

# Disruption of brain white matter microstructure in women with anorexia nervosa

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**Background:** The etiology of anorexia nervosa is still unknown. Multiple and distributed brain regions have been implicated in its pathophysiology, implying a dysfunction of connected neural circuits. Despite these findings, the role of white matter in anorexia nervosa has been rarely assessed. In this study, we used diffusion tensor imaging (DTI) to characterize alterations of white matter microstructure in a clinically homogeneous sample of patients with anorexia nervosa. **Methods:** Women with anorexia nervosa (restricting subtype) and healthy controls underwent brain DTI. We used tract-based spatial statistics to compare fractional anisotropy (FA) and mean diffusivity (MD) maps between the groups. Furthermore, axial (AD) and radial diffusivity (RD) measures were extracted from regions showing group differences in either FA or MD. **Results:** We enrolled 19 women with anorexia nervosa and 19 healthy controls in our study. Patients with anorexia nervosa showed significant FA decreases in the parietal part of the left superior longitudinal fasciculus (SLF;  $p_{FWE} < 0.05$ ), with increased MD and RD but no differences in AD. Patients with anorexia nervosa also showed significantly increased MD in the fornix ( $p_{FWE} < 0.05$ ), accompanied by decreased FA and increased RD and AD. **Limitations:** Limitations include our modest sample size and cross-sectional design. **Conclusion:** Our findings support the presence of white matter pathology in patients with anorexia nervosa. Alterations in the SLF and fornix might be relevant to key symptoms of anorexia nervosa, such as body image distortion or impairments in body–energy–balance and reward processes. The differences found in both areas replicate those found in previous DTI studies and support a role for white matter pathology of specific neural circuits in individuals with anorexia nervosa.

## Introduction

Anorexia nervosa is an eating disorder characterized by disturbing preoccupations about body self-image, weight and dieting. Patients with anorexia nervosa engage in intense food restrictions that, in some cases, are accompanied by binge eating and

purging episodes (restricting subtype v. binge eating/purging subtype), with severe and enduring physical and psychological consequences.<sup>1</sup> Although the etiology of anorexia nervosa is unknown, there is consensus regarding its multifactorial origin and the contribution of neurobiological factors in the vulnerability, onset and maintenance of the disorder.<sup>2,3</sup>

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Data from neurocognitive and imaging studies suggest that patients with anorexia nervosa have impairments in neural systems implicated in executive functions, visuospatial processes, self-image perception, emotional regulation and reward processing.<sup>2</sup> Consistent with these data, studies with currently ill patients with anorexia nervosa have shown widespread grey matter decreases in the neocortex and in areas linked to emotion regulation and reward, such as the anterior cingulate, orbito-frontal cortex, insular cortex, hippocampus/parahippocampus, amygdala and striatum.<sup>4-6</sup> However, other studies have reported grey matter increases in neocortical<sup>6-8</sup> and limbic regions.<sup>7,8</sup> Moreover, although some of these alterations may normalize in recovered patients,<sup>4</sup> other studies have shown the persistence of volume alterations in recovered patients.<sup>7,9,10</sup> The distributed nature of these changes implies disturbances of interregional brain connectivity, as has been observed in most other psychiatric disorders.<sup>11,12</sup>

At the same time, the major reorganization in white matter that the brain undergoes during adolescence and early adulthood is thought to be relevant to the development of some psychiatric disorders, including anorexia nervosa.<sup>3,13</sup> Normal maturation and organization of white matter might be affected in patients with anorexia nervosa, particularly when considering that most of the identified factors contributing to the development of the disorder — including psychological, social and biological factors — have their major impact in this period. Despite all this, white matter alterations remain rather unexplored in anorexia nervosa. Of the few published studies exploring regional volumetric differences, most showed white matter decreases in fronto-temporo-parietal and sensorimotor regions in adult patients,<sup>7,14,15</sup> although 3 others found either no differences<sup>16,17</sup> or white matter increases in temporal and hippocampus regions in adolescent patients.<sup>8</sup> Regional white matter alterations were also reported in recovered patients,<sup>7</sup> although other studies found no differences in volume.<sup>18-20</sup>

In this context, diffusion tensor imaging (DTI) affords a new and highly suitable approach to assess microstructural white matter alterations that may putatively characterize anorexia nervosa. It provides information of white matter organization based on the analysis of the brain's water diffusion.<sup>21</sup> Specifically, water diffusion is typically quantified using 2 main compound measures: fractional anisotropy (FA) and mean diffusivity (MD), or the closely related apparent diffusion coefficient (ADC). Fractional anisotropy provides information about the maximum direction of water diffusion and the degree to which it is constrained by tissue barriers, such as axonal fibres, whereas MD indexes the overall degree of water diffusion, regardless of direction.<sup>22,23</sup> Both measures are typically negatively correlated and are considered to be an indicator of white matter integrity (FA is decreased in pathological white matter). Nevertheless, FA and MD are broad measures that could be driven by a number of factors (e.g., axonal ordering, density, degree of myelination).<sup>22,23</sup> Indirect measures of these changes include axial (AD;  $\lambda_{||}$ ) and radial diffusivity (RD;  $\lambda_{\perp}$ ), which contribute to the computation of aggregate measures, such as FA and MD, and represent relatively specific aspects of water diffusivity, such as the average diffusion parallel (AD) and perpendicular (RD) to axonal fibres.<sup>24</sup> For this reason, AD and RD are thought

