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Pediatrics 2008;122;e426-e437
DOI: 10.1542/peds.2008-0170

ARTICLE

Cognitive Function and Brain Structure in Females With a History of Adolescent-Onset Anorexia Nervosa

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The authors have indicated they have no financial relationships relevant to this article to disclose.

What's Known on This Subject

Abnormal brain structure and cognitive impairments have been found in acutely ill females with adolescent-onset AN, but the reversibility of these abnormalities is unclear. The degree of weight restoration remains a controversial predictor of brain structure and cognitive function.

What This Study Adds

Female subjects with adolescent-onset AN showed cognitive impairments and larger ventricles compared with control subjects. We report a relationship between menstrual function and cognitive function in clinical subjects, necessitating additional examination of sex hormones and cognitive functioning.

ABSTRACT

OBJECTIVE. Abnormalities in cognitive function and brain structure have been reported in acutely ill adolescents with anorexia nervosa, but whether these abnormalities persist or are reversible in the context of weight restoration remains unclear. Brain structure and cognitive function in female subjects with adolescent-onset anorexia nervosa assessed at long-term follow-up were studied in comparison with healthy female subjects, and associations with clinical outcome were investigated.

PATIENTS AND METHODS. Sixty-six female subjects (aged 21.3 ± 2.3 years) who had a diagnosis of adolescent-onset anorexia nervosa and treated 6.5 ± 1.7 years earlier in a tertiary care hospital and 42 healthy female control subjects (aged 20.7 ± 2.5 years) were assessed. All participants underwent a clinical examination, magnetic resonance brain scan, and cognitive evaluation. Clinical data were analyzed first as a function of weight recovery (n = 14, <85% ideal body weight; n = 52, ≥85% ideal body weight) and as a function of menstrual status (n = 18, absent/irregular menses; n = 29, oral contraceptive pill; n = 19, regular menses). Group comparisons were made across structural brain volumes and cognitive scores.

RESULTS. Compared with control subjects, participants with anorexia nervosa who remained at low weight had larger lateral ventricles. Twenty-four–hour urinary free-cortisol levels were positively correlated with volumes of the temporal horns of the lateral ventricles and negatively correlated with volumes of the hippocampi in clinical participants. Participants who were amenorrheic or had irregular menses showed significant cognitive deficits across a broad range of many domains.

CONCLUSIONS. Female subjects with adolescent-onset anorexia nervosa showed abnormal cognitive function and brain structure compared with healthy individuals despite an extended period since diagnosis. To our knowledge, this is the first study to report a specific relationship between menstrual function and cognitive function in this patient population. Possible mechanisms underlying neural and cognitive deficits with anorexia nervosa are discussed. Additional examination of the effects of estrogen on cognitive function in female subjects with anorexia nervosa is necessary. *Pediatrics* 2008;122:e426–e437

ANOREXIA NERVOSA (AN) is a severe eating disorder that typically begins during adolescence. It is the third most common chronic illness affecting adolescent females[1] and has the highest mortality rate among all psychiatric disorders.[2] Chronic starvation associated with AN results in medical complications that affect every organ

www.pediatrics.org/cgi/doi/10.1542/peds.2008-0170

doi:10.1542/peds.2008-0170

This work was presented in part at the annual meeting of the Society for Adolescent Medicine; March 27–31, 2007; Denver, CO.
This article is based on Mr Chui's thesis submitted to fulfill, in part, requirements for a University of Toronto Master of Science degree.
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Key Words
adolescent-onset anorexia nervosa, cognitive function, brain structure, menstrual function

Abbreviations
AN—anorexia nervosa
MR—magnetic resonance
UFC—urinary free cortisol
Ccr—creatinine clearance
IBW—ideal body weight
OCD—obsessive-compulsive disorder
OCP—oral contraceptive pill
HSD—honestly significant difference

Accepted for publication Mar 27, 2008

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PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

system in the body.[3] In adolescent-onset AN, a particularly concerning complication involves abnormalities in brain structure and cognitive function. Dynamic structural brain changes[4] and cognitive maturation[5] that occur during adolescence may be compromised by AN with long-term consequences. Cognitive deficits may also hinder treatment efforts and contribute to illness chronicity.[6,7]

Although acutely ill patients with AN show smaller brain tissue volumes[8–17] and deficits in broad neuropsychological functioning,[16–26] the current literature is inconclusive about the temporal course, mechanisms, and reversibility of these abnormalities.[27] Inconsistent findings may be attributed to study design limitations, such as small sample size, short follow-up periods, and the lack of appropriate control groups. The lack of a consistent definition of recovery also makes cross-study comparisons difficult.

Our previous magnetic resonance (MR) studies[11,12] demonstrated the persistence of gray-matter deficits and cerebrospinal fluid (CSF) elevations but normal white-matter volumes among weight-restored participants with adolescent-onset AN. These findings suggest incomplete reversibility of structural brain abnormalities in this patient group. Moreover, gray-matter volumes were positively associated with BMI and negatively associated with 24-hour urinary free cortisol (UFC) when patients were acutely ill with AN.[8] Examination of these clinical variables at follow-up could help clarify their roles in mediating neural abnormalities in adolescent-onset AN.

