$), longer disease duration ($p = 0.053$), lower VO2max ($p = 0.066$), shorter 6MWT distance ($p = 0.050$) and longer T25FW ($p = 0.080$) (Table 1).

Baseline VBM findings

Compared to HC, MS patients had a widespread pattern of GM atrophy, including sensorimotor, cerebellar, occipital areas, bilateral insula and deep GM nuclei ($p < 0.05$, FWE) (Fig. 1). No significant differences were found between MS-AT and MS-C or between MS-WL and MS-WOL.

Correlations between baseline clinical and MRI findings

In MS patients, a higher left insular T2-hyperintense WM LV correlated with a higher HRR ($r = 0.27$, $p = 0.042$).

A trend towards a correlation between higher right insular T2-hyperintense WM LVs and higher HRR ($r = 0.23$, $p = 0.083$) was also found. No other significant correlations among clinical variables and MRI measures were found (Table 2).

The GM volume of clusters in fronto-temporo-parietal cortices and the cerebellum, but not in the insula, was significantly correlated with cardiopulmonary variables (r values ranging from 0.434 to 0.504) (Table 3).

Longitudinal clinical and MRI findings

All MS patients completed the training period. No relapses or adverse events were recorded during the training period. Table 4 summarizes longitudinal clinical changes in MS subgroups.

At follow-up, all clinical and cardiovascular measures showed a substantial improvement in MS-AT, even though only the 6MWT distance increase was statistically significant (median 6MWT delta = $+5.7\%$, in MS-AT), confirmed by group ($p < 0.01$) and time-group interaction significance level ($p < 0.05$). Within MS-AT, only 6MWT distance
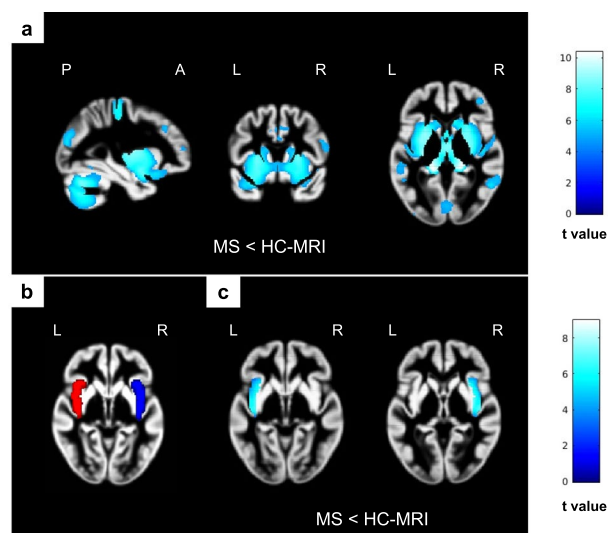

Fig. 1 Baseline voxel-based morphometry results. SPM analysis showing significant differences in GM volume (lower volume encoded in blue/light blue color-coded, $p < 0.05$ family-wise corrected, cluster extent = 10) superimposed on the custom GM template. (**a**) Clusters showing widespread pattern of lower GM volume in MS patients compared to HC-MRI. (**b**) The insula masks used for local VBM analysis (left mask in red and right mask in blue), (**c**) clusters showing lower GM volume within bilateral insula areas in MS compared to HC-MRI. *GM* gray matter, *HC-MRI* healthy control group for baseline magnetic resonance imaging comparison, *MS* multiple sclerosis, *SPM* statistical parametric mapping

showed a significant improvement for MS-WOL (median 6MWT delta = $+6.4\%$) compared to MS-WL (median 6MWT delta = $+3.1\%$) patients ($p < 0.05$) (Table 4).

At follow-up, no new T2-hyperintense WM lesions were found in MS patients and PBVC was $-0.19\% \pm 0.76\%$. PBVC did not differ between MS-AT ($-0.91\% \pm 0.82\%$) and MS-C ($-0.31\% \pm 0.47\%$) nor between MS-WL-AT and MS-WL-C ($p \geq 0.133$). Within MS-WOL, MS-AT showed a lower brain atrophy (mean PBVC = $0.08\% \pm 1.01\%$) compared to MS-C patients ($-0.46\% \pm 0.54\%$), although not significant ($p = 0.067$). Insular volume change did not differ within and between MS-AT and MS-C groups ($p \geq 0.231$).

