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Mammillary Body Volume Abnormalities in Anorexia Nervosa

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Abstract

Objective—Several case reports of Wernicke's Encephalopathy in AN due to thiamine deficiency have described mammillary body (MB) injury, but systematic studies are lacking. Here we evaluated whether underweight and weight-restored individuals with AN demonstrate evidence of abnormal MB morphology, via retrospective examination of a previously collected data set.

Method—Using standard-resolution T1-weighted magnetic resonance imaging at 3 Tesla, we measured MB volume and fornix area in a cross-sectional study of 12 underweight AN, 20 weight-restored AN, and 30 age- and sex-matched healthy comparisons. Due to the small size of these structures, a manual tracing approach was necessary to obtain accurate measurements. A blinded expert rater manually traced MB and fornix structures in each participant.

Results—We observed significantly smaller MB volumes in the underweight AN group. However, the weight-restored AN group exhibited significantly larger MB volumes. The right fornix was smaller in the weight-restored AN group only.

Discussion—These findings suggest the possibility that MB volume and fornix area could represent potential biomarkers of acute weight loss and restoration, respectively. Verification of

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SSK and RK designed the study. JDF collected the data. SSK, RK and VP analyzed the data. SSK principally wrote the paper with the help of RK, MS and JDF.

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this finding through prospective studies evaluating MB morphology, cognition, and thiamine levels longitudinally across individual illness trajectories might be warranted.

INTRODUCTION

Converging evidence suggests that the prolonged food restriction and emaciation occurring in anorexia nervosa (AN) are associated with cerebral atrophy (1–3). For example, global reductions of cortical gray matter (4), subcortical white matter (5, 6) and corresponding ventricular enlargements (7) have been observed consistently in the underweight state. Regional cortical volume reductions have also been reported, though to a lesser extent. For instance, underweight individuals with AN have demonstrated regional volume decreases in hippocampal (8, 9), anterior cingulate (10), cerebellar, occipital and somatosensory (11) gray matter. Regional increases in brain volumes have also been described, for example, in the orbitofrontal and insular cortices (12). These volumetric differences have been associated with clinically relevant features such as a negative correlation between orbitofrontal gray matter volume and ratings of sweet taste pleasantness (12). In other studies volumetric gray matter changes have not correlated with clinical measures (8, 13), suggesting that additional delineation of illness cerebropathology is needed.

Focal abnormalities in several white matter tracts have also been reported in AN. These have most commonly focused on the fornix, a fiber bundle that has been shown to play key roles in regulating feeding and drinking patterns, memory, anxiety expression, and reward sensitivity in animals (14–16). Since these processes are also frequently disturbed in AN, several studies have examined fornix morphology in humans. Using diffusion tensor imaging, Kazlouski et al (17) identified reduced fractional anisotropy (FA) in the bilateral fimbria-fornix, fronto-occipital fasciculus, and the posterior cingulum in adults with AN. Frank et al (18) subsequently described reduced fornix FA and increased fornix apparent diffusivity, a measure of water diffusion that can reflect cellular damage(19), in a sample of adolescents with AN. More recently, Via et al (20) also observed reduced fornix FA and increased mean diffusivity in the fornix in underweight AN.

Nutritional deficiencies could be a potential source for some of the clinical symptoms and brain abnormalities observed in underweight AN, particularly of essential micronutrients. Although some individuals prone to AN may preemptively take multivitamin supplementation during self-starvation(21), many studies continue to identify micronutrient abnormalities in eating disorder populations. For instance, individuals with AN have reported reduced vitamin intake(22, 23), and exhibited serum deficiencies of several essential vitamins in the underweight state including thiamine (vitamin B1)(22, 24) and vitamin D (25).

