Title: Normal white matter microstructure in women long-term recovered from anorexia nervosa: A diffusion tensor imaging study

Running Title: Normal white matter

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ABSTRACT

Objective: Studies point to white matter (WM) microstructure alterations in both adolescent and adult patients with anorexia nervosa (AN). These include reduced fractional anisotropy in several WM fiber tracts, suggesting reduced WM integrity. The extent to which these alterations are reversible with recovery from AN is unclear. There is a paucity of research investigating the presence of WM microstructure alterations in recovered AN patients, and results are inconsistent. This study aimed to investigate the presence of WM microstructure alterations in women long-term recovered from AN. **Method:** Twenty-one adult women who were recovered from AN for at least one year were compared to 21 adult comparison women. Participants were recruited via user-organizations for eating disorders, local advertisements, and online forums. Diffusion tensor imaging was used to compare WM microstructure between groups. Correlations between WM microstructure and clinical characteristics were also explored. **Results:** There were no statistically significant between-group differences in WM microstructure. These null-findings remained when employing liberal alpha level thresholds. Furthermore, there were no statistically significant correlations between WM microstructure and clinical characteristics. **Discussion:** Our findings showed normal WM microstructure in long-term recovered patients, indicating the alterations observed during the acute phase are reversible. Given the paucity of research and inconsistent findings, future studies are warranted to determine the presence of WM microstructure alterations following recovery from AN.

Keywords: Anorexia nervosa, white matter microstructure, magnetic resonance imaging, neurobiology, diffusion tensor imaging.

INTRODUCTION

Anorexia nervosa (AN) is a potentially fatal eating disorder that predominantly develops in adolescents (Association, 2013). The hallmark features include a relentless pursuit of thinness, body image disturbances, and severe food-restriction leading to extreme weight-loss. A wealth of studies has shown that the brain is affected by AN-related emaciation and malnourishment. Studies typically show that acutely ill patients with AN exhibit major global reductions of gray and white matter (WM) brain volumes (Titova, Hjorth, Schioth, & Brooks, 2013), but larger focal gray matter volumes among patients have also been reported (Frank, Shott, Hagman, & Yang, 2013; Brooks et al., 2011). These alterations appear to be at least partially reversible with weight-restoration and recovery; several studies show that brain volumes are completely normalized following recovery (Bang, Rø, & Endestad, 2016; Bernardoni et al., 2016; Seitz, Herpertz-Dahlmann, & Konrad, 2016). However, some do report persistent gray matter volume alterations following recovery (Frank et al., 2013; Friedrich et al., 2012), which may reflect irreversible tissue damage (e.g. microstructural neuronal damage) or trait-characteristics.

Beyond WM volume, investigators have recently used diffusion tensor imaging (DTI) to investigate microstructural WM characteristics. DTI quantifies the diffusion of water molecules in the brain, and can be used to measure WM fiber tracts and their microstructural properties, including axonal coherence, density, or degree of myelination (O'Donnell & Westin, 2011). Water diffusion is highly directional (anisotropic) along myelinated WM fiber tracts, and undirectional in areas where there are few barriers to constrict the diffusion of water (e.g. in cerebrospinal fluid). DTI generates several scalars, including fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD). FA is the most commonly

reported scalar, and reflects the degree of directionality in a voxel. It is a summary measure sensitive to the presence - but not nature - of microstructural changes, and is often interpreted as reflecting degree of WM integrity (although this interpretation has been criticized, see Jones, Knösche, & Turner, 2013). MD reflects the overall degree of water diffusion, and is typically inversely correlated with FA (see Alexander, Lee, Lazar, & Field, 2007; O'Donnell & Westin, 2011 for in-depth descriptions of the scalars).

