RESEARCH ARTICLE

White matter microstructure in anorexia nervosa

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Abstract

The neurobiological underpinnings of anorexia nervosa (AN) are unclear. White matter deficits have been described in the illness, but findings are inconsistent between studies. The aim of this study was to investigate differences in white matter microstructure in AN using diffusion-weighted imaging (DWI). It was hypothesised that people with AN, relative to a healthy control (HC) group, would show decreased functional anisotropy (FA) and increased mean diffusivity (MD) in the fornix and superior longitudinal fasciculus, consistent with previous literature. Analyses were conducted on 23 females with AN and 26 age- and gender-matched HCs using tract-based spatial statistics (TBSS). The results revealed widespread FA decreases and MD increases in the AN group. Our hypothesis was largely supported, although FA differences were not specifically found in the fornix. The findings suggest extensive differences in white matter structure in AN, which may contribute to AN pathophysiology.

KEYWORDS

diffusion, eating disorder, fractional anisotropy, magnetic resonance imaging, neurobiology

1 | INTRODUCTION

Anorexia nervosa (AN) is a psychiatric illness of unknown aetiology; although, biological, psychological and sociocultural factors all contribute to the illness. A number of psychological and sociocultural factors thought to be involved in AN have been identified (e.g., perfectionism, living in a Western culture) [Bastiani, Rao, Weltzin, and Kaye (1995); Miller and Pumariega (2001)]. However, the biological mechanisms involved in the illness are still poorly understood (Phillipou, Rossell, & Castle, 2014). Both functional and structural brain markers have been reported in AN, but with little consensus on the specific brain regions involved in the illness. Functional brain imaging typically involves identifying differences in response to certain stimuli, thereby not necessarily providing sufficient

insight into the neurobiological underpinning of dysfunction. Analysis of structural differences in grey and white matter may, therefore, provide a greater understanding of the neurobiological contributions to AN. Grey matter deficits have been long described in AN (i.e., reduced grey matter volumes) [e.g., Castro-Fornieles et al. (2009); Phillipou et al. (2018)]. Reductions in white matter volumes have also been described in AN (e.g., Boghi et al., 2011), but their interpretation is limited given their nonspecificity in describing the white matter tracts affected. The use of diffusion-weight imaging (DWI) to identify differences in white matter tracts of the brain in AN has the potential to inform our understanding of the illness.

DWI allows the assessment of microstructural white matter alterations based on the brain's water diffusion, typically through fractional

anisotropy (FA) and mean diffusivity (MD). FA is an index of the amount of diffusion asymmetry within a voxel and indicates the direction and degree of water diffusion. MD, conversely, is an index of overall diffusivity irrespective of direction. FA and MD are typically negatively correlated, with *decreased* FA and *increased* MD being indicative of white matter pathology. Both FA and MD represent aggregate measures, contributed to by axial diffusivity (AD) and radial diffusivity (RD). AD and RD represent parallel and perpendicular diffusivity directions in white matter tracts, respectively (for a review, see Alexander et al., 2011). *Decreases* in AD are associated with axonal injury (Sun et al., 2006), whereas *increases* in RD are associated with dysmyelination or demyelination (Song et al., 2002).

DWI studies have identified a number of differences in white matter in AN. Decreased FA, relative to healthy controls (HCs), has been reported in a number of regions with fibre tracking techniques including bilateral segments of the fimbria-fornix and superior longitudinal fasciculus (Travis et al., 2015), the fimbria-fornix (Frank, Shott, Hagman, & Mittal, 2013; Kazlouski et al., 2011), posterior thalamic radiation (Frieling et al., 2012), fronto-occipital fasciculus and posterior cingulum (Kazlouski et al., 2011). Utilising tract-based spatial statistics (TBSS) specifically, a number of studies have reported differences in FA and the other measures of diffusivity. A recent study by Gaudio et al. (2017) reported decreased FA in treatment-naïve adolescents with AN in the left anterior and superior corona radiata and superior longitudinal fasciculus; and decreased AD in the superior longitudinal fasciculus bilaterally and left superior and anterior corona radiata; but no difference in RD or MD to HCs. The authors suggest that alterations in the microstructural properties of these brain regions may reflect a vulnerability for impaired cognitive flexibility and body image distortion that are characteristic of AN.