Thiamine deficiency can induce a neuropsychiatric syndrome called Wernicke's Encephalopathy (WE), which is characterized by a classical triad of symptoms that includes ataxia, ocular nystagmus and a confusional state sometimes characterized by memory loss, confabulation or psychosis. In severe cases, WE can result in permanent cognitive dysfunction or fatality. The most common neuropathological finding in WE is damage to the mammillary bodies (MB)(26) and fornix fibers (27). While the most common causes of WE

neuropathology are from chronic malnutrition in the setting of alcohol dependence (28), some studies have demonstrated evidence of MB injury due to non-alcoholic starvation (29, 30). Furthermore, numerous case reports (including in recent years), have documented instances of WE and mammillary body damage due to AN (31–34), or a combination of anorexia and alcohol abuse (35). These reports could provide a potential link between thiamine deficiency and some of the subcortical neuropathological changes observed in neuroimaging studies of AN.

The complete triad of WE symptoms is rarely observed, and often only in the most severe cases. WE more commonly presents with early symptoms that are nonspecific, such as fatigue, abdominal discomfort, and headache, leading to the suggestion that WE is prone to under-diagnosis by clinicians(26). The fact that these early WE symptoms are highly overlapping with those frequently endorsed by AN patients raises the possibility that a continuum of neuropathology related to thiamine deficiency may also exist in AN. It also begs the question of whether such symptoms could reflect thiamine deficiency, at least in some cases.

At present no studies at the group level have evaluated MB or fornix pathology in AN. To address this gap in the literature, in the current study we evaluated whether underweight and weight-restored individuals with AN demonstrated evidence of structural differences in the MB and fornix compared with healthy individuals, using standard-resolution T1-weighted MRI. We hypothesized that MB volume and fornix area would be abnormally decreased in the underweight AN group, but would not be different in the weight restored AN group. The fornix hypothesis was derived from diffusion tensor imaging studies in AN and altered fornix pathology in WE.

METHODS AND MATERIALS

Participants

Twelve underweight females with current AN (mean age 19.4 years, mean illness duration 5.4 years), 20 weight-restored AN (18 female, mean age 22.4 years, mean illness duration 6.6 years), and 30 age- equivalent healthy comparisons (HC) were studied. AN participants were diagnosed during a clinical evaluation by a psychiatrist or psychologist with eating disorders expertise, and were considered eligible if they met DSM-IV criteria for AN (excluding the requirement of amenorrhea). Participants were categorized as underweight if they demonstrated a BMI < 18.5 during measurement on the day of the study, which is consistent with the minimum BMI threshold for mild AN under DSM-5. Participants were categorized as weight-restored if they demonstrated a BMI ≥ 18.5 on the day of the study. AN participants were recruited from the UCLA inpatient Eating Disorders Program, UCLA partial hospitalization program, community outpatient eating disorders centers, and via advertisements. All participants were screened for current major psychiatric illnesses including AN using the Mini International Neuropsychiatric Interview (36), and were specifically excluded if they endorsed any substance dependence (including alcohol dependence). To obtain a sample representative of the clinical population, AN participants were not excluded if they met criteria for comorbid dysthymia, major depressive disorder, panic disorder, generalized anxiety disorder, or social anxiety disorder. However, patients

were excluded if they met criteria for a psychotic disorder, bipolar disorder or body dysmorphic disorder. To maximize sample size, Selective Serotonin Reuptake Inhibitor (SSRI) monotherapy was allowed provided the dose was stable for >4 weeks. No other medications were allowed. Exclusion criteria for all participants included suicidal ideation, pregnancy, claustrophobia, or presence of a metal (e.g. braces, permanent retainer) or implanted devices that may induce MRI artifacts precluding visualization of the mammillary bodies.

The protocol was approved by the Institutional Review Board at UCLA. All participants gave written and informed consent before the study.

Magnetic resonance imaging

Whole-brain images were acquired from all participants using a 3 Tesla MRI scanner (Siemens Magnetom Allegra-Trio, Erlangen, Germany), while participants lay supine. Head movement was minimized with foam padding on both sides of the head and by securing tape to the forehead. One T1-weighted magnetization prepared rapid gradient-echo (MP-RAGE) sequence was collected to provide a standard-resolution structural anatomical image of the entire brain (field of view= 250×250 mm, flip angle= 9° , slice thickness= 1 mm, TR= 1900 ms, TE= 2.26, voxel size= 1 mm^3).

