White matter integrity is disrupted in adolescents with acute anorexia nervosa: A diffusion tensor imaging study

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A R T I C L E   I N F O

Keywords:
Diffusion tensor imaging
Reward circuitry
Starvation
FA
MD

A B S T R A C T

Anorexia nervosa (AN) is a highly debilitating mental illness with multifactorial etiology. It oftentimes begins in adolescence, therefore understanding the pathophysiology in this period is important. Few studies investigated the possible impact of the acute state of illness on white matter (WM) tissue properties in the developing adolescent brain. The present study expands our understanding of the implications of AN and starvation on WM integrity. 67 acutely ill adolescent patients suffering from AN restricting type were compared with 32 healthy controls using diffusion tensor imaging assessing fractional anisotropy (FA) and mean diffusivity (MD). We found widespread alterations in the vast majority of the WM regions with significantly decreased FA and increased MD in the AN group. In this highly selective sample in the acute stage of AN, the alterations are likely to be the consequence of starvation. Still, we cannot rule out that some of the affected regions might play a key role in AN-specific psychopathology.

1. Introduction

Anorexia nervosa (AN) is a highly debilitating mental illness characterized by intensive concern about weight and body shape, restrictive eating behavior, severe underweight and malnutrition (Treasure et al., 2015). The disease typically emerges in adolescence and young adulthood and affects mainly females with a ratio of 9:1 (Zipfel et al., 2015). Genetic effects and neurodevelopmental risk factors have been identified, and the somatic consequences have been studied (Treasure et al., 2015). Still, the neuronal basis of the disease remains unclear (Milos Gabriella 2021).

Potential mechanisms that underlie structural brain alterations in patients suffering from AN are hypovolemia due to dehydration or loss of electrolytes, changes of oncotic pressure due to reduced albumin, levels, deficiency of polyunsaturated fatty acids and insufficient intake of micronutrients. Albumin is considered a late indicator for malnutrition (Keller, 2019), and can be used for monitoring severely ill patients.

Further factors that may contribute to microstructural brain alterations, ie., small axonal size, density and dendritic branching alterations, are suppression of leptin and brain-derived neurotrophic factor (BDNF), suppression of the hypothalamic-pituitary-adrenal axis and the hypothalamic-pituitary-gonadal axis, and elevation of ghrelin, the hunger hormone, and cortisol (King et al., 2018; Treasure et al., 2020).

Diffusion tensor imaging (DTI) is a magnetic resonance imaging (MRI) technique, that probes molecular displacement of water (Filler, 2009; Wandell, 2016) to indirectly assess white matter (WM) architecture of the human brain noninvasively. DTI allows to capture a variety of metrics which inform about the local tissue integrity and its changes on a microstructural level. Along axons and WM tracts, water movement is anisotropic, which is described by the index fractional anisotropy (FA). Higher FA values suggest greater fiber integrity, as water molecules in brain tissue migrate rather alongside an axon than perpendicular. FA is therefore interpreted as marker for white matter integrity as it is associated with axon diameter, fiber packing density, membrane permeability and myelination. Another relevant measure is the mean diffusivity (MD). DTI measures are influenced by multiple factors e.g.

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crossing fibers (Jones and Cercignani, 2010). Further confounding factors are nutritional status and hydration (King et al., 2018).

DTI study results in adults with AN are highly inconsistent. Studies have mostly demonstrated a decrease in FA in the thalamic region, cerebellar areas, in the cingulum, the insula and fronto-occipital pathways (Frank et al., 2016; Frieling et al., 2012; Hayes et al., 2015; Hu et al., 2017; Kazlouski et al., 2011; Nagahara et al., 2014; Via et al., 2014), while others found increased FA values in the corona radiata, the corpus callosum and fronto-occipital pathways (Cha et al., 2016; Frank et al., 2013; Vogel et al., 2016). One study observed no significant FA changes between patients and healthy controls (Bang et al., 2018).

Since AN originates in the majority of patients in adolescence, young patients are especially important to be studied. The brain and WM structure develops across the life span (Qiu et al., 2008; Shaw et al., 2008). WM volume increases dramatically up to adolescence (12–15 years) and continues to increase at a slower rate until the 4th decade of life (Courchesne et al., 2000). The period from adolescence to young adulthood is critical for emotional and cognitive development, and it is also a period where AN originates. It is agreed, that WM maturation during this period is characterized by widespread changes of MD and FA. Young adults (18.6–26.1 years) compared to young adolescents (9.4–11.5 years) had increased FA in multiple regions including cerebellum, cerebral peduncles, mid and posterior temporal lobe, anterior and posterior parts of the internal capsules, anterior part of the external capsule, superior corona radiata, cingulum, parts of the frontal and parietal lobes. No age-related FA changes were found in the Corpus Callosum (CC) (Qiu et al., 2008).