Although weight restoration is an important goal of treatment and 1 indicator of recovery from AN,[28] menstrual function may remain abnormal in some weight-recovered patients and normal in some low-weight patients.[29] These observations highlight the possibility that weight and menstrual function may have independent effects on brain structure and cognitive function in AN. Research has shown that low circulating estrogen levels, coupled with amenorrhea, have been associated with cognitive impairment in animals and humans.[30] To date, no studies have examined the specific relationship between menstrual function and brain structure or cognitive function in female subjects with adolescent-onset AN.

The purpose of this study was to compare brain structure and cognitive function between female subjects with a history of adolescent-onset AN and healthy female control subjects. We also examined specific clinical indices, such as weight, menstrual function, and cortisol levels, and their relationships with brain structure and cognitive function to identify mechanisms that may underlie the structural brain and cognitive findings in adolescent-onset AN.

METHODS

Participants

Participants With AN

Female subjects who were registered in the Eating Disorders Program at the Hospital for Sick Children between 1993 and 1998 and met the *Diagnostic and Statistical Manual for Mental Disorders, Fourth Edition*[31] (DSM-IV) criteria for AN according to the *Diagnostic Interview for Children and Adolescents-Revised*[32], a structured interview that identifies diagnosis on the basis of DSM-IV criteria, were located using addresses and telephone numbers in hospital files. Potential participants were contacted by letter or telephone and invited to participate in this follow-up study between 2001 and 2003. Exclusion criteria included self-reported diagnosis of past or current learning disability, psychotic disorder, substance abuse or substance dependence disorder, head trauma with loss of consciousness for >30 minutes or significant neurologic sequelae, glucocorticoid therapy, chronic medical illness unrelated to AN, pregnancy, and metallic implants or injuries that precluded MR scanning. The psychiatric diagnosis at the time of study was established for each participant on the basis of interviews by a physician and a trained research assistant using the *Structured Clinical Interview for the DSM-IV Axis I Disorders-Patient Edition* (SCID-I/P).[33]

Control Participants

Healthy female subjects were recruited through posters and newspaper advertisements from the community. The advertisement called for healthy young women to participate in a study on brain structure and cognitive function. There was no mention of weight or eating concerns to preclude participation from individuals with biased interests in eating behaviors and/or body shape and size. Where possible, control subjects were matched with AN participants for age (±6 months), socioeconomic status (SES) (A. B. Hollingshead, PhD, *Four-Factor Index of Social Status*, unpublished manual, 1975), and education level. In addition to the exclusion criteria listed for clinical participants, control subjects also could not have a past or current eating disorder, as determined by the SCID-I/P.[33]

This study was approved by the research ethics board at the Hospital for Sick Children. Informed written consent was obtained from participants ≥16 years of age. Participants <16 years old gave verbal assent, and a parent or caregiver provided informed written consent.

Measures and Procedures

Clinical Assessment

Sociodemographic Status

Participants' age and the number of years of education received were recorded at the time of study. The education level and occupation of the parents or caregivers were also recorded to determine participants' SES according to the Hollingshead *Four-Factor Index of Social Status*.

Physical Variables

Weight and height were measured in all of the participants at the time of study, and their BMI was calculated (weight [kilograms]/height [meters squared]). Systolic and diastolic blood pressures and sexual maturity ratings[34] for breasts and pubic hair were also recorded.

Laboratory Indices

Blood samples were collected and analyzed for levels of serum urea nitrogen, creatinine, total protein, and albumin. Twenty-four–hour urine samples were also collected where possible to determine 24-hour UFC, creatinine, and creatinine clearance (Ccr). Cortisol level was expressed as the UFC/Ccr ratio to correct for decreased Ccr and filtered cortisol in patients with AN.[8,35]

Symptom Measures for Depression and Obsessive-Compulsive Disorder

The Beck Depression Inventory-II[36] (BDI-II) and the Florida Yale-Brown Obsessive-Compulsive Scale[37,38] (FLY-BOCS) were administered. These instruments were chosen because of the high prevalence of comorbid depression and obsessive-compulsive disorder (OCD) in patients with AN.[39]

Illness Characteristics

Data on the history of AN were collected from patient charts for all of the clinical participants. These included the AN subtype, lowest recorded BMI, number of hospitalizations, and age of onset. Age of onset of AN was defined as the age reported at the time of treatment when weight concerns and dietary restrictions began. An objective estimate of illness duration, the length of follow-up, was also calculated. This is defined as the time interval between the present study and the initial diagnosis of AN.

Determination of Weight Recovery

The ideal body weight (IBW) for each clinical participant was calculated as the weight corresponding with the median BMI for age according to the Centers for Disease Control and Prevention growth charts.[40] These growth charts provide information for individuals up to the age of 20 years. For participants whose age exceeded this limit at the time of study, their IBWs were estimated from the median BMI for the age of 20 years. In addition, where premorbid weights and heights were available, consideration was given to an individual's linear growth trajectory according to the Centers for Disease Control and Prevention growth charts when determining the IBW.

The percentage IBW was then calculated (%IBW = weight at study/IBW × 100).[41]Participants were considered weight recovered if they were >85% of their IBW, whereas those who were <85% of their IBW were considered low weight.[31] There is currently no standard or consensus for determining IBW in individuals with adolescent-onset AN among experts. The approach here is believed to be a reasonable approximation.