DTI studies of acutely ill patients with AN have generally shown microstructural WM alterations (Monzon, Hay, Foroughi, & Touyz, 2016), although specific findings are mixed. For adult patients, studies point to decreased FA (Frieling et al., 2012; Hayes et al., 2015; Kazlouski et al., 2011; Nagahara et al., 2014; Via et al., 2014), which in some instances is accompanied by increased MD (Nagahara et al., 2014; Via et al., 2014). For adolescents, the emerging findings are more inconsistent; both reduced (Frank et al., 2013; Travis et al., 2015) and increased (Cha et al., 2016; Travis et al., 2015; Vogel et al., 2016) FA have been reported. Also, a recent investigation found no evidence of any WM microstructure alterations in adolescent AN patients (Pfuhl et al., 2016). It is important to acknowledge that adolescence is a period where considerable development of WM occurs; with increases to both WM volume (Mills et al., 2016) and FA in WM fiber tracts (Barnea-Goraly et al., 2005; Moon et al., 2011). In light of this, AN may impact WM microstructure differentially in adolescents and adults.

Which specific WM fiber tracts are affected varies between studies, and includes the fimbria-fornix, fronto-occipital fasciculus, superior longitudinal fasciculus, posterior cingulum, posterior thalamic radiation, and medio-dorsal thalamus. The mechanisms underlying these alterations in WM microstructure are not known. One study

excluded dehydration as the possible culprit (Vogel et al., 2016). It is known that WM microstructure can be affected by a variety of factors, including sleep deprivation (Elvsåshagen et al., 2015) and fasting (Bakan et al., 2015). In sum, despite inconsistencies studies strongly suggest the presence of WM microstructure alterations in acutely ill patients. Whether these alterations are secondary to emaciation and malnourishment, or reflect primary AN pathophysiology is unclear.

To shed light on this, several studies have investigated the extent to which alterations in WM microstructure reverse following weight-gain and recovery. Findings from such studies are inconsistent. In a longitudinal investigation (Vogel et al., 2016), increased FA in frontal, temporal, and parietal fibers among adolescent patients were observed to *partially* normalize following weight-restoration. In another longitudinal study, Cha and colleagues (2016) observed increased FA in fibers connecting the orbitofrontal cortex and the nucleus accumbens among adolescent and adult patients with AN, which persisted following weight-restoration with no indication of partial normalization.

Further evidence of WM microstructure alterations following recovery from AN have emerged from cross-sectional studies where adult patients have maintained normal body weight for an extended period of time. One such study (Shott, Pryor, Yang, & Frank, 2016) reported decreased FA in WM fiber tracts connecting the insula and fronto-striatal regions, and in frontal and cerebellar tracts. Reduced FA was also reported by Frieling and colleagues (2012), but in the posterior thalamic radiation and mediodorsal thalamic nuclei. However, their study was based on a mixed sample of ill and recovered patients, and only nine recovered patients were included. In contrast, another study found normal FA, but lower MD in frontal, parietal, and cingulum WM among recovered women (Yau et al., 2013). Furthermore, one recent investigation

found no evidence of altered WM microstructure among recovered patients (Pfuhl et al., 2016). Their recovered sample included both adolescents and adults, but null-findings remained when they restricted their analyses to the adults.

Thus, the few studies that have investigated the presence of WM microstructure alterations following recovery have yielded inconsistent findings. The reason for these discrepant findings is not known, but small sample size, variations in sample characteristics (e.g. age and clinical characteristics) and different analytical approaches could be potential contributors. It remains unclear if individuals recovered from AN are characterized by alterations in WM microstructure similar to the ones observed during the acute phase. If present, such alterations could reflect irreversible damage secondary to emaciation, or alternatively vulnerability traits preceding the onset of the disorder. Given the paucity of research investigating the presence of these alterations in patients following recovery, and the contradicting findings, more studies are warranted. The aim of our study was to investigate the presence of WM microstructure alterations in a sample of adult women long-term recovered from AN.

