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Years of play alter MRI measures of brain health in former Canadian Football League athletes: a pilot study

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Abstract

Introduction: Concussive and sub-concussive blows are commonly sustained during contact sports. Through a detailed neuroimaging analysis, this pilot study aimed to determine if a history of sport-related concussions exacerbated cognitive decline later in life. It was hypothesized that clinical health assessments and magnetic resonance imaging (MRI) techniques would provide insight into lasting health and well-being, structural, microstructural, and functional alterations caused by a history of concussive injuries. **Materials and Methods:** Twenty aging, retired Canadian Football League (rCFL) players (aged 56.9 ± 6.9) had clinical testing and MRI data acquired. Cortical thickness, voxel-wise diffusion tensor imaging (DTI), and Default Mode Network (DMN) connectivity data was collected for each subject and compared against healthy controls. Retired athlete age, playing position, and career length were also examined. **Results:** This study found widespread cortical thinning, significantly increased mean diffusivity, increased axial diffusivity, and both hyperactivity and hypoactivity within the DMN. Athlete age, position, and career length all influenced microstructural integrity. On average, retired athletes scored about 4 times greater depression-like and concussion-related symptoms and scored significantly lower in all health categories compared to healthy controls. **Conclusions:** These findings suggested that lasting signs of neurological injuries were present years after retiring from professional play.

Keywords

magnetic resonance imaging (MRI), sport-related concussion, default mode network (DMN), cortical thinning, diffusion tensor imaging (DTI)

Introduction

Sport-related concussions affect about 1.6 to 3.8 million athletes in the United States annually.[1] A concussive injury often comes with a host of post-concussive symptoms including fatigue, dizziness, headaches, depression, irritability, and deficits in memory and executive function.[2] In addition to traditional concussive injuries, athletes participating in contact sports are prone to sub-concussive traumas as well. These sub-concussive incidents, potentially occurring in large numbers throughout an athlete's career, often do not present with common observable signs and symptoms.[3,4] Similar to concussions, these repetitive sub-concussive blows place an athlete at higher risk for developing persistent post-concussive symptoms, structural brain alterations, and neurodegenerative diseases.[5–8] The past decade has seen a steady rise in reported sport-related concussions.[9–11] However, this increase is likely a result of

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heightened concussion awareness efforts, improvements in injury identification, and changes in symptom reporting; rather than an indication of increased aggression in sports.[9] Sports that consistently report the highest concussion exposure rates include high intensity collision and contact sports such as American football, ice hockey, lacrosse, rugby, and soccer.[9–11]

Diffusion tensor imaging (DTI), functional MRI (fMRI), and high-resolution anatomical data have demonstrated capacity for detection and characterization of minute brain changes common to concussions.[12] Extensive research has been focused on the neuroimaging-based assessment of subjects in the acute (hours to days) and sub-acute (days to weeks) periods post-concussion.[12–14] Studies have shown that participation in collision sports can lead to widespread grey and white matter abnormalities,[15,16] however, neuroimaging research into the chronic effects of a history of concussion in older adults has produced variable and potentially contradictory results. For example, Terry et al. (2019) found only minimal white matter damage in the internal capsule in former high school football players with a history of at least two concussions, now aged between 40 to 65,[17] compared to Guell et al. (2020) who found widespread functional connectivity dysfunction in the cerebrum and cerebellum in aging, retired athletes[18] or Clark et al. (2018) who found white and grey matter alterations that were associated with number of concussions and career length in former collegiate athletes.[19] Thus, more research is required to determine if specific brain regions or athlete sub-populations are particularly vulnerable to long-term brain abnormalities.

With the recent discovery of an overwhelming presence of chronic traumatic encephalopathy (CTE) in retired National Football League (NFL) athletes (110 of 111 NFL players, and 87% of all athletes tested), it is essential to identify a potential imaging-based indicator for poor later-life neurological outcome, CTE existence, and neuropathology progression.[20] Furthermore, as there is variability in frequency and magnitude of head contact, concussion incidence and long-term brain health are based on a player's position.[20–24] A recent study of NFL players separated player positions into three profiles based on magnitude and frequency of repetitive head impact exposure.[23] It was determined that the quarterback, running back and defensive back positions experienced the fewest, albeit significantly highest magnitude, impacts with the largest time interval between impacts, whereas the offensive and defensive linemen positions experienced the highest frequency of impacts, with the least time interval between impacts, and primarily with low magnitude.[23]

The diverse findings of recent neuroimaging studies involving retired athletes with a history of sport-related concussion have uncovered the inhomogeneity of these traumatic biomechanical events and made it challenging to determine a common injury signature.[25–29] To computationally model a common injury site, sport-related concussions were simulated and found the corpus callosum (CC) to be a common site of high strain during concussive events.[30,31] The CC was also indicated as a common location of compromised microstructural integrity and structural thinning in retired NFL players.[26–29] Altered functional connectivity, measured using resting state fMRI (rsfMRI), has also been detected in retired professional athletes.[30,32,33] The default mode network (DMN), a robust network activated during wakeful rest, appears supressed or dysfunctional in former athletes compared to healthy controls.[33–35]

Our work focused on a quantitative neuroimaging analysis of former professional athletes of the Canadian Football League (CFL), compared to age/sex-matched healthy controls. Objective *in-vivo* measures must be applied to distinguish brain alterations exceeding the normal effects of aging in order to better understand the implications associated with repetitive sport-related head impacts. Cortical thickness measurements, DTI, and functional connectivity analyses were used to comprehensively investigate structural, microstructural, and functional brain alterations. The goal of this pilot cohort study was to examine brain health and potential long-term deleterious effects of a professional career in a high-impact sport such as American-style football. It was hypothesized that alterations would be present relative to healthy controls exhibited as cortical thinning (i.e., structural), reduced fractional anisotropy (FA) and axial diffusivity (AD) and increased mean diffusivity (MD) and radial diffusivity (RD) (i.e., microstructural), and reduced fMRI signal and network dysfunction (i.e., functional). From an exploratory standpoint, the effects of career length and playing position on brain health were also examined. The expectation was that a longer career and playing positions known to include a high frequency of low-magnitude head impacts would be associated with more severe brain alterations.

Materials and methods

Subjects

The local institutional research ethics board approved our study which was in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. Twenty retired CFL athletes (rCFL) (age 56.9 ± 6.9; **Table 1**), having partaken in at least one season of professional play, were locally recruited. Specific exclusion criteria for recruitment included a documented history of neurological or psychiatric illness, documented substance abuse, recent concussion not related to sports (within the past five years) or any medical condition requiring daily medication/therapy (i.e., malignant cancer, diabetes, hypertension, and cardiovascular disease). A neuroradiologist also reviewed the routine MRI scans of participants (i.e., T1-weighted, T2- weighted, T2*, susceptibility weighted imaging) to rule out any undiagnosed neuropathology (e.g., microbleeds, T2 hyperintensities, profound ventricular hypertrophy and brain atrophy (beyond that of normal aging), brain lesions visually causing structural damage). No former player included in the study had brain abnormalities visible at the time of the study.

For exploratory analyses, the rCFL sample was further subdivided (**Table 2**) based on years of professional play (i.e., less or greater than 10 years), frequency of contact by position (i.e., high frequency on offense: center, running back, full back, guard and tackle; high frequency on defense: defensive tackle, linebacker and defensive end; low frequency on offense: quarterback, wide receiver and tight end; low frequency on defense: safety and cornerback),[23] and on age at the time of the study (age <58 or age ≥58).