Only a few studies investigated WM microstructural changes using DTI in adolescent patients suffering from AN. Study samples were heterogeneous (restrictive type AN, binge purging Type AN, unspecified eating disorder), sample sizes were low (n < 35 patients) and results are highly controversial (Cha et al., 2016; Frank et al., 2013; Gaudio et al., 2017; Olivo et al., 2017; Pfuhl et al., 2016; Travis et al., 2015; Vogel et al., 2016). Some authors reported decreased FA in widespread areas of the brain (Breithaupt et al., 2020; Frank et al., 2013; Gaudio et al., 2017; Olivo et al., 2017; Travis et al., 2015), others found increased FA in the patient group (Cha et al., 2016; Vogel et al., 2016), whilst one study group found preserved WM structure and no group differences (Pfuhl et al., 2016).

A meta-analysis by Barona et al. was published, to better understand this between-study variability (Barona et al., 2019). They included studies with adult samples as well as adolescent samples and found decreased FA in the posterior areas of the CC, the left superior longitudinal fasciculus II, and the left precentral gyrus, as well as increased FA in the right cortico-spinal projections and lingual gyrus in AN compared to healthy controls (HC). And again, in a recent thorough review, the discrepant results were a matter of discussion (Kappou et al., 2021).

Considering the heterogeneity in the existing literature this study aimed to assess WM microstructural alterations in a large sample of exclusively adolescent patients with acute AN, restricting type. Based on the existing literature we hypothesized that during the acute stage of AN, patients showed widespread alterations affecting different WM regions compared to age matched HC. Secondly, we aimed to investigate correlations between illness severity using the Eating Disorder Examination (Hilbert et al., 2013) and microstructural changes. Thirdly, we hypothesized a correlation between malnutrition by assessing albumin blood levels and WM integrity.

2. Methods

2.1. Subjects

Consecutive patients admitted to inpatient treatment from 2011 to 2017 were asked to participate in the study if they (1) had a diagnosis of restrictive anorexia nervosa (AN-r) based on the criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV; Association, 2000) and (2) were 12 – 18 years old. Exclusion criteria were (1) a comorbid neurological disease or brain injury or (2) metallic implants or claustrophobic anxieties prohibiting MRI. Written informed consent was obtained from patients and one of their legal guardians in accordance to the Declaration of Helsinki prior to the assessment. The protocol was approved by the local ethics committee (Nr.246/2011).

77 participants admitted to inpatient treatment were asked to participate in the study. Two patients were excluded due to metallic implants prohibiting MRI, and eight had to be excluded from the analysis due to incomplete DTI data. The final sample size consisted of 67 participants.

A subsample of 37 adolescents agreed to participate in an additional clinical interview assessing eating disorder psychopathology and 33 patients had routinely blood examination within days of MRI. These subsamples were used to examine the association between clinical variables (AN symptom severity, illness duration) and albumin levels and FA and MD respectively. (For more details about the measurements see section ‘Clinical data and albumin’).

Data from the AN sample was compared to a HC group. We searched two online, open-access databases: The Autism Brain Imaging Data Exchange (ABIDE) and the Mind Research Network (MRN) whether participants met the following eligibility criteria: (1) no previous or current eating disorder, (2) no other previous or current psychiatric illness, (3) age between 10 and 24 years and (4) normal weight. Twelve participants from the MRN Network (http://fcon_1000.projects.nitrc.org/indi/C0R8/html/mrn_1.html) and 20 participants from the ABIDE II database (http://fcon_1000.projects.nitrc.org/indi/abide/abideII.html) (Trinity centre for Health Sciences subcohort) met the eligibility criteria. The final sample size of the HC group consisted of 32 participants.

2.2. Clinical data and albumin

Diagnosis of AN according to diagnostic criteria of the DSM-IV and illness duration was assessed via clinical interview at admission by child and adolescent psychiatrists. The Eating Disorder Examination (Hilbert et al., 2013), a semi-structured interview carried out by trained members of the research team, was used for the assessment of eating disorder symptoms. The EDE global score is an indicator for severity of ED with higher scores indicating higher ED psychopathology. As part of inpatient treatment, weight and height was measured by nursing staff on the day of the MRI. Age adjusted BMI percentile was calculated.