Menstrual Function

As part of the evaluation, all of the participants completed the Morgan-Russell outcome criteria[42,43] with a trained interviewer. Menstrual function was determined by using scores on the menstrual pattern subscale of the Morgan-Russell outcome criteria (0, 4, 8, and 12, ranging from "no menstrual loss at any time" to "regular and cyclical throughout"), in conjunction with the reported use of the oral contraceptive pill (OCP) over the past 6 months. Participants who received a score of 0, 4, or 8 were considered to have absent or irregular menses. Those who received a score of 12 and did not report OCP use were considered to have regular menses. Those who reported OCP use were categorized as such. The reasons for OCP use were not elucidated in this study.

Volumetric Measures of Brain Structure

MRI Acquisition

T1-weighted neuroimages were acquired with a 1.5-T MRI system (General Electric, Milwaukee, WI) at the Toronto General Hospital. A three-dimensional, inversion recovery prepared, fast spoiled-gradient echo sequence was applied (time to inversion = 300 milliseconds; repetition time = 12 milliseconds; echo time = 5 milliseconds; flip angle = 20°; field of view = 20 cm; matrix = 256 × 256 pixels), yielding 124 contiguous 1.5-mm-thick coronal sections.

Image Processing

Images were first corrected for intensity nonuniformity.[44] To correct for head size and orientation differences, images were normalized to the Montreal Neurologic Institute standard space and resampled to 1.0-mm^3 isotropic voxels.[45] Each voxel was then classified as a gray-matter, white-matter, or CSF voxel using Intensity Normalized Stereotaxic Environment for the Classification of Tissue.[46] Nonbrain structures, such as the skull and scalp, were removed using an iterative extraction algorithm.[47] All of the images were assessed visually; any scalp and skull that remained were manually masked before the determination of regional brain volumes.

Classified images were subdivided into 39 distinct regions using Automated, Nonlinear, Image Matching and Anatomical Labeling.[48] The reference brain volume used for automated labeling was manually segmented by a neuroanatomist (Dr Kabani). The hippocampus and the inferior horn of the lateral ventricle (ie, temporal horn) were also manually delineated by 2 experienced neuroimage analysts as regions of interest for volumetric examination. The hippocampus was delineated according to Pruessner et al,[49] and the temporal horn was demarcated as the region of the lateral ventricle that lies below the parietoccipital/calcarine border and in front of the corpus callosum border.

The analysts trained on a different data set where the hippocampus and temporal horn had been labeled by an expert neuroanatomist. Each analyst rated brain structures in 5 participants at 5 different time points, with a 2-week interval between ratings. On the basis of the calculated intraclass correlations,[50] intrarater reliabilities were, on average, .90 for the hippocampus and .90 for the temporal horn. Interrater reliabilities (with respect to the expert) were, on average, .90 for the hippocampus and .90 for the temporal horn. The volume of each brain region was defined as the number of 1.0-mm^3 voxels present in that segmented region, expressed in cubic centimeters (1 mL = 1000 mm^3).

Each processed image was examined, and suboptimal images were excluded from statistical analyses. Reasons for elimination included irregularities in tissue classification

and the presence of substantial amounts of nonbrain tissue (eg, dura mater) after masking. In total, scans from 6 clinical participants and 5 control subjects were removed from volumetric analyses. They were all right-handed. Additional examination using *t* tests revealed no difference between excluded and included participants in current weight and height. Excluded participants were younger than included participants (excluded: 19.6 ± 2.5 years; included: 21.2 ± 2.3 years; 2-tailed $P = .03$).

Measures of Cognitive Functioning

Participants completed a comprehensive neuropsychological evaluation using the Standard Battery of the Woodcock-Johnson III (WJ-III),[51] the Hopkins Verbal Learning Test-Revised (HVLT-R),[52] and the visual reproduction subscale of the Wechsler Memory Scale-Revised.[53] These tests were selected for their excellent psychometric properties and the availability of age-normed scores for analyses. Furthermore, cluster scores computed from subtest scores on the WJ-III[54] were used for analyses to minimize α inflation secondary to multiple family wise comparisons. Cluster scores also have higher reliabilities than individual subtest scores and were previously validated using factor analytic methods to reduce overlap between neuropsychological domains.[55]

Six participants did not complete all of the subtests needed to calculate cluster scores on the WJ-III. Their missing scores were predicted from regression equations computed for participants (clinical participants and control subjects) with complete data by using the missing subtest as a dependent variable and other subtests forming the same cluster as independent variables. One control subject did not complete the HVLT-R and was excluded from the verbal memory analyses.

Data Analysis

Random checks were first performed to ensure that data from patient charts and neuropsychological test reports were correctly entered into the password-protected database. Approximately 10% of data were checked. All of the statistical procedures were performed by using SPSS 14.0 for Windows (SPSS Inc, Chicago, IL).

Preliminary Analyses

Data from clinical and control groups were first screened separately for outliers ($z > |3.29|$),[56] which were excluded from the relevant analyses. Outliers included 1 control for serum albumin, 1 control for UFC, 2 control subjects, 1 clinical participant for UFC/Ccr, 1 clinical participant for frontal and parietal white-matter volumes, 1 clinical participant for lateral ventricle volumes, and 1 control and 1 clinical participant for temporal horn volumes. Temporal horn outliers were also removed in hippocampal analyses because of anatomic proximity. High psychiatric symptom scores were not excluded, because they are clinically relevant.