METHODS

Participants

We recruited 22 adult women long-term recovered from AN (RAN) and 22 age-matched, healthy comparison women (CW) via patient-organizations for eating disorders (i.e. supportive interest groups for individuals affected by eating disorders), local advertisements, and online forums. Current and lifetime DSM-IV diagnoses were evaluated with the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I/P, First, Spitzer, Gibbon, & Williams, 2002). RAN women had a lifetime history of AN according to the DSM-IV criteria (Association, 2000), excluding the amenorrhea criterion. Women were considered recovered if they had maintained a body mass index (BMI, kg/m^2) above 18.0 and had neither engaged in binging and purging behavior, nor severely restricted food intake for the past year. Exclusion criteria for these women included: lifetime history of a psychotic disorder, substance abuse or dependence, or the presence of any Axis-I disorder the past year.

Differentiating between lifetime history of AN restricting and AN binge-eating/purging subtype proved difficult considering the diagnostic interview was done retrospectively. Several participants experienced several episodes (i.e. relapses) of AN, and several had difficulty recalling the frequency and timing of any potential binge-eating/purging episodes. However, based on the diagnostic interviews we broadly characterized participants as either having had lifetime AN restricting subtype (with minimal occurrences of binge-eating/purging behaviors during AN episode) or AN binge-eating /purging subtype (having frequent occurrences of binge-eating/purging behaviors during AN episode).

We included comparison women who had no lifetime history of any Axis-I disorder and who were not currently taking psychoactive medications. We excluded CW who reported any binging and purging behavior, excessive and compulsive exercising, severely restricted food-intake, or a body mass index below 18.0 for the past 12 months. Women in both groups were excluded if they reported any major medical illnesses, a history of severe head trauma, or any MRI-contraindications. Three RAN women were currently taking psychoactive medications (one an antidepressant for mood, one an antipsychotic for insomnia, and one an antiepileptic and an antidepressant for mood/anxiety). This study was approved by the Regional Ethics Committee in Norway (reference number 2012/1386), and all participants provided written informed consent prior to onset of the study.

Behavioral measures

All participants completed the following questionnaires; Eating Disorder Examination-Questionnaire (Fairburn & Beglin, 2008), Spielberger State-Trait Anxiety Inventory (Spielberger, Gorsuch, & Lushene, 1970), Beck Depression Inventory-II (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961), and Difficulties in Emotion Regulation Scale (Gratz & Roemer, 2004). Participants were weighed in order to calculate their current BMI.

Diffusion weighted image acquisition

Diffusion weighted images (DWIs) were acquired with a 3 Tesla Achieva MRI scanner (Philips, Eindhoven), using a single-shot echo-planar sequence with 32 diffusion directions (b weighting = 1000). An additional non-diffusion weighted image

(b = 0) was acquired for each participant. The following acquisition parameters were used: voxel size = 2 x 2 x 2 mm, matrix size = 112 x 112, FOV = 224 x 224 x 124, TE = 54 ms, TR = 10342 ms. DWIs were acquired in axial anterior-posterior commissure orientation.

DWI analysis

DWI data were processed using the FSL's Diffusion Toolbox (FDT), part of the FSL software suite (FMRIB Software Library version 5.0.9, http://fmrib.ox.ac.uk/fsl, Smith et al., 2004). All DWI images were visually inspected for data quality and artifacts. Data was corrected for eddy current-induced distortions and participant movement, followed by B-matrix rotation to ensure orientational information was correctly preserved. Head motion was then extracted and inspected for excessive movement. Two participants (one in each group) exhibited considerable head motion, which was associated with corrupted DWIs. These participants were excluded from all analyses (resulting in n = 21 for each of the two groups). There were no group differences in movement for the final sample (t-test, p = .81). Tensors were then fitted to the data, and voxel-wise maps of FA, MD, AD, and RD was generated.