Blood samples were collected after overnight fasting by clean and atraumatic venipuncture using a 21-gage butterfly needle (0.8 × 19 mm; Greiner Bio-One, Kremsmuenster, Austria) for routine laboratory. Albumin was quantitatively measured with the bromocresol green binding method (Hill, 1985).

2.3. MRI acquisition

MRI data acquisition was performed on a 3 Tesla scanner (Philips, ACHIEVA). All patients were scanned after at least one week of inpatient treatment following a standardized nutrition regimen. The MRI examination comprised a high resolution structural T1-weighted sequence (TR/TE: 8.2/3.7 ms, FOV 240 × 240 × 190 mm, voxel size: 1 × 1 × 1 mm), and a diffusion tensor sequence (TR/TE: 6438/70 ms, FOV 224 × 224 × 120 mm, voxel size: 2 × 2 × 2 mm, b-value = 1000, 32 gradient encoding directions).

The HC group consisted of two subcohorts with following acquisition parameters: The MRN dataset was acquired on a 3 Tesla scanner (Siemens, TrioTrim) comprising a diffusion tensor sequence (TR/TE: 9000/64 ms, FOV 256 × 256 × 144 mm, voxel size: 2 × 2 × 2 mm, b-value = 800, 35 gradient encoding directions). The HC subcohort from the ABIDE dataset was acquired on a 3 Tesla scanner (Philips, Achieva) comprising a diffusion tensor sequence (TR/TE: 20,244/79 ms, FOV 248 × 248 × 130 mm, voxel size: 1.94 × 1.94 × 2 mm, b-value = 1500, 61 gradient encoding directions).
2.4. Pre-processing

All preprocessing steps were carried out with the FSL toolbox (Jenkinson et al., 2012). DTI images were brain extracted and distortion corrected for eddy currents. A diffusion tensor model was fitted using FSL’s dtifit at each voxel, obtaining voxelwise FA and MD values.

2.5. Tract based spatial statistics

A whole-brain, voxel-wise Tract-based Spatial Statistics (TBSS) (Smith et al., 2006) analysis was performed using FSL. FA images were aligned to the MNI 152 standard space (Montreal Neurological Institute, McGill, USA) using nonlinear registration. FA images were averaged to create a mean FA image, then a skeleton was created using a threshold of FA > 0.2 to include only major fiber bundles and to restrict analysis to white matter. FA maps of each subject were then projected onto the skeleton. The same procedure was applied to MD maps.

2.6. Statistical analysis

We conducted t-tests for independent samples and Chi²-tests to compare the AN subsamples with the AN total group as well as the HC group with the AN total group in terms.

Statistical analysis was conducted with a permutation test implemented in FSL’s randomize, using the TFCE (Threshold-Free Cluster Enhancement) option which is a cluster-based multiple comparisons correction. The voxel count of the DTI skeleton is approximately 130,000.

We compared the FA and MD values between AN patients and healthy controls, statistically adjusted for sex and age. The mean age was comparable, but we wanted to correct for individual differences. Since randomize provides no effect sizes we used the contrast of parameter estimates (COPE) to demonstrate the size of the effects.

Furthermore, to examine the relationship between FA and MD values and clinical variables we performed multiple regression for a subgroup of AN patient cohort where these variables were available. In detail, we conducted multiple regression for age, duration of illness and EDE total score, as well as for age and Albumin. We quantified the affected white matter regions with the Johns Hopkins ICBM-DTI-81 white-matter labels atlas (Mori et al., 2005; Oishi et al., 2008).