The superior longitudinal fasciculus has been implicated in a number of these studies in AN and describes a major association fibre that connects the frontal cortex to parietal, temporal and occipital cortices. A study by Via et al. (2014) also reported decreased FA of the left superior longitudinal fasciculus, but with increased MD and RD, and no difference in AD. This study also reported decreased FA of the fornix that was associated with increased MD, RD and AD. The fornix is particularly related to reward processing, and deficits in this region may reflect the reduced capacity for reward, or anhedonia, reported in AN (Tchanturia et al., 2012). Similarly, Nagahara et al. (2014) reported differences in the fornix in AN, namely increased MD; as well as reduced FA of the left cerebellum in the AN group, that was positively correlated with body mass index (BMI). The study by Via et al. (2014), however, did not find correlations between BMI and any of the diffusion measures. In addition, behavioural findings from our recent eye movement research have suggested brainstem (specifically, superior colliculus) dysfunction in AN (Phillipou, Abel, et al., 2015; Phillipou, Rossell, Castle, Gurvich, & Abel, 2014; Phillipou, Rossell, Gurvich, Castle, & Abel, 2016; Phillipou, Rossell, Gurvich, Castle, Troje, et al., 2016; Phillipou, Rossell, Gurvich, Hughes, et al., 2016), although, to date, no DWI studies have specifically focused on brainstem differences.

Overall, studies to date are, somewhat limited given the small sample sizes (typically < 20 per group) as well as the failure to report correlations between the diffusion parameters and clinical measures to elucidate the effects of starvation and relationship to clinical

symptoms. The aim of the study reported here was to utilise TBSS to identify differences in diffusion measures between individuals with a current diagnosis of AN and HCs utilising a whole brain approach. A further exploratory aim was to correlate these measures with BMI and eating disorder symptomatology measures. It was hypothesised that individuals with AN would show reduced FA and increased MD in the fornix and superior longitudinal fasciculus, consistent with previous studies. No specific hypotheses were made in relation to RD and AD contributions. A further exploratory aim was to utilise a region-of-interest approach to identify if white matter microstructure of the brainstem differs in AN.

2 | METHOD

The investigation was carried out in accordance with the latest version of the Declaration of Helsinki. The study was granted independent ethics approval by the Human Research Ethics committees at St Vincent's Hospital, Austin Health and The Melbourne Clinic (HREC-A 057/12; NDSAC-HREC 2012/04646; TMC-HREC 235). In addition, the study received expedited ethics approval from Swinburne's Human Research Ethics Committee (SUHREC 2012/277) and was registered with The University of Melbourne Health Sciences Human Ethics Sub-Committee (HESC 1239068), on the basis of the prior St Vincent's Hospital review. Written informed consent was obtained from all participants.

2.1 | Participants

Twenty-six right-handed females with AN and 27 female HC participants underwent a magnetic resonance imaging (MRI) scan. Due to circumscribed excessive signal loss that could not be recovered, three AN and one HC participants were excluded, allowing analyses on 23 AN and 26 HC participants. Groups were matched on age and premorbid intelligence as determined by the Wechsler Test of Adult Reading (WTAR) (Wechsler, 2001). HCs were recruited through public advertisements, whereas AN participants were recruited through public advertisements as well as from the Body Image and Eating Disorders Treatment and Recovery Service at the Austin and St Vincent's Hospitals, and The Melbourne Clinic; all in Melbourne, Australia. All participants were English speaking and had no history of significant brain injury or neurological condition. HC were required to have no history of an eating disorder or other current mental illness.

