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Repairing Myelin After Irradiated Pediatric Brain Tumor: A Magnetization Transfer Imaging Analysis

Éloïse Baudou[1] | Jennifer L. Ryan[1] | Julie Tseng[1] | Éric Bouffet[2] | Ute Bartels[2] | Brian W. Timmons[3] | Cynthia B. de Medeiros[1] | Donald J. Mabbott[1,4]

[1]Neurosciences and Mental Health Program, Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada | [2]Divisions of Hematology/Oncology, The Hospital for Sick Children, Toronto, Ontario, Canada | [3]Child Health and Exercise Medicine Program, Department of Pediatrics, McMaster University, Hamilton, Ontario, Canada | [4]Department of Psychology, University of Toronto, Toronto, Ontario, Canada

Correspondence: Donald J. Mabbott (donald.mabbott@sickkids.ca)

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Keywords: brain tumor | children | exercise | magnetization transfer imaging | metformin

ABSTRACT

Background: While exercise training and metformin treatment have demonstrated preliminary cognitive improvements in pediatric brain tumor (PBT) survivors, the neuronal mechanisms underlying their cognitive improvements are unclear. Diffusion-weighted metrics (e.g., fractional anisotropy [FA]) are commonly used to evaluate remyelination, but magnetization transfer imaging is thought to be more sensitive to myelin plasticity.

Methods: We compared white matter changes after exercise and metformin interventions by evaluating magnetization transfer ratio (MTR) and FA changes in irradiated PBT survivors who completed either an exercise (NCT01944761) or metformin pilot trial (NCT02040376) (30 participants: exercise $n = 11$, metformin $n = 12$, and control $n = 7$). Then, we explored correlations between MTR and cognitive outcomes.

Results: There were significant MTR changes in three brain regions (right forceps minor in both interventions, right inferior and superior longitudinal fasciculi in the exercise group), but no significant FA changes. MTR increases occurred in the right forceps minor in the exercise and metformin groups compared with the control group ($p < 0.033$), and in the superior longitudinal fasciculus in the exercise group compared with the control group ($p = 0.016$). Preliminary correlations between MTR and cognitive changes were not significant after correcting for multiple comparisons.

Conclusions: Our results suggest that 12 weeks of exercise or metformin intervention may promote remyelination in PBT survivors in brain regions involved in memory and executive function, and there may be differences in the brain regions affected by each

Abbreviations: ANCOVA, Analysis of covariance; CANTAB, Cambridge Neuropsychological Test Automated Battery; DTI, Diffusion tensor imaging; EPI, Echo planar imaging; FA, Fractional anisotropy; FA_{diff}, Change in fractional anisotropy from timepoint 1 to timepoint 2; FDR, False discovery rate; FOV, Field of view; MRI, Magnetic resonance imaging; MTI, Magnetization transfer imaging; MTR, Magnetization transfer ratio; MTR_{diff}, Change in magnetization transfer ratio from timepoint 1 to timepoint 2; MTR_{tp1}, Magnetization transfer ratio from timepoint 1; MTR_{tp2}, Magnetization transfer ratio from timepoint 2; PBT, Pediatric brain tumor; TBSS, Tract-based spatial statistics; TE/TR, Echo time/repetition time.

Éloïse Baudou and Jennifer L. Ryan have contributed equally to this work.

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intervention. This work sets the stage for larger clinical trials to identify definitive differences in MTR and validate their association with cognition.

1 | Introduction

The neuropsychological deficits and diffuse white matter damage induced by pediatric brain tumor (PBT) and their subsequent treatment are well-documented [1–5]. Cognitive impairment after PBT and its treatment is associated with disrupted neural transmission [2, 3, 6, 7], which is due, in part, to changes in the presence and quality of myelin in the brain [8]. These pervasive long-term deficits have prompted trials aimed at improving cognitive function and neuroplasticity in PBT survivors, with promising improvements observed with physical exercise [9] and metformin [10] interventions. While this preliminary research suggests that there are different ways to foster brain repair and cognitive change, it is unclear whether exercise and metformin interventions have similar effects on cognition and white matter microstructure, which is critical for determining the most appropriate cognitive intervention following medical treatment for PBT.

Remyelination occurs through the activation of oligodendrocyte precursor cells that migrate to demyelinated areas and differentiate into oligodendrocytes that produce and deposit myelin around the demyelinated axon [11]. While exercise and metformin interventions likely induce remyelination by stimulating stem cell niches, the cellular processes may differ between interventions and have differential effects on brain regions. Exercise promotes the production of neurotrophic factors, such as brain-derived neurotrophic factor and insulin growth factor, which leads to proliferation of the oligodendrocytes [12]. Metformin activates the atypical protein kinase C-CREB–binding protein pathway in endogenous neural precursor cells that differentiate into oligodendrocytes [13, 14].