3. Results

3.1. Demographic and clinical assessment

Table 1 shows the clinical characteristics of the AN sample, the HC sample as well as the two AN subsamples. There were no significant differences between the subsamples and the AN total sample in terms of age, BMI, illness duration (all p-values above 0.073). The time between admission and MRI was significantly lower in the AN Albumin subsample compared to the AN total sample, whereas no significant differences were found between the AN EDE sample and the AN total sample. The control group consisted of 32 participants (28.1% females, 71.9% males) of normal weight, who are comparable to the AN sample.

| Table 1 Sociodemographic and clinical characteristics of the AN and HC samples. (AN = anorexia nervosa, HC = healthy controls). |
| Sample | AN total n= 67 | AN EDNOS n= 37 | AN albumin n= 33 | HCN = 32 | Statistics | AN vs. HCN(t, p) | AN EDE vs. AN total(c, p) | AN Alb vs. AN total(c, p) |
|--------|----------------|----------------|----------------|---------|-----------|-----------------|-----------------|-------------------|
| Mean Age in years (SD) | 15.1 (1.5) | 15.1 (1.4) | 14.8 (1.6) | 15.6 (3.4) | 0.808, 0.424 | 0.333, 0.129 | 1.325, 0.190 |
| Sex; female (%); male (%) | 67 (100%); | 37 (100%); | 33 (100%); 0 (0%) | 9 (28.1%); 23 (71.9%) | n.a | n.a | n.a |
| Mean BMI (SD) | 14.7 (1.3) | 14.9 (1.3) | 14.7 (1.3) | n.a | 1.431, 0.164 | 0.007, 0.994 |
| BMI < 3rd percentile (%); BMI > 3rd percentile (%) | (88.2%); 11.8% | (86.5%); 13.5% | (87.9%); 12.1% | n.a | n.a | 0.397, 0.529 | 0.307, 0.579 |
| Mean Illness duration in months (SD) | 13.3 (9.7) | 11.7 (6.7) | 10.7 (7.5) | n.a | n.a | 1.437, 0.169 | 1.843, 0.073 |
| Mean time between admission and MRI in days (SD) | 18.8 (12.4) | 19.9 (14.0) | 15.3 (10.6) | n.a | 0.946, 0.348 | 2.403, 0.019 |

| Table 2 Regions of significantly decreased FA in AN compared to HC (FA = fractional anisotropy, AN = anorexia nervosa, HC = healthy controls). |
| Region | Hemisphere | Peak voxel (MNI coordinates) | X | Y | Z | T | P |
|--------|------------|-----------------------------|---|---|---|---|---|
| Genus of corpus callosum | Both | −4 | 30 | 6 | 11.42 | 0.0002 |
| Body of corpus callosum | Both | −1 | 19 | 17 | 7.52 | 0.0002 |
| Splenium of corpus callosum | Both | −11 | −41 | 19 | 5.98 | 0.0004 |
| Fornix (column and body) | Both | −1 | −9 | 16 | 4.93 | 0.0018 |
| Cerebral peduncle | Left | −16 | −21 | −12 | 3.39 | 0.0406 |
| Anterior limb of internal capsule | Right | 13 | 7 | 5 | 6.62 | 0.0256 |
| Anterior limb of internal capsule | Left | −12 | 6 | 4 | 5.42 | 0.0016 |
| Posterior limb of internal capsule | Right | 11 | −10 | −4 | 3.37 | 0.0110 |
| Posterior limb of internal capsule | Left | −23 | −21 | −2 | 4.57 | 0.0012 |
| Retroventricular part of internal capsule | Right | 17 | 36 | 6 | 7.35 | 0.0002 |
| Anterior corona radiata | Right | −15 | 36 | 6 | 7.18 | 0.0002 |
| Anterior corona radiata | Left | 22 | −9 | 35 | 5.95 | 0.0146 |
| Superior corona radiata | Right | −21 | −9 | 34 | 5.72 | 0.0012 |
| Posterior corona radiata | Right | 22 | −30 | 30 | 4.11 | 0.0228 |
| Posterior corona radiata | Left | −19 | −35 | 34 | 4.31 | 0.0034 |
| Posterior thalamic radiation | Right | 33 | −68 | 4 | 4.74 | 0.0466 |
| Posterior thalamic radiation | Left | −34 | −62 | −1 | 3.46 | 0.0148 |
| Sagittal stratum | Left | −44 | −26 | −14 | 4.12 | 0.0016 |
| External capsule | Right | 26 | −31 | −20 | 5 | 4.18 | 0.0012 |
| External capsule | Left | 31 | −10 | −13 | 3.4 | 2.42 | 0.0088 |
| Cingulum (cingulate gyrus) | Right | 21 | −32 | 9 | 4.02 | 0.0044 |
| Fornix (cres) | Right | −27 | −25 | −4 | 6.14 | 0.0012 |
| Fornix (cres) | Left | 46 | −9 | 25 | 2.86 | 0.0108 |
| Superior longitudinal fasciculus | Left | −32 | −29 | 37 | 3.53 | 0.0134 |
| Superior longitudinal fasciculus | Right | −21 | 3 | 24 | 2.76 | 0.0150 |
| Superior fronto-occipital fasciculus | Right | 33 | 5 | −12 | 2.06 | 0.0150 |
in terms of age ($p = 0.424$).