Voxel-wise statistical analyses of these maps were carried out using the Tract-Based Spatial Statistics (TBSS, Smith et al., 2006) pipeline, as implemented in FSL. FA maps were nonlinearly registered to 1 x 1 x 1 mm MNI152 standard space, averaged, and a WM skeleton was created using a FA threshold of 0.2 to exclude areas of gray matter. Finally, individual FA maps were projected onto the skeleton. The registration and skeletonisation derived from FA maps were then applied to MD, AD, and RD maps.

Voxel-wise comparisons of FA, MD, AD, and RD maps were performed using a non-parametric permutation based algorithm as implemented in FSL's randomise (Winkler, Ridgway, Webster, Smith, & Nichols, 2014). Between-group differences were tested using unpaired t-tests with age, BMI, and head motion as covariates of no interest (with 5000 permutations). We also performed the same unpaired t-tests *without* BMI as a covariate, to investigate if this had any impact on the results. Significant clusters were identified using the threshold-free cluster enhancement approach (Smith & Nichols, 2009), at a threshold of $p < .05$ family-wise error corrected for multiple comparisons.

To further characterize potential differences in WM microstructure between groups, we extracted the global FA, MD, AD, and RD averages of the entire WM skeleton (restricted to tracts with FA > 0.2, as in the main TBSS analyses), and compared these between our groups using a one-way ANOVA with age, BMI, and head motion as covariates. As in the main TBSS analyses, we also investigated the impact of excluding BMI as a covariate on these analyses.

Because previous studies have reported significant associations between WM microstructure and BMI (Nagahara et al., 2014) and AN severity (Nagahara et al., 2014; Shott et al., 2016; Yau et al., 2013), we also performed exploratory correlations between FA maps and BMI, AN duration, age of AN onset, and lowest lifetime weight in the RAN group. FSL's randomise were used to test for positive and negative correlations, with age and head motion as covariates of no interest (with 5000 permutations). Similar to the main analyses, significant clusters were identified using the threshold-free cluster enhancement approach, but a liberal threshold of $p < .001$ uncorrected for multiple comparisons was used. We have previously reported that the

RAN and CW groups are similar in total brain size, global gray matter, and global WM volumes (Bang et al., 2016), so these measures are not considered here.

RESULTS

Participant characteristics

Participant characteristics are presented in Table 1. Groups were of similar age, but BMI was significantly lower for the RAN women. Relative to CW, RAN women reported higher levels of emotion dysregulation, anxiety, depression, and eating disorder psychopathology. All participants scored below the empirically established Norwegian global EDE-Q clinical threshold of 2.5 (Rø, Reas, & Stedal, 2015). Of the RAN women, 11 were characterized as having had a history of AN binge-eating/purging subtype, while the remaining 10 were characterized as having had a history of AN restricting subtype. Clinical characteristics of the RAN women are presented in Table 1.

INSERT TABLE 1 ABOUT HERE

DWI results

There were no significant differences between groups in FA, AD, MD or RD in the main TBSS analyses. This indicates that WM microstructure is normal in RAN women. These null-findings remained when using a more liberal statistical threshold of $p < .001$ uncorrected for multiple comparisons. Omitting BMI as a covariate in the analyses had no impact on the results.

Analyses of global FA, AD, MD, and RD averages showed that the CW group had marginally higher average global values on all diffusivity measures, but these differences were non-significant, in line with the main TBSS analyses. Specifically, in the CW compared to the RAN group, mean FA was 0.38% higher ($F[1] = 0.46$, $p =$

.50), mean MD was 0.18% higher (F[1] = 0.00, p = 1.00), mean AD was 0.31% higher (F[1] = 0.42, p = .52), and mean RD was 0.05% higher (F[1] = 0.00, p = 1.00). Omitting BMI as a covariate in the analyses had no impact on the results.

Excluding the three RAN women who were currently taking psychoactive medications did not alter our findings. Furthermore, the correlation analyses did not show any statistical significant associations between FA maps and BMI, AN duration, age of AN onset, and lowest lifetime weight.