TBM findings

At follow-up, MS-AT showed increased GM volume in the right middle frontal gyrus and decreased GM volume in cerebellar, temporal and occipital areas. MS-C showed increased GM volume in few clusters within cerebellar and frontal areas and decreased GM volume in several clusters of occipital–temporal, cerebellar and frontal areas as well as bilateral insula ($p < 0.01$, uncorrected). Compared to MS-C, MS-AT showed significant GM volume increase in frontotemporal regions and GM volume decrease in parieto-occipital areas (Fig. 2).

Table 2 Baseline correlations between MRI measures and clinical cardiopulmonary variables in MS patients

		VO2max	HRR	HRrest	MWT6	T25FW
T2-hyperintense WM LV	*r*-value	0.057	− 0.010	− 0.162	− 0.0204	0.094
	p-value	0.668	0.941	0.220	0.125	0.506
NGMV	*r*-value	0.245	0.052	0.170	0.259	− 0.164
	p-value	0.077	0.714	0.199	0.061	0.240
Left Insula T2-hyperintense LV	*r*-value	− 0.219	**0.268**	− 0.079	− 0.028	0.038
	p-value	0.098	**0.042**	0.551	0.837	0.791
Right Insula T2-hyperintense LV	*r*-value	0.074	0.230	− 0.123	0.081	0.112
	p-value	0.579	0.083	0.354	0.547	0.430
Left Insula volume	*r*-value	− 0.480	0.239	0.047	− 0.40	0.148
	p-value	0.719	0.071	0.723	0.768	0.294
Right Insula volume	*r*-value	− 0.069	0.207	0.054	− 0.046	0.148
	p-value	0.606	0.119	0.687	0.743	0.294

Statistically significant comparisons are shown in bold

bpm beats per minute, *HRrest* heart rate at rest, *HRR* heart rate reserve, *IQR* interquartile range, *Kg* kilograms, *min* minutes, *mL* milliliters, *MS-A* Multiple sclerosis (MS) patients that performed aerobic training, *MS-C* MS group that performed motor control training without direct involvement of cardiopulmonary system, *MS-WL-A* MS patients with baseline insula lesions that performed aerobic training, *MS-WOL-A* MS patients without baseline insula lesions that performed aerobic training, *MS-WL-C* MS patients with baseline insula lesions that performed motor control training, *MS-WOL-C* MS patients without baseline insula lesions that performed motor control training, *WM* white matter, *NGMV* normalized gray matter volume, *T25FW* timed 25-foot walk test, *VO2max* maximal oxygen consumption, *6MWT* six-minute walk test

Table 3 Baseline correlations between voxel-based morphometry results and clinical data (*p* < 0.001 uncorrected, cluster extension kE ≥ 10)

Variable	Side	Area	BA	MNI (*x y z*)	kE	*T*-value	*R*-value
VO2max	L	Cerebellum (Crus2)	NA	− 34 − 80 − 34	724	3.98	0.504
	L	Middle frontal gyrus	46	− 39 56 14	26	3.83	0.489
	R	Postcentral gyrus	43	57 − 14 36	74	3.73	0.480
	R	Fusiform gyrus	37	33 − 57 − 9	22	3.64	0.471
	R	Superior temporal pole	48	60 4 3	33	3.59	0.465
	R	Middle temporal gyrus	21	57 − 46 − 3	17	3.54	0.461
	L	Rolandic operculum°	48	− 57 12 4	21	3.5	0.457
		Vermis (7)	18	− 3 − 75 − 18	16	3.38	0.444
HRR	L	Middle frontal gyrus	46	− 32 20 38	43	3.31	0.437
	R	Hippocampus	20	34 − 12 − 22	16	3.21	0.425
6MWT	R	Postcentral gyrus	43	60 − 10 33	310	4.08	0.503
	R	Rolandic operculum	48	62 8 4	81	3.97	0.492
	L	Inferior parietal lobule	2	− 51 − 28 48	56	3.52	0.449
	L	Postcentral gyrus	2	− 44 − 33 50		3.38	0.434
	L	Rolandic operculum	48	− 57 9 4	22	3.48	0.444
	R	Superior parietal lobule	7	26 − 63 54	48	3.46	0.442
T25FW	R	Inferior frontal gyrus (Orb)	11	27 30 − 24	35	3.93	− 0.496
	R	Inferior parietal lobule	40	32 − 46 54	43	3.49	− 0.451