3.2. Group comparison AN-HC

In the DTI analysis we found statistically significant reduced FA values in the patient sample, after correcting for age and sex in the following WM regions: CC (bilateral, B, the body, genu and splenium), Fornix (B, column and body of fornix), the cerebral peduncle (left, L), the anterior, posterior and superior corona radiata (B), the anterior thalamic radiation including the optic radiation (B), the external capsule (B), cingulate gyrus (right, R), superior longitudinal fasciculus (B), the superior fronto-occipital fasciculus (L) and the uncinate fasciculus (R). Details are listed in Table 2 and demonstrated in Fig. 1.

The MD values were found significantly higher in the following brain regions: The middle cerebral peduncle (B), pontine crossing tract (B), genu, body and splenium of the CC (B), fornix (B), corticospinal tract (L), the medial lemniscus (B), inferior and superior cerebellar peduncle (B), internal capsule (B), anterior and superior corona radiata (B), superior corona radiate (L), posterior thalamic radiation (L), external capsule (B), cingulum (hippocampus) (L), superior longitudinal fasciculus (L), superior fronto-occipital fasciculus (B) and uncinate fasciculus (B). Details are listed in Table 3 and demonstrated in Fig. 2.

The size of the effect of FA being greater in HC than in AN and of MD being greater in AN than in HC is shown in the supplement Figs. 1 and 2 (Supp. 1, Supp. 2).

3.3. Associations with clinical characteristics and albumin

We conducted a multiple regression analysis in a subsample of the AN group ($n = 37$) to test for possible correlations between FA, MD, EDE total score and illness duration. The mean EDE total score in this sample was 2.75 (SD = 1.42) and the duration of illness prior to admission was on average 11.7 months (SD = 6.7). After correcting for age, there was no significant correlation between EDE total score, illness duration and FA as well as MD.

In a second multiple regression analysis using a subsample of 33 ANs we examined the effect of albumin levels on FA and MD. Average albumin level in this group was 48.0 g/L. 90.9% ($n = 30$) of the participants had albumin levels within the normal range (32.0 – 52.0 g/L), while 9.1% ($n = 3$) had elevated levels. After correcting for age, we found no significant correlation between albumin level and DTI measures.

4. Discussion

This is, to the best of our knowledge, the largest homogenous sample of AN patients ($n = 67$) to be investigated for WM microstructural changes using DTI. Our sample is homogenous as we included consecutive adolescents in the acutely ill state, that were admitted to our specialized inpatient treatment. MRI was done after at least one week of re-alimentation with an inward nutritional regimen. Patients were all still severely underweight, with the BMI below the third percentile for their age.

With this study, we want to shed light on the so far highly controversial results reported in literature. We found widespread alterations in the vast majority of all WM regions in the ill brain, with significant FA reductions compared to HCs. Regarding existing literature, the following studies are not comparable due to patient characteristics: Cha and colleges (Cha et al., 2016) found increased WM anisotropy in the AN sample which was a mixed sample of 12 patients with restrictive AN and 10 with binge purging AN, the age (mean age 19.5) and the mean BMI (mean BMI of 17.3) was higher than in our sample. Another study (Olivo et al., 2017) reported reduced FA in the CC, the corona radiata and the posterior thalamic radiation, which is in line with our results. They included only 3 patients with a BMI below the 3rd percentile and included mainly patients with an eating disorder, not otherwise specified (EDNOS). In the follow up scan, patients had normalized BMI, and no differences were found.

Frank et al. (Frank et al., 2013) reported lower FA in adolescents with AN (17 restricting, 2 binge purging type) in the fornix, posterior frontal and parietal areas, in line with our results, but higher FA in anterior frontal, orbitofrontal and temporal lobes. They analyzed 19
patients at an age comparable to our patient group, but with a higher mean BMI (16.2), which might have led to the partly differing results. Another study (Gaudio et al., 2017) reported lower FA values mainly in the body of the corpus callosum and posterior limb of internal capsule as well as the left inferior longitudinal fasciculus. These surprising findings are not in line with most existing literature, and authors are discussing the impact of illness duration and age, but these parameters did not differ in our investigation. Another study reported preserved WM microstructure in 35 AN patients in late adolescence (mean age 16.1) (Pfuhl et al., 2016) being in contrast to most DTI studies. The different results may also be attributed to different methodological approaches utilized for data analyses. VBM (voxel based morphometry) was used (G.K. Frank et al., 2013; Gaudio et al., 2017) as well as TBSS (Vogel et al., 2016) and tractography (Pfuhl et al., 2016; Travis et al., 2015).