to index more specific aspects of white matter pathology, being more sensitive to changes in integrity and myelination, respectively.<sup>21-25</sup> Decreases in FA, for instance, might derive both from a decreased AD due to axonal impairment or an increased RD due to myelination changes, as observed in animal models.<sup>23-25</sup> Therefore, the combined quantification of such measurements is recommended to better characterize any putative changes in white matter microstructure.<sup>23,25</sup>

Two previous studies have explored white matter microstructure with DTI in adult patients with anorexia nervosa.<sup>14,26</sup> Kazlouski and colleagues<sup>14</sup> studied a group of 16 patients with anorexia nervosa with acute symptoms (10 patients with restricting subtype and 6 with binge eating/purging subtype); they found FA decreases in the bilateral fimbria-fornix, the fronto-occipital fasciculus and the posterior cingulum as well as ADC/MD increases in frontoparietal and parieto-occipital bundles. Comorbid depression and anxiety diagnoses were not excluded (8 participants per diagnosis), although both diagnoses are often comorbid with anorexia nervosa. In the second study, Frieling and colleagues<sup>26</sup> included a sample combining acute ( $n = 12$ ) and recovered ( $n = 9$ ) patients with anorexia nervosa; they found FA reductions in thalamic regions, the posterior corona radiata, the left middle cerebellar peduncle and parts of the left superior longitudinal fasciculus. Methodological limitations, such as lack of sample power or mixed samples of patients, might account for differences between these 2 studies. In this sense, although comparisons between subgroups of patients were conducted in both studies, splitting an already modest sample size may have limited statistical power.

The objective of the present study was to identify differences in white matter microstructure in a homogeneous sample of patients with anorexia nervosa. To this end, we recruited a phenotypically well-characterized group of currently ill patients with anorexia nervosa, restricting subtype. The inclusion of a homogeneous sample was designed to reduce some of the variability typically found in clinical samples, such as the one associated with a subgroup of patients with impulsive behaviours (binge/purging subtype).<sup>6,27</sup> Unlike previous studies, we also aimed to provide a full characterization of white matter microstructure abnormalities across the aforementioned diffusivity measures (i.e., FA, MD, AD, RD). This was conducted to derive a more comprehensive understanding of potential alterations, as suggested.<sup>25</sup> In addition, to explore whether the results were modulated by potential confounders or explained by symptoms and psychological factors related to anorexia nervosa, we assessed correlations between clinical variables and DTI-derived parameters. Finally, we explored whether differences in diffusivity were accompanied by differences in grey or white matter volumes.

Based on previous findings, we hypothesized that patients with anorexia nervosa would present decreases in FA and/or increases in MD in long-range connections between frontal and temporoparietal or occipital areas. We also expected these changes to correlate with clinical variables, such as duration and severity of the disease, or with personality traits thought to be associated with anorexia nervosa, such as harm avoidance.<sup>14,28</sup>

## Methods

### Participants

The study was conducted between 2011 and 2012. We consecutively recruited women with anorexia nervosa fulfilling DSM-IV-TR criteria for anorexia nervosa, restricting subtype,<sup>1</sup> from the Eating Disorders Unit of Bellvitge University Hospital (day hospital), Barcelona, Spain. Diagnoses were made using the Structured Clinical Interview for DSM-IV Axis I Disorders.<sup>29</sup> Comorbid psychiatric disorders, any neurologic condition and abuse of any substance with the exception of nicotine were exclusion criteria. Before scanning, all patients must have had at least 1 week of supervised meals and hydration during their day hospital admission. By ensuring normal hydration, we minimized putative biases that dehydration may cause in brain measurements.<sup>30</sup>

We recruited healthy controls, matched for sex, mean age, handedness and mean educational level, from the same socio-demographic area as patients. Controls were screened to exclude any psychiatric or other medical condition by means of the General Health Questionnaire<sup>31</sup> and a clinical semistructured interview.<sup>32</sup> We also ensured that controls had a body mass index (BMI) within the healthy range and that they did not present unhealthy eating behaviours (e.g., constant dieting) or subthreshold symptoms of any eating disorder.