Deviation from normality (2-tailed $P < .001$ in tests of skewness/kurtosis)[56] was then checked and detected in current BMI, urine creatinine, UFC, and UFC/Ccr, as well as scores on the BDI-II and FLY-BOCS. Nonnormal variables were square-root transformed for parametric tests. The Mann-Whitney *U* test was used to analyze scores on the FLY-BOCS, because transformation did not normalize the distribution. Subsequent parametric *t* tests applied assumed equal variance, unless significant inequality of variances were found (2-tailed $P < .05$ in Levene's test).[57]

Clinical Participants Versus Control Subjects

Student's *t* test was used to compare total and regional brain volumes and cognitive test scores between groups. As a preliminary analysis, significance level was set at $P < .05$ (2-tailed). Effect sizes (Cohen's *d*) were also calculated.

Comparisons According to Weight-Recovery Status

One-way analysis of variance (ANOVA) was used to compare brain volumes and cognitive scores among low-weight participants, weight-recovered participants, and healthy control subjects. To reduce multiple comparisons, only brain regions that differed significantly between clinical participants and control subjects were analyzed, and the bilateral volumes (ie, left plus right) were used. The hippocampus was also included as a region of interest.[58,59] Tukey honestly significant difference (HSD) was applied on significant ANOVA findings ($P < .05$) to determine where omnibus differences lie.

Clinical Correlates of Brain Structure and Cognitive Function

Pearson product-moment correlations were computed between brain volumes/cognitive scores and current BMI, cortisol levels (UFC/Ccr), and psychiatric symptom scores (BDI-II and FLY-BOCS) in clinical participants.

Comparisons by Menstrual Function

Many participants with a history of AN continue to experience amenorrhea and/or menstrual irregularity, including a number who were considered to be weight recovered. One-way ANOVA was used to compare brain volumes and cognitive scores among participants who had amenorrhea or irregular menses, participants who had regular menses or OCP, and healthy control subjects. Equivalent brain volumes and cognitive scores found between clinical participants who were on OCP and those who had regular menses during initial analyses justified that they may be collapsed and compared against participants with absent or irregular menses as a way to examine the association between abnormal menstrual function and brain structure or cognitive function in AN.

RESULTS

Participant Characteristics

There were 252 female subjects diagnosed with adolescent-onset AN who were registered in the Eating Disorder Program at the Hospital for Sick Children between 1993 and 1998. Eighty-nine (35%) of these patients could not be reached at the time of recruitment. Of the remaining 163 potential participants, 66 (41%) agreed to participate and met inclusion criteria for this study.

TABLE 1 **Participant Characteristics**

Characteristics	Adolescent AN ($N = 66$)	Control ($N = 42$)
Age at study, mean ± SD (range), y	21.3 ± 2.3 (15–26)	20.7 ± 2.5 (15–26)
Education, mean ± SD (range), y	14.5 ± 2.0 (10–19)	14.3 ± 2.3 (10–18)
SES score, mean ± SD (range)[a]	50.1 ± 10.4 (23–66)	48.5 ± 11.2 (21–66)
BMI at study, mean ± SD (range), kg/m^2	21.8 ± 3.4 (15–31)[b]	24.3 ± 4.3 (19–37)
Lowest BMI, mean ± SD (range), kg/m^2	15.3 ± 1.7 (12–20)[c]	NA
AN subtype at diagnosis, *n* (%)		
Restricting	54 (81.8)	NA
Binge/purge	12 (18.2)	NA
Age of AN onset, mean ± SD (range), y	13.3 ± 1.5 (9–16)	NA
No. of hospitalizations, mean ± SD (range)	2.2 ± 2.4 (0–12)	NA
Length of follow-up, mean ± SD (range), y	6.4 ± 1.7 (4–10)	NA
Weight recovery, *n* (%)		
Low weight	14 (21.2)	NA
Weight recovered	52 (78.8)	NA
Menstrual function, *n* (%)		
Irregular/absent menses	18 (27.3)	4 (9.5)
Regular menses	19 (28.8)	17 (40.5)
Oral contraceptive pill	29 (43.9)	21 (50.0)

NA indicates not applicable.

[a] SES indicates SES according to A. B. Hollingshead, PhD (*Four-Factor Index of Social Status*, unpublished manual, 1975), with adolescent AN ($n = 65$) and control ($n = 38$) subjects.

[b] $P = .001$ (2-tailed).

[c] Data are for adolescent AN ($n = 65$).

TABLE 2 **Laboratory and Psychiatric Measures**

Variable	Adolescent AN		Control	
	n	Mean ± SD	*n*	Mean ± SD
Serum				
Creatinine, μmol/L	63	65.9 ± 8.6	42	68.1 ± 8.0
Urea nitrogen, mmol/L	63	3.5 ± 1.0	42	3.7 ± 0.9
Total protein, g/L	63	75.1 ± 4.0	42	76.1 ± 3.6
Albumin, g/L	63	41.3 ± 2.8	41	41.2 ± 2.5
Urine				
Creatinine, μmol/L	52	5780 ± 3799[a]	41	10367 ± 5420
Ccr, mL/s per 1.73 m^2	47	1.1 ± 0.5[b]	37	1.4 ± 0.5
UFC, nmol/d	51	119.3 ± 84.5	39	115.1 ± 54.3
UFC/Ccr, (nmol/d)/(mL/s per 1.73 m^2)	45	106.3 ± 52.2[c]	34	80.1 ± 39.1
Psychiatric symptom measures				
BDI-II	66	12.2 ± 12.4[a]	40	4.2 ± 4.2
FLY-BOCS	58	2.7 ± 7.3[b]	39	0.2 ± 1.4

[a] $P < .001$ (2-tailed).