The Mini International Neuropsychiatric Interview, 5.0.0 (MINI; Sheehan et al., 1998) was used to screen all participants for Axis I psychiatric disorders (including recent substance abuse) according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). The MINI was also used to confirm diagnoses of AN (restrictive AN, $n = 22$), with the exception of the amenorrhea criterion which is not included in DSM-5. AN was required to be the primary diagnosis of the AN group. AN participants with comorbid psychiatric conditions, other than psychotic conditions, were not excluded as this would not have represented a typical AN sample. The following comorbid conditions were identified in the AN group: current depressive episode ($n = 17$), dysthymia ($n = 15$), current manic/hypomanic episode

($n = 1$), current panic disorder ($n = 4$), social anxiety disorder ($n = 10$), obsessive compulsive disorder ($n = 13$), posttraumatic stress disorder ($n = 4$), alcohol dependence ($n = 1$), substance dependence ($n = 1$), bulimia nervosa ($n = 4$) and generalised anxiety disorder ($n = 17$). Eating disorder symptomatology was assessed with the Eating Disorder Examination Questionnaire (EDE-Q) (Fairburn, 2008). Participant characteristics are presented in Table 1.

2.2 | MRI image acquisition parameters

Images were acquired in 60 diffusion gradient directions (b -value = 2,000 s/mm²) and 10 nondiffusion weighted reference images using a sequence optimised to collect diffusion weighted images [repetition time (TR) = 9,200 ms, echo time (TE) = 102 ms, voxel size = $2 \times 2 \times 2$ mm³], on a Siemens Tim Trio 3 Tesla system with a 32 channel head coil at Swinburne University of Technology (Melbourne, Australia).

2.3 | MRI image preprocessing and statistical analysis

DWI data were processed and analysed using FMRIB Software Library (FSL) 5.0.10 (Smith et al., 2004). Diffusion-weighted images were initially corrected for subject motion and eddy current-induced

distortions using 'eddy' as implemented in FSL (Andersson & Sotiropoulos, 2016), with non-brain tissue removed from the eddy-corrected images using a binary brain-mask created with FSL's Brain Extraction Tool (Smith, 2002). Subsequently, FA and MD maps were estimated for each participant using the DTIFIT option within the FMRIB Diffusion Toolbox (FDT), by fitting a tensor model to the eddy-corrected and brain-masked diffusion data. AD (λ_1) and RD ($(\lambda_2 + \lambda_3)/2$) maps were also estimated using the eigenvalues associated with the fitted tensor model.

Tract-based spatial statistics (TBSS) was used to test for between-group differences in FA. Each participant's FA images were aligned to the $1 \times 1 \times 1$ mm standard space through registering them to the FMRIB58_FA template provided in FSL using the FMRIB nonlinear registration tool 'FNIRT'. A mean FA map was generated and subsequently a mean FA skeleton map created, representing the centre of all the tracts common to the group. The mean skeleton map was thresholded to the standard value of 0.2 to exclude any peripheral areas with high variability and/or partial volume effects, with each participant's FA map then projected onto the skeleton. Mean MD/AD/RD skeleton maps were created by applying the original nonlinear registration to the MD/AD/RD data for each participant, which was then merged together and projected onto the original mean FA skeleton.

Between-group comparisons of the resulting FA skeleton were performed using FSL's nonparametric permutation tool 'randomise' (Winkler, Ridgway, Webster, Smith, & Nichols, 2014), with 5,000 permutations and age included as a nuisance variable, with the following contrasts: HC > AN and AN > HC. Threshold-Free Cluster Enhancement (TFCE; Smith & Nichols, 2009) was used to obtain clusters and a family-wise error-corrected p value (p_{FWE}) of < .05. These preprocessing and thresholding procedures were repeated for the MD images. Anatomical localisation of the clusters was based on the John Hopkins University (JHU) White Matter Labels (1 mm) Atlas provided in FSL (Mori & Van Zijl, 2007). For each cluster found to have a significant group difference in FA or MD, cluster-averaged voxel values were calculated to obtain a summary measure of the diffusion properties in these regions for each participant. To this end, clusters of statistically significant voxels ($p < .05$, FWE) were identified with FSL's 'cluster' command (see Figure 1 for FA and MD cluster-averaged voxel values for significant clusters). To better characterise microstructural changes underlying the between-group differences in FA and MD, mean component diffusion measures (AD and RD) were examined within significant clusters. Post hoc two-sample t -tests compared the study groups on each component measure within these regions-of-interest.