Diffusion-weighted magnetic resonance imaging (MRI) is a common method for indirectly exploring changes in white matter microstructure and its association with cognitive function [7], where diffusion-weighted outcomes are a proxy for changes in myelin. In previous evaluations of white matter changes in PBT survivors following exercise or metformin intervention, we evaluated fractional anisotropy (FA) [9] and axonal water fraction [10], respectively. While both interventions increased their respective diffusion metrics in the corpus callosum, exercise also increased FA in the cingulum, superior longitudinal fasciculi, right corticospinal tract, and inferior frontal occipital fasciculus [9, 10]. However, we have yet to directly compare the impact of each intervention on white matter using the same metric within the same analysis. Further, diffusion-weighted imaging is not specific to changes in myelin, as it measures water molecule diffusion in response to an applied magnetic gradient and is influenced by the presence of any physical barrier (e.g., cell membranes, microtubules, and myelin) [15–17]. Thus, to understand the differential impact of these interventions on remyelination, a more specific measure is required.

Magnetization transfer imaging (MTI) is an MRI modality that measures the exchange of magnetization between free-water protons and lipid-bound protons [18, 19]. As such, MTI should be more sensitive to myelin than diffusion tensor imaging (DTI). Magnetization transfer ratio (MTR) is the percentage change in signal with and without a saturation pulse and has been sensitive to short-term brain changes in conditions such as relapsing-remitting multiple sclerosis [20]. Further, MTR changes are correlated with behavioral changes in people with multiple sclerosis [20] and, as such, is the recommended method for exploring the impact of interventions on white matter microstructure in this clinical population [21–23]. Similarly, our team observed decreased MTR in normal-appearing white matter in PBT survivors that correlated with abnormalities in DTI metrics [24]. While we collected MTI data in our metformin and exercise studies, we have yet to analyze them.

Given the increased specificity of MTR over DTI-based measures of white matter, MTR is ideal for identifying similarities and differences in white matter tract changes between PBT survivors who have received either exercise or metformin. Ideally, exercise and metformin interventions would be compared within the same randomized control trial, but, to date, no such study has taken place. As such, this study draws upon data from separate exercise and metformin pilot trials for PBT survivors and provides a preliminary comparison of the changes in white matter microstructure and cognition between interventions to, ultimately, support a future definitive clinical trial in this area.

This study explored MTR changes in irradiated PBT survivors who either completed an exercise training program (our "exercise group") [9], took metformin (our "metformin group") [10], or did not receive any intervention (our "control group") with the aim of determining whether: (1) the interventions induced white matter changes in similar or different regions of the brain, (2) one intervention elicited more white matter changes than the other, and (3) MTR changes were correlated with cognitive changes. We expected that MTR would increase post-intervention in both the exercise and metformin groups compared with the control group, that there would be similarities and differences in white matter tract changes between the exercise and metformin groups, that MTR changes may occur in locations where FA changes did not, and that increased MTR would be correlated with improved cognition.

2 | Methods

Trial designs and participants: The pilot exercise trial explored the impact of a 12-week group aerobic training program (ClinicalTrials.gov, NCT01944761) on cognitive function and brain repair in children 6–17 years old recruited from The Hospital

for Sick Children (Toronto, Ontario, Canada) and McMaster Children's Hospital (Hamilton, Ontario, Canada) who were 1–10 years from the conclusion of PBT treatment and had received cranial radiotherapy. Children with severe neurological or motor dysfunction that precluded safe participation in an exercise program were excluded. A cross-over design with a 12-week intervention period and a 12-week comparative period of no intervention was used with participants randomly allocated to receive intervention in the first or second period. The exercise intervention consisted of three 90-minute sessions of aerobic activities each week with the goal of maintaining participants' heart rates at 80% of their baseline peak heart rate for at least 30 minutes per session [9]. During the no-intervention period, participants continued with their normal physical activity routines.

The placebo-controlled pilot metformin trial explored the impact of metformin treatment (ClinicalTrials.gov, NCT02040376) in PBT survivors 5–21 years old recruited at The Hospital for Sick Children (Toronto, Ontario, Canada) on cognitive recovery and brain repair. Inclusion criteria were: ≥ 2 years from PBT diagnosis, not receiving active treatment, ≤ 15 years since radiotherapy, not palliative, able to swallow pills, no liver or renal dysfunction (or history), no unstable and/or insulin-dependent (type 1) diabetes, no metabolic acidosis and/or lactic acidosis, no history of congestive heart failure requiring pharmacologic treatment, and no known hypersensitivity to metformin hydrochloride. A cross-over design with a 12-week intervention period and a 12-week comparative period of no intervention was also used in this study. Participants were randomly allocated to receive the intervention in the first or second period. The study was double blinded, with a 10-week washout period between the intervention and/or no-intervention periods. During the no-intervention period, participants received placebo pills containing nonactive ingredients following the same protocol.