All rCFL subjects received an MRI and clinical testing to assess overall brain, mental and physical well-being. The clinical tests were the Beck Depression Inventory II (BDI II), Short Form Health Survey (SF-36), and the Post-Concussion Symptom Scale (PCSS). The SF-36 consists of self-reported questions regarding a vast range of health criteria offering insight into a person's mental state and perception of themselves. Twenty age/sex matched healthy controls were recruited from the community to complete the clinical tests but due to funding constraints did not undergo MRI scanning.

The rCFL subjects of this current study were also part of research exploring concussions and brain health using electroencephalography (EEG) and event-related brain potentials (ERPs). Three EEG-based articles were published from this dataset that yielded evidence of an overall decrease in brain health detectable many years after their professional football career.[36–38]

Table 1. Athlete and healthy control subject data.

(Years)	Athlete Retired CFL (n = 20)	Healthy Control fCONN (fMRI) (n = 19)	Healthy Control ADNI (Anat/ DTI) (n = 26)
Subject Age	56.9 ± 4.7	63.1 ± 4.8	
CFL Career	7.8 ± 4.1	0	0
Years Since Retiring	28.2 ± 8.0	0	0

MRI data acquisition and pre-processing

Subjects were scanned using a GE MR750 Discovery 3 T MRI scanner with a 32-channel RF receiver head coil (General Electric Healthcare, Milwaukee, WI). Following a routine 3-plane localizer a 3D inversion recovery-prepped T1-weighted anatomical data set was acquired. This was followed with a resting state fMRI scan (eyes closed) and lastly a diffusion tensor imaging scan. All scanning acquisition details are provided in **Table 3**.

Healthy age/sex matched control data ($n = 19$, age 52.0 ± 4.7) for rsfMRI was obtained from the 1000 functional Connectome Project (fCONN) database[39] **Table 1**. They were classified as having no history of neurological or psychiatric disorders and acquired using a 3 T GE scanner (parameters in **Table 3**). Healthy age/sex matched subjects ($n = 25$, age 63.1 ± 4.8) with 3D anatomical and diffusion data was downloaded from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database[40] **Table 1**. The anatomical and diffusion data was acquired at 14 different sites across North America using 3 T GE Healthcare MRI scanners. The MRI parameters for the rCFL players, fCONN dataset and ADNI dataset are detailed in **Table 3**. Although they are not identical, the differences are very minor and would not impact the data or make the datasets too dissimilar to compare. There was no significant difference in age between the rCFL players and the healthy controls used in the rsfMRI analyses ($p = 0.17$), but the healthy controls used in the DTI analyses were significantly older ($p = 0.000051$). This should not affect the results but is a potential limitation to this study.

Image data pre-processing

Image data pre-processing was performed using *FSL*.[41–43] In order to allow for more involved analyses to be implemented, all T1-structural images were first brain extracted

Table 2. Subdivisions of retired athletes. Half of the subjects played less than 10 years while half played more than 10 years (mean age differentiating the groups was 58yrs). Player position was taken into consideration as either those who most likely experienced frequent contact (e.g., offensive players: center, running back, full back, guard and tackle; Defensive players: defensive tackle, linebacker and defensive end) or those less likely to experience frequent contact (e.g., Offensive players: quarterback, wide receiver and tight end; Defensive players: safety and cornerback). YOP = Years of play; YSR = years since retirement.

(Years)	Age < 58	Age ≥ 58	YOP < 10	YOP ≥ 10	Frequent Contact	Less Frequent Contact
Number of players	10	10	12	8	13	7
Age	50.8 ± 4.7	62.4 ± 2.5	56.0 ± 6.1	58.0 ± 7.6	56.8 ± 7.1	57.1 ± 7.9
YOP	6.10 ± 4.0	9.3 ± 3.7	4.6 ± 2.2	12.0 ± 1.3	8.5 ± 4.2	8.0 ± 3.5
YSR	23.4 ± 8.2	32.6 ± 4.3	30.1 ± 7.1	25.8 ± 8.3	27.7 ± 8.0	27.7 ± 10.3

Table 3. A summary of the magnetic resonance imaging (MRI) scan parameters for the three acquisition techniques of 3D anatomical, diffusion tensor imaging (DTI), and resting state functional MRI (rsfMRI) for both the control data and the retired Canadian football league (rCFL) players.

	3D anatomical scan		DTI		Resting state	
	Controls (ADNI)	rCFL data	Controls (ADNI)	rCFL data	Controls (fCONN)	rCFL data
Sequence details	- Inversion-recovery (IR) prepped - Fast SPGR - axial acquisition	- Inversion-recovery (IR) prepped - Fast SPGR - axial acquisition	- dual echo spin echo EPI - 41 non-coplanar directions (b = 1000 s/mm^2) - 5 b = 0 s/mm^2 images	- dual echo spin echo EPI - 60 non-coplanar directions (b = 1000 s/mm^2) - 6 b = 0 s/mm^2 images	- eye closed - gradient echo EPI - axial acquisition - 128 temporal points	- eyes closed - gradient echo EPI - axial acquisition - 175 temporal points
Acquisition matrix	- 256 × 256 - 25.6 cm FOV - 1.2 mm thick	- 256 × 256 - 25.6 cm FOV - 1 mm thick	- 96 × 96 - 25.9 cm FOV - 2.7 mm thick	- 122 × 122 - 24.4 cm FOV - 2 mm thick	- 64 × 64 - 25.6 cm FOV	- 64 × 64 - 22 cm FOV - 3 mm thick
Resolution	1 mm in-plane 1.2 mm thick	- 1 mm isotropic	- 2.7 mm isotropic	- 2 mm isotropic	- 4 mm isotropic	- 3.44mm^2 - 3 mm thick
Timing	TE = 2.85ms TR = 6.98ms TI = 400ms flip = 11°	TE = 4.25ms TR = 11.36ms TI = 450ms flip = 12°	TE = 87ms TR = 9000ms	TE = 87ms TR = 8800ms	TE = 50s TR = 2000ms flip = 90°	TE = 35ms TR = 2000ms flip = 90°
Slices	130 contiguous slices	140 contiguous slices	55 contiguous slices	70 contiguous slices	23 contiguous slices	35 contiguous slices

using the Brain Extraction Tool (*BET2*).[44,45] BOLD rsfMRI data was motion corrected using *MCFLIRT*.[46] DTI images were corrected for eddy-current induced distortions and head motion through the use of the FSL tool *eddy*,[47] and non-brain tissue was removed using *BET2*.[44,45]

Cortical thickness measures

Segmentation and measurement of cortical structures across the subjects was performed using FreeSurfer (version 6.0.0).[48,49] Quality control was performed through manual inspection of individual images. Images were smoothed with a 5 mm full-width half-maximum (FWHM) kernel. Voxel-wise cortical thickness maps were generated using group (rCFL vs controls) as a factor, and age as a covariate. Correction for multiple comparisons was performed using the false discovery rate (FDR, $p < 0.05$).

