Cortical grey matter sodium accumulation is associated with disability and secondary progressive disease course in relapse-onset multiple sclerosis

WJ Brownlee MD, PhD[1], BS Solanky PhD [1], F Prados PhD[1,2], MC Yiannakas PhD[1], P Alves Da Mota MSc[1], F Riemer PhD[3], MJ Cardoso PhD[2], S Ourselin PhD[2], X Golay PhD[4], CAM Gandini Wheeler-Kingshott PhD[1, 5, 6], O Ciccarelli MD, PhD [1, 7]

[1] Queen Square MS Centre, Department of Neuroinflammation, UCL Institute of Neurology, London, United Kingdom

[2] Translational Imaging Group, Centre for Medical Image Computing (CMIC), Department of Medical Physics and Bioengineering, University College London, London, United Kingdom

[3] Department of Radiology, University of Cambridge School of Clinical Medicine, Cambridge, United Kingdom

[4] Department of Brain Repair and Rehabilitation, UCL Institute of Neurology, London, United Kingdom

[5] Brain MRI 3T Research Centre, IRCCS Mondino Foundation, Pavia, Italy

[6] Department of Brain and Behavioural Sciences, University of Pavia, Pavia, Italy

[7] National Institute for Health Research (NIHR) University College London Hospitals Biomedical Research Centre, London, United Kingdom

Corresponding author:
Dr Wallace Brownlee

NMR Research Unit, Queen Square Multiple Sclerosis Centre, UCL Institute of Neurology, Box 112, London WC1B 5EH, United Kingdom

w.brownlee@ucl.ac.uk+442031087409

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ABSTRACT

Objective: Sodium (^{23}Na)-MRI is an emerging imaging technique to investigate *in-vivo* changes in tissue viability, reflecting neuroaxonal integrity and metabolism. Using an optimised ^{23}Na-MRI protocol with smaller voxel sizes and improved tissue contrast, we wanted to investigate whether brain total sodium concentration (TSC) is a biomarker long-term disease outcomes in a cohort of patients with relapse-onset multiple sclerosis (MS), followed from disease onset.

Methods: We performed a cross-sectional study in 96 patients followed up ~15 years after a clinically isolated syndrome (CIS), and 34 healthy controls. Disease course was classified as CIS, relapsing-remitting MS or secondary progressive MS. We acquired ^{1}H-MRI and ^{23}Na-MRI, and calculated the TSC in cortical grey matter, deep grey matter, normal-appearing white matter and white matter lesions. Multivariable linear regression was used to identify independent associations of tissue-specific TSC with physical disability and cognition, with adjustment for tissue volumes.

Results: TSC in all tissues was higher in MS patients compared with healthy controls and patients who remained CIS; with differences driven by secondary progressive MS patients. Higher cortical grey matter TSC was independently associated with Expanded Disability Status Scale (R^2=0.26), timed 25-foot walk test (R^2=0.23), 9-hole peg test (R^2=0.23), Paced Auditory Serial Addition Test (R^2=0.29), Symbol Digit Modalities Test (R^2=0.31) and executive function (R^2=0.36) test scores, independent of grey matter atrophy.

Conclusions: Sodium accumulation in cortical grey matter reflects underlying neuroaxonal metabolic abnormalities relevant to disease course heterogeneity and disability in relapse-

onset MS. TSC and should be considered as an outcome measure in future neuroprotection trials.

INTRODUCTION

Energy failure is thought to be central to the pathophysiology of multiple sclerosis (MS), and may provide a link between demyelination and neurodegeneration[1], the two pathological hallmarks of MS. Following inflammatory demyelination, compensatory electrophysiological changes occur in demyelinated axons in order to preserve function, at least in the short-term, with up-regulation and redistribution of sodium channels in order to maintain conduction.[2] The high energy requirements of the Na^+/K^+-ATPase pump, potentially compounded by primary mitochondrial dysfunction in MS[3], overwhelm neuronal ATP production, resulting in failure of the Na^+/K^+-ATPase pump and intracellular sodium accumulation. Increased intracellular sodium concentration triggers a cascade of events that ultimately results in neuroaxonal loss, the major substrate for disease progression in MS.