2.4 | Brainstem sub-analysis

The region-of-interest analysis focussing on the brainstem was conducted using a more conventional voxel-based-morphological approach due to the skeletonisation process restricting inference to a limited 'skeleton' of white matter regions within the brainstem. The previously estimated FA map for each participant was registered to the FMRIB58_FA template using FSL's nonlinear registration tool FNIRT. The nonlinear warp was initialised with an affine registration generated by the linear registration tool FLIRT (Jenkinson & Smith, 2001), with the resulting warping transformations used to resample

TABLE 1 Participant characteristics

	AN M (SD) [range]	HC M (SD) [range]	F	p
Age	22.01 (5.42) [16.16–26.30]	22.61 (3.12) [18.83–26.75]	0.24	.629
WTAR	105.26 (7.87) [87.00–117.00]	106.00 (7.19) [87.00–120.00]	0.12	.733
BMI	16.71 (1.18) [14.39–17.71]	22.83 (3.38) [18.21–25.89]	67.92	<.001
Illness duration	5.35 (4.62) [1.00–20.00]	–	–	–
Age of illness onset	16.35 (2.90) [12.00–23.00]	–	–	–
EDE-Q restraint	3.81 (1.45) [0.60–6.00]	0.60 (0.63) [0–0.24]	96.97	<.001
EDE-Q eating concern	3.73 (1.27) [1.20–6.00]	0.25 (0.32) [0–1.4]	170.04	<.001
EDE-Q shape concern	4.99 (0.92) [3.13–6.00]	1.18 (0.86) [0–3.75]	206.11	<.001
EDE-Q weight concern	4.47 (1.37) [2.20–6.00]	0.69 (0.83) [0–3.00]	128.56	<.001
EDE-Q global score	4.25 (1.13) [2.08–5.69]	0.68 (0.54) [0–2.29]	189.04	<.001

Note. Data reported for 23 anorexia nervosa and 26 healthy control participants included in the diffusion analysis; AN = Anorexia nervosa; HC = Healthy control; WTAR = Wechsler Test of Adult Reading; BMI = Body mass index; EDE-Q = Eating Disorder Examination Questionnaire; Age, Illness duration and age of illness onset reported in years.

FIGURE 1 Fractional anisotropy and mean diffusivity values for anorexia nervosa (AN) and healthy control (HC) participants. Bars represent 95% confidence intervals

the FA, MD, AD and RD images into the FMRIB58_FA space. This resampling step was implemented with FSL's 'applywarp' command. Each image was visually inspected at each stage to ensure accuracy of registration and were then smoothed with an isotropic Gaussian kernel with full-width half-maximum of approximately 2 mm. The smoothed images of all participants were then merged into a single 4D image. Between-group comparisons and cluster enhancement were conducted as per above. HC > AN and AN > HC contrasts were performed to analyse group differences in FA and MD at each voxel comprising a liberal white matter mask of the brainstem that was delineated by the binarisation of a probabilistic subcortical structural atlas provided by FSL.

2.5 | Correlational analyses

Pearson's correlation analyses were performed separately for AN and HC groups between FA, MD, AD and RD, and BMI, EDE-Q scores and illness duration. Given these analyses involved exploratory correlations, a stringent alpha of .01 was selected as it is acknowledged that Bonferroni corrections in such circumstances are too stringent (Perneger, 1998).

3 | RESULTS

3.1 | Diffusion analyses

Group comparisons revealed a significant decrease in FA in the AN group relative to the HC group in a single WM cluster. The cluster encompassed the corpus callosum, the left and right corona radiata and superior longitudinal fasciculus, as well as the right posterior thalamic radiation (Figure 2a, Table 2). There were no WM regions in which the HC group had significantly lower FA values than the AN group. Post hoc examination of the component measures revealed that within this region-of-interest there was a trend for increased RD in the AN group ($p = .05$), with no between-group differences in AD detected.