For all participants, the presence of symptoms and psychological features involved in eating disorders were assessed with the self-reported Eating Disorder Inventory-2 (EDI-2) scale.<sup>33</sup> We assessed depressive and anxiety symptoms using the Hamilton Rating Scale for Depression (HAM-D)<sup>34</sup> and the Hamilton Rating Scale for Anxiety (HAM-A).<sup>35</sup> The harm avoidance personality trait from the Temperament and Character Inventory—Revised<sup>36</sup> was also collected. The ethical committee of clinical research of the Bellvitge University Hospital approved the study protocol. All participants gave written informed consent after a detailed description of the study.

### Imaging protocol — DTI

#### Acquisition

Scanning was performed with a GE Signa Excite scanner at 1.5 T (Medical Systems) equipped with an 8-channel phased-array head coil. We used a single-shot, spin echo, echo planar imaging sequence to obtain 26 consecutive axial diffusion-weighted images for each participant (repetition time [TR] 8300 ms; echo time [TE] 95 ms; thickness 5 mm, no gap; pulse angle 90°; field of view 26 cm; 128 x 128 acquisition matrix reconstructed to a 256 x 256 matrix). Twenty-five diffusion-weighted volumes were acquired along noncollinear directions using a b value of 1000 s/mm<sup>2</sup>. A single non-diffusion weighted volume was also acquired.

#### Preprocessing

Imaging data were processed on a Macintosh computer running FMRIB's Software Library (FSL), developed by the Analysis Group at the Oxford Centre for Functional MRI of

the Brain (FMRIB).<sup>37</sup> Diffusion-weighted images were corrected for possible eddy current distortions ("Eddy Current Correction" option in the FMRIB Diffusion Toolbox [FDT] version 2.0 in FSL), and a brain mask was applied using the FSL Brain Extracting Tool. Subsequently, we estimated FA and MD maps using FDT in FSL by fitting a tensor model to the eddy-corrected and brain-masked diffusion data. We also estimated AD and RD maps using the eigenvalues associated with the fitted tensor model. Data from 2 participants (1 patient and 1 control) were excluded owing to excessive signal loss in the orbitofrontal cortex.

#### Processing and statistical analyses

We used tract-based spatial statistics (TBSS)<sup>38</sup> in FSL to test for between-group differences in FA on a voxel-wise basis. First, all FA images were aligned to the 1 x 1 x 1 mm standard space provided in FSL (FMRIB58 FA) using the FMRIB nonlinear image registration tool (FNIRT). Next, we created a mean FA skeleton map (together with a mean FA map), which represents the centre of all the tracts common to the group. Finally, the skeleton was thresholded at a standard intensity value of 0.2, which is recommended in TBSS analyses to avoid areas of high variability,<sup>38</sup> and each participant's FA map was projected to this skeleton. The resulting FA data were analyzed using the Randomize permutation-based program in FSL<sup>39</sup> with the following contrasts: controls > patients and patients > controls. The results were cluster-wise thresholded using a primary *t* statistic of  $t > 2$  and a family-wise error (FWE)-corrected *p* value of  $p_{\text{FWE}} < 0.05$ . These preprocessing and thresholding procedures were repeated for the MD images. Anatomic localization of the clusters was based on the white matter atlas of the Johns Hopkins University White Matter Labels (1 mm) Atlas available in the FSL software library.<sup>40</sup>

For each cluster showing a significant group difference in FA or MD, we extracted voxel values and averaged them to obtain a summary measure of diffusion properties in these regions for each participant. We calculated Spearman correlations between these diffusion measures and demographic and clinical variables (EDI-2, duration of illness, harm avoidance, age, BMI, HAM-D and HAM-A) in the patient group alone. We also assessed between-group differences in the component AD and RD measurements, which were additionally extracted from each cluster with significant between-group differences in FA or MD. See the Appendix, available at [jpn.ca](http://jpn.ca), for details of the acquisition, preprocessing and analysis of structural images (voxel-based morphometry).

## Results

### Participants

We recruited 20 women with anorexia nervosa, but 1 had to be excluded for the technical reason of excessive signal loss in the orbitofrontal cortex, for a final patient sample of 19 women (mean age  $28.37 \pm 9.55$  yr). Five (26%) patients were on pharmacological treatment (3 on selective serotonin reuptake inhibitors, 1 on a tricyclic antidepressant and 1 on

a combination of low doses of a sedative antipsychotic treatment plus a tricyclic antidepressant) owing to the presence of past ( $n = 4$ ) or current ( $n = 1$ ) depressive and anxiety symptoms. However, at the time of examination, none of the patients fulfilled criteria for any comorbid psychiatric disorder. We recruited 20 healthy controls, but 1 had to be excluded after the preprocessing of the images, leaving a final sample of 19 controls (mean age  $28.63 \pm 8.58$  yr). Table 1 summarizes the demographic and clinical characteristics of the sample.

### Clinical and demographic variables

There were no statistical differences in age, handedness or educational level between the anorexia nervosa and control groups. As expected, BMI and EDI-2 measures were significantly different between the groups, with lower mean BMI and higher mean EDI-2 scores in patients with anorexia nervosa. Depressive and anxiety symptoms were also higher in the patients than the controls, although differences in anxiety did not reach statistical significance after correction for multiple comparisons. Harm avoidance scores were no different between the groups (Table 1).