A recent meta-analysis (Barona et al., 2019) pointed out mixed findings for adolescent AN patients. Most AN studies reported a reduction of FA in various regions, but two out of thirteen studies found also increased FA values. Barona et al. attribute these differences in directionality to a possible unmasking effect caused by crossing fibers. FA is naturally low in normal white matter areas where fibers cross (Jones et al., 2013). Despite the heterogenous literature offering room for discussion, our findings support the main outcome of the meta-analysis.

In this highly selected sample in the acute stage of AN, the alterations are still likely to be the consequence of acute starvation (King et al., 2018). In this stage of disease direct effects of loss of electrolytes, low oncoptic pressure and elevated cytokines lead to microstructural deficits (Anderson et al., 2020; Fugate and Rabinstein, 2015; Solmi et al., 2015). At the time of the MRI scanning we screened the nutritional status in a subsample of patients via albumin blood levels. Albumin levels were mostly in the normal range, maybe since patients had at least a week of standardized nutrition. Indeed, we cannot rule out that some of the regions do play a key role in the psychopathology of AN. In the following we will discuss some of the affected regions that might play a key role in AN specific functioning but cannot be differentiated here from severe microstructural defects due to starvation processes.

We found significantly decreased FA in the AN group in the genu, the body and the splenium of the CC and increases of MD in the genu and body (Catani and Thiebaut de Schotten, 2008). This is in line with existing literature with adolescent patients (Gaudio et al., 2017; Olivo et al., 2017) and adults (von Schwabenflug et al., 2019; Zhang et al., 2020).

CC is the fundamental structure for inter-hemispheric connections. The anterior fibers transfer motor information interconnecting the frontal lobes, and the posterior fibers are responsible for the somatosensory (body), auditory (thalamus) and visual (splenium) information as these fibers connect parietal, temporal and occipital lobes (Fabri and Polonara, 2013). Taste stimuli activate the anterior CC, the genu is activated by sweet stimuli. It was speculated that lower FA in the body of the CC reflecting prefrontal interhemispheric connectivity may contribute to body image distortion (Zhang et al., 2020). The body of the corpus callosum also connects supplementary motor areas, that are involved in planning and controlling motor actions and it has been speculated that impaired white matter integrity might lead to cognitive-behavioral inflexibility (Zhang et al., 2020).

FA values in the uncinate fasciculus were significantly lower in the patient sample than in the HC group. It was increased. This structure is considered to belong to the limbic system and is involved in emotion processing (Gaffan and Wilson, 2008).

The internal capsule and the corona radiata are a complex projection system containing fibers from the thalamus to the cerebral cortex and from the fronto-parietal cortex to subcortical nuclei (basal ganglia and brainstem nuclei) and spinal cord (Glickstein and Berlucchi, 2008). It is important for perceptual and motor functions and other higher cognitive functions. The internal capsule is also part of the gustatory pathway.
The fornix is part of the limbic system and is involved in emotion processing. Further it connects the hippocampus to the nucleus accumbens and the hypothalamus playing an important role in controlling food intake. This area was recently studied in patients with AN and a typical AN and results were correlated to ghrelin total levels and pubertal stage, finding no significant differences between AN and controls, but fasting ghrelin levels being negatively associated with FA in the fornix (Breithaupt et al., 2020; Schalla and Stengel, 2018). We found bilateral significant FA decreases and MD increases in our patient sample. This is a finding described by others previously (Frank et al., 2013; Gaudio et al., 2017; Travis et al., 2015).

The internal capsule, the fornix and the thalamic radiation are part of the reward circuitry (Koch et al., 2014). These areas may play a key role in AN-specific alterations in reward regulation. In the analysis within the patient sample we could not find correlations between DTI indices and EDE total score and illness duration after correcting for age and age adjusted BMI percentile. This might reflect our homogenous sample being scanned in the severely ill state.