DISCUSSION

We investigated WM microstructure in an adult sample of women long-term recovered from AN. Our study found no between-group differences in any of the DTI indices (FA, MD, AD, and RD). Furthermore, there were no correlations between WM microstructure and clinical characteristics. Our findings indicate normal WM microstructure among women recovered from AN, suggesting the observed alterations in the acute phase are reversible.

In contrast to our findings, some previous studies have found alterations in WM microstructure following long-term recovery (Shott et al., 2016; Yau et al., 2013). However, the specific WM microstructure alterations reported differ. Shott and colleagues (2016) reported reduced FA in in WM tracts connecting the insula to striatal and prefrontal circuits, as well as in frontal and cerebellar tracts. However, they did not find alterations in MD, AD, or RD. Another study (Yau et al., 2013) found no evidence of altered FA, but did report *reduced* MD in frontal, parietal and cingulum WM pathways. These findings are somewhat inconsistent with results of the Shott et.al study, as FA and MD are typically negatively correlated, and MD has been found to be *increased* in ill patients (Frank et al., 2013; Nagahara et al., 2014; Via et al., 2014). Additionally, one study (Frieling et al., 2012) reported reduced FA in the posterior thalamic radiation and mediodorsal thalamic nuclei in a mixed sample of ill and recovered AN. Although the authors were unable to detect group differences between the ill and recovered individuals, only nine recovered patients were included, making it difficult to ascertain whether true WM microstructure alterations were present in the recovered group.

These discrepant findings could be attributable to confounding factors including sample characteristics (e.g. age, clinical characteristics), sample size, and analytical

approach. The studies by Frieling et.al, Shott et.al, and Yau et.al all included adult patients similar in age to our own sample, and all used conservative criteria to operationalize recovery from AN. Some of the previous studies included small samples (n < 13) which could be sensitive to outliers. Also, studies vary in their analytical approach (e.g. type of analysis, software package, inclusion of covariates in the models), which could contribute to the inconsistent findings.

Our results are in line with a recent study (Pfuhl et al., 2016) comprising the largest sample of both ill (n = 35) and recovered (n = 32) AN patients to date. Using a robust method for WM fiber reconstruction, they failed to find any alterations to WM microstructure in adult recovered patients. Mean age of this group was slightly lower compared to other studies of recovered patients. Interestingly, they also failed to show any WM alterations in acutely ill patients as well, in contrast to previous studies. The authors showed that these results were not affected by potential confounders including age, IQ, and psychiatric symptoms. This study, along with our own, cast doubts on the presence of WM microstructure alterations following recovery from AN.

It is possible that WM microstructure alterations are only observed in a subgroup of recovered patients, for example those with a history of a particular severe or long-lasting AN. Indeed, some have reported that altered WM microstructure is correlated with clinical characteristics. In our study, we failed to find any correlations between FA and clinical characteristics, including current BMI, AN duration, age of AN onset, and lowest lifetime weight. Such correlations have previously been reported, both in ill (Nagahara et al., 2014) and recovered (Shott et al., 2016; Yau et al., 2013) patients. However, others have also failed to detect similar associations (Travis et al., 2015). It should be noted that in our study these correlations were explored on a whole-brain level as opposed to a regions-of-interest level, which reduces the

statistical power. It is also unclear whether AN subtype impacts findings in DTI studies of AN. If some of the reported WM microstructure alterations observed during the acute phase of AN reflect trait dispositions, it is possible that different WM microstructure characteristics are observed in the AN restricting and AN binge-eating/purging subtypes. Given the paucity of studies, and contradictory findings, more studies are needed to determine the presence of WM microstructure alterations following recovery from AN, and the associations between clinical characteristics and WM microstructure. There is a particular need for well-powered longitudinal studies with both short and long follow-ups, in order to characterize WM microstructure over time, from the acute to the recovered phase. It is possible that for some individuals, for example those with a particular severe AN, more time is needed for full restoration of WM microstructure. These issues are particularly important to consider when adolescent samples are recruited, for which WM microstructure is still under development, and could be stunted due to malnutrition and emaciation.