Sodium (^{23}Na) MRI is an emerging imaging technique able to quantify brain tissue sodium concentration *in-vivo*, providing information on tissue viability, reflecting neuroaxonal integrity and metabolic function.[4 5] The measured total sodium concentration (TSC) using ^{23}Na-MRI reflects a composite of intracellular sodium (10-15mM) and extracellular sodium (~140mM). Previous ^{23}Na-MRI studies have found evidence of increased TSC in white matter (WM) lesions and normal-appearing tissues in MS[6-10], reflecting either increased intracellular sodium, expansion of the extracellular space (due to neuroaxonal loss), or both. In cross-sectional studies, increases in TSC are most marked in progressive MS[8] and in patients with greater physical disability[7 8] and cognitive impairment[10].

^{23}Na-MRI is challenging due to the lower tissue concentrations of sodium compared with hydrogen, and a lower signal-to-noise ratio. Previous studies have been affected by hardware/software limitations of clinical scanners and have acquired ^{23}Na-MRI with large voxel sizes ($4x4x4mm^3$)[6-10], thereby increasing partial volume contamination, especially from

cerebrospinal fluid (CSF), which contains a higher sodium concentration (~140mM) than tissues. We recently optimised a ^{23}Na-MRI protocol with smaller voxel size (3x3x3mm^3 vs 4x4x4mm^3 in previous studies), and a 3D-cones acquisition trajectory technique with improved tissue contrast, signal-to-nose ratio and reproducibility, in clinically-acceptable scanning times.[11]

We applied this optimised ^{23}Na-MRI protocol in a cross-sectional study involving a unique and homogeneous cohort of patients followed over ~15 years following presentation with a clinically isolated syndrome (CIS). The aims of the study were: (1) to investigate the relationship of tissue TSC with long-term disease course in a group of patients well-matched for disease onset and (2) to investigate the relationship of tissue TSC with physical disability and cognitive impairment.

METHODS

Patients

This is a cross-sectional study carried out at the last visit of a longitudinal clinical-MRI study. Briefly, patients were initially recruited into the study within 3 months of a CIS and invited to return for scheduled clinical and MRI follow-up, irrespective of clinical status.[12] The clinical and MRI assessments presented here were done ~15 years after CIS. We diagnosed MS using the McDonald 2010 criteria at follow-up.[13] Disease course at ~15 years was classified as CIS, relapsing-remitting MS (RRMS) and secondary progressive MS (SPMS).[14] We retrospectively reviewed the clinical details and MRI scans of patients diagnosed as CIS in light of the revised McDonald 2017 criteria and no additional patients were identified as having MS.

We assessed physical disability using the Expanded Disability Status Scale (EDSS), timed 25-foot walk test (TWT) and 9-hole peg test (9HPT).[15] [16] The TWT raw scores were transformed into an inverse to determine walking speed (in feet/second) and 9HPT times were transformed into a *Z*-score using normative values.[16] Cognition was assessed using: (1) tests of verbal and visual memory from the Adult Memory and Information Processing Battery[17]; (2) tests of information processing speed, the Paced Auditory Serial Addition Test (PASAT)[16] and the Symbol Digit Modalities Test (SDMT)[18]; and (3) tests of executive function, the Hayling and Brixton tests.[19] Premorbid intellectual function was estimated using the National Adult Reading Test (NART).[20] The raw test scores for each of the cognitive tests were transformed into a *z*-score using published age-matched normative data.[16-19] Patients with a *z*-score ≤ -2 were considered impaired on that test. Self-reported fatigue was measured using the Fatigue Severity Scale.[21] None of the patients had a relapse or received treatment with corticosteroids within 3 months of the clinical assessment and MRI.

A group of healthy control subjects with no known neurological disorder underwent the same MRI protocol. All subjects provided written informed consent and the study was approved by the institutional Research Ethics Committee.

MRI acquisition

The MRI scans were obtained on the same 3.0 T scanner (Achieva, Phillips Healthcare Systems). Using a 32-channel receiver head coil the following ^{1}H-MRI scans were obtained: (i) a proton density(PD)/T2-weighted fast-spin multi-echo scan for identification of T2-hyperintense lesions; (ii) a 2D T1-weighted spin echo scan for identification of T1-hypointense lesions; and (iii) a magnetization-prepared 1mm^3 isotropic 3D T1-weighted turbo field echo scan for tissue segmentation. The 32-channel head coil was changed to a ^{23}Na TX/RX volume body coil (RAPID Biomedical, Germany) and the subject was returned to

the scanner with two calibration agar phantoms placed in a pre-defined position, containing NaCl with a sodium concentration of 40mM and 80mM respectively. The NaCl concentration in the calibration phantoms was chosen to encompass the range of physiological values expected in brain parenchymal tissues. Following the ^{23}Na-MRI with the subject remaining in the same position, a low-resolution ^{1}H-PD/T2-weighted scan was obtained using the body coil, for registration purposes. Details of the MRI acquisition protocol are shown in Supplementary Table 1. Representative images from a healthy control subject and each of the patient groups studied is shown in Figure 1.