### Group comparison of the FA and MD diffusivities

A significant FA reduction in patients relative to controls was localized to a large cluster (512 voxels;  $p_{\text{FWE}} < 0.05$ ) that extended across the parietal region of the left superior longitudinal fasciculus (SLF), including its portion II (SLF II) and the arcuate fasciculus (AF). This area included the temporoparietal junction and surrounded the posterior insular cortex and the temporal and parietal opercula. The results extended into the inferior longitudinal fasciculus and inferior fronto-occipital fasciculus (Fig. 1, Table 2). No significant increases in FA were identified in the patient group.

In addition, patients showed increased MD in a cluster encompassing the fornix and extending to the anterior thalamic radiations bilaterally (266 voxels;  $p_{\text{FWE}} < 0.05$ ; Fig. 2,

Table 2). There was no decrease in MD in patients.

To test if potential confounding factors (e.g., age, depression and anxiety symptoms, medication and nicotine use) accounted for a significant percentage of variability in the diffusivity results, we conducted 2 separate hierarchical multiple regression models, with mean FA and MD measures from the previously mentioned clusters as the dependent variables. The inclusion of potential confounders did not significantly increase the percentage of variability explained by group, which was the only variable that significantly predicted FA and MD. Specifically, group accounted for 62% of the variance of FA in the SLF ( $p < 0.001$ ) and 69% of the variance of MD in the fornix ( $p < 0.001$ ; see the Appendix, Table S1). In addition, we repeated our analyses by excluding 1 participant with a late-onset disorder (aged 48 yr at onset). All differences remained significant after excluding this participant.

### Clinical correlations and component measures

There were no significant correlations between the extracted FA/MD measurements and the clinical variables examined (Appendix, Fig. S1).

Analysis of the component measures extracted from the clusters showing significant FA/MD differences indicated that SLF alterations were predominantly driven by an increase in RD accompanied by an MD increase (RD:  $t_{36} = 7.24$ ; FA:  $t_{36} = 7.73$ ; MD:  $t_{36} = 5.54$ ; all  $p < 0.001$ ). In the fornix, changes were driven by increases in both AD and RD accompanied by an FA decrease (AD:  $t_{36} = 4.39$ ; RD:  $t_{36} = 9.37$ ; FA:  $t_{36} = 7.93$ ; MD:  $t_{36} = 8.96$ ; all  $p < 0.001$ ; Fig. 3).

### Group differences in volume

There were no between-group differences in grey or white matter volumes either at the whole brain level or in specific analyses aimed at the regions of significant between-group differences in DTI measurements. Further information is provided in the Appendix.

**Table 1: Demographic and clinical characteristics of the study sample**

Variable	Group; mean $\pm$ SD (range)*		Between-group differences	
	Anorexia nervosa, $n = 19$	Control, $n = 19$	$t$	$p$ value
Age, yr	28.37 $\pm$ 9.55 (18–49)	28.63 $\pm$ 8.58 (19–52)	0.09	0.93
Handedness, no. right:left	18:1	18:1	—	—
Education level, yr	15.47 $\pm$ 3.22 (12–23)	16.58 $\pm$ 2.46 (10–21)	1.19	0.24
Age at onset, yr	21.84 $\pm$ 9.19 (12–48)	—	—	—
Duration of the illness, mo	78.32 $\pm$ 72.37 (12–240)	—	—	—
BMI	17.03 $\pm$ 1.09 (14–18)	21.09 $\pm$ 1.80 (18–25)	8.45	< 0.001†
EDI-2 total scores	66.79 $\pm$ 44.28 (13–178)	13.53 $\pm$ 7.37 (3–28)	5.17	< 0.001†
Harm avoidance (TCI-R)	106.89 $\pm$ 20.26 (75–144)	98.63 $\pm$ 10.83 (78–119)	1.57	0.13
HAM-D	3.26 $\pm$ 3.02 (0–10)	0.84 $\pm$ 1.07 (0–3)	3.30	0.003†
HAM-A	5.11 $\pm$ 5.91 (0–22)	1.63 $\pm$ 1.42 (0–4)	2.50	0.025

BMI = Body Mass Index; EDI-2 = Eating Disorders Inventory-2; HAM-A = Hamilton Rating Scale for Anxiety; HAM-D = Hamilton Rating Scale for Depression; SD = standard deviation; TCI-R = Temperament and Character Inventory, revised edition.

\*Unless otherwise indicated.

†Significant  $p$  values after Bonferroni correction ( $p < 0.007$ ).