For both trials, participants had to either speak English as their native language or have had at least two years of schooling in English (for the neuropsychological assessment) and be able to participate in neuroimaging without sedation. Both trial protocols were approved by institutional review boards. Either written informed consent or assent and parental consent (where applicable) was obtained.

Present study: We included participants who had both pre- and post-intervention MRI collected on a 3 Tesla MRI whose scans passed quality assurance by É. Baudou. If participants had enrolled in both studies, data were used only from the first study they completed. Initially, the metformin study did not involve MRI and neuropsychological assessment immediately following the first intervention period but was amended mid-study. Thus, some study participants were excluded due to the lack of immediate post-intervention data.

Exercise study participants who were allocated to the no-intervention arm for the first period of the study were used as a control group. Their data from the second period of the study (i.e., the exercise intervention) were not included in the exercise group analysis. Unfortunately, there was no control group from the metformin study due to the aforementioned issue with post–first-period assessment. While there are considerable limitations to using a control group solely from the exercise group, particularly due to baseline differences that can arise from differing study eligibility criteria, it permitted an important preliminary comparison between intervention and no intervention over the same 12-week period.

Neuropsychological assessment: As previous studies have shown an association between reaction time and white matter microstructure [25, 26], the Cambridge Neuropsychological Test Automated Battery (CANTAB) [27] was used in both trials to obtain mean reaction times and accuracy in subtests measuring attention (rapid visual information processing, match to sample visual search), processing speed (simple reaction time, choice reaction time), and short-term memory (delayed matching to sample).

Imaging: Images were acquired at The Hospital for Sick Children on a Siemens Tim Trio 3T MRI scanner with a 12-channel head coil (Siemens Canada Ltd., Mississauga, ON). The scanner was upgraded to a Siemens Prisma Fit scanner with a 20-channel head coil (Siemens Canada) during the metformin trial. The same sequences were used for both trials.

Diffusion-weighted images were acquired using a single-shot spin-echo DTI sequence with EPI readout. Parameters were 30 directions, 1 b0 image, b = 1000 s/mm^2, TE/TR = 90/9000 ms, flip angle = 90°, 70 contiguous axial slices, 122 × 122 matrix, interpolated to 244 × 244, FOV = 24.4 × 24.4 cm, voxel size = 2 mm isotropic. $MTR_{on/off}$ were acquired using a T1 Axial FL3D Grappa 2 protocol. Parameters were TE/TR 5/34 ms; 104 contiguous axial slices; flip angle 10°; 128 × 128 matrix, interpolated to 256 × 256; FOV 192 × 192, voxel size = 1.5 mm isotropic (post-upgrade: voxel size = 0.8 × 0.8 × 1.5 mm).

We performed voxel-wise analyses of FA and MTR maps across subjects and time points using tract-based spatial statistics (TBSS) [28] using a method adapted for longitudinal data [29]. Diffusion-weighted images were first processed using either FSL (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki) or PyDESIGNER to generate FA maps using DTIFIT or PyDESIGNER (v1.0 March 2022), as some data had already been processed as part of the original pilot studies, for time points 1 and 2 [30]. Then, using FSL's SIENA, one FA map in halfway space was generated for each participant, representing the average between time points 1 and 2. These steps are reported in a TBSS analysis of FA for the exercise trial [9]. TBSS with the study-specific template option was used on FA halfway space data to produce a white matter skeleton for metric comparison across participants.

MTR maps for time points 1 and 2 were generated using FSL's flirt and fslmaths: first, MTR_{on} images were linearly registered to MTR_{off} images, then MTR maps were calculated with the following formula: $(MTR_{off} - MTR_{on})/(MTR_{off}) \times 100$. MTR maps were coregistered to diffusion images using AIR image registration [31] or FSL's flirt/fnirt, then into halfway space using the transformation matrices produced by SIENA. Finally, the MTR map from time point 2 (MTR_{tp2}) was subtracted from time point 1 (MTR_{tp1}) to obtain one change in MTR map (MTR_{diff}) in halfway space for each participant, i.e., "MTR_{diff} map = MTR_{tp2} − MTR_{tp1} map." The MTR_{diff} maps for each participant were analyzed using TBSS, which tested for differences in the same

white matter skeleton produced in the FA halfway data analysis described above.

For quality control, É. Baudou visually checked each participant's data for alignment between the MTR halfway at time point 1 and the FA halfway, and between the MTR halfway at time point 2 and the FA halfway.