[b] $P < .05$ (2-tailed).

[c] $P < .01$ (2-tailed).

Fifteen (9%) could not participate either because of medical reasons (eg, pregnancy precluding MRI) or relocation to another city. Seven (4%) agreed to participate but did not appear for their scheduled appointment. Thirteen (8%) indicated interest but could not be scheduled for a convenient time. Sixty-two (38%) did not provide a reason why they were not interested in participating in this study. Forty-two healthy female control subjects met the inclusion criteria and were recruited.

Clinical and control participants were comparable in age, education, and SES (Table 1). Clinical participants had lower current BMI than control subjects ($t_{106} = -3.4$; $P = .001$), but their mean BMI was within the reference range.[60] Sixty clinical participants were right handed, and 6 were left handed. Forty-one control subjects were right handed, and 1 was left handed.

Clinical Characteristics

At the time of study, 10 clinical participants continued to meet criteria for AN (6 restricting and 4 binge-eating and/or purging) according to the SCID-I/P.[33] One clinical participant had binge-eating disorder. Thirty were in partial remission (showing residual physiologic or psychological symptoms). Twenty-five were free from any symptoms of AN. None of the control subjects had an eating disorder.[33] Clinical participants and control subjects had comparable blood pressures and sexual maturity ratings.

Compared with control subjects, clinical participants had lower urine creatinine concentration ($t_{91} = -4.8$; $P < .001$) and Ccr ($t_{82} = -2.1$; $P = .04$) and higher Ccr -adjusted cortisol levels (UFC/Ccr; $t_{77} = 2.55$; $P = .01$; Cohen's $d = 0.56$; Table 2). Clinical participants also reported more symptoms of depression ($t_{104} = 4.2$; $P < .001$) and OCD (Mann-Whitney $U = 982$; $P = .04$), but they did not differ from control subjects in levels of serum creatinine, serum urea nitrogen, total protein, and albumin.

Structural Brain Volumes and Cognitive Functioning

Clinical Participants Versus Control Subjects

Compared with control subjects, AN participants had larger third ventricles ($t_{95} = 2.34$; $P = .02$; Cohen's $d = 0.49$), right lateral ventricles ($t_{94} = 2.27$; $P = .03$; Cohen's $d = 0.48$), and right temporal horns ($t_{93} = 2.50$; $P = .01$; Cohen's $d = 0.53$). They also showed a trend toward larger left lateral ventricles ($t_{94} = 1.94$; $P = .06$; Cohen's $d = 0.41$) and left temporal horns ($t_{92.3} = 1.61$; $P = .11$; Cohen's $d = 0.30$). Volumes of total gray matter, white matter, and global CSF were comparable (Table 3).

Clinical participants performed more poorly than healthy control subjects across almost all of the tested neuropsychological domains (Table 4). Moderate effect sizes (Cohen's $d \geq 0.5$) were detected in verbal ability, cognitive efficiency, broad reading, broad math, and delayed verbal recall. No correlation was found between neuropsychological test scores and measures of brain volumes among clinical participants or control subjects.

Comparison According to Weight-Recovery Status

Low-weight participants, weight-recovered participants, and control subjects differed significantly in volumes of the third ventricle ($F_{2,94} = 3.07$; $P = .05$), lateral ventricles ($F_{2,93} = 3.30$; $P = .04$), and temporal horns ($F_{2,92} = 3.80$; $P = .03$; Table 5). Posthoc Tukey HSD revealed that low-weight participants had larger lateral ventricles and temporal horns than control subjects (Cohen's $d = 0.8$); weight-recovered participants did not differ from either