Diffusion tensor imaging

To perform whole-brain, voxel-wise, connectivity based parcellation analysis of DTI data, the FMRIB Diffusion Toolbox (*FDT*) and Tract-Based Spatial Statistics (*TBSS*) programs were used.[41–43,50–55] Through *FDT*, diffusion tensors were reconstructed by estimation and fitting of a tensor model to the raw diffusion data, resulting in images of fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD) and radial diffusivity (RD). Axial diffusivity is the principal diffusion eigenvector ($AD=\lambda_1$) while RD was calculated from averaging the second and third tensor eigenvectors ($RD = (\lambda_2+\lambda_3)/2$). From here, a common registration target was created using non-linear transformation matrices and an affine transform. Then, using *TBSS*, each subject's aligned FA image was projected onto a tract-invariant skeleton target, representative of all included subjects' white matter (WM) anatomy. This skeleton represents voxels with the highest and most consistent FA measurements across all subjects. To reduce the inclusion of grey matter and CSF voxels in the skeleton, the threshold of the FA skeleton was set to 0.35. Voxel-wise statistics were performed, in addition to cluster-based identification of significant regions of interest (ROI) based on the ICBM-DTI-81 white-matter labels atlas; this atlas consists of 48 WM tracts that have been identified by averaging diffusion MRI maps from 81 subjects.[56] Group differences in DTI measures were probed through permutation testing methods, and the use of the *FSL Randomise* program.[57] The design of a linear model placing patients and controls in different sub-categories, including subject age as a covariate in the analysis, and subsequent application of Threshold-Free Cluster Enhancement (TFCE) provided the basis for observing and quantifying any significant group differences in structural integrity between the former-athlete and healthy control populations. Further statistics were performed between different groupings within the rCFL population, as noted in **Tables 1 and 2**.

Resting state fMRI

To perform seed-based ROI rsfMRI analysis, the data was first pre-processed as described above. The overall analysis pipeline followed the procedure detailed by Uddin and colleagues, with slight modifications to the masking and statistical methods.[58,59] An ROI within the posterior cingulate cortex (PCC) was created on a standard MNI 1 mm atlas through placement of a 6 mm sphere. The sphere for the PCC ROI was centered at MNI coordinates $x,y,z = 2, -60,36$. Masks of cerebral spinal fluid (CSF) and white matter (WM) regions were created for each subject's anatomical data through *FMRIB's Automated Segmentation Tool* (*FAST*).[60] Next, *FMRIB*'s *FEAT* analysis package was used to filter and spatially smooth the functional datasets (5 mm FWHM), along with registration of each subject's functional data to their anatomical data using the Boundary-Based Registration (BBR) algorithm, and finally to a standard MNI152 1 mm average image.[61] The output transformation matrices for each subject (from anatomical to standard space) were used to fit the previously created CSF and WM masks to a patient's individual atlas data. Time series data was then extracted from the standard space functional data through the CSF and WM masks and normalized using R.[62] These normalized time series were used as regressors within a higher-level analysis in *FEAT* through a General Linear Model (GLM). A similar process was used for implementation of the PCC ROI, where the time series data was extracted and normalized, and used within a higher-level regression analysis in *FEAT* through design of a GLM. Group statistics were done using *FMRIB*'s Local Analysis of Mixed Effects (*FLAME*).[63] The *FLAME* program estimates the true degrees of freedom and random effects at each voxel, resulting in Z-maps of regions of significant positive and negative correlation with the PCC seed region.[63] Detrimental effects of WM and CSF regions were removed by including their masks and normalized time series information as regressors within the *FLAME* analysis. Correction for multiple comparisons was performed through *randomise* within *FSL*, with the minimum cluster significance set to $Z = 2.0$.[57]

Imaging technique statistics

A series of Pearson correlations were performed to determine if there were any connections between cortical thickness, DTI and rsfMRI results. Correlations were made using the voxel-wise outliers for each technique and each subject, which was calculated from Z-scored MRI data in R.[62] Only the DTI and rsfMRI outliers were included in these statistical analyses to narrow the scope of the analyses and explore the relationship between outliers representative of concussion-related brain abnormalities across several neuroimaging techniques. Based on findings of previous research, it was expected that DMN rsfMRI and FA values would be decreased, and MD values increased, with injury.[27,35] Therefore, only Z-score outliers less than two standard deviations lower than the control mean (i.e., $Z \leq -2$) were considered for correlations between rsfMRI and FA, and only Z-score outliers greater than two standard deviations from the control mean (i.e., $Z \geq 2$) were considered for MD. Outliers both greater than and less than two standard deviations from the control mean (i.e., $-2 \geq Z \geq 2$) were used for cortical thickness as some brain regions may have thickened, and some may have thinned.

For the exploratory within-group analyses for playing position and career length, age was not applied as a covariate in the general linear modelling due to the small sample size that under-powered the analysis, and the results are presented and interpreted as preliminary findings. Also, no family-wise error correction was applied to these analyses because of the exploratory nature of these comparisons.

Clinical testing statistics

A series of two-tailed Student's T-tests were used to compare the clinical test scores between the 20 rCFL players and the 20 age-sex matched healthy control community members. A Bonferroni correction was applied to apply a family-wise error correction, which lowered the p-value for the clinical test statistical analyses to 0.005 based on the 10 categories (BDI-II, PCSS, SF-36: physical functioning, physical health, emotional health, energy & fatigue, emotional well-being, social functioning, pain, and general health).

Results

Clinical testing results

The rCFL players had a BDI-II score of 8.15 ± 7.03, which was significantly higher ($p = 0.0021$) compared to healthy age/sex matched controls (2.2 ± 2.68). The rCFL group mean score of 8.15 fell within the none to minimal depression category (0-13), with only some rCFL players scoring mild depression (10-18). But on average, the rCFL population reported almost 4 times greater levels of depression-like symptoms than controls. The highest recorded BDI-II score was 25, which placed that rCFL subject within the moderate depression category.

The rCFL players also scored more than 4 times higher with PCSS compared to healthy controls (13.35 ± 12.29 compared to 2.8 ± 5.55) and scored significantly higher on the PCSS test ($p = 0.0017$). The most frequent high scoring categories for rCFL subjects were irritability (80%, 16 of 20), difficulty remembering (70%, 14 of 20), and difficulty sleeping (45%, 9 of 20), consistent with previous work.[2]

From the SF-36 clinical test, the rCFL players reported lower scores in all eight categories. Furthermore, rCFL players self-reported significantly lower social function ($p = 0.0035$) and pain ($p = 0.00046$) scores than the healthy controls (**Table 4**).

Cortical thickness measures

Voxel-wise cortical thickness analysis revealed significant cortical thinning in the rCFL group compared to healthy age/sex matched controls. When comparing group means, 13 clusters survived the correction for multiple comparisons (**Table 5**). Regions of cortical thinning in players compared to controls included the precuneus, inferior and superior parietal, lateral occipital, caudal middle frontal, inferior and superior temporal, lingual, superior temporal, and paracentral gyri (**Figure 1**). No significant differences in measures of cortical thickness were found when looking within the rCFL population at sub-groupings based on length of professional career, position played or age.

Diffusion tensor imaging

The ICBM-DTI-81 white-matter labels atlas was used to identify 48 individual WM structures with notable local differences in voxel-wise FA, MD, AD and RD measures compared to controls. After correcting for multiple comparisons and including subject age as a covariate, regions or clusters along WM tracts were extracted that demonstrated significant deviation ($p < 0.05$) from their respective group control mean (**Figure 2**).

The rCFL sample exhibited significant increases in MD and AD compared to the control population as revealed through group-wise *TBSS*. The ROIs with increased MD in rCFL subjects compared to controls included the genu

Table 4. Results of SF-36 behavioural testing. rCFL players versus age/sex matched healthy controls. rCFL players demonstrate significantly lower measures in social function and pain compared to controls (* = $p < 0.005$).