Analysis: First, analysis of variance was used to examine baseline differences (sex, age at baseline, handedness, maternal and paternal education, age at diagnosis, tumor type, location, presence of hydrocephalus and the need for treatment, mutism after surgery, extent of resection, number of surgeries, type of radiotherapy, type of chemotherapy, time since diagnosis, and time since end of radiotherapy) between exercise, metformin, and control groups.

To determine where white matter changes occurred in the exercise and metformin groups (objective 1), TBSS was used to examine voxel-wise differences in the MTR_{diff} and FA_{diff} maps within each treatment group using two-sample independent *t*-tests, assuming remyelination would correspond to an increase in MTR/FA ($MTR_{diff}/FA_{diff} > 0$). Within-group analyses for the exercise and metformin groups were performed with and without the following covariates: age at baseline, sex, handedness, age at diagnosis, and delay from diagnosis. The control group was not part of this analysis. A family-wise error correction was applied with a permutation methodology using threshold-free cluster enhancement; the null distribution of the cluster-size statistic was built up over 5000 random permutations. Cluster size was thresholded at $p < 0.05$, which was corrected for multiple comparisons. To anatomically label the areas of white matter where differences were evident, clusters of significance that were greater than 10 voxels were identified and labeled using the Johns Hopkins University's ICBM-DTI-81 white matter and the Johns Hopkins University Tractography atlases [32].

To determine whether white matter changes in one intervention were greater than the other intervention (objective 2), we extracted mean MTR_{diff}/FA_{diff} for each cluster of significant within-group difference ($MTR_{diff}/FA_{diff} > 0$). The clusters were labeled depending on the white matter tracts involved. For example, if a cluster of significant change was found in the exercise group for MTR in the corpus callosum, the mean MTR_{diff} in this cluster was extracted for all groups (i.e., exercise, metformin, and control groups) and the cluster was labeled "corpus callosum"). Then, we compared the mean MTR_{diff} between the exercise, metformin, and control groups for each cluster identified above, using analysis of covariance (ANCOVA) with MTR_{diff} as a dependent variable, group as a fixed factor, and the following covariates: age, sex, handedness, delay from the end of treatment, age at diagnosis, and mean MTR_{tp1}. When significant group differences were identified, pairwise comparisons (exercise–control, metformin–control, and exercise–metformin) were completed with post-hoc Tukey tests to determine where the differences occurred.

Finally, to determine whether MTR changes were correlated with cognitive changes (objective 3), we tested for group differences on change in CANTAB subtest reaction time and accuracy scores using ANCOVAs with subtest score at the first time point as a covariate, and planned to calculate pairwise comparisons for any significant findings, as described for objective 2. Subsequently, Spearman's rank correlation coefficients were used to explore the association between mean MTR_{diff} within each cluster and neuropsychological score changes for each CANTAB subtest.

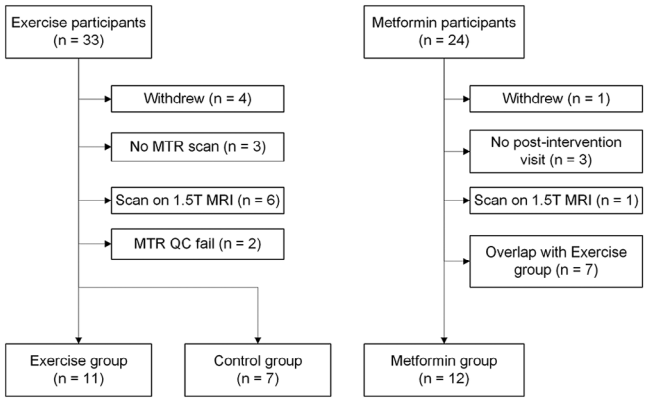

FIGURE 1 | Flow chart of participants included in the analysis and reasons for exclusion.

3 | Results

Thirty participants were included in this analysis: 11 in the exercise group, 12 in the metformin group, and 7 in the control group (Figure 1). There was a group difference in age at baseline ($F(29) = 4.42$, $p = 0.022$) with the metformin group being older than the exercise group (post-hoc Tukey: $p = 0.019$). There were also group differences in tumor location ($F(29) = 3.67$, $p = 0.039$) between the exercise, metformin, and control groups, with more supratentorial locations in the metformin group and infratentorial locations in the exercise group (post-hoc Tukey: $p = 0.033$). Table 1 details participants' medical and demographic characteristics.

3.1 | MTR Change as a Function of Intervention

Using TBSS, we identified three clusters of white matter where MTR increased before and after the intervention period within groups and no clusters with increased FA. In the metformin group, change in MTR (i.e., MTR_{diff}) was significantly greater than zero in a cluster of 96 voxels in the right frontal lobe corresponding to the forceps minor (Figure 2A). In the exercise group, MTR_{diff} was significantly greater than zero in two clusters of 140 and 14 voxels in the right temporal lobe, corresponding to the inferior and superior longitudinal fasciculi, respectively (Figure 2B). The addition of covariates to the TBSS analysis did not alter the clusters with significant change.