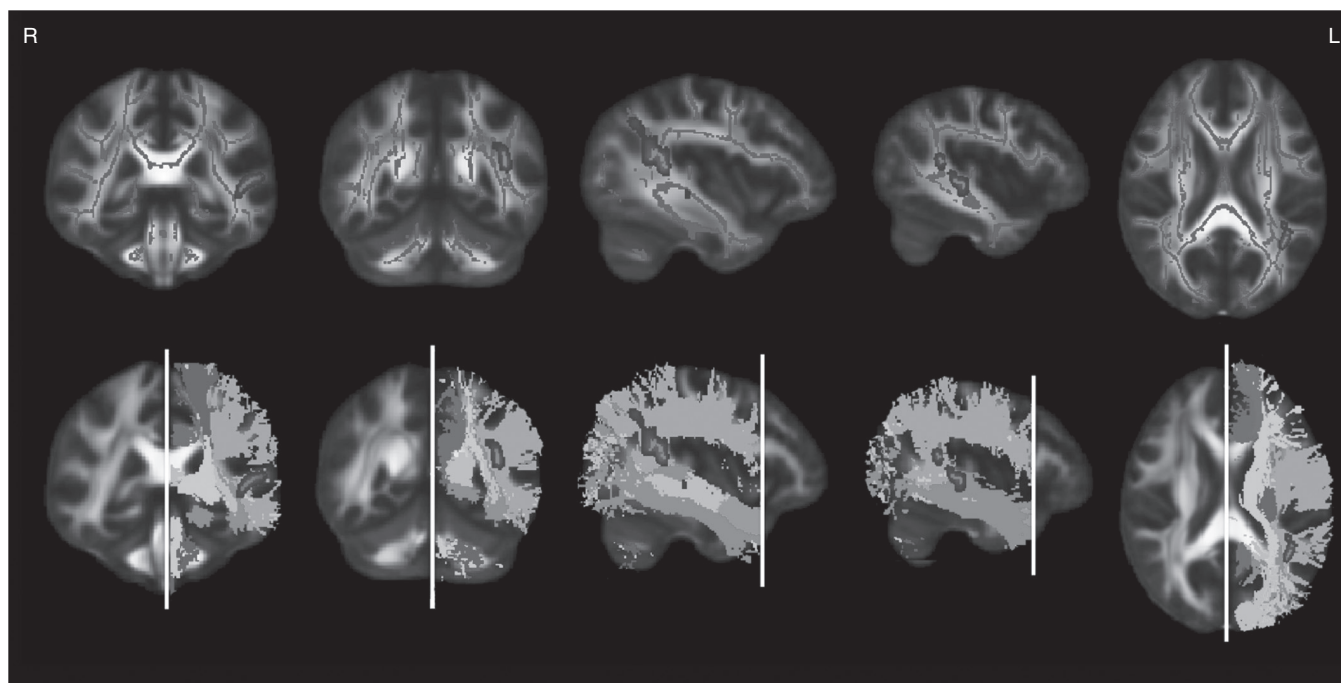
**Discussion**

Using DTI in a well-characterized sample of women with anorexia nervosa, we identified axonal abnormalities that highlight the relevance of specific brain systems in the pathophysiology of this disorder. Overall, patients with anorexia nervosa demonstrated alterations of white matter microstructure in the parietal component of the superior longitudinal fasciculus as well as the fornix. The nature of these alterations varied for each fibre tract: decreased FA in the SLF was largely driven by increased RD, whereas increased MD in the fornix was driven by a combination of increased AD and RD. These results were not found to be attributable to potential confounding factors.

*Changes in the left SLF*

The SLF is a major association fibre, connecting frontal to parietotemporal and occipital areas. Although it comprises several portions, our results were mainly located in the parietal parts of SLF II and AF components, fibres that connect dorsolateral and ventrolateral prefrontal areas to the angular gyrus and posterior parts of the superior temporal cortex.<sup>41</sup>

Alterations in the structure of the SLF are consistent with grey matter decreases found in patients with anorexia nervosa in both frontal and parietotemporal regions<sup>4</sup> and replicate part of the findings of a previous DTI study of anorexia nervosa,<sup>26</sup> which found a decrease in FA in the parietal parts



**Fig. 1:** Map of the fractional anisotropy (FA) differences between groups (patients < controls). The FMRIB58\_FA (1 mm thick) template provided in FSL was used in all the figures. Top row: results are shown on top of the mean FA skeleton template created from all the participants' images for this study. Bottom row: results are shown on top of the atlas of tracts probability Johns Hopkins University–International Consortium for Brain Mapping (threshold 0 mm and 1 mm thick) provided in FSL; different shades correspond to specific probability tracts in the left hemisphere: superior longitudinal tract, corticospinal tract, cingulum, anterior thalamic radiation, splenium of the corpus callosum, inferior longitudinal tract and inferior fronto-occipital tract.