Image analysis

A single rater (WJB) manually outlined T2-hyperintense and T1-hypointense white matter lesions on using a semi-automated edge-finding tool (JIM6.0, Xinapse Systems, UK) to obtain T2 lesion volume (T2LV) and T1 lesion volume (T1LV). The T2-hyperintense lesion masks were co-registered to the 3D T1-weighted scan. White matter lesions were filled using a patch-based lesion filling technique.[22] Brain extraction and probabilistic tissue segmentation of the lesion-filled 3D-T1w scans were done using Geodesic Information Flows [23] to create cortical grey matter (CGM), deep grey matter (DGM) and normal-appearing WM (NAWM) masks. The brain parenchymal fraction (BPF), grey matter fraction (GMF) and WM fraction (WMF) were calculated, by dividing the tissue-specific volumes by the total intracranial volume.

A fully-automated image analysis pipeline was used to determine the TSC in tissues from the sodium images.[24] The signal intensity in calibration phantoms was used as a reference to quantify the TSC on a voxel-by-voxel basis.[6] A series of registration steps were then used and concatenated to quantify TSC in each tissue type: (1) the sodium scan was registered to the PD/T2-weighted scan obtained using the body coil; (2) the PD/T2-weighted scan

obtained using the body coil was registered to the PD/T2-weighed scan obtained using the 32-channel head coil; and (3) the PD/T2-weighted scan obtained using the 32-channel head coil was registered to the 3D T1-weighted scan. Tissue mask were resampled using a point-spread-function scaling factor into sodium space to calculate the TSC (in mM) for each tissue.[25] Using the probabilistic masks partial volume correction was performed for each voxel.[8] Finally, using a voxel-wise partition-based correction method, we removed CSF contamination.[8]

Statistical analysis

All clinical, ^{1}H-MRI and ^{23}Na-MRI quantitative data are presented as mean (SD) unless otherwise stated.

Differences in clinical measures between groups were compared using univariable linear regression, except for EDSS, which was compared using the non-parametric Wilcoxon rank-sum test. Differences in ^{1}H-MRI findings between groups were compared using univariable linear regression, with age and sex included as covariates when comparing brain volumetric measures. Differences in TSC in CGM, DGM, NAWM and lesions between groups was compared using univariable linear regression using GMF or WMF as a covariate depending on the tissue type being examined.

Multivariable linear regression was used to identify independent associations between tissue-specific TSC and clinical measures in all patients and in the subgroup with MS. Significant univariable predictor variables were entered into the model and removed by stepwise backward manual elimination of variables with $p>0.08$. Each of the omitted variables was then tested in the final models. Because EDSS is not normally distributed, the

final regression model was checked using non-parametric bias-corrected and accelerated bootstrap with 1000 replicates to confirm associations. Tissue-specific brain atrophy was included as a covariate in all models, and the NART was included as a covariate in models examining associations with cognition. Age, sex and disease duration had no material effect on regression coefficients and were not retained as covariates.

Statistical analyses were done using SPSS 21. Significance is reported at the level of $p<0.05$.

RESULTS

Demographic and clinical characteristics

130 subjects were studied: 34 healthy controls and 96 patients from the CIS follow-up study (Table 1). Over a mean follow-up period of 14.7 years (range 11.2–19.2 years), 78 (81%) patients developed MS and 18 (19%) remained CIS. Among the MS patients disease course was classified as RRMS in 65 and SPMS in 13 patients. 20 (26%) MS patients were receiving (or had received) disease-modifying therapy.

Compared with the patients who remained CIS at 15 years, the MS patients had more physical disability, worse performance on cognitive tests (with the exception of visual memory) and greater fatigue ($p<0.05$ for all comparisons). The SPMS patients had worse performance on all clinical measures compared with the RRMS patients ($p<0.05$ for all comparisons), except verbal memory.