Time series review with boxplots of the 4D group MD image post skeletonisation resulted in the identification of a single HC outlier. Participant outlier status was confirmed following review of whole skeleton-averaged MD values and subsequently removed from between-group comparisons. Group comparisons of MD revealed a single, widespread cluster in the AN group displaying significantly increased MD relative to the HC group (Figure 2b, Table 2). The significant cluster comprised almost the entire WM skeleton, including the corpus callosum, fornix, the left and right internal capsule, corona radiata, posterior thalamic radiation, sagittal stratum, external capsule, fornix (cres)/stria terminalis, cingulum, uncinate fasciculus and the superior longitudinal fasciculus. There were no WM regions in which the HC group had significantly higher MD values than the AN group. Post hoc examination of the component measures revealed that within this region-of-interest, the AN group had significantly increased RD and AD compared to the HC group.

As a consequence of the size of the significant cluster found in the MD analysis, a conventional VBM-based approach was used on the MD data set to verify the widespread result. The results of the VBM-based approach were parallel to that found using TBSS, with a single widespread cluster of significantly increased MD detected in the AN group relative to HCs (see Supporting Information).

3.2 | Brainstem sub-analysis

Significantly reduced FA in a cluster encompassing the superior colliculus (Figure 3a), and significantly increased MD in a cluster containing the superior colliculus and the posterior brainstem (Figure 3b) were found in the AN group relative to HC. Within the significant FA and MD clusters, both RD and AD indices were significantly increased in the AN group relative to the HC group (Table 3).

3.3 | Correlational analyses

We found no significant correlations between any FA, MD, AD or RD cluster and BMI, EDE-Q scores or illness duration, for either the AN or HC group (whole-brain or sub-analysis). The brainstem sub-analysis

FIGURE 2 (a) Tract-based spatial statistics (TBSS) results comparing fractional anisotropy, anorexia nervosa < healthy controls. (b) TBSS results comparing mean diffusivity, anorexia nervosa > healthy controls [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 Clusters of significant group differences observed using tract-based spatial statistics

Cluster	Voxels	AN	HC	<i>p</i>	MNI coordinates			<i>t</i> ^a
					<i>x</i>	<i>y</i>	<i>z</i>	
FA	12,863	0.528 (0.015)	0.554 (0.012)	.002	-8	6	26	4.54
RD		0.420 (0.016)	0.402 (0.031)	.051 ^b				
AD		1.061 (0.025)	1.055 (0.026)	.400 ^b				
MD	60,726	0.652 (0.015)	0.630 (0.014)	<.001	-28	26	-12	2.60
RD		0.476 (0.015)	0.453 (0.014)	<.001 ^b				
AD		1.004 (0.020)	0.980 (0.016)	<.001 ^b				

Note. FA = fractional anisotropy; MD = mean diffusivity; RD = radial diffusivity; MNI = Montreal Neurological Institute; AN = anorexia nervosa; HC = healthy control;

^a Corresponds to the region with maximal *t*-value ($p_{FWE} < .05$ for all cases);

^b Result of post hoc two-sample *t*-test; MD, RD and AD values are $\times 10^{-3}$.

did reveal a trend for a negative relationship between the EDE-Q shape concern subscale and AD within the significant MD cluster for the AN group only ($r = -.552$, $p = .02$).

4 | DISCUSSION

The aim of this study was to identify differences in white matter microstructure between individuals with a current diagnosis of AN and HC. A further exploratory aim was to correlate these measures with BMI and eating disorder symptomatology. It was hypothesised that individuals with AN would show reduced FA and increased MD in the fornix and superior longitudinal fasciculus. This hypothesis was largely supported, although FA differences were not specifically found in the fornix.