**Table 2: Coordinates of the between-group differences in diffusion tensor measures**

Diffusion measures	Geometrical centre and extension of the cluster	Pathway/region	Cluster size	MNI coordinates			t*
				x	y	z	
FA	Patients < controls	Left superior longitudinal tract (parietal pars) extended to fibres of the inferior longitudinal fasciculus and inferior fronto-occipital fasciculus	512	-43	-42	15	4.49
MD	Patients > controls	Fornix extended to bilateral anterior thalamic radiations	266	0	-7	11	4.92

FA = fractional anisotropy; MD = mean diffusivity; MNI = Montreal Neurological Institute.  
\*Corresponds to the region with maximal t value ( $p_{FWE} < 0.05$  in all cases).

of the left SLF. Moreover, changes in this white matter tract might be present irrespective of the phase of the disorder, as suggested by findings reported in 2 recent DTI studies involving adolescent patients<sup>8</sup> and recovered patients.<sup>28</sup>

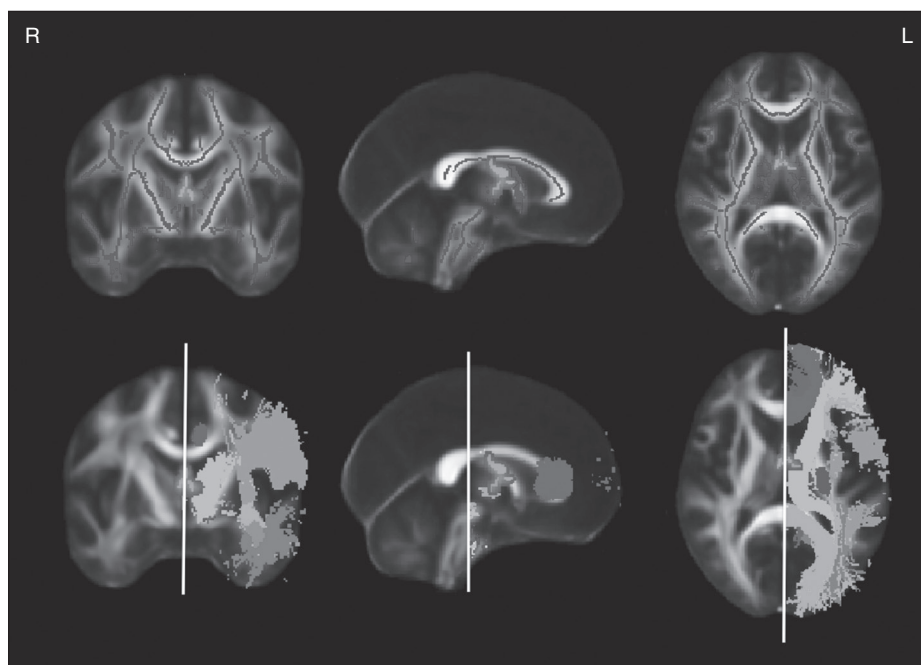
The results found in the left SLF seem functionally relevant to one of the characteristic features of anorexia nervosa, body image distortion. The medial and inferior parietal areas are involved in proprioception, size and spatial judgment, visual imagery and the integration of visual information — all of which are processes that conform the neural basis for the representation of the body self-image.<sup>42</sup> In turn, body-image perception is integrated in a functional network connecting prefrontal and parietal areas,<sup>43</sup> with the SLF being the major white matter tract connecting these regions. Accordingly, several studies have found differences between patients with anorexia nervosa and controls in the activity of the parietal cortex and prefrontal areas during the visualization of their own body image (see the review by Gaudio and Quattrocchi<sup>42</sup>). In addition, the unilateral location of our findings might relate to previously observed lateralization of self-image distortions in patients with anorexia nervosa to the left hemisphere.<sup>43</sup>

Superior longitudinal fasciculus alterations may also influence other cognitive processes. Some studies have demonstrated that both currently ill patients with anorexia nervosa and recovered patients have a specific perceptual cognitive style, the so-called “weak central coherence,” which de-

scribes enhanced attention to local details at the expense of global processing.<sup>44</sup> These abnormalities are thought to rely on alterations of long-range connections between prefrontal and parieto-occipital areas in other disorders,<sup>45</sup> and these same areas also seem to be implicated in weak central coherence in patients with anorexia nervosa.<sup>46</sup> However, the specific role of the SLF in these deficits in patients with anorexia nervosa remains to be further investigated.

The pattern of alterations observed in the SLF, consisting of decreases in FA, increases in MD and RD and no modification of AD, seems consistent with a reduction in the degree of myelination in this area, as shown in animal models.<sup>24</sup> Given that long-range associative connections, such as the SLF, continue their myelination into adulthood,<sup>47</sup> it is possible that these areas might be more vulnerable to factors involved in the onset and development of anorexia nervosa during adolescence and early adulthood. In turn, this vulnerability might be greater in some individuals, such as those with greater harm avoidance, given the correlation found between this personality trait and MD in this area in recovered patients.<sup>28</sup> Harm avoidance, however, was not correlated with either FA or MD results in our study.