TABLE 3 **Total and Regional Brain Volumes**

Variable	Mean ± SD volume/mL		*P* (2-tailed)	Effect Size (Cohen's *d*)
	Adolescent AN (*N* = 60)	Control (*N* = 37)		
Gray matter				
Frontal, L	157.2 ± 10.6	159.2 ± 11.4	0.38	−0.19
Frontal, R	152.9 ± 11.3	153.2 ± 13.4	0.91	−0.02
Parietal, L	83.6 ± 5.7	83.3 ± 7.7	0.83	0.05
Parietal, R	85.4 ± 10.8	84.6 ± 8.3	0.63	0.11
Temporal, L	108.8 ± 10.9	107.0 ± 10.7	0.44	0.16
Temporal, R	110.6 ± 10.6	107.1 ± 10.7	0.12	0.33
Occipital, L	50.2 ± 4.6	49.9 ± 4.9	0.74	0.07
Occipital, R	44.8 ± 4.3	44.9 ± 4.8	0.98	−0.01
Insula, L	12.8 ± 0.9	13.0 ± 1.1	0.33	−0.21
Insula, R	12.5 ± 0.9	12.4 ± 1.1	0.76	0.07
Cingulate region, L	21.7 ± 2.2	22.3 ± 1.8	0.15	−0.30
Cingulate region, R	22.8 ± 2.1	23.6 ± 1.7	0.07	−0.38
Caudate nucleus, L	6.7 ± 0.5	6.7 ± 0.5	0.45	0.16
Caudate nucleus, R	6.7 ± 0.5	6.6 ± 0.6	0.25	0.24
Putamen, L	8.6 ± 0.9	8.8 ± 1.1	0.36	−0.19
Putamen, R	8.9 ± 1.0	9.3 ± 1.2	0.09	−0.36
Globus pallidus, L	1.6 ± 0.2	1.7 ± 0.3	0.28	−0.23
Globus pallidus, R	1.6 ± 0.2	1.6 ± 0.3	0.17	−0.32
Thalamus, L	11.0 ± 0.7	10.7 ± 0.8	0.10	0.37
Thalamus, R	10.7 ± 0.6	10.5 ± 0.7	0.07	0.38
Subthalamic nucleus, L	0.1 ± 0.0	0.1 ± 0.0	0.53	−0.13
Subthalamic nucleus, R	0.1 ± 0.0	0.1 ± 0.0	0.57	−0.12
Hippocampus, L[a]	3.6 ± 0.4	3.7 ± 0.5	0.34	−0.20
Hippocampus, R[a]	3.6 ± 0.4	3.7 ± 0.5	0.15	−0.31
White matter				
Frontal, L[b]	95.0 ± 8.0	95.2 ± 9.0	0.93	−0.02
Frontal, R[b]	90.8 ± 7.6	89.9 ± 9.6	0.61	0.11
Parietal, L[b]	44.2 ± 5.1	43.6 ± 6.2	0.43	0.17
Parietal, R[b]	45.9 ± 5.8	45.3 ± 6.3	0.68	0.09
Temporal, L	47.7 ± 3.9	48.3 ± 6.0	0.52	−0.14
Temporal, R	49.4 ± 4.5	49.0 ± 5.4	0.69	0.08
Occipital, L	18.3 ± 4.5	16.1 ± 3.7	0.93	0.05
Occipital, R	19.8 ± 4.9	19.9 ± 4.4	0.81	−0.02
Fornix, L	1.0 ± 0.1	1.0 ± 0.1	0.06	−0.11
Fornix, R	1.0 ± 0.1	1.0 ± 0.1	0.60	−0.40
Corpus callosum	16.6 ± 2.7	17.1 ± 1.8	0.25	−0.22
Brainstem/cerebellum[c]	193.3 ± 13.7	190.1 ± 16.9	0.30	0.22
CSF				
Third ventricle	2.0 ± 0.4	1.8 ± 0.5	0.02	0.49
Fourth ventricle	0.1 ± 0.0	0.1 ± 0.0	0.74	−0.07
Subarachnoid space	147.3 ± 19.4	150.4 ± 17.8	0.44	−0.16
Lateral ventricle, L[b]	7.8 ± 3.9	6.6 ± 3.1	0.06	0.41
Lateral ventricle, R[b]	7.5 ± 3.2	6.1 ± 2.5	0.03	0.48
Temporal horn, L[a]	1.4 ± 0.6	1.3 ± 0.3	0.11	0.30
Temporal horn, R[a]	1.8 ± 0.7	1.4 ± 0.5	0.01	0.53
Total gray matter	919.4 ± 55.1	916.5 ± 62.7	0.82	0.14
Total white matter[b]	429.1 ± 35.6	428.4 ± 44.8	0.93	0.21
Total CSF[a]	164.7 ± 19.9	165.3 ± 19.9	0.89	−0.03

Only optimally processed images were included; *N* applies unless otherwise indicated; L indicates left; R, right.

[a] Data show adolescent AN (*n* = 59) and control (*n* = 36) subjects.

[b] Data show adolescent AN subjects (*n* = 59).

[c] Data are not included in total tissue volume calculations.

TABLE 4 **Neuropsychological Performance**

Variable	Adolescent AN Mean ± SD (*N* = 66)	Control Mean ± SD (*N* = 42)	*P* (2-tailed)	Effect Size (Cohen's *d*)
WJ-III clusters				
Tests of cognitive abilities				
Verbal ability	95.4 ± 9.5	100.9 ± 10.6	.006	0.55
Thinking ability	107.3 ± 10.7	111.9 ± 11.2	.04	0.42
Cognitive efficiency	106.1 ± 15.1	114.2 ± 16.5	.01	0.52
Working memory	102.3 ± 12.4	107.5 ± 14.8	.05	0.39
Tests of achievement				
Oral language	98.8 ± 11.0	103.0 ± 13.8	.10	0.35
Broad reading	108.4 ± 10.3	114.6 ± 14.3	.02	0.52
Broad math	98.3 ± 11.5	105.1 ± 13.8	.006	0.55
Broad written language	111.2 ± 12.1	115.1 ± 12.7	.11	0.32
HVLT-R[a]				
Total recall	41.6 ± 12.1	43.6 ± 9.4	.37	0.18
Delayed recall	41.9 ± 12.7	49.1 ± 10.8	.003	0.60
Recognition	44.2 ± 12.0	48.9 ± 11.0	.05	0.40
WMS-R				
Visual reproduction	61.0 ± 25.7	67.9 ± 22.9	.16	0.28
Delayed visual reproduction	54.3 ± 29.1	66.6 ± 27.1	.03	0.43

[a] Data show control subjects (*n* = 41).

groups. Hippocampal volumes were comparable among groups.