Data analysis

All standard-resolution T1-weighted brain images of AN and comparison participants were visually assessed for any major brain pathology such as cystic lesions, infarcts or other lesions, and for the presence of any mass lesion near the hypothalamus that might alter mammillary body or fornix morphology. These images were also visually examined to ensure the absence of motion artifacts or ghosting due to retainers or dental braces. One healthy control brain was excluded due to significant ghosting artifact, leaving 30 complete healthy comparison brain volumes. Image processing was conducted using the statistical parametric mapping package SPM5 (Wellcome Department of Cognitive Neurology, UK; <http://www.fil.ion.ucl.ac.uk/spm/>), and Matlab-based (The MathWorks Inc, Natick, MA) custom software.

Total intracranial volume calculation

Standard-resolution T1-weighted images were reoriented (without warping) into a common space. These images were segmented into gray matter, white matter, and cerebrospinal fluid (CSF), resulting in different tissue probability maps using the unified segmentation approach (37) implemented in SPM 5 [Gaussian per class = (3, 2, 2, 5); bias full-width-at-half-maximum = 70 mm cutoff; sampling distance = 2]. Voxels with a probability value ≥ 0.5 in gray, white, and CSF probability maps were counted, and whole brain gray matter, white matter, and CSF volumes were calculated by multiplying the number of voxels by the volume of each voxel. Total intracranial volume (TIV) was calculated as the sum of gray, white, and CSF volumes.

Mammillary body volume quantification

Using the reoriented T1-weighted image volumes, brain sections containing both mammillary bodies were oversampled to a resolution of $0.2 \times 0.2 \times 0.2 \text{ mm}^3$. A single investigator, blinded to subject group assignment, performed manual tracing using MRICron (<http://www.sph.sc.edu/comd/rorden/mricron/install.html>). Mammillary bodies were distinguished from surrounding tissue by its lighter hue, and traced after determining the brain midline from coronal and axial views (figure 1). Medial borders were defined by tracing tissue in the sagittal plane to the left or right of the midline. Using a central slice in the coronal plane, we determined and connected the superior and inferior mammillary notches that defined superior and lateral boundaries of the structure, respectively. Body outlines were traced in the sagittal plane using the central coronal slice as a guideline, and continued through successive sagittal slices laterally from the medial slice. Lateral boundaries were defined by disappearance of the central coronal slice. Trace edges were smoothed, and voxels filled where appropriate while moving from anterior to posterior limits in the coronal plane; actions were repeated for the axial view, if necessary. We repeated the procedure for the other mammillary body. Traced voxels in the left and right mammillary bodies were counted, and volumes calculated by multiplying the number of traced voxels by the volume of each voxel. Bilateral mammillary body volumes were determined by summing left and right mammillary body volumes (see(38, 39) for previous demonstrations of this method in other clinical samples).

Fornix area quantification

The fornix is a small fiber bundle with a complex three-dimensional course. We chose to measure the cross-sectional area of this structure at a point where it could be most confidently visualized and measured: namely, at the point before it enters the septum parallel to the anterior commissure. Reoriented T1-weighted images were oversampled ($0.2 \times 0.2 \times 0.2 \text{ mm}$) in brain areas immediately dorsal to the septum containing the columns of the fornix. Left and right fornix fibers were visualized using a coronal view and marked before entering the septum parallel to the anterior commissure. These tags provided boundaries for the fibers in the medial-lateral direction. Caudal-rostral borders were visualized using a sagittal view and marked for both fornix columns. These tags provided boundaries for the fibers in the anterior-posterior direction. Both tags were used to define the medial-lateral and anterior-posterior extent of the left and right fornix, separately. Using an axial view, fornix cross-sectional areas were outlined and calculated by multiplying the voxel count by the voxel area, for both the left and right fornix separately (figure 1). Total fornix cross-sectional area was determined by summing left and right fornix areas.

Intra-tracer reliabilities

To assess the reliability of the manual tracing measurements of the mammillary bodies, a subset of the original sample (24%) was retraced and intra-tracer reliabilities were calculated using intraclass correlation coefficients (two way, mixed model, absolute agreement).