Behavioural metric	Controls	rCFL	p-value
Physical Function	83.75 ± 27.57	78.25 ± 19.21	0.47
Limitations due to Physical Health	98.75 ± 5.59	88.75 ± 23.61	0.080
Limitations due to Emotional Health	98.33 ± 7.45	81.67 ± 36.64	0.060
Energy/Fatigue	72 ± 11.74	62.25 ± 20.29	0.073
Emotion Well-Being	87.6 ± 6.86	77.8 ± 18.28	0.034
Social Function	97.5 ± 5.13	79.38 ± 24.09	0.0035*
Pain	84.5 ± 17.6	61.13 ± 20.72	0.00046*
General Health	81 ± 12.73	72.75 ± 16.34	0.083

Table 5. Cluster information from cluster wise correction for multiple comparisons of the voxel-wise cortical thickness measures. All rCFL players were placed against controls, with age as a covariate.

Cluster No.	Size (mm^2)	MNI Coord. (X Y Z)	Peak p-value	Region (lobe/gyrus)
1	1646.27	−17, −70, 32	0.0002	Precuneus L
2	970.97	−26, −82, 13	0.0002	Superior Parietal L
3	875.37	−37, −79, −14	0.0006	Lateral Occipital L
4	746.62	−27, −82, 13	0.002	Caudal Middle Frontal L
5	546.81	−39, −80, 6	0.02	Lateral Occipital L
6	7243.99	43, −61, 0	0.0002	Inferior Temporal R
7	1729.70	25, −5, 0	0.0002	Lingual R
8	1663.08	49, 20, 11	0.0002	Pars Opercularis R
9	1564.72	48, −11, −16	0.0002	Superior Temporal R
10	1091.17	9, −38, 58	0.0002	Paracentral R
11	914.21	43, 4, 22	0.0002	Pars Opercularis R
12	575.14	47, −63, 32	0.017	Inferior Parietal R
13	502.59	41, −35, 39	0.036	Supramarginal R

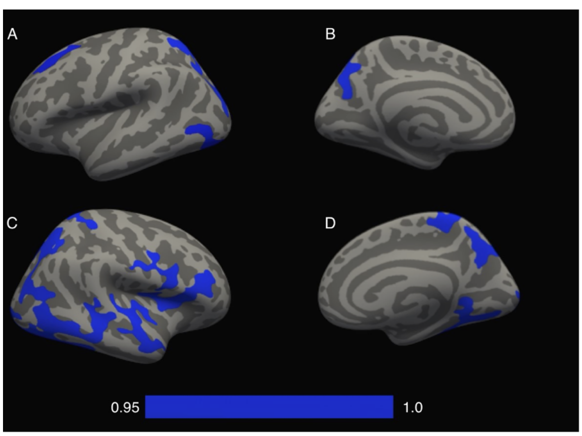

Figure 1. Cluster corrected voxel-wise cortical thickness measures comparing rCFL and control groups. Blue regions indicate areas of significant cortical thinning in the rCFL group compared to the controls ($p < 0.05$), including age as a covariate. Images A and B show the outer and inner cortical surfaces of the left hemisphere. Images C and D demonstrate inner and outer regions of the right hemisphere.

of the CC and left anterior corona radiata (ACR). As for AD, the ROIs that were increased in rCFL subjects compared to controls included the left optic radiation (OR), left ACR, left inferior longitudinal fasciculus (ILF) and splenium and genu of the CC. The differences detailed above included microstructural changes within commissural tracts (connecting the same cortical region within left and right hemispheres), namely the CC, along with projection tracts (tracts inter-connecting regions of the cortex with other parts of the CNS) such as the left OR and left ACR. In comparing DTI results with behavioural scores, it was noted that the PCSS score of 'feelings of sadness' correlated with increases in MD ($r = 0.656$) and AD ($r = 0.650$), while 'sensitivity to light' correlated with decreased measures of FA ($r = 0.677$). Measured with the SF-36 test, limitations due to emotional health correlated with increases in MD ($r = 0.645$) and AD ($r = 0.648$).

Exploratory within rCFL group results

Statistical analyses performed within the rCFL subject group revealed significant differences in all three group-wise comparisons. In the division of players based on position, athletes who played in a position with more frequent contact exhibited a small region of decreased FA within the genu of the CC ($p < 0.05$) (**Figure 3**). No other significant differences in MD, AD or RD were noted in the comparison between subject position. Based on CFL career length, increased measures of RD ($p < 0.05$) were found in athletes with professional career lengths greater than 10 years compared to those who played less than 10 years (**Figure 4**). This indicated that athletes with longer professional careers exhibited changes in core WM microstructure when compared to those with shorter careers. These differences appeared within the body of the CC, superior corona radiata (SCR), and superior longitudinal fasciculus (SLF). In the comparison based on rCFL subject age, significant and diffuse changes were noted for several different DTI metrics ($p < 0.05$) (**Figure 5**). Decreased FA in older players was observed bilaterally in the ACR, SLF, OR, external capsule, and genu and body of the CC. Interestingly, there were also increases in measures of MD and RD in the right external capsule, right OR, and splenium and body of the CC measured in older rCFL players, compared to the younger rCFL cohort.

DMN in rCFL and control samples

Several significant differences in DMN connectivity were noted between the rCFL and control samples (**Figure 6 & Figure 7**). The rCFL group exhibited decreased connectivity within the paracingulate gyrus (frontal pole) portion of

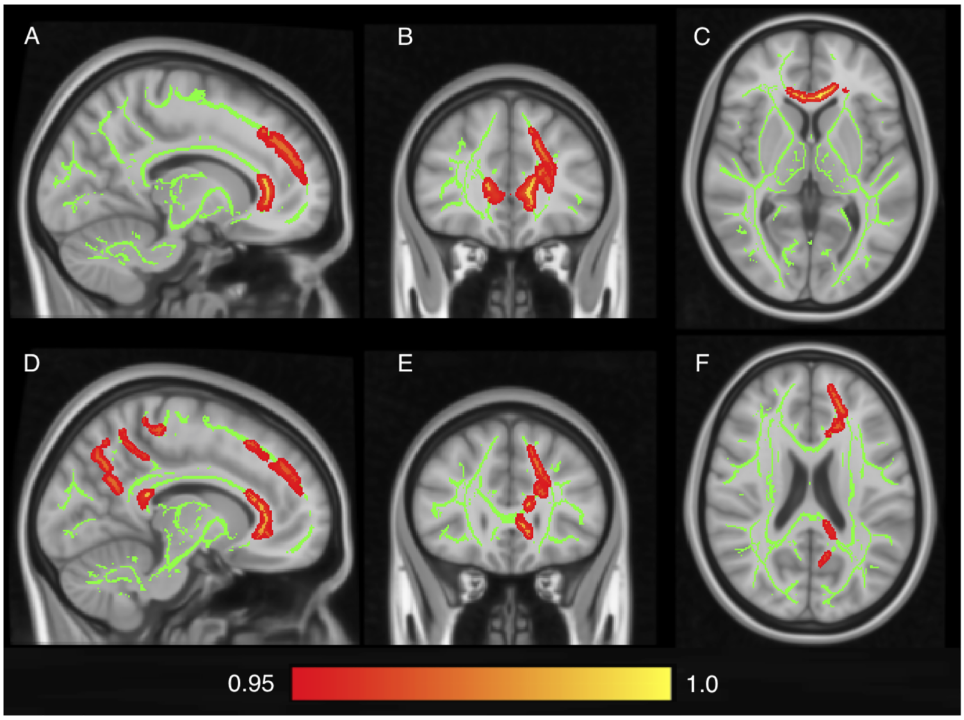

Figure 2. DTI TBSS results of MD (A, B, C) and AD (D, E, F) measures for rCFL group compared to control comparisons, shown projected onto a standard MNI 1 mm template and tract-invariant FA skeleton. Regions appearing in red-yellow indicate a significant increase in that measure in the rCFL group compared to controls. All images are FWE rate corrected ($p < 0.05$) for multiple comparisons, including age as a covariate.