Weight-recovered participants and control subjects also differed significantly in scores of verbal ability ($F_{2,105} = 3.95$; $P = .02$), cognitive efficiency ($F_{2,105} = 3.47$; $P = .04$), broad reading ($F_{2,105} = 3.45$; $P = .04$), broad math ($F_{2,105} = 4.44$; $P = .01$), and delayed verbal recall ($F_{2,104} = 4.66$; $P = .01$). However, the differences between low-weight participants and control subjects were not statistically significant in any of the domains (Fig 1).

Clinical Correlates of Brain Structure and Cognitive Function

Clinical participants' cortisol levels (UFC/Ccr) were positively correlated with volumes of the temporal horns ($r = 0.37$; $P = .02$) and negatively correlated with volumes of the hippocampi ($r = -0.32$; $P = .04$). Current BMI and severity of depression and/or OCD were not associated with abnormal regional brain volumes (ie, third ventricle, lateral ventricles, and temporal horns).

No significant association was found between clinical participants' neuropsychological test scores and cortisol levels or severity of depression or OCD. Modest correlations were found between BMI and scores of the HVLT-R (total recall: $r = 0.26$, $P = .04$; recognition: $r = 0.26$, $P = .04$).

Comparison According to Menstrual Function

Significant differences were detected in volumes of the lateral ventricles ($F_{2,91} = 3.06$; $P = .05$) and temporal horns ($F_{2,89} = 3.05$; $P = .05$) among clinical participants with amenorrhea or irregular menses, clinical participants with regular menses or OCP, and healthy control subjects (Table 6). No differences were found among groups on measures of the third ventricle and hippocampus. Posthoc Tukey HSD showed that par-

TABLE 5 **Comparison of Regional Brain Volumes According to Weight Recovery**

Brain Region	Mean ± SD, Volume per mL			ANOVA, *P*	Effect Size (Cohen's *d*)		
	Low-weight AN (*n* = 12)	Weight-Recovered AN (*n* = 48)	Control (*n* = 37)		1 vs 3	2 vs 3	1 vs 2
Third ventricle	2.1 ± 0.4	2.0 ± 0.4	1.8 ± 0.5	.05	0.64	0.43	0.30
Lateral ventricles[a]	17.0 ± 6.6	14.9 ± 5.2	12.7 ± 5.4	.04	0.75	0.41	0.38
Temporal horns[b]	3.6 ± 1.8	3.1 ± 0.9	2.7 ± 0.8	.03	0.79	0.45	0.46
Hippocampi[b]	7.2 ± 0.7	7.1 ± 0.8	7.3 ± 0.9	.43	−0.16	−0.29	0.13

Compared with control subjects, low-weight AN has greater lateral ventricle volumes ($P = .05$), and temporal horn volumes ($P = .03$) (Tukey's HSD).

[a] Data show weight-recovered AN subjects ($n = 47$).

[b] Data show weight-recovered AN ($n = 47$) and control ($n = 36$) subjects.

ticipants with amenorrhea or irregular menses did not have systematically elevated CSF volumes across the lateral ventricle and temporal horns. Thus, menstrual function did not seem to be specifically related to structural brain measures in adolescent-onset AN.

In contrast, significant omnibus between-group differences in test scores were observed in verbal ability ($F_{2,101} = 3.80$; $P = .03$), cognitive efficiency ($F_{2,101} = 3.57$; $P = .03$), oral language ($F_{2,101} = 5.02$; $P = .008$), broad reading ($F_{2,101} = 3.19$; $P = .05$), broad math ($F_{2,101} = 3.87$; $P = .02$), and delayed verbal recall ($F_{2,101} = 4.12$; $P = .02$). Posthoc Tukey HSD procedures showed that participants with amenorrhea or irregular menses had significantly lower scores than control subjects across these domains (Fig 2). They also had lower oral language scores compared with participants with regular menses or OCP ($P = .02$). Participants with regular menses or OCP did not differ significantly from control subjects in any neuropsychological measure.

DISCUSSION

In this study, female subjects with a history of adolescent-onset AN had larger lateral and third ventricles, along with a broad range of neuropsychological deficits compared with healthy control subjects. They also had elevated UFC levels and reported more symptoms of depression and OCD. Additional analyses revealed that low weight and high cortisol levels were associated with greater structural brain abnormalities, whereas abnormal menstrual function was associated with more severe cognitive deficits. Cognitive deficits were not associated with abnormal measures of brain volumes. This is the first study to report the dissociable influence of various