Statistical analysis

Statistical analysis of demographic data and mammillary body volumes and fornix cross-sectional areas were performed using SPSS v. 22.0 (IBM Corp, Armonk, NY). Demographic data and clinical characteristics were compared using independent samples t-tests separately for the 1) underweight AN versus comparisons and 2) weight restored AN versus comparisons. Mammillary body volume and fornix cross-sectional area differences were evaluated with independent samples t-tests separately for the 1) underweight AN versus comparisons and 2) weight restored AN versus comparisons. Analysis of covariance (ANCOVA), controlling for head size (covariate; TIV) was conducted separately for the 1) underweight AN versus comparisons and 2) weight restored AN versus comparisons. We purposefully did not enter all of the groups into single ANCOVA, as such a test would be inappropriate for testing our second hypothesis that the weight restored group would show normal MB volumes. Since the samples did not differ significantly in mean age or sex proportion, these variables were not included. Body mass index was also not included as a covariate, since this unit is a fundamental biomarker of AN and could therefore partial out effects of interest related to the illness.

RESULTS

Demographics and clinical variables

The groups did not differ with respect to age or sex (table 1). Both groups exhibited significantly lower BMI than the healthy comparisons ($p < 0.01$), with the underweight AN group showing BMIs in the abnormally low range, and the weight restored group showing BMIs in the normal range. Both groups exhibited significantly higher levels of depression and anxiety with respect to healthy comparisons ($p < 0.01$). Eating disorder severity was in the moderate range for both groups, as evidenced by Eating Disorder Examination global score.

Mammillary body volume

Total MB volumes were lower in the underweight AN group compared with healthy comparisons ($p = 0.017$; table 1, figure 1). Left and right MB volumes were also significantly smaller in the underweight AN group (left: $p = .036$, right: $p = 0.044$). Significant disparities in total MB volume remained between groups when evaluated with ANCOVA using total intracranial volume as a covariate ($p < 0.048$), but not when assessed individually (left: $p = 0.08$, right: $p = 0.10$).

In contrast, we observed increased total MB volumes in the weight-restored AN group ($p = .002$; table 1, figure 1). Left and right MB volumes were also significantly larger in the weight-restored AN group (left: $p = 0.005$, right: $p = 0.009$). Disparities in total MB volume remained when evaluated with ANCOVA using total intracranial volume as a covariate ($p < 0.001$), and also when MB volumes were assessed individually (left: $p = 0.002$, right: 0.003). Intra-tracer reliabilities were excellent for the total ($r = .97$, $p < .001$), left ($r = .92$, $p < .001$) and right ($r = .98$, $p < .001$) volumes.

Fornix area

The underweight AN group did not show significant between group differences in total fornix area ($p = 0.68$), or for the left ($p = 0.92$) and the right ($p = 0.28$) fornix. The weight-restored AN group showed a significant difference in the right fornix ($p = 0.044$), indicating a smaller right fornix area. There was no significant difference in the left fornix ($p = 0.42$) or total fornix area ($p = 0.26$).

DISCUSSION

This study represents the first systematic group-level demonstration of abnormal mammillary body volume and fornix cross-sectional area in AN. Consistent with extant case reports, we observed reduced total mammillary body volume in the underweight AN group. Surprisingly, we observed increased total mammillary body volume in the weight-restored AN group. We did not find any differences in fornix area in the underweight AN group, but observed decreased right fornix area in the weight-restored group.

Reduced mammillary body volume in underweight AN is consistent with previous reports of generalized cerebral atrophy in AN(1–4), and with prior case reports of acute WE in AN (31–33, 35). It seems possible that decrements in mammillary body volume in underweight AN could reflect generalized atrophy due to overall reductions in energy availability, might reflect a specific sensitivity to thiamine deficiency, or alternatively, pathology due to overlapping nutritional deficiencies. We cannot substantiate or differentiate these possibilities from the current study, as this was a retrospectively evaluated data set, and we did not measure serum thiamine or other vitamin levels. Therefore, while we interpret the current results as preliminary evidence of a potentially interesting biomarker effect, verification through further testing will be required to confirm the finding.