^{1}H-MRI findings

The GMF and WMF were similar in patients who remained CIS and health controls (Table 2). We observed significant differences in the MS patients in GMF (adjusted difference -0.011 [95%CI -0.016, -0.006], p<0.001) and WMF (adjusted difference -0.009 [95%CI -0.015, -0.003], p=0.002) compared with healthy controls, and significant differences in the MS patients in GMF (adjusted difference -0.008 [95%CI -0.014, -0.002], p=0.010) and WMF (adjusted difference -0.012 [95%CI -0.020, -0.004], p=0.002) compared with the CIS patients (Table 2). Within the MS group, the SPMS patients showed significant differences in GMF (adjusted difference -0.009 [95%CI -0.016, -0.001], p=0.021), WMF (adjusted difference -0.010 [95%CI -0.020, -0.001], p=0.028), T2LV (17.97 vs 9.81 ml, p=0.026) and T1LV (5.87 vs 1.90 ml, p<0.001) compared with RRMS patients (Table 2).

^{23}Na-MRI findings

TSC in CGM, DGM and NAWM was similar in the patients who remained CIS and healthy controls (Table 2). In all MS patients together, there were significant differences in TSC in CGM (adjusted difference 2.12mM [95%CI 0.66, 3.57], p=0.005), DGM (adjusted difference 1.90mM [95%CI 0.33, 3.47], p=0.018) and NAWM (adjusted difference 1.99mM [95%CI 0.45, 3.53], p=0.012) compared with healthy controls (Table 2). These changes were driven by SPMS patients (Table 2).

In MS patients, there were significant differences in TSC in T2-hyperintense lesions compared with NAWM (adjusted difference 11.42mM [95%CI 9.53, 13.3], p<0.001), and T1-hypointense compared with T1-isointense white matter lesions (adjusted difference 8.14mM [95%CI 5.12, 11.17], p<0.001). Significant differences in TSC in T2-hyperintense lesions in the SPMS patients compared with RRMS patients (adjusted difference 7.54mM [95%CI 2.61, 12.46], p=0.003), mainly driven by the difference in TSC in T1-isointense lesions in the SPMS group (adjusted difference 6.58mM [95%CI 2.29, 10.87], p=0.003)

Associations between tissue sodium concentrations and disability

CGM sodium concentration was independently associated with EDSS (R^2=0.26), TWT (R^2=0.23) and 9HPT (R^2=0.23) (Table 3). CGM sodium concentration was also independently associated with PASAT (R^2=0.29), SDMT (R^2=0.31), and the Brixton test (R^2=0.36). No independent association was seen with tissue-specific TSC and memory tests, the Hayling test and fatigue.

The multivariable linear regression analyses were repeated after excluding patients who remained CIS (Table 3), firstly to confirm findings in the MS group and secondly to examine associations between TSC in white matter lesions with disability (none of the CIS patients at 15 years had typical demyelinating white matter lesions). In the MS-only model (n=78), CGM and T1-hypointense lesion TSC were associated with EDSS (R^2=0.25), TWT (R^2=0.24) and 9HPT (R^2=0.32). The associations with the cognitive tests was similar: CGM TSC was associated with PASAT (R^2=0.29), SDMT (R^2=0.31) and the Brixton test (R^2=0.38), and no association was seen with memory, the Hayling test or fatigue.

DISCUSSION

In a unique, homogeneous cohort of patients with relapse-onset MS we found a consistent association of CGM TSC with disease course, physical disability and cognition when assessed cross-sectionally 15 years after disease onset. We aimed to overcome some of the key limitations of early applications of ^{23}Na-MRI in MS, including: (1) an optimised ^{23}Na-MRI protocol with the smaller voxel sizes (effective voxel size 27 mm^3 versus 64 mm^3 in earlier studies), to reduce the impact of partial volume effects from CSF; (2) a new post-

processing pipeline with a cutting-edge tissue segmentation algorithm[23]; and (3) adjustment of statistical analysis for tissue-specific volume loss.

We found a higher TSC in both CGM and DGM in MS patients compared with healthy controls. In WM, a gradient was seen in MS patients with the highest TSC in T1-hypointense lesions, followed by T1-isointense lesions and then NAWM. These differences were mainly driven by patients with SPMS, who showed higher TSC in these regions compared with RRMS, suggesting a greater degree of neuroaxonal metabolic dysfunction, neuroaxonal loss, or both in SPMS. These findings are consistent with a greater extent of brain atrophy and microstructural damage detected using ^{1}H-MRI in SPMS.[26 27] In contrast, no increase in tissue-specific TSC was seen in the patients who remained CIS after 15 years. We also found no difference in brain volume measurements in the patients who remained CIS compared with healthy controls. These findings are supported by previous studies showing that patients who remain CIS in the long-term do not have detectable structural[26 27] or metabolic[28] abnormalities seen in people with MS, and MRI findings are similar to healthy controls.