The subsequent ANCOVA for mean MTR_{diff} at each significant cluster indicated that MTR at time point 1 was a significant covariate for the right forceps minor cluster ($p < 0.001$). No other covariates were significant for any of the three clusters. For the right forceps minor cluster, significant differences between groups were found ($F(21) = 5.37$; $p = 0.013$), with pairwise differences between the control and the exercise (post-hoc Tukey: $p =$

TABLE 1 | Medical and demographic characteristics of exercise, metformin, and control groups.

	Exercise	Metformin	Control	*P*- value
Number of participants	11	12	7	
Age at baseline (years)				
Mean (M)	M = 10.88	M = 14.96	M = 12.16	
Standard deviation (SD) Range (R)	SD = 2.88 R = 7.67–16.41	SD = 4.04 R = 7.87–20.88	SD = 2.66 R = 9.75–16.85	*0.022**
Sex (male:female)	5:6	6:6	4:3	0.900
Handedness (right:left)	10:1	9:3	5:2	0.543
Maternal education (years)				0.528
Num. data points (*n*)	n = 7	n = 11	n = 6	
Mean (M)	M = 15.86	M = 14.54	M = 15.00	
Standard deviation (SD)	SD = 2.91	SD = 2.11	SD = 2.10	
Range (R)	R = 12–20	R = 10–17	R = 12–17	
Paternal education (years)				0.237
Num. data points (*n*)	n = 7	n = 11	n = 6	
Mean (M)	M = 16.86	M = 14.82	M = 14.00	
Standard deviation (SD)	SD = 2.54	SD = 3.34	SD = 3.16	
Range (R)	R = 13–20	R = 12–22	R = 10–18	
Age at diagnosis (years)				0.401
Mean (M)	M = 5.57	M = 7.32	M = 6.19	
Standard deviation (SD) Range (R)	SD = 2.63 R = 1.92–9.33	SD = 3.92 R = 0.96–12.48	SD = 1.79 R = 2.92–8.25	
Time since diagnosis (years)				0.238
Mean (M)	M = 5.23	M = 7.65	M = 5.90	
Standard deviation (SD) Range (R)	SD = 2.88 R = 1.08–10.25	SD = 4.01 R = 4.00–16.10	SD = 3.05 R = 2.08–10.42	
Time since end of radiotherapy (years)				0.207
Mean (M)	M = 4.39	M = 7.12	M = 5.18	
Standard deviation (SD) Range (R)	SD = 3.16 R = 0.92–10.00	SD = 4.20 R = 3.17–15.83	SD = 3.37 R = 1.83–10.17	
Type of tumor				0.316
Medulloblastoma	7	7	5	
Ependymoma	3	1	1	
Craniopharyngioma	0	1	0	
Astrocytoma	1	0	0	
Germinoma	0	2	0	
Pineoblastoma	0	1	0	
High-grade astroblastoma	0	0	1	
Localization				*0.039**
Infratentorial	11	7	6	
Supratentorial	0	5	1	
Hydrocephalus				0.391
No hydrocephalus	2	3	0	
Hydrocephalus w/o treat	2	2	1	
Hydrocephalus w/ treat	7	7	6	

(Continues)

TABLE 1 | (Continued)

	Exercise	Metformin	Control	*P*- value
Surgery				0.267
Biopsy or partial resection	4	4	1	
Gross total resection	7	8	6	
Mutism after surgery				0.862
Mutism	4	5	2	
No mutism	4	7	2	
Number of surgeries				0.192
0	0	1	0	
1	7	9	6	
2	2	2	1	
3	2	0	0	
Radiotherapy				0.259
Focal	3	4	2	
Craniospinal reduced dose + tumor bed boost	3	2	2	
Craniospinal reduced dose + posterior fossa boost	2	1	3	
Craniospinal standard dose + tumor bed boost	2	4	0	
Craniospinal standard dose + posterior fossa boost	1	1	0	
Chemotherapy				0.182
None	1	1	2	
ACNS-0121 (carboplatin, cyclophosphamide, vincristine, and etoposide)	3	0	0	
ACNS-0332 (carboplatin, cyclophosphamide, vincristine, cisplatin, G-CSF, and isotretintoin)	0	1	0	
POG9631 (etoposide, cisplatin, cyclophosphamide, and vincristine)	0	1	1	
COG9961 (vincristine, lomustine, and cisplatin)	2	1	1	
COG99703 (thiotepa, carboplatin, and cisplatin; cyclophosphamide; vincristine; etoposide)	1	0	0	
SJMB96 and SJMB03 (vincristine, cisplatin, and cyclophosphamide)	4	5	3	
Other	0	3	0	

Significant group differences (*) determined by analysis of variance.