The increased mammillary body volume in weight-restored AN was unanticipated, but is not unprecedented. Increased gray matter volumes have been previously observed in weight-restored AN in cortical regions such as the insula and orbitofrontal cortex (12), using manual tracing methods similar to those employed in the current study, as well as using automated methods. Furthermore, some global volumetric changes also appear to be reversible (4, 40–42), indicating that the brain responds dynamically to changes in caloric and nutrient availability. For example, recent studies have shown that cortical thickness can rapidly improve during weight restoration in AN independently of illness duration, hydration status, or symptom improvement (43) (for a review, see (44)). Despite such malleability, the presence of residual cognitive deficits (45), and other persisting volumetric reductions following treatment and weight restoration (12, 46) indicates that some forms neuroanatomical pathology can linger. The significance of enlarged MB volumes in weight-restored states is unclear. While we might speculate that this could reflect morphological reorganization following injury to this neural structure, we are unable to determine this possibility from the current study, as we did not employ a longitudinal design. We also did not utilize other measures of cerebral pathology, such as fractional anisotropy and diffusivity. Replication of the current findings in larger samples, in studies utilizing a longitudinal pre- and post-treatment design, and incorporating detailed assessments of clinical characteristics and cognitive functions related to MB (e.g. measures of memory)

would provide key clarification of these possibilities. Controlling for potential acute influences on brain volume, such as dehydration(47) or excessive exercise(48) will also be important(49). The current study did not assess these factors or other acute indices of dehydration (e.g. urine specific gravity, as in(42)).

Since the current study was cross-sectional, we also cannot discount an alternate interpretation: that individuals born with larger MB may be better predisposed for weight restoration recovery from AN, or perhaps buffered somehow from the effects of acute illness. The MB constitutes an integral component of the Papez circuit (50). Accordingly, it has been implicated in homeostatic regulation of hypothalamic and hippocampal processing, and associated emotional experiences, learning, and memory. While speculative, it may be possible that individuals with larger MB might be more capable of integrating emotional experience with learning and memory, which could possibly enable them to respond more adaptively to psychotherapeutic processes (and hence weight restoration). Or at least, to have the cognitive and emotional resources to do so on their own.

Despite the cross-sectional comparison of these data sets, we feel it is important to emphasize that the observed MB volume differences do not appear to be related to global atrophy (in the case of underweight AN) or global cerebral enlargement (in the case of weight restored AN). This is because there were no volume differences in GM, WM, or CSF between either AN group and the healthy comparisons. Also, the fornix was not smaller in the underweight sample while the MBs were smaller, providing further evidence suggesting that the underweight state does not necessarily induce global atrophy. However, both AN groups had volumes that in some cases bordered on significant (WM for underweight, TIV for weight restored; Table 1). At this point it is also important to mention that most findings on WM volumes in AN come from studies with small sample sizes, which suggest mixed findings, and limited evidence for regional differences. Castro-Fornieles et al (4) did not see any white matter volume differences at acute inpatient admission (12 AN vs. 9 HC) or at 7 month follow up. Katzman et al (5) observed WM volume increases in 6 weight restored AN patients. Swayze et al (6) found lower WM volume during inpatient admission vs. increased WM volume after weight normalization (13 patients). Meta-analyses of studies using pooled data have been conducted in the past several years. These suggested that there are global reductions in GM and WM, and increased CSF (Titova et al (1), n=228 patients; Van den Eynde et al, (2), n=236 patients). Despite a substantial overlap of the studies that were reviewed, each meta-analysis reached somewhat different conclusions about WM differences: global reductions in the former, and inconclusive evidence in the latter. Larger prospective studies are desperately needed in this area, and would provide some much needed progress in understanding the underlying cerebral pathology of AN.