Independent associations were observed between ^{23}Na-MRI abnormalities and clinical impairment. TSC in CGM was associated with EDSS, walking speed and upper limb dexterity. Previous studies have also reported an association of total grey matter TSC with measures of physical disability.[6-8] CGM TSC was also associated with performance on tests of information processing speed and executive function. Cortical grey matter pathology is recognised as being a major substrate for both physical disability and cognitive impairment[29], including ^{1}H-MRI detected grey matter lesions[30], atrophy[27] and microstructural damage[26]. Although we observed a consistent association of CGM TSC with clinical measures, the associations were only moderate. This highlights that while brain ^{23}Na-MRI is sensitive to the

detection of neuroaxonal metabolic changes and/or tissue loss, other disease mechanisms that contribute to disability and cognitive performance are not captured by this technique (e.g. spinal cord damage, changes in structural and functional connectivity).

Sodium channel blockade has been proposed as a neuroprotective strategy in MS based on results of animal studies showing that sodium channel blocking agents reduced axonal loss in experimental autoimmune encephalomyelitis models.[31] These observations are supported by a recent phase II showing a neuroprotective effect of phenytoin on retinal nerve fibre layer thinning acute optic neuritis.[32] In view of our findings (and previous studies highlighting the sensitivity of ^{23}Na-MRI measures of clinical impairment[4-8]), and the reproducibility of this technique (including in multi-centre studies[33]), TSC should be added as an exploratory endpoint to neuroprotection trials that aim to preserve neuroaxonal viability using sodium channel blockade. We have included ^{23}Na-MRI as an endpoint in the Protective Role of Oxcarbazepine in Multiple Sclerosis (PROXIMUS) study – a phase IIa randomised controlled trial of oxcarbamazepine in people with relapsing MS with worsening disability while receiving disease-modifying therapies (https://clinicaltrials.gov/ct2/show/NCT02104661).

Some possible limitations of this work should be noted. The healthy control group were younger than the patient group. However, we found no relationship between age and tissue-specific TSC, in keeping with previous studies.[8] It is unlikely that the increased TSC in MS patients is due to older age, particularly since there was no difference between healthy control and CIS patients. Although we studied a large cohort (this study is largest application of ^{23}Na-MRI in MS to date), the number of patients with SPMS who were scanned as part of the 15 year follow-up was small (n=13). Despite the small number clear differences were seen, particularly in grey matter, between the SPMS and RRMS group, findings that are consistent with a previous study with a larger sample of patients with SPMS.[8]

Although ^{23}Na-MRI is a promising new imaging technique with the potential to provide information on tissue viability, including neuroaxonal integrity and function, there are a number of technical challenges and limitations in previous studies using 3T clinical scanners. The concentration of ^{23}Na in tissues is significantly lower than ^{1}H with a much lower signal-to-noise ratio. Consequently the voxel size required is significantly larger, typically 4x4x4mm^3.[6-10] A larger voxel size increases the risk of partial volume effects, especially from CSF, which contains a much higher sodium concentration than tissues (~140mM). We took a number of steps to reduce the effects of partial volume, representing an innovative methodology. Firstly, the scans were acquired using an optimised ^{23}Na-MRI protocol that included a higher resolution (nominal 3x3x3mm^3) and more efficient sampling of *k*-space using a 3D-cones technique.[11] These changes have been shown to improve tissue contrast, increase signal-to-noise ratio, and provide a better point spread function with voxel sizes closer to the nominal value.[11] Secondly, the image-analysis pipeline included a partition-based method to correct for CSF contamination. Thirdly, the tissue masks were resampled to sodium space using a point spread function scaling factor.[25] Finally, as an additional precaution all of the analyses were adjusted for tissue-specific atrophy (GMF, WMF or both), which has not been done in previous studies.