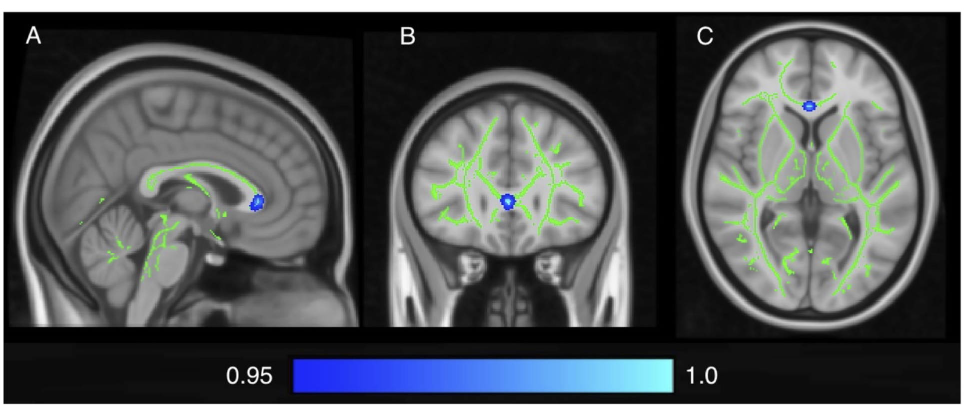

Figure 3. DTI TBSS results of FA measures for within rCFL group comparisons based on position, shown projected onto a standard MNI 1 mm template and tract-invariant FA skeleton. Regions appearing in blue-light blue indicate a significant decrease in that measure. All images are FWE corrected for multiple comparisons $p < 0.05$,. In the division of players based on position, athletes who played in a position where contact was more frequent exhibited a small region of decreased FA within the genu of the CC ($p < 0.05$).

the DMN, compared to controls ($p < 0.05$). Also, there was increased functional connectivity within the precuneus of rCFL subjects relative to controls ($p < 0.05$).

Imaging technique correlations

Pearson's r correlations were performed to determine if there were any relationships between imaging techniques that would aid in the development of a concussion-specific damage profile. Positive and negative outliers (i.e., $-2 \geq Z \geq 2$) for cortical thickness, decreased DMN fMRI and FA outliers (i.e., $-2 \geq Z$), and increased MD outliers (i.e., $2 < Z$) were considered. A strong, significant correlation was found between FA outliers and MD outliers ($r = 0.709$, $p = 0.001$) (**Figure 8**). The remaining correlations were weak (i.e., $-0.299 \leq r \leq 0.299$).

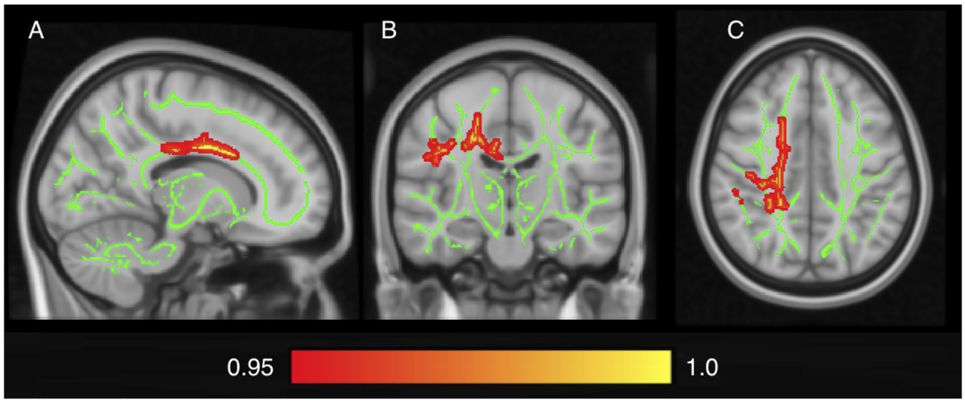

Figure 4. DTI TBSS results of RD (A, B, C) measures for within rCFL group comparisons based on career length, shown projected onto a standard MNI 1 mm template and tract-invariant FA skeleton. No other significant changes were noted in measures of FA, MD or AD. Regions appearing in red-yellow indicate a significant increase in that measure. All images are FWE corrected for multiple comparisons $p < 0.05$. Areas of higher RD were observed in the athletes with professional career lengths greater than 10 years in length compared to those who played less than 10 years.

Discussion

Comparison of neuroimaging between rCFL subjects and healthy controls

In this pilot study we showed that retired CFL players exhibited significant alterations to structural and micro-structural integrity and functional connectivity when compared to a group of age matched healthy controls. In addition, retired athletes who had a longer professional career, and those who participated in a position involving more frequent contact, exhibited a poorer microstructural outcome. Age at the time of scanning comparisons also found significant and diffuse alterations to microstructural integrity observed in the older subjects (62.4 ± 2.5yrs) relative to the younger subjects (mean age 50.8 ± 4.7yrs).

Prior neuroimaging studies examining retired athlete populations have expressed mixed results and offer conflicting insights as to a common neuroimaging-based indicator for long-term concussion outcome assessment. However, the inclusion of differing imaging scans could offer improved capabilities. Recently, a study of 19 retired CFL athletes reported significant cortical thinning in the anterior temporal lobe and orbitofrontal cortex, negative correlations of uncinate fasciculus (UF) AD with error rates and aggression, and increased resting-state functional connectivity between the anterior temporal lobe and orbitofrontal cortex.[32] A similar population of 18 retired CFL athletes revealed increased AD measures in the SLF, corticospinal tract (CST) and anterior thalamic radiations (ATR) in retired CFL athletes compared to controls.[25] These findings contributed to the hypothesis that the health of associative WM tracts, such as UF and SLF, could be a determinant in behavioural and cognitive outcome of retired athletes.[25,32] Our study corroborated these previous findings and adds to the growing coherency of concussion injury profile. It also suggests the idea that a combination of increased measures of AD in association tracts (SLF, ILF, UF), cortical thinning in temporal regions, and altered functional connectivity could represent injury patterns that are indicative of lasting repetitive sport-related concussion abnormalities.

Other research into neuroimaging and behavioural changes in retired athletes has found more diffuse DTI changes.[26,64,65] One study found that aging former athletes with a history of sport-related concussion had decreased FA, along with increased MD and RD relative to healthy controls.[26] Increased MD values were found within the anterior body and genu of the CC, association tracts (ILF, SLF), and projection tracts (external/internal capsules, ACR, CST).[26] The regions of increased MD observed in our study agree with those above involving measured changes in primarily commissural and projection tracts.[26] Another study found decreased measures of FA in frontal and parietal regions, the CC, and left temporal lobe within a population of 34 retired NFL players.[64] Moreover, 45 retired NFL players (age = 30-60yrs) in another study had decreased FA and a higher depression incidence than the normal population.[65] In relation to normal, healthy aging brains there have been heterogenous findings between studies. A few larger-scale neuroimaging studies on healthy older adults demonstrated an increase in FA and AD with decreased RD in healthy older adults.[66,67] Contradictory to these findings, several studies have exhibited a gradient of change in healthy aging adults with brain-wide decreased FA and increased RD in frontal WM regions and the CC.[68–70] Similarly, we observed significant and diffuse decreased FA and increases in MD and RD in older rCFL athletes relative to the younger rCFL cohort.