0.033) and the control and metformin ($p = 0.031$) groups. There were no significant pairwise differences between the exercise and metformin ($p = 0.984$) groups in the right forceps minor. For the 14-voxel right temporal cluster (superior longitudinal fasciculus), there were significant group differences ($F(21) = 4.69$, $p = 0.021$) with pairwise differences between the control and exercise groups ($p = 0.016$) but not between the control and metformin groups ($p = 0.593$) or the exercise and metformin groups ($p = 0.263$). For the 140-voxel right temporal cluster (inferior longitudinal fasciculus), there were no group differences ($F(21) = 2.37$, $p = 0.12$). Figure 3 illustrates the change in MTR from time 1 to time 2 for each group and cluster.

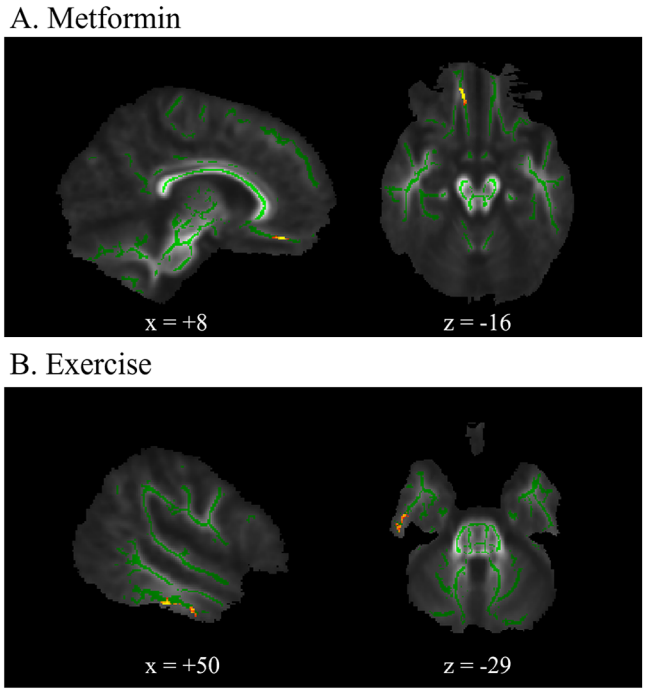

FIGURE 2 | MTR changes identified. (A) Post metformin and (B) Post exercise. Red-yellow identifies voxel clusters with significant MTR change (i.e., $MTR_{diff} > 0$) ($p < 0.05$, corrected for multiple comparisons), overlaid on the white matter skeleton shown in green and mean fractional anisotropy image in the study-specific template space.

3.2 | Correlations Between Change in Cognitive Scores and MTR

There were no significant group differences in CANTAB subtest change scores for reaction time and accuracy in tests of attention, processing speed, and short-term memory ($p > 0.153$). Thus, pairwise comparisons were not performed. For the metformin group, MTR increase in the right forceps minor voxel cluster was correlated with an increase in accuracy on the Choice Reaction Time subtest (Spearman's rho: 0.62, $p = 0.044$ [uncorrected], $p = 0.254$ [FDR-corrected], Figure 4). For the exercise group, MTR increase in the same cluster was associated with increased accuracy on the Rapid Visual Information Processing subtest (Spearman's rho: 0.73, $p = 0.010$ [uncorrected], $p = 0.184$ [FDR-corrected]). In the exercise group, MTR_{diff} in the 140-voxel right temporal lobe cluster was also correlated with increased reaction time for the Simple Reaction Time subtest (Spearman's rho: 0.78, $p = 0.004$ [uncorrected], $p = 0.081$ [FDR-corrected]) and the Choice Reaction Time subtest (Spearman's rho: 0.68, $p = 0.021$ [uncorrected], $p = 0.188$ [FDR-corrected]). There were no significant uncorrected correlations between the control group and cognitive test change scores.

4 | Discussion

The results from this small convenience sample demonstrate the potential for MTI to detect white matter changes that are not identified with DTI and support the ongoing use of MTI to evaluate white matter changes in PBT survivors. While the preliminary similarities and differences in brain regions that were affected by the exercise and metformin interventions identified in this study are not conclusive, they should serve to stimulate larger-scale clinical trials in PBT survivors that definitively determine the differential impact of either intervention and, ultimately, indicate when one or both treatment approaches are clinically warranted.