BA Brodmann area, *kE* cluster extension, *L* left, *MNI* Montreal Neurological Institute, *R* right, *T25FW* timed 25-foot walk test, *VO2max* maximal oxygen consumption, *6MWT* six-minute walk test

Table 4 Longitudinal clinical and cardiopulmonary results in MS subgroups, focus on lesion-based groups in those variables where longitudinal changes or baseline correlations were statistically significant

	Group (n)	Timepoint			p-value		
		Baseline	Follow-up	Delta %	Group	Time	Interaction
VO2max Median (IQR) [mL/Kg/min]	MS-AT (31)	15.7 (11.8;18.7)	14.6 (11.3;19.0)	5.5 (−17.5;15.7)	0.91	0.39	0.64
	MS-C (30)	16.1 (11.8;18.6)	15.3 (12.0;15.3)	4.7 (−12.9;13.3)			
HRR Median (IQR) [bpm]	MS-AT (31)	53 (34;64)	52 (38;67)	−2.8 (−21.4;24.8)	0.93	0.29	0.44
	MS-C (30)	46 (34;63)	49 (37;61)	11.7 (−9.9;37.8)			
6MWT Median (IQR) [meters]	MS-AT (31)	314 (175;420)	317 (134;424)	5.7 (−3.3;13.7)	**0.01**	0.89	**0.05**
	MS-C (30)	367 (180;420)	328 (215;443)	1.4 (−13.0;6.1)			
T25FW Median (IQR) [seconds]	MS-AT (31)	7.44 (5.31;10.45)	6.62 (5.50;11.36)	−2.0 (−9.6;8.8)	0.40	0.67	0.09
	MS-C (30)	6.16 (5.31;10.34)	6.81 (5.15;9.71)	0.4 (−6.3;10.2)			
6MWT Median (IQR) [meters]	MS-WL-AT (13)	252 (162;417)	210 (85;340)	3.1 (−3.4;13.5)	**0.04**	0.36	**0.05**
	MS-WOL-AT (18)	347 (188;420)	385 (191;471)	6.4 (3.2;14.9)			
6MWT Median (IQR) [meters]	MS-WL-C (14)	377 (197;414)	340 (193;363)	−5.7 (−14.6;1.8)	0.15	0.15	0.06
	MS-WOL-C (16)	351 (185;420)	370 (267;460)	4.6 (−5.8;6.1)			
HRR Median (IQR) [bpm]	MS-WL-A (13)	64 (37;65)	60 (41;73)	−6.3 (−10.1;23.0)	0.67	0.64	0.49
	MS-WOL-A (18)	46 (34;64)	49 (36;56)	1.1 (−21.8;24.8)			
HRR Median (IQR) [bpm]	MS-WL-C (14)	39 (26;57)	41 (38;56)	2.9 (−8.9;41.9)	0.47	0.84	0.42
	MS-WOL-C (16)	46 (32;63)	51 (31;64)	−0.8 (−8.5;20.4)			

Statistically significant comparisons are shown in bold. Delta% = [(follow-up) – (baseline)/(baseline)] × 100

bpm beats per minute, *HRR* heart rate reserve, *IQR* interquartile range, *Kg* kilograms, *min* minutes, *mL* milliliters, *MS-AT* Multiple sclerosis (MS) patients that performed aerobic training, *MS-C* MS group that performed motor control training without direct involvement of cardiopulmonary system, *MS-WL-AT* MS patients with baseline insula lesions that performed aerobic training, *MS-WOL-AT* MS patients without baseline insula lesions that performed aerobic training, *MS-WL-C* MS patients with baseline insula lesions that performed motor control training, *MS-WOL-C* MS patients without baseline insula lesions that performed motor control training, *T25FW* timed 25-foot walk test, *VO2max* maximal oxygen consumption, *6MWT* six-minute walk test

Correlations between longitudinal clinical and MRI findings

In MS-AT, greater improvement in 6MWT distance was correlated with more limited left anterior insula volume loss ($r = 0.651$, $p < 0.001$ uncorrected). No other correlations were found.