In another study, a population of 54 NFL alumni aged 20–50yrs had hyperactive dorsolateral prefrontal cortex (DLPFC) during planning and retrieval tests, and

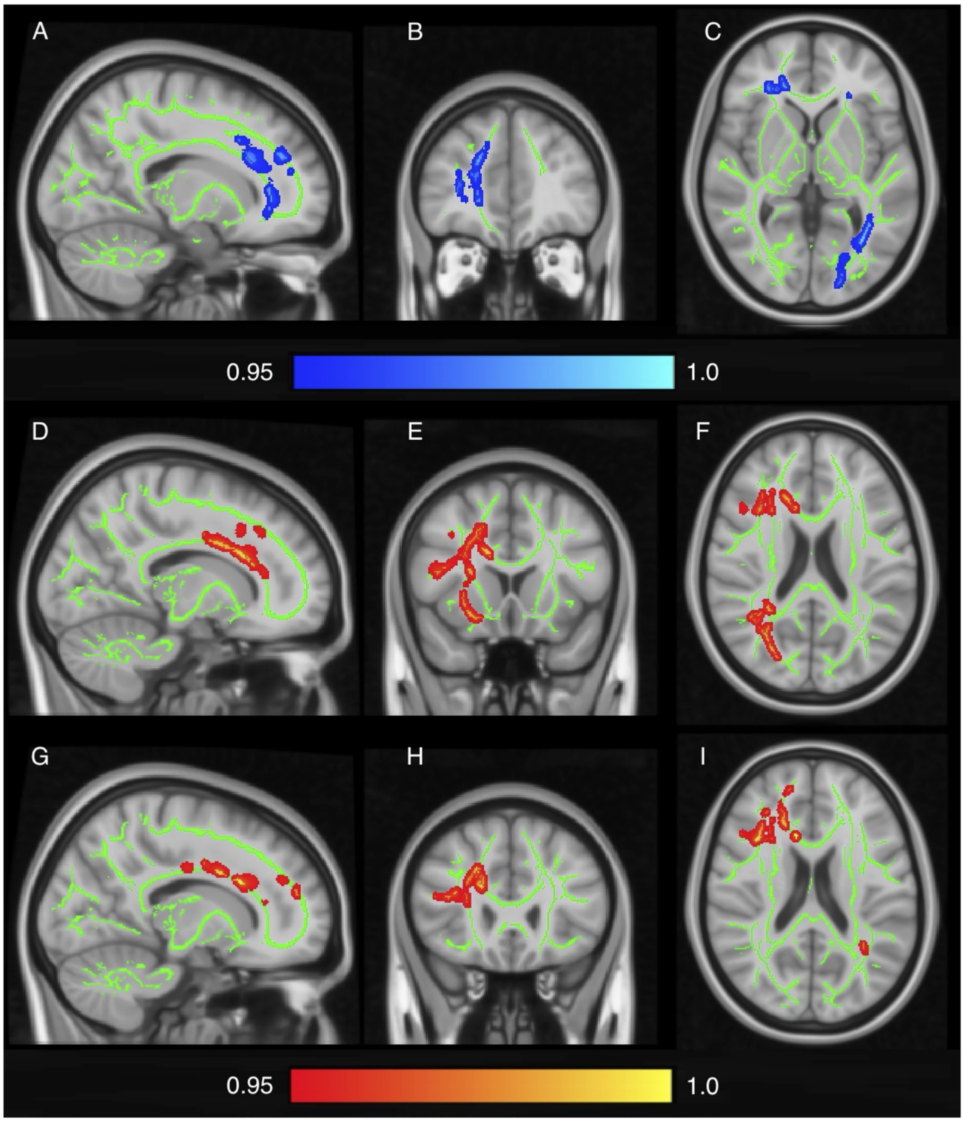

Figure 5. DTI TBSS results of FA (A, B, C), MD (D, E, F), and RD (G, H, I) measures for within rCFL group comparisons based on subject age. Regions appearing in blue-light blue indicate a significant decrease in that measure and red-yellow indicate a significant increase in that measure. Images A, B, and C show lower FA measures in the older rCFL group as compared to the younger rCFL group. Images D, E, and F indicate increased measures of MD and images G, H, and I detail increases in RD in the older rCFL athletes as compared to the younger rCFL population. All images are FWE corrected for multiple comparisons $p < 0.05$.

hypoconnectivity of the frontopolar cortex (FPC) when compared to healthy controls.[29] This result agrees with our findings of decreased connectivity within the vmFPC in rCFL subjects. These relatively consistent findings between studies suggest that changes in frontal region functional connectivity could be a sensitive neuroimaging-based indicator signifying lasting sport-related concussive injuries.

Finally, correlations between MRI methods produced interesting results based on the total voxel outlier count for each subject. The strong, significant correlation between FA and MD was a noteworthy finding as those two metrics were expected to be reciprocally representative of concussion-related abnormalities. The weak negative correlation between decreased DMN functional connectivity and decreased cortical thickness ($r = -0.200$) was surprising as it was expected that the DMN would be decreased in subjects with thinning cortical grey matter. Although each quantitative technique used in this study provided information on different parts of the brain, it was unfortunate we did not find an MRI measure that correlated with the other two (i.e., cortical thickness with fMRI and DTI).

Behavioural changes measured by clinical tests

It is interesting to note that our MRI findings were supported by changes in behavioural metrics. Lower scores in the SF-36 indicated overall poorer general health in the rCFL

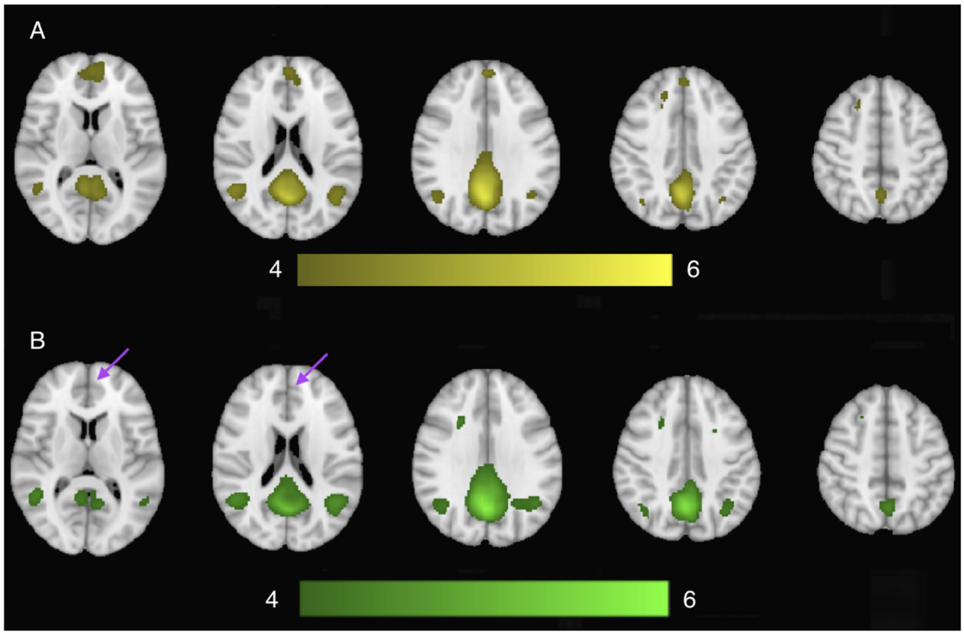

Figure 6. Results of rs-fMRI analysis, probing the DMN through seed-based correlation. The yellow regions indicate mean activation pathways of the DMN for the control population (A). Green regions show activation pathways for the rCFL subjects (B) ($4 < Z < 6$). Notice the lack of vmPFC recruitment in the rCFL population indicated by the purple arrow. All images are corrected for multiple comparisons, including age as a covariate.