The results of the study revealed reduced FA in the AN group in a large cluster encompassing the bilateral superior longitudinal fasciculus, in addition to the bilateral corona radiata, the right posterior thalamic radiation and the corpus callosum; but no reduction of FA in the fornix, as we had predicted. Further, the FA cluster was associated

with a trend for increased RD in the AN group suggesting dysmyelination or demyelination in these regions. As predicted, the MD analysis revealed increased MD in AN of the superior longitudinal fasciculus and the fornix, in addition to widespread increases comprising almost the entire white matter skeleton. Increases in MD are thought to be indicative of increased water content or extracellular fluid (Pfefferbaum & Sullivan, 2005), and may be indicative of widespread

FIGURE 3 Brainstem region-of-interest voxel-based morphometry (VBM) results comparing (a) fractional anisotropy, anorexia nervosa < healthy controls, and (b) mean diffusivity, anorexia nervosa > healthy controls [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Clusters of significant group difference within the brainstem using a region-of-interest voxel based morphometry (VBM) approach

Cluster	Voxels	AN	HC	<i>p</i>	MNI coordinates			<i>t</i> ^a
					<i>x</i>	<i>y</i>	<i>z</i>	
FA	648	0.196 (0.010)	0.210 (0.001)	.003	−1	−30	−7	5.70
RD		0.841 (0.061)	0.774 (0.046)	<.001 ^b				
AD		1.081 (0.050)	1.021 (0.049)	<.001 ^b				
MD	4,101	0.863 (0.030)	0.815 (0.026)	.003	−5	−36	−17	4.51
RD		0.708 (0.029)	0.664 (0.024)	<.001 ^b				
AD		1.173 (0.036)	1.116 (0.034)	<.001 ^b				

Note. FA = fractional anisotropy; MD = mean diffusivity; RD = radial diffusivity; AD = axial diffusivity; MNI = Montreal Neurological Institute; AN = anorexia nervosa; HC = healthy control;

^a Corresponds to the region with maximal *t*-value ($p_{FWE} < .05$ for all cases);

^b Result of post hoc two-sample *t*-test; MD, RD and AD values are $\times 10^{-3}$.

inflammation or atrophy in AN. Increased MD in this cluster was also associated with both increased AD and RD in the AN group, suggestive of superior parallel diffusivity but inferior perpendicular diffusivity (dysmyelination or demyelination) in AN. Our findings are consistent with previous studies reporting decreased FA in similar white matter tracts in AN (Nagahara et al., 2014; Via et al., 2014). Consistent with the study of Via et al. (2014) in particular, we found increased MD accompanied by increased RD and AD. This pattern is difficult to interpret, as the reasons for increases in AD are unclear from animal models. Studies have suggested that increased AD may result from greater extracellular water as a result of fibre atrophy (Della Nave et al., 2011), or from axonal reorganisation (Xu, Li, Lin, Sinha, & Potenza, 2013).

The main regions in which white matter microstructure differed between groups included the corona radiata, corpus callosum and superior longitudinal fasciculus. Widespread reductions in grey matter volume have been consistently reported in AN and may be contributed to by deficits in these key white matter tracts (Joos et al., 2010; Phillipou et al., 2018). Deficits involving the corona radiata that connects the cortex to the basal ganglia and brainstem may reflect these widespread grey matter deficits in AN and may be involved in the eye movement deficits reported in AN which involve cortical and subcortical interactions (Phillipou, Abel, et al., 2015; Phillipou, Rossell, Castle, et al., 2014; Phillipou, Rossell, Gurvich, Castle, et al., 2016; Phillipou, Rossell, Gurvich, Castle, Troje, et al., 2016; Phillipou, Rossell, Gurvich, Hughes, et al., 2016). Deficits in the corpus callosum suggests reduced communication between the two hemispheres and may contribute to the cognitive deficits experienced in AN. In particular, the corpus callosum has been implicated in cognitive flexibility (Papathanasiou, Messinis, Zampakis, & Papathanasopoulos, 2017), a key cognitive and behavioural deficit reported in AN; although, other areas such as frontostriatal regions are more often associated with cognitive inflexibility (Eslinger & Grattan, 1993). Corpus callosum deficits may also be indicative of reduced cortical connectivity in the illness. The superior longitudinal fasciculus, conversely, is a major association fibre that connects frontal to parietotemporal and occipital areas. We found widespread deficits in the white matter microstructure of this tract, suggestive of poor integration between frontal, parietal, temporal and occipital regions of the brain. Poor integration of these regions has been reported in resting state functional MRI (fMRI) in AN. In

particular, reduced functional connectivity between occipital and parietal regions has been suggested to contribute to the distorted perception of body size in AN, that is, a discrepancy between perceived size and 'felt' size (Phillipou, Abel, et al., 2016).