The frontal and temporal lobes, brain regions with known sensitivity to radiotherapy [26, 33], had increased MTR post-intervention, which suggests that these brain regions are also amenable to repair. Both interventions had increased MTR in the frontal lobe (forceps minor) compared with our control group, and only exercise had increased MTR in the temporal lobe (superior longitudinal fasciculus), which could either be unique to our study sample or support the different cellular processes involved in remyelination between exercise and metformin. These findings, in combination with the lack of significant differences in MTR change between exercise and metformin groups, emphasize the need to compare these interventions within the same clinical trial. Similarly, the positive correlations between MTR in the right forceps minor and accuracy in processing speed and attention tests require a clinical trial that is powered to detect cognitive change and validate the association between MTR and cognition.

This exploratory work has limitations related to sample size and heterogeneity. There were significant baseline differences in age and tumor location between the exercise, metformin, and control groups. While myelination is age-dependent [19], tumor location and PBT treatment (radiotherapy) disrupt typical myelin changes, which makes it difficult to determine the relative importance of the participant's age over age at the time of/since PBT treatment. Baseline MTR was the only significant covariate in our analysis, which suggests that the combined impact of age, age at diagnosis, tumor location, PBT treatment, and time since the end of treatment on the white matter was likely the most meaningful indicator in this sample. Nevertheless, study sample heterogeneity combined with small group sizes may have skewed our interpretation of the results, either by overemphasizing the brain regions affected by metformin/exercise or overlooking other brain regions affected by the intervention(s).

Additionally, while changes in MTR correlate with de/remyelination processes in multiple sclerosis [20], MTR could also be sensitive to other glial cells, edema, inflammation, or axon density. To address this concern, researchers have proposed inhomogeneous MTI, an imaging technique that isolates dipolar order in macromolecules, as an even more specific tool for myelin assessment [34]. Finally, our small control group was obtained only from the exercise study. While this control group was not ideal, it allowed us to compare the two treatment approaches to a PBT survivor who did not receive intervention.

In rare diseases, such as PBT, multisite research is necessary to obtain sample sizes that are powered to detect changes in cognitive outcomes and permit the generalization of results beyond the study sample. Given the proposed differences in cellular mechanisms for remyelination between exercise and metformin, similar increases in MTR in the right forceps minor between interventions, and differences in MTR changes in the

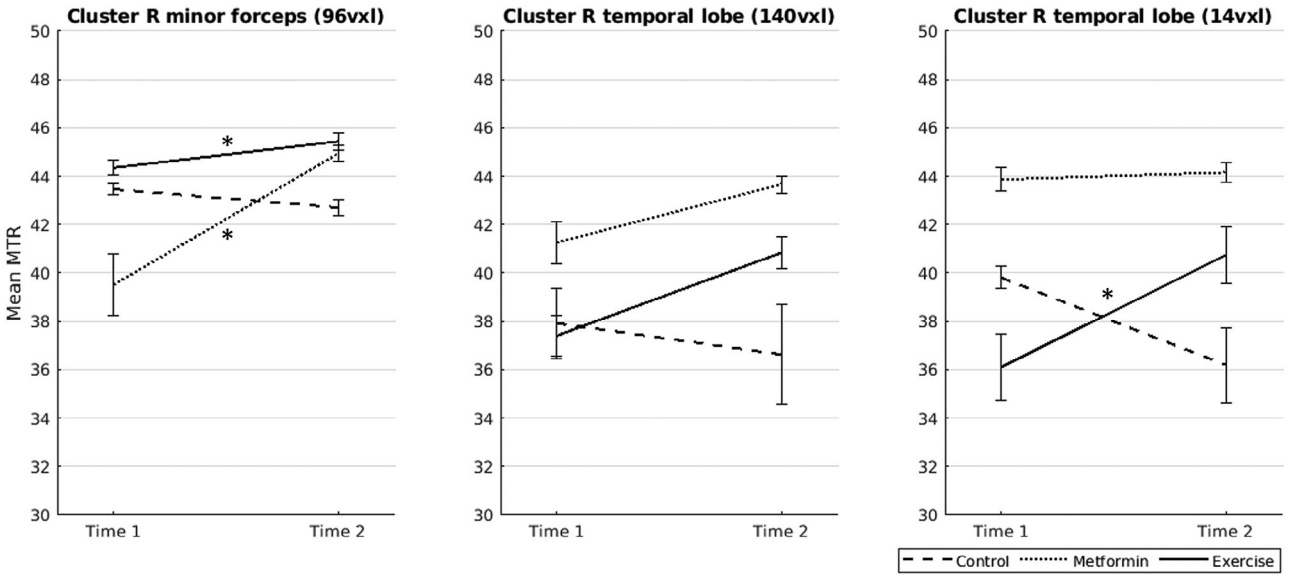

FIGURE 3 | Mean MTR at time points 1 and 2 for each tract-based spatial statistics cluster. Vertical bars indicate standard error, and asterisks (*) denote significant differences from the control group in MTR_{diff} ($p < 0.05$). vxl = voxel, R = right.