group as compared to the control group. A history of concussive head trauma often correlates with higher depression incidence,[71–73] and this correlation becomes stronger with increased lifetime number of concussions.[74] In addition, retired professional football players who suffered one or two previous concussions were nearly 1.5 times more likely to be diagnosed with depression, and those with 3 or more were 3 times more likely.[73] Furthermore, of the 2552 retired professional football players in that study, those with a history of concussion and depressive episodes were also more likely to experience general pain (muscle and joint), declining health conditions and a feeling of helplessness.[73]

We reported significant cortical thinning, microstructural anomalies, and functional abnormalities. However, when performing the clinical testing correlative analyses, there were no strong correlations observed between any of the behavioural tests, rsfMRI results or cortical thickness measures. Moderately strong correlation, although not significant, was found between decreased measures of FA and sensitivity to light as measured by PCSS. Moderately strong correlations were also noted between increases in AD and MD, sadness scoring within the PCSS, and limitations due to emotional health scoring in the SF-36.

Exploratory: DTI differences based on position and career length

Comparisons based on player position offered interesting exploratory insights into potential long-term neurological differences due to frequent sub-concussive impacts, or less frequent higher force impacts. It was found that FA was decreased within the genu of the CC in players who participated in a position more prone to frequent impacts. This finding agrees with prior work investigating distribution of injury force that applied finite element modelling techniques and found that the CC was a site of consistently high strain in sport-related concussion patients.[31,75,76] A strong correlation between model predicted strain in the CC and CC microstructural outcome has been measured using DTI.[31] Further, data from this field suggests that impacts to the temporal region of the brain, as would be typically experienced with contact between linebackers, caused coronal rotations leading to high CC strain.[75] Therefore, the observed abnormal DTI measures (i.e., decreased FA) in rCFL subjects who played in a frequent contact position was corroborated by the literature.

In addition to playing position, a recent study used DTI to examine the relationship between age of first exposure to repetitive head impacts (through tackle football) and later-life CC microstructural integrity in 40 retired NFL players, 40-60yrs old.[77] Athletes who began playing football before the age of 12 demonstrated decreased measures of FA in the genu and body of the CC, and increased measures of RD in the CC genu.[77] In our study, it is likely that rCFL subjects who participated in a position more susceptible to frequent head contact may have been exposed to greater total number and frequency of head impacts. Moreover, head impact could have begun at a younger

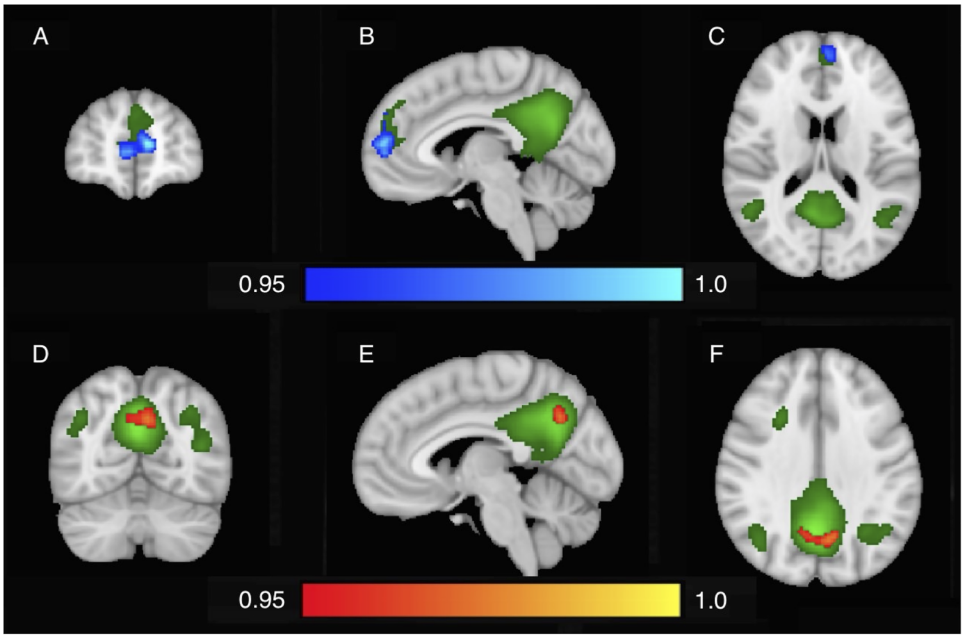

Figure 7. Results of rs-fMRI analysis, probing the DMN through seed-based correlation. The green regions indicate mean activation pathways of the DMN for the control (A, B, and C) and rCFL (D, E, and F) populations ($4 < Z < 6$). Regions in red-yellow indicate significant increases in network activity in rCFL compared to control populations, whereas regions in blue-light blue show significant decreases in activation in rCFL subjects ($p < 0.05$). All images are corrected for multiple comparisons, including age as a covariate.

age (i.e., linemen always experience contact, even in minor leagues), which supports the differences in FA within the CC that we observed based on playing position.

The exploratory career length comparison also yielded noteworthy results. With the understanding that increased RD is associated with demyelination,[78] and increased AD is associated with neuronal loss,[79] increased measures of RD, in absence of increased AD, within the CC, SCR and SLF could potentially be indicative of concussion-related demyelination within those regions.[80] However, this interpretation relies on the assumption that there are no WM fiber crossings within the outlying voxels.[81] Referring to the previous section discussing trauma induced in the coronal rotations of typical football impacts, the genu of the CC was identified as a primary region of potential high strain.[31,75,76] Therefore, it is well-founded that our study observed heightened measures of RD within the CC, and presumably decreased microstructural integrity, in the group of athletes who played longer professional careers. Further research should be performed on a larger sample of retired athletes, but our findings suggest that longer professional careers may have translated into more overall head impacts, and thus increased potential to sustain injury.

Exploratory: DTI differences in relation to age

Some of the most interesting findings from this MRI study surrounded the differences measured between the younger rCFL subject group and the older athletes. It is important to note that the sample sizes for the older rCFL group ($n = 11$) and younger rCFL group ($n = 9$) were both small and should be interpreted with that understanding. The older rCFL athlete group demonstrated significant abnormalities along commissural, association, and projection tracts as measured by regions of decreased FA, alongside increased MD and RD. Previous work investigating age related changes through DTI metrics reported a general pattern of decreased FA, along with increased MD and RD, with increasing age.[82,83] These aging observations traditionally begin to accelerate in subjects 50 to 60 years old.[82] However, our findings indicate a very significant decline in fiber integrity between the younger rCFL ($n = 9$, $\text{age} = 52.2 \pm 4.9$) and older rCFL ($n = 11$, $\text{age} = 62.7 \pm 2.2$) subjects. This result could point to accelerated, as well as more pronounced, age-related changes in those who have history of sport-related concussion. Another interesting factor to consider, however is that younger retired athletes played in the 1980-1990s, while the older retired athletes played throughout the 1970-1980s. At the end of the 1975 season, rules were set in place to prevent the use of the head as the initial point of contact during a tackle.[84,85] This change to the game, alongside technological advancements to helmet technology, could have had an impact on the severity of head trauma experienced by athletes during play, further contributing to the white matter differences observed between younger and older rCFL populations.