TSC in tissues represents a composite of intracellular and extracellular sodium. Tissue sodium accumulation in MS could be due to an increase in intracellular sodium due to axonal metabolic dysfunction, an increase in extracellular sodium due to expansion of the extracellular space arising from neuroaxonal loss, or both. A number of approaches have been described for measuring intracellular sodium concentration *in vivo*. A recent study at 7 T used triple quantum filtering to exploit the differences in the correlation time of sodium nuclei in intracellular and extracellular space to effectively separate them.[34] This technique allows quantification of TSC, intracellular sodium concentration and the intracellular sodium

volume fraction (a surrogate measure of the extracellular sodium). This approach has been applied in a study of 19 patients with RRMS. Compared with healthy controls, the RRMS patients showed a significant increase in intracellular sodium concentration and decrease in intracellular sodium volume fraction.[33] These findings suggest that both an increase in intracellular sodium concentration and a reduction in the intracellular volume (due to axonal loss) contribute to the increase in TSC in MS.

CONCLUSIONS

^{23}Na-MRI detects pathological abnormalities that are relevant to long-term disease progression, physical disability and cognitive impairment in relapse-onset MS. Metabolic abnormalities in CGM may be an important mechanism contributing to disease progression and disease course heterogeneity in relapse-onset MS. ^{23}Na-MRI is an emerging outcome measure for novel neuroprotection interventions in MS, and has started to be translated into neuroprotection trial design.

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DISCLOSURES

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FIGURE LEGENDS

Figure 1. Raw sodium images, sodium concentration maps, and ^{1}H T2-weighted and T1-weighted images (obtained using the 32 channel head coil), in healthy controls and patients with CIS, RRMS and SPMS.

Table 1 Demographic characteristics of healthy controls and patients grouped by clinical status at 15 years.

			MS patients		
	Healthy controls (n=34)	CIS patients (n=18)	All MS (n=78)	RRMS (n=65)	SPMS (n=13)
Age, years	35.5 (10.1)	49.0 (7.2)	47.0 (7.5)	46.8 (7.6)	47.8 (7.8)
Female, n (%)	23 (66)	12 (67)	59 (86)	50 (77)	9 (69)
Disease duration, years		14.5 (2.1)	14.7 (2.4)	14.6 (2.4)	15.0 (2.8)
Disease-modifying therapy, n (%)		0 (0)	0 (0)	18 (28)	4 (31)
EDSS, median (range)		0 (0-1)	2 (0-7)^^	2 (0-6.5)	6 (3.5-7)$^{\#\#}$
Walking speed, seconds		0.24 (0.03)	0.18 (0.06)^^	0.20 (0.04)	0.09 (0.05)$^{\#\#}$
9-Hole peg test, *z* score		0.78 (0.70)	0.23 (0.91)^	0.50 (0.61)	-1.16 (0.82)$^{\#\#}$
Verbal memory, *z* | n impaired (%)		0.21 (0.60) | 0 (0)	-0.32 (0.58)^^ | 1 (1)	-0.27 (0.54) | 1 (2)	-0.59 (0.74) | 0 (0)
Visual memory, *z* | n impaired (%)		0.24 (0.49) | 0 (0)	-0.06 (0.65) | 2 (3)	-0.01 (0.63) | 1 (2)	-0.35 (0.76)$^{\#\#}$ | 1 (8)
PASAT, *z* | n impaired (%)		0.21 (0.68) | 0 (0)	-0.48 (1.09)^ | 7 (9)	-0.32 (0.99) | 3 (5)	-1.32 (1.25)$^{\#\#}$ | 4 (30)
SDMT, *z* | n impaired (%)		0.31 (0.90) | 0 (0)	-0.65 (1.38)^^ | 11 (14)	-0.34 (1.18) | 3 (5)	-2.12 (1.38)$^{\#\#}$ | 8 (62)
Brixton test, *z* | n impaired (%)		-0.19 (0.98) | 0 (0)	-1.06 (1.18)^ | 12 (15)	-0.93 (1.09) | 7 (11)	-1.74 (1.44)$^{\#\#}$ | 5 (38)
Hayling test, *z* | n impaired (%)		0.20 (0.86) | 0 (0)	-0.63 (1.04)^^ | 3 (4)	-0.56 (1.01) | 1 (2)	-0.97 (1.15)$^{\#}$ | 2 (15)
Fatigue severity score		2.4 (1.3)	4.3 (3.80)^^	3.5 (1.8)	5.6 (1.5)$^{\#\#}$

All data presented as mean (SD) unless otherwise indicated. Impairment on cognitive tests was defined as a *z* score ≤2.0 standard deviations below published normative data.