Discussion

By evaluating cardiopulmonary and MRI data from a cohort of MS patients who underwent rehabilitation, we investigated the role of insular damage in modulating CF and training responses in this condition.

At baseline, compared to HC, MS patients showed significantly lower VO2max and 6MWT, higher HRR, especially in the presence of insular lesions, as well as widespread GM atrophy, including the bilateral insula. After training, MS-AT patients showed greater improvement in 6MWT compared to MS-C, especially in MS patients without insular lesions, and a significant association between a longer 6MWT distance and a more limited left anterior insula volume loss.

Our study showed that, compared to HC, MS patients had significantly lower VO2max and 6MWT, higher HRR and a widespread pattern of GM atrophy, suggesting that the disease is associated with worse aerobic fitness and walking capacity [23] together with irreversible neurodegeneration [24].

Consistently with previous literature [15, 16], our study shows a crucial role of insular damage on CF, despite the fact that no correlation has been found with HR at rest, but only with HRR, a measure of heart function during maximal physical effort. In MS patients, higher T2-hyperintense WM LV in the left insula, but not global T2-hyperintense WM LV or volumetric data, was significantly associated with higher HRR, indicating worst cardiopulmonary performance. Moreover, although not reaching the statistical significance, MS patients with insular lesions tended to have lower insular volumes, lower VO2max and shorter 6MWT distance, suggesting that insular damage may negatively influence maximal and submaximal indicators of aerobic fitness. Our results should be interpreted cautiously, since MS patients with lesions had more severe disability and structural brain damage that may also contribute to worse CF. However, they may explain, at least partially, the attenuated
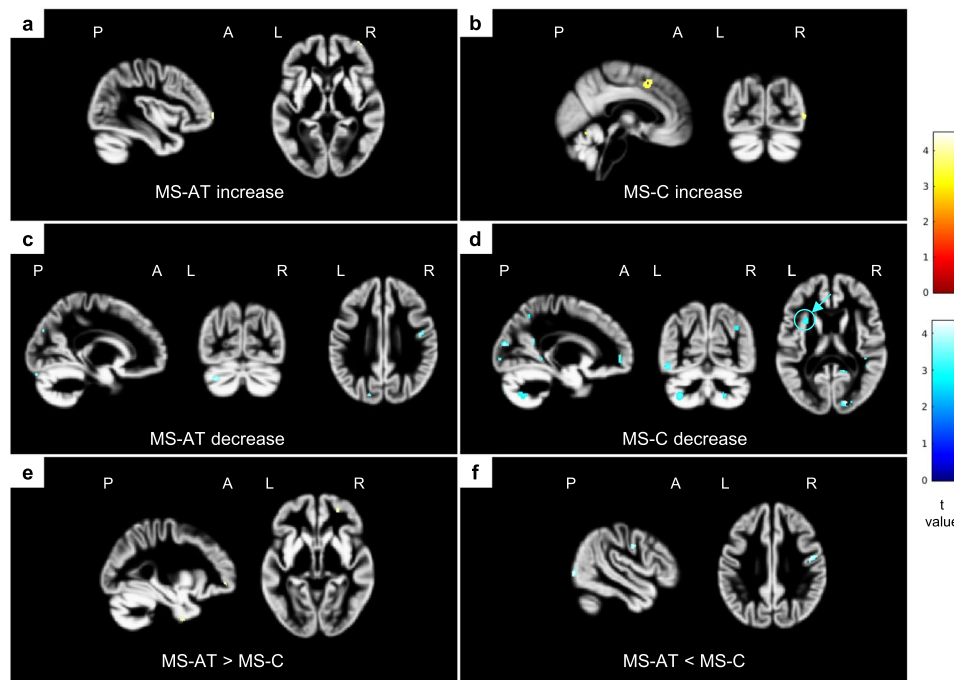
Fig. 2 Longitudinal tensor-based morphometry results. SPM analysis showing significant GM volume changes (increased volumes are encoded in red–yellow, while decreased volumes are encoded in blue–light blue, $p < 0.001$ uncorrected, cluster extent = 10). (**a**) Clusters showing GM volume increase within MS-AT group after training. (**b**) Clusters showing GM volume increase within MS-C group after training. (**c**) Clusters showing GM atrophy within MS-AT group after training. (**d**) Clusters showing GM atrophy within MS-C group after training. (**e**) Clusters showing greater GM volume increase in MS-AT compared to MS-C. (**f**) Clusters showing greater GM atrophy in MS-AT compared to MS-C. *GM* gray matter, *MS* multiple sclerosis, *MS-AT* MS group that performed aerobic training, *MS-C* MS group that performed motor control training without direct involvement of cardiopulmonary system, *SPM* statistical parametric mapping. The arrow in (**d**) points out the atrophy process experienced by MS-C within left insula area

cardiovascular system response to exercise that is typically seen in MS patients compared to HC [3].