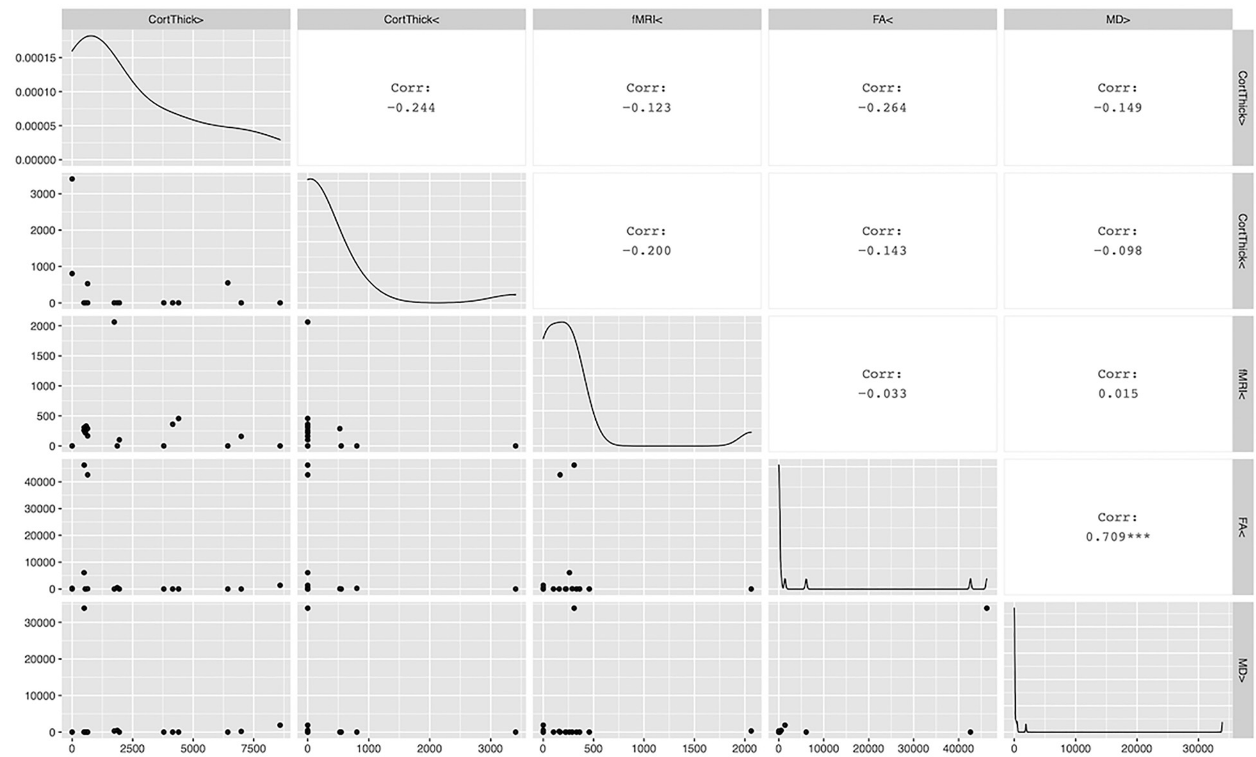

Figure 8. A correlation matrix of the five MRI metrics related to expected concussion induced abnormalities. These values were based on the number of Z-score outlier voxels that were either greater than or less than two standard deviations from the control mean, per subject for each metric. The nomenclature for this figure is as follows: "CortThick>": cortical thickness outliers greater than the control mean; "CortThick<": cortical thickness outliers less than the control mean; "fMRI<": functional MRI outliers less than the control mean; "FA<": fractional anisotropy outliers less than the control mean; "MD>": mean diffusivity outliers greater than the control mean. A strong, significant correlation was calculated between FA and MD ($r = 0.709$, $p = 0.001$).

Limitations

This pilot study utilized advanced MR imaging techniques to quantify brain health in retired professional athletes. Our work is not presented without limitations. First, the number of rCFL subjects was only twenty. Although this cohort was relatively homogenous as all players in the CFL are male and the subjects in our study were within a similar age range, more subjects would have bolstered our findings and provided us with the statistical power to sub-divide the rCFL subjects based on playing position and age groups. Therefore, this work should be considered as a pilot study. The results of this study will be used to inform the effect size and sample size calculations for future research on retired collision sport athletes. Despite the sub-division of subjects based on playing position being under-powered and had very limited group differences, we reported these exploratory results because there is evidence of different amounts and types of head impacts experienced by professional football players in relation to position.[21,23,24] It is extremely difficult to know the actual number of sub-concussive and concussive head impacts the rCFL subjects in our study sustained over their football careers. Therefore, unless we included substantially more subjects for each football playing position and knew exactly how many concussions each subject had sustained, the metric of previous concussions sustained should be used as an informative, but not conclusive, demographic metric.

Additionally, this study could have been made more robust with the inclusion of a greater number of controls to better approximate a healthy age/sex matched control distribution. Due to the nature of online MRI data repositories, information about demographics, health and lifestyle of healthy controls was unavailable for the downloaded datasets. Therefore, we could not match the controls with the rCFL subjects based on factors such as education, fitness level or possible substance abuse. The inclusion of downloaded healthy control MRI data from online repositories is increasingly common, but not without limitations. This study incorporated healthy control data from three different groups of cohorts collected on GE Healthcare, Philips Medical Systems, or Siemens Medical Solutions 3 Tesla magnets, but the MRI data was acquired using very similar scan parameters that would not effect the calculations of this study.[86] Furthermore, all the data was processed the same way (i.e., motion and eddy current correction, and warping into the MNI standard brain space), thus the datasets were comparable.

Another potential limitation is the application of a DTI analysis that assumes a single diffusion direction within voxels. The analysis technology for fixel-based measurements have made it possible for the assessment of fibre crossing, branching, and fanning within voxels.[87,88] A future direction for this work would be to apply a fixel-based analysis to corroborate the findings calculated using this DTI analysis.[88] Finally, although the DMN is a reliable and thoroughly researched functional brain network, limiting our analyses exclusively to the DMN may have resulted in missed functional abnormalities present for other networks in our subjects. Further research should explore other network connectivity measures such as the salience or fronto-parietal functional networks in a larger sample of retired athletes with a history of concussion.

Conclusions

Our pilot study found structural, microstructural, and functional alterations present in retired professional football players decades after their retirement from play. Along with being significantly distinguishable from healthy controls as a cohort of retired athletes, the various exploratory analyses of the rCFL subjects based on their age, position of play and the duration of their professional careers revealed interesting differences. To our knowledge, this was the first time this combination of factors has been examined. For many of the athletes in our study, it had been more than a decade since retiring from play. Our results, however, point to the discovery that many of these players are still suffering the effects of their (presumed) concussive past. In the performed behavioural testing, rCFL subjects reported 4 times greater levels of depression (in the BDI-II), as well as 4 times greater levels of symptoms in categories of irritability, sadness, difficulty sleeping, sensitivity to light, and difficulty remembering (in the PCSS) than those without a history of sport-related head injury. In addition, rCFL subjects reported significantly greater pain and reduced social function (in the SF-36) when compared to controls. These results reinforce the idea that all is not well for many of these former athletes. This study provides further evidence of the detrimental long-term effects of playing contact sports and the insightful ability of advanced MRI techniques to objectively identify and quantify concussion-related brain injuries.

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Declaration of conflicting interests

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