It is also important to acknowledge the possibility that some of the previous alterations in WM microstructure among patients may have been biased by the ventricular enlargements often observed in these individuals. A recent study (Kaufmann et al., 2017) showed that differences in FA of the fornix between ill patients and healthy controls were biased by ventricular volumes, and that these group differences disappeared after correcting for cerebrospinal-fluid partial volume effects. In light of this, it is possible that some of the prior findings of reduced FA among ill patients with AN – particularly in areas close to ventricles – are biased. Future studies investigating WM microstructure in patients should take such partial volume effects into account (Seitz, 2017).

Strengths of the current study include the thorough diagnostic evaluation, strict inclusion criteria, the use of well-established DTI analytical approach, and the inclusion of relevant covariates such as age, BMI, and head motion. Main limitations of our study include its cross-sectional design and the modest sample size. As we only included recovered patients, we don't know the extent to which there was WM microstructure alterations when these individuals were ill. Also, we cannot rule out that differences in WM microstructure would emerge with larger sample sizes, although our null-findings indicate that if such alterations are present, they are small. Lastly, we only included RAN women who did not fulfill criteria for any Axis-I disorder the previous year, to avoid confounding effects of other mental disorders. However, this might have resulted in the exclusion of individuals who suffered the most damage during the course of AN, and who possibly would be most likely to exhibit persistent WM microstructure alterations following recovery from AN. There is a need to better understand how clinical characteristics (AN severity, comorbidities) impacts WM microstructure.

We have previously reported that the recovered AN women in the current study have normal gray and WM volumes (Bang et al., 2016). Along with findings from the present study, and in line with recent work by others (Bernardoni et al., 2016; Pfuhl et al., 2016), this indicates that the structural brain changes induced by acute AN are reversible, and do not persist following recovery. Given the inconsistent findings, further well-powered studies are needed to determine if WM microstructure alterations are present following recovery from AN. Longitudinal studies that can characterize WM microstructure over time, during both the acute and recovered phase are particularly needed.

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Table 1. Participant characteristics

	Recovered AN (n = 21)	CW (n = 21)	Two sample t-test		
Characteristic	Mean ± SD	Mean ± SD	*t (df)*	*p*	*d*
Age	27.62 ± 5.06	26.10 ± 4.75	1.01 (40)	.32	0.31
Body Mass Index (kg/m^2)†	20.45 ± 1.69	21.83 ± 1.80	-2.44 (36)	.02	-0.79
STAI trait	38.56 ± 11.84	28.90 ± 6.50	3.84 (32.00)	.002	1.01
STAI state	32.06 ± 8.37	26.40 ± 5.16	3.03 (33.86)	.005	0.81
DERS total	75.39 ± 23.51	61.30 ± 14.33	2.92 (40)	.006	0.72
BDI	6.61 ± 8.47	1.90 ± 2.79	2.59 (24.62)	.02	0.75
EDE-Q global	0.87 ± 0.77	0.18 ± 0.16	4.33 (22)	<.001	1.24
Lowest lifetime weight‡	71.58 ± 9.51 (range: 47 – 85)				
Age of AN onset	17.25 ± 4.36 (range: 11 – 32)				
Duration of illness (months)§	33.90 ± 27.75 (range: 6 – 120)				
Duration of recovery (months)§	53.60 ± 42.80 (range: 12 – 192)				

Abbreviations - AN, anorexia nervosa; CW, comparison women; STAI, Spielberger state-trait inventory; DERS, Difficulties with emotional regulation; BDI, Beck depression inventory; EDE-Q, Eating disorder examination-questionnaire; *d*, Cohen's *d* effect size.

† Data not available for three recovered anorexia nervosa women and one comparison woman.

‡ Expressed as percentage of ideal weight, taking into account height, age and gender.

§ Data not available for one recovered anorexia nervosa woman.