Another point worth discussing is the potential difference in lowest BMI reported between the underweight and weight-restored AN groups (14.2 ± 2.0 vs. 15.8 ± 1.7 , respectively). This might raise the possibility that there were less severely affected patients in the restored group, and that a milder illness phenotype would explain the larger MB volumes in this group. However, this logic would seem to be contradicted by an opposing qualitative observation that the weight-restored group had longer illness durations. Furthermore, we did not perform any comparisons of brain volume or clinical demographics between the

underweight and weight restored as the reference of interest in this cross-sectional study was healthy comparisons. Longitudinal comparisons would help to address this possibility.

Although there is some convergence in the DTI literature suggestive of fornix pathology in underweight AN (51), this region is particularly prone to artifacts (52) which provided some justification for the targeted approach to manually evaluating fornix area. Our observation of reduced right fornix area in weight-restored AN provides some suggestive macroscopic evidence of the relevance of this region that could potentially corroborate with the microstructural differences observed in diffusion tensor imaging studies (17, 18, 20). However, the unilateral finding and absence of corroboration with clinical symptoms in this study limit further speculation as to the clinical relevance of this finding. It is also possible that the lack of differences in fornix area in the underweight AN group, while unpredicted, could be explained by a small sample size. Or, alternatively, it could be due to the incomplete measurement of this structure in the current study. A high-resolution volumetric study, or a study employing multiple morphological measurements of the fornix (e.g., multiple cross sections) would help to address this limitation.

One noteworthy aspect of the current study relates to the fact that our MRI measurements of MB volumes for the healthy participants are in good agreement with large studies using MRI measurement as well as the gold standard method of post mortem evaluation. For example, in a normative study directly evaluating MB volumes, Sheedy et al (53) reported unilateral MB volumes of $63.4 \pm 19.8 \text{ mm}^3$ in a postmortem sample of 2048 neurologically healthy individuals.¹ To compare the volume differences between these two methods, we calculated error scores for the healthy samples using the formula: (normative study mean – current study mean)/normative study mean $\times 100\%$. This yielded a combined measurement error of less than 5.5% for the healthy total MB (3.3% for the left MB and 7.7% for the right MB). Given the small size of this structure and difficulty tracing it using automated methods, we interpret this as evidence positively supporting the validity of the manual tracing approach.

Limitations and future directions

1) The sample sizes of the AN subgroups were not large by current normative standards. This is a general limitation common to many AN studies, and may be in part influenced by a low prevalence rate in the population. Thus while our sample sizes are equivalent to, and in several cases larger than many of the prior studies cited here, replication is warranted to confirm the finding. 2) We did not ascertain cellular estimates of neuropathology in the current study. Such measures could be obtained via diffusion tensor imaging (e.g., fractional anisotropy, diffusivity). Other measures, such as positron emission tomography or magnetic resonance spectroscopy would allow assessments of receptor density and glucose metabolism and metabolite levels. Unfortunately such approaches are currently unfeasible due to the poorer spatial resolution of these methods (compared with MRI), and the small size of the MB and fornix. 3) We did not assess or control for several acute factors that could potentially affect cerebral volume, such as the duration of the underweight or weight

¹In this normative study only unilateral MB volumes, left or right, were selected for measurement based on perceived ease of assessment for each case by the experimenter. This is the typical approach employed by most investigators using postmortem methodology (C. Harper, personal communication).

restored state, hydration status, or the refractory period associated with acute exercise. We recommend that future studies incorporate such measures prospectively. 4) We were unable to characterize nutritional intake patterns, hydration status, or serum vitamin levels (e.g., thiamine), as this study was conducted retrospectively on a pre-existing neuroimaging data set. Such measurements at the time of scanning would have been helpful in discerning whether the observed differences relate to a thiamine or other vitamin deficiency, and we recommend that future studies incorporate such measures prospectively. 5) We did not obtain intratracer reliability estimates for the fornix measurements. 6) Three of the underweight patients were taking serotonergic medications. This could have affected the observed group results. 7) Participant recruitment for the current study was heterogeneous, including individuals from different treatment centers, acuity levels, and stages of recovery. This clinical variability might have increased the variability of biomarkers measured in this study. Replicating this finding in a more homogeneous sample would provide evidence against this notion. 8) We did not utilize a longitudinal design. This would substantially improve the ability to make causal inferences about the relationships between changes in body weight and changes in mammillary body size. It would especially allow for predictive analyses of the relationship between changes in volume and illness trajectory. We highly recommended this approach for future studies in this area.