Regarding the albumin level we also could not find any significant correlations. This is most likely due to the fact that most of our severely ill patients had levels in the normal range. The strength of this study is clearly the large sample size. Furthermore, our sample is homogenous, since we included patients with AN, restricting type, in the acute stage of illness. All patients were severely underweight, but with a standardized nutritional regimen for at least one week prior to MRI. During the inpatient treatment all subjects were abstinent for alcohol, caffeine or tobacco use. Considering the development of the adolescent brain we used an age matched control group.

Our sample was characterized by low BMI, but data on BMI was not available in the healthy control sample. We can rule out that subjects with a current eating disorder were included, so we don’t suspect any HC to be underweight. But since we have no exact data on BMI, adolescents with obesity could be included. According to a prevalence study among youth, 17 to 19% of the male and 11% of the female adolescents in Austria were overweight or obese (WHO, 2012). We could speculate therefore that about 15% of the adolescents could have been overweight or obese. Research in adults and adolescents indicate that obese and overweight individuals have poorer WM integrity in several tracts (Carbine et al., 2020; Karlsson et al., 2013; Kullmann et al., 2015; Marks et al., 2011; Mueller et al., 2011; Stanek et al., 2011; Verstynen et al., 2012). In obese adolescents results are mixed with decreased WM integrity in many areas (the superior frontal corpus callosum, left and right uncinate fasciculi, left corticospinal tract and the left inferior fronto-occipital fasciculus), but also few regions with increased WM integrity (corpus callosum, right inferior fronto-occipital fasciculus, the left cingulum and the left corticospinal tract) (Carbine et al., 2020). In sum, we can assume that WM integrity is mostly decreased by an under- and overweight status in comparison to normal weight adolescents, and a fraction of overweight adolescents in the HC group would not interfere with our results of significantly decreased FA in AN compared to HC.

There are other limitations that have to be kept in mind. First, we did not have a control group that was tested at our clinic, and thus an external sample with similar image acquisition parameters had to be used. The prominent sex in the HC group was male. Findings regarding sex differences in adolescence are inconsistent (Kaczkurkin et al., 2019): Some studies found greater FA values in males in many regions, but interestingly an exception is the CC, where adult females had higher FA than males. Sex difference was tied to hormonal levels, ie., estradiol had a negative correlation with FA in female youth. In our female AN sample we found as expected for reduced adipose tissue low levels of estradiol. There are several studies that showed no sex differences in FA in youth (Eluvathingal et al., 2007; Krogshud et al., 2016). A longitudinal study showed differences in childhood and adulthood, but equivalent values in adolescence (Kaczkurkin et al., 2019). Sample size is an important matter, when detecting sex differences. In order to achieve sufficient power to detect differences between boys and girls a rather large sample size (>100) should be utilized (Schmithorst et al., 2008). Overall, studies of gender differences in neuroimaging remain inconclusive, and again meta-analyses suggest a possible publication bias, where findings are mostly driven by small sample sizes (David et al., 2018; Eliot et al., 2021). And even in well-designed large-scale studies, only a small effect size (average cohens d of 0.19) is observed for sex differences in DTI.
tactography in adults (Ritchie et al., 2018). In our control group, males and females are combined (9 female, 23 male), in order to minimize the effect of sex.

Another limitation is that we could not include information on handedness in our analysis. Else we are in line with latest recommendations (G. K. W. Frank et al., 2018) as we analyzed a large homogenous sample, and all patients were hospitalized and had similar nutritional conditions. Since this study has a cross sectional design we cannot draw any conclusion whether our findings are state or trait alterations. Longitudinal data are needed to address this question. Favorable would be a prospective study of high-risk adolescents prior to any weight loss.

5. Conclusion

Patients suffering from AN in the acute stage of disease show widespread alterations in WM integrity. These deficits are likely to be a consequence of starvation, malnutrition and dehydration. Areas with high neuropsychological significance in the course of disease might be the CC, the fornix and the internal capsule. Therefore, starvation is likely to lead to higher severity of anorexia nervosa symptoms and re-alimentation needs to be the first therapeutic goal.

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Conception and design of the study: A.K., G.K., DP; Acquisition and analysis of data: C.L., K.-H. N., T.W., V.S., J.S., E.S.M. G.W., S.S., J.P.; Drafting the manuscript or figures: C.L., K.-H. N., T.W. All authors have reviewed and approved the final manuscript.

Declaration of Competing Interest

The authors declare no conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jspsychiatr.2021.111427.

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