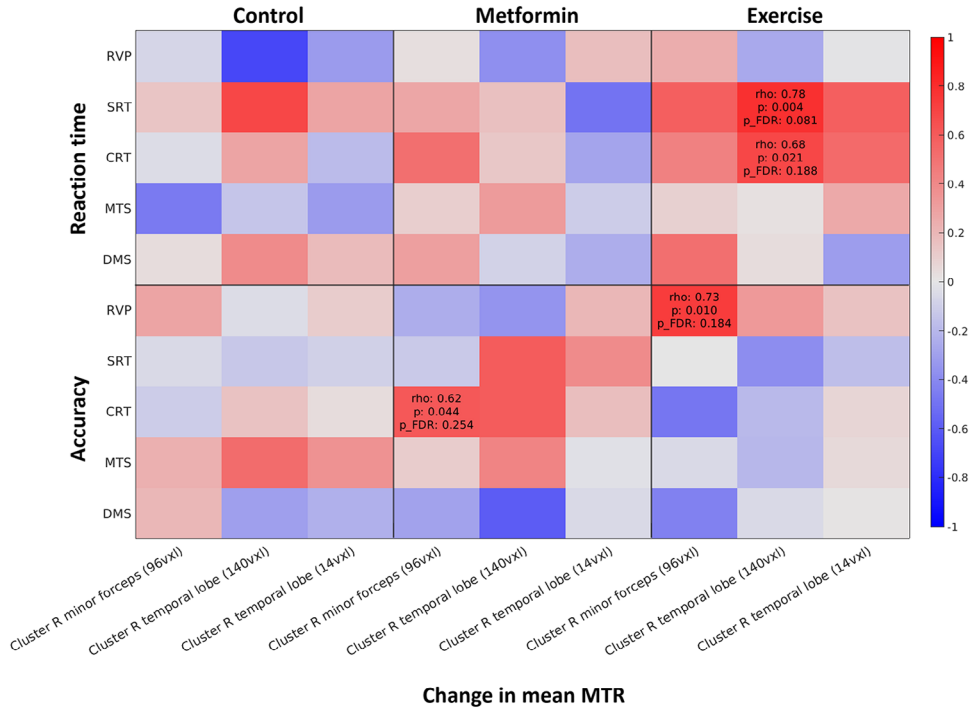

FIGURE 4 | Spearman correlations between Cambridge Neuropsychological Test Automated Battery (CANTAB) subtest change scores and change in MTR within three voxel clusters identified through tract-based spatial statistics analysis. Subtest scores are clustered by type (reaction time or accuracy) and correlations were carried out for each group separately (columns). Spearman's rho, uncorrected and false discovery rate (FDR)-corrected *p*-values are provided for correlations that had significant uncorrected correlations. RVP = rapid visual information processing, SRT = simple reaction time, CRT = choice reaction time, MTS = match to sample visual search, DMS = delayed matching to sample, vxl = voxel, R = right.

inferior longitudinal fasciculus between interventions, a multisite randomized control trial should evaluate the impact of exercise, metformin, "exercise plus metformin," and no intervention on white matter microstructure and cognition in PBT survivors. Finally, the nonsignificant decreases in MTR in the control group over the 12-week period could be attributed to the small sample size, a relatively short reassessment period, or actual null findings. To better understand white matter change after PBT and determine the optimal timing for interventions, a longitudinal evaluation of MTR in PBT survivors is warranted. Together, these trials will enable the identification of at-risk PBT survivors based on their cognitive profile and white matter integrity, and refine the type and timing of cognitive interventions to optimize outcomes.

5 | Conclusion

Due to its increased specificity and sensitivity to white matter compared with diffusion-weighted metrics, MTR provides important insights into the remyelination processes associated with interventions that aim to improve cognitive function in PBT survivors. This exploratory work identified increased white matter in the temporal and frontal brain regions using MTR after 12 weeks of exercise training or metformin treatment in a small sample of PBT survivors, with similarities and differences between interventions. While our correlations between MTR changes and cognitive scores were not significant after corrections for multiple comparisons, preliminary positive correlations between MTR in the anterior corpus callosum and accuracy in processing speed and attention tests, as well as correlations between MTR in the inferior longitudinal fasciculus and reaction times in tests of processing speed, suggest that further work is required to determine the validity of these associations. Future research should continue to explore the brain changes in larger samples of PBT survivors and evaluate the separate and combined effects of metformin and exercise on MTR and cognition.

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Conflicts of Interest

The authors have no conflicts of interest to declare.

Data Availability Statement

Research data are not shared.

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