Alternatively, considering that myelination is a dynamic process<sup>48</sup> and that changes in RD have been observed even after a short period of cognitive training<sup>49</sup> or meditation,<sup>50</sup> these white matter changes may reflect a plastic response to



**Fig. 2:** Map of the mean diffusivity (MD) differences between groups (patients > controls). The FMRIB58\_FA (1 mm thick) template provided in FSL was used in all the figures. Top row: results are shown on top of the mean FA skeleton template created from all the participants' images for this study. Bottom row: results are shown on top of the atlas of tracts probability Johns Hopkins University–International Consortium for Brain Mapping (threshold 0 and 1 mm thick) provided in FSL; shades correspond to specific probability tracts in the left hemisphere: superior longitudinal tract, corticospinal tract, cingulum, anterior thalamic radiation, splenium of the corpus callosum, inferior longitudinal tract and inferior fronto-occipital tract. L = left; R = right.

the cognitive distortions and symptoms of anorexia nervosa as well as to nutritional problems. However, we found no correlations with clinical variables to support these speculations. Different design approaches, such as the inclusion of a high-risk group or a longitudinal cohort, would be particularly informative in this context to further understand the nature of these alterations.

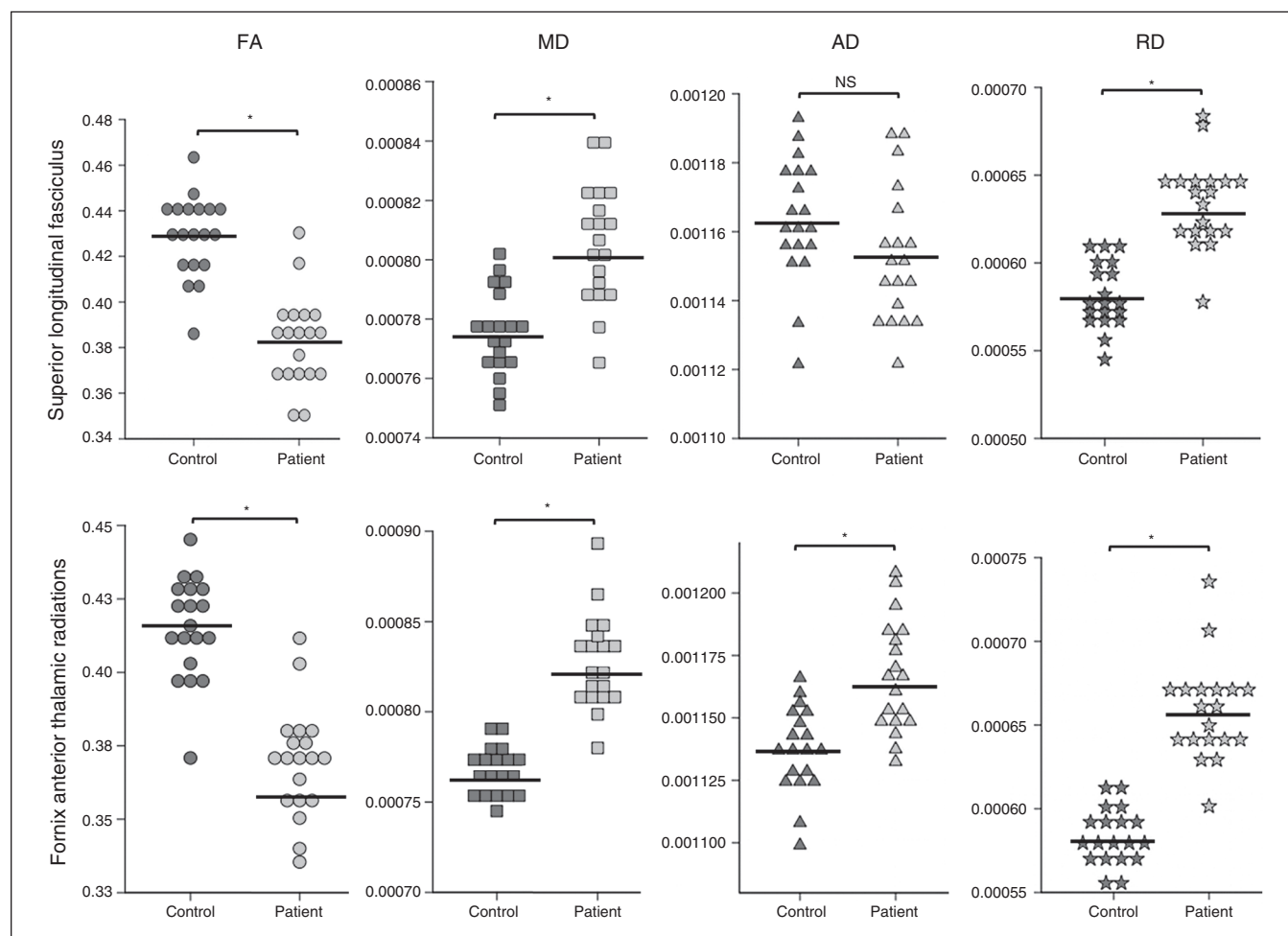
### Changes in the fornix

The fornix is the main white matter tract connecting the hippocampus to the hypothalamus; it also connects the hippocampus to the ventral striatum and prefrontal areas, including the orbitofrontal and anterior cingulate cortices. Through the connections it forms between these structures, the fornix is a key structure involved in the regulation of body-energy balance and processing of reward responses.<sup>51</sup> Although these results should be interpreted with some caution owing to putative specific preprocessing problems of this area (i.e., misalignment),<sup>52</sup> it is interesting to note that they replicate the

findings of several previous studies in currently ill patients with anorexia nervosa that showed either FA decreases or ADC/MD increases in the fornix<sup>8,14</sup> or in closely related areas, such as the mediodorsal thalamus.<sup>26</sup>

Two studies have reported hippocampal volume reductions in patients with anorexia nervosa,<sup>53,54</sup> and it is likely that some of the metabolic alterations implicated in the disorder might impair this structure and its connections. For example, the typically hyperactive hypothalamic-pituitary-adrenal axis observed in anorexia nervosa can lead to hippocampal atrophy in animal models.<sup>3</sup> The hippocampus has been implicated in weight-regulation processes,<sup>55</sup> and animal models have shown that both lesions of the hippocampus and fornix transections lead to alterations in eating behaviours.<sup>56,57</sup>

The reward system is also intrinsically related to body-energy regulation. This system is thought to underlie alterations in some eating behaviours,<sup>58,59</sup> and it has received increasing interest in recent years, being considered a key element in the development and maintenance of anorexia nervosa.<sup>2,60</sup> Actually, several studies have shown that patients