A further exploratory aim of the study was to investigate white matter microstructural differences specifically of the brainstem in AN. Similar patterns of reduced FA and increased MD, together with increased AD and RD, were found in the superior colliculus in the AN group, relative to HC. In addition to its role in the inhibition and initiation of eye movements, the superior colliculus is particularly involved in multisensory integration (Meredith & Stein, 1986), and deficits in this area may be involved in disturbed perception of body size in AN (Phillipou, Rossell, Castle, et al., 2014; Phillipou, Rossell, Gurvich, Castle, Troje, et al., 2016).

The current study also aimed to determine whether diffusivity measures correlated with BMI, illness duration and EDE-Q scores. We found no significant correlations between any FA, MD, AD or RD in any of the clusters identified, and the clinical measures. This suggests that white matter microstructure in AN may not be directly related to starvation-related effects (i.e., reduced BMI and illness duration), or to eating disorder symptomatology, but instead to trait characteristics of the disorder. However, to confirm whether white matter microstructural differences are a trait feature of AN, a healthy-weight group of AN participants would need to be assessed.

A number of potential limitations of this study must be noted. The current study assessed individuals with a diagnosis of AN based on DSM-IV criteria, with the exception of the amenorrhea criterion. As the DSM-5 utilises broader criteria for the diagnosis of AN, our sample may be representative of a more severe spectrum of AN. In addition, although including psychiatric comorbidities represents a more typical AN sample, the range of comorbidities has the potential to have influenced the results. The findings are also somewhat limited by the omission of a weight-restored AN group. Studies in recovered AN patients have reported no differences in DWI parameters (Bang, Rø, & Endestad, 2018; Yau et al., 2013). However, it remains unclear whether these findings reflect weight-restoration or psychological recovery. Although no direct correlations were found between the diffusivity measures and BMI in our study, starvation-related effects are still likely to contribute to these results, as BMI is not a direct measure of starvation. Although investigating individuals who are weight-restored from

AN may be beneficial for this purpose, the long-term effects of starvation remain unclear; in addition, weight-restored individuals typically demonstrate remitting psychological symptoms. Therefore, longitudinal structural imaging studies in individuals at risk of AN or those with 'atypical' AN would be valuable. Atypical AN patients share the same psychological symptoms but do not reach a severely underweight state, thus, examining these individuals may shed light upon microstructural differences inherent to AN. In addition, correlating the white matter microstructure results with performance on cognitive assessments related to AN such as cognitive flexibility and visuospatial integration would be valuable in future research. Further, longitudinal studies in AN would be valuable to identify the long-term effects of white matter microstructural changes on cognition and other functions.

A major strength of this study is the sample size recruited; one of the largest DWI samples in AN to date. The results are suggestive of widespread white matter deficits in those currently ill with AN, which are neither related to BMI or eating disorder symptomatology. These deficits in diffusivity may play a role in the maintenance of AN by contributing to cognitive inflexibility and poor cognitive integration, including visuospatial integration relating to body image distortion. However, the effects of starvation remain unclear and require further investigation.

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CONFLICT OF INTERESTS

Prof. Castle reports grants and personal fees from Eli Lilly, Janssen-Cilag, Roche, Allergan, Bristol-Myer Squibb, Pfizer, Lundbeck, AstraZeneca and Hospira; personal fees from Organon, Sanofi-Aventis, Wyeth and Servier, during the conduct of the study and outside the submitted work. A/Prof Abel reports personal fees from Actelion Pharmaceuticals, Switzerland, outside the submitted work. Dr Phillippou, Mr Carruthers, Dr Di Biase, A/Prof Zalesky, Dr Gurvich and Prof Rossell report no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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