Abbreviations: EDSS = Expanded Disability Status Scale; PASAT = Paced Auditory Serial Addition Test; SDMT = Symbol Digit Modalities Test.

^p<0.05 compared with CIS; ^^p<0.01 compared with CIS; $^{\#}$p<0.05 compared with RRMS; $^{\#\#}$p<0.01 compared with RRMS.

Table 2 Total sodium concentrations in healthy controls and subjects grouped by clinical status at 15 years.

			MS patients		
	Healthy controls (n=34)	CIS patients (n=18)	All MS (n=78)	RRMS (n=65)	SPMS (n=13)
^{1}H MRI					
Brain parenchymal fraction	0.768 (0.009)	0.767 (0.008)	0.747**, ^^ (0.028)	0.751 (0.018)	0.731$^{\#\#}$ (0.028)
Grey matter fraction	0.458 (0.009)	0.453 (0.006)	0.445**, ^ (0.014)	0.447 (0.012)	0.438$^{\#\#}$ (0.014)
White matter fraction	0.310 (0.011)	0.314 (0.010)	0.302**, ^^ (0.016)	0.303 (0.016)	0.293$^{\#\#}$ (0.016)
T2 lesion volume, ml			11.08 (12.00)	9.81 (11.60)	17.97$^{\#\#}$ (12.28)
T1 lesion volume, ml			2.54 (3.52)	1.90 (2.91)	5.87$^{\#\#}$ (4.53)
^{23}Na MRI					
Cortical grey matter	40.63 mM (2.33)	40.68 mM (1.74)	42.82 mM** (3.53)	42.30 mM (3.29)	45.45 mM$^{\#}$ (3.69)
Deep grey matter	34.83 mM (2.79)	36.13 mM (1.69)	36.69 mM* (3.74)	36.14 mM (3.47)	39.47 mM$^{\#}$ (3.98)
Normal-appearing white matter	32.03 mM (3.44)	32.92 mM (2.43)	34.85 mM* (4.05)	34.43 mM (3.95)	36.95 mM (4.04)
White matter lesions					
All T2-hyperintense lesions			45.58 mM (8.53)	44.27 mM (8.27)	51.99 mM$^{\#\#}$ (6.92)
T1-isointense lesions			43.93 mM (5.83)	42.87 mM (7.04)	49.30 mM$^{\#\#}$ (5.83)
T1-hypointense lesions			52.08 mM (7.43)	51.02 mM (11.56)	57.45 mM (7.43)

All data presented as mean (SD).

*p<0.05 compared with healthy controls; **p<0.01 compared with healthy controls; ^p<0.05 compared with CIS; ^^p<0.01 compared with CIS;

$^{\#}p<0.05$ compared with RRMS; $^{\#\#}p<0.01$ compared with RRMS.

Table 3 Statistically significant associations between tissue-specific total sodium concentrations and clinical measures

	All patients (n=96)					MS patients (n=78)				
Clinical measure	TSC	β	95% CI	*p*	R^2	TSC	β	95% CI	*p*	R^2
EDSS	CGM	0.174	0.061, 0.287	0.003	0.26	CGM	0.128	0.010, 0.246	0.035	0.25
						T1-hypointense lesions	0.020	0.000, 0.041 (0.002, 0.037*)	0.055 (0.023*)	
Walking speed	CGM	-0.006	-0.010, -0.002	0.003	0.23	CGM	-0.005	-0.009, -0.001	0.023	0.24
						T1-hypointense lesions	-0.001	-0.002, -0.0005	0.032	
z 9HPT	CGM	-0.096	-0.148, -0.051	0.001	0.23	CGM	-0.091	-0.152, -0.030	0.004	0.32
						T1-hypointense lesions	-0.014	-0.025, -0.004	0.008	
z PASAT	CGM	-0.105	-0.177, -0.032	0.005	0.29	CGM	-0.101	-0.180, -0.023	0.012	0.29
z SDMT	CGM	-0.156	-0.246, -0.066	0.001	0.31	CGM	-0.143	-0.240, -0.046	0.005	0.31
z Brixton test	CGM	-0.067	-0.120, -0.014	0.014	0.36	CGM	-0.066	-0.118, -0.015	0.013	0.38

*Bootstrap confidence interval and p-value.

Abbreviations: 9HPT = 9 Hole peg test; EDSS = Expanded Disability Status Scale; PASAT = Paced Auditory Serial Addition Test; SDMT = Symbol Digit Modalities Test.

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