**Fig. 3:** Diffusivity measures' eigenvalues for patients and controls in the 2 regions with significant differences in fractional anisotropy (FA) and mean diffusivity (MD). Black horizontal lines represent the mean of the values represented in each plot. \*Significant between-group differences of the extracted measures, all  $p < 0.001$ . AD = axial diffusivity; NS = nonsignificant comparison; RD = radial diffusivity. \* $p < 0.05$ .

with this disorder present deviant responses to the reinforcing characteristics of several types of disorder-relevant and nonrelevant stimuli and that such abnormal responses are probably implicated in the persistence of the pathological behaviour.<sup>60</sup>

While our observed MD and RD increases and FA decreases putatively relate to myelin decreases in the fornix, the biological significance of AD increases is still unclear. Some possibilities include increased extracellular water resulting from fibre atrophy, breakdown of axonal flux of water<sup>61</sup> and/or axonal reorganization.<sup>62</sup> These mixed results might reflect the effects of overlapping pathophysiological mechanisms in the fornix. It is also interesting to note that these mechanisms might be nonspecific to patients with anorexia nervosa, restricting subtype, since a similar pattern of microstructural alterations in the fornix has been found in patients with bulimia nervosa<sup>63</sup> and in overweight and obese individuals.<sup>64</sup> Even if some of these conditions have opposite behavioural consequences, some shared biological effects are suggested,<sup>65</sup> and it is likely that extreme weight conditions might have similar structural effects in this energy balance system.

### Limitations

Our sample size was relatively modest and replication is required. However, we sought to improve on previous DTI studies in adults with anorexia nervosa by providing a more homogeneous sample, comprising currently ill patients with the restricting subtype of the disorder. Second, we included patients receiving current pharmacological treatment. Since the interaction between medications and DTI measures is still unknown, such an effect cannot be ruled out. Nevertheless, between-group differences persisted even after taking treatment status into account. Third, while the precise biological interpretations of the diffusion measures remain a topic of debate,<sup>25</sup> the detailed characterization of our results has provided testable hypotheses concerning regional pathophysiological alterations. In addition, the use of a 1.5 T MRI system limited our sensitivity to group differences compared with higher-field imaging techniques. In this regard, our findings may reflect a conservative estimate of the extent of white matter abnormalities in patients with anorexia nervosa. Finally, our results are limited to a cross-sectional design. It would be interesting to test whether these alterations might persist in a longitudinal assessment of the same participants — especially after recovery — or, alternatively, in comparison to a group of recovered individuals.

### Conclusion

Our results provide new insight into the nature of white matter microstructural alterations in patients with anorexia nervosa. Alterations found in the SLF and the fornix were found to result from different microstructural changes, such that demyelination may be more prominent in the SLF and a combination of altered white matter myelination and integrity may characterize changes in the fornix. Taken together, alterations in these areas are consistent with previous findings

and with prevailing hypotheses regarding the neurobiological basis of anorexia nervosa, as well as with core symptoms of the disorder, such as body distortion, dysfunctions in weight regulation and altered reward processing.

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