Conclusions

In summary, this is the first study to systematically evaluate mammillary body morphology in AN. Our results suggest that in AN the underweight state may be associated with reductions in mammillary body volume, and that weight restoration may be conversely associated with increases in mammillary body volume and a reduced fornix area. Further study appears warranted to determine whether these limbic regions respond dynamically to changes in weight and illness state in AN, and to identify whether such changes are related to nutrient intake, clinical characteristics, or therapeutic outcomes.

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Figure 1.

Figure 2.

Table 1

Demographic and clinical characteristics, mammillary body volumes, fornix area and intracranial volumes for the underweight AN, weight-restored AN and healthy comparison groups

	Underweight AN (UW_AN)		Healthy comparison (HC)		Weight-restored AN (WR_AN)		UW_AN vs. HC		WR_AN vs. HC	
							t_{40}/χ^2	P	t_{48}/χ^2	P
Age (years)	19.4 +/- 7.3		22.5 +/- 4.8		22.4 +/- 3.9		-1.61	.12	-0.08	.94
Sex	12 F		24 F, 6 M		18 F, 2 M		2.8	.10	0.89	.89
Body Mass Index (BMI)	16.2 +/- 2.0**		22.6 +/- 3.4		20.2 +/- 1.6**		-6.44	<.001	-3.34	.002
Education (years)	11.3 +/- 3.3**		14.3 +/- 2.9		14.5 +/- 2.9		-2.95	.007	0.22	.82
HAM-A	7.2 +/- 5.6**		2.0 +/- 1.9		8.6 +/- 7.2**		4.50	<.001	4.00	<.001
MADRS	12.3 +/- 9.5**		0.87 +/- 1.3		12.7 +/- 9.8**		4.13	.002	5.33	<.001
EDE – global score	3.1 +/- 1.4		-		3.2 +/- 1.3					
AN restricting subtype	100%		-		95%					
Illness duration (years)	5.4 +/- 6.6		-		6.6 +/- 5.0					
Lowest BMI	14.2 +/- 2.0		-		15.8 +/- 1.7					
% medicated	25 (n=3)		-		0					
Total MB volume (mm ³)	115.6 +/- 21.4*		133.8 +/- 21.4		154.8 +/- 23.5**		-2.49	.017	3.27	.002
Left MB	57.0 +/- 11.4*		65.5 +/- 11.6		75.1 +/- 10.7**		-2.16	.036	2.97	.005
Right MB	58.6 +/- 14.7*		68.3 +/- 13.1		79.7 +/- 16.3**		-2.08	.044	2.73	.009
Total fornix area (mm ²)	12.5 +/- 3.3		12.9 +/- 3.1		12.0 +/- 2.4		-.41	.68	-1.15	.26
Left fornix	6.5 +/- 1.1		6.0 +/- 2.5		6.2 +/- 1.5		-.10	.92	-.81	.42
Right fornix	6.6 +/- 1.7		6.8 +/- 1.8		5.8 +/- 1.1*		-1.10	.28	-2.1	.044
Total intracranial volume (L)	1.591 +/- 0.128		1.730 +/- .315		1.593 +/- 0.153		-1.48	.15	-1.80	.08
Gray matter	0.717 +/- 0.091		0.779 +/- 0.127		0.727 +/- 0.063		-1.54	.13	-1.70	.10
White matter	0.434 +/- .047		0.490 +/- 0.100		0.451 +/- .039		-1.85	.07	-1.66	.10
CSF	0.443 +/- 0.060		0.458 +/- 0.129		0.415 +/- 0.091		-.39	.70	-1.29	.20

Means +/- standard deviation. MADRS: Montgomery-Asberg Depression Rating Scale. HAM-A: Hamilton Anxiety Rating Scale. EDE: Eating Disorder Examination Scale. MB: mammillary body.

* p < .05,

** p < .001.