Disappointingly, insular volume was not associated with CF. The inclusion of MS patients with heterogeneous disease course and severity and the small sample size may contribute to explain this negative finding. However, it is tempting to speculate that insular focal WM lesions may be more relevant than atrophy in influencing CF. A disconnection of the insula from other brain regions involved in cardiac autonomic control may be one of the underlying phenomena. This process has been described in other neurological conditions, such as Parkinson disease [25], and it has been shown to contribute to cognitive impairment in MS [26].

Our analysis of the effects of AT confirmed that this rehabilitation strategy may significantly ameliorate aerobic and walking capacity in MS patients [27]. Indeed, MS-AT patients showed a significant improvement in 6MWT compared to MS-C and a trend toward improvement in all the other variables. It is noteworthy that the insula showed a key role in modulating the effects of AT. In particular, MS patients without insular lesions experienced a greater improvement in cardiopulmonary and walking capacity (i.e., 6MWT) after AT compared to those having insular lesions.

It is also interesting that in patients who did not perform AT the presence of insular lesions led to a faster process of cardiopulmonary deconditioning over a period of three months (6MWT: −5.7% vs +4.6%, HRR: +2.9% vs −0.8%). Accordingly, the presence of insular lesions seems to impair the physiological improvement of cardiovascular fitness promoted by AT and may worsen CF in those MS patients not performing aerobic activity.

Consistently with previous studies [28], following rehabilitation both groups of MS patients showed dynamic volume modifications of GM areas involved in sensorimotor control, visual processing, and cognitive functions. Several physiological processes, such as synaptic sprouting and plasticity, angiogenesis, as well as changes at axonal, myelin, and non-neuronal cell levels, may concur to explain these volumetric changes following rehabilitative trainings [29]. In line with previous studies [23, 27], these longitudinal MRI results endorse the possible neuroprotective role of AT with a reduced atrophy progression rate in MS patients who performed this type of training.

Noteworthy, longitudinal volumetric analysis further supported the relevant interplay between insular structural integrity, CF modulation and response to AT. MS patients

who performed AT did not experience insular atrophy and their greater improvement in 6MWT distance was correlated with a more limited progression of insular volume loss. Conversely, MS patients who performed motor training not involving the cardiopulmonary system showed a significant progression of left insular atrophy.

The prevailing role of the left compared to the right insula is in line with previous literature [30, 31] showing a stronger link between left rather than right insular lesions after a stroke and cardiovascular dysfunction in human and experimental models, supporting a lateralization of functions in the insula [6]. Moreover, the dominant hemisphere might be more vulnerable to the accumulation of damage for increased excitability and consequent overuse [32].

Our study is not without limitations. We evaluated a relatively small number of MS patients with some heterogeneities in the training protocol, thus possibly limiting our statistical power. However, given the nature of the study, the concomitant COVID-19 pandemic and the time commitment required, it was difficult to enroll a desirable larger number of subjects. For these reasons, our exploratory results should be confirmed on a larger sample. The lack of clinical and MRI longitudinal comparisons from matched HC did not allow to explore the potential effect of different training on HC's CF and brain structure. Finally, although cortical lesions have been consistently reported in the insular cortex, a specific MRI sequence for their assessment was not available for our patients.

In conclusion, this study provides evidence that AT is an effective tool to improve cardiovascular function and walking capacity and to exert neuroprotective effects in MS patients. The insula, especially the left one, can modulate CF and AT responses in MS patients, thus its integrity should be taken into account to optimize the efficacy of AT treatments. Determining insula integrity using MRI can be informative of the possible results the patient can expect from moderate AT. However, further studies are needed to determine the best tailored treatment for patients also taking into account the insula status.

Declarations

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