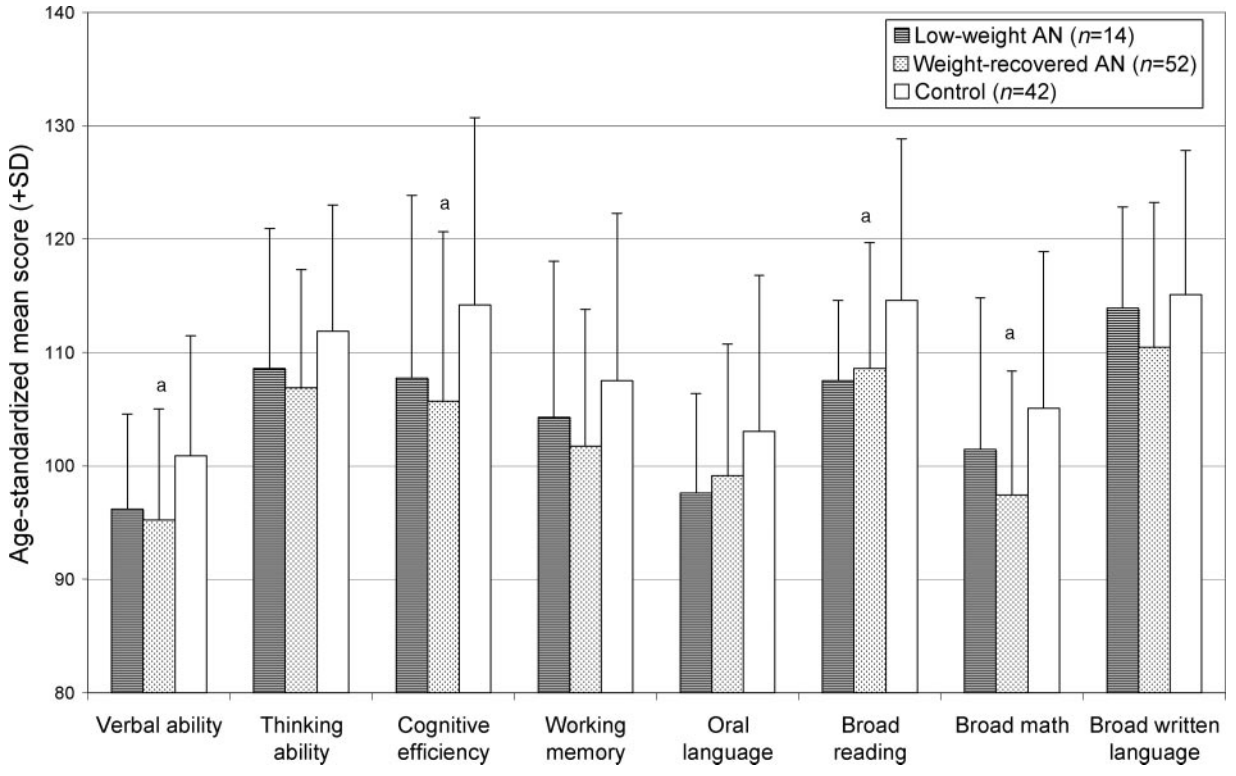

FIGURE 1
Performance on the WJ-III according to weight recovery. [a] $P < .05$ compared with control subjects.

TABLE 6 **Comparison of Regional Brain Volumes According to Menstrual Function**

Brain Region	Mean ± SD, Volume per mL			ANOVA, *P*	Effect Size (Cohen's *d*)		
	Amenorrhea or Irregular Menses AN ($n = 14$)	Regular Menses or OCP AN ($n = 46$)	Control ($n = 34$)[a]		1 vs 3	2 vs 3	1 vs 2
Third ventricle	2.1 ± 0.5	2.0 ± 0.4	1.8 ± 0.5	.10	0.55	0.39	0.26
Lateral ventricles[b]	15.1 ± 5.0	15.4 ± 5.7	12.4 ± 5.2	.05	0.51	0.54	−0.06
Temporal horns[c]	3.6 ± 1.4	3.1 ± 1.0	2.8 ± 0.8	.05	0.78	0.35	0.41
Hippocampi[c]	7.1 ± 0.8	7.1 ± 0.7	7.3 ± 0.9	.64	−0.24	−0.18	−0.08

Compared with control subjects, regular menses or OCP AN has greater lateral ventricle volumes ($P = .05$), and amenorrhea or irregular menses AN has greater temporal horn volumes ($P = .05$) (Tukey's HSD).

[a] Only control subjects who had regular menses or who were on OCP were included.

[b] Data show regular menses or OCP AN subjects ($n = 45$).

[c] Data show regular menses or OCP AN ($n = 45$) and control ($n = 33$) subjects.

clinical indices on brain structure and cognitive function in adolescent-onset AN.

Brain Structure

Despite a long time interval (>6 years) since the initial diagnosis of AN, clinical participants exhibited ventricular enlargements, which are commonly reported during the acute stages of AN.[8–17] Additional examination revealed that only participants who remained low weight had these anomalies; weight-recovered participants had intermediate ventricular volumes that did not differ from either low-weight participants or control subjects. This observation is consistent with previous reports of ventricular volume reduction to normal levels with weight recovery.[11–13,17]

Volumes of total gray matter, white matter, and CSF were comparable between clinical participants and control subjects. These results replicate some studies[61] but contradict others.[11,12] Mixed findings may be attributed to differences in patient variables, such as length of follow-up. In a previous study by our group that reported reductions in gray matter and white matter volumes in recovered patients,[11] the follow-up period was 2.7 ± 0.3 years compared with 6.5 ± 1.7 years in the present study. Another explanation may relate to differences in the ability to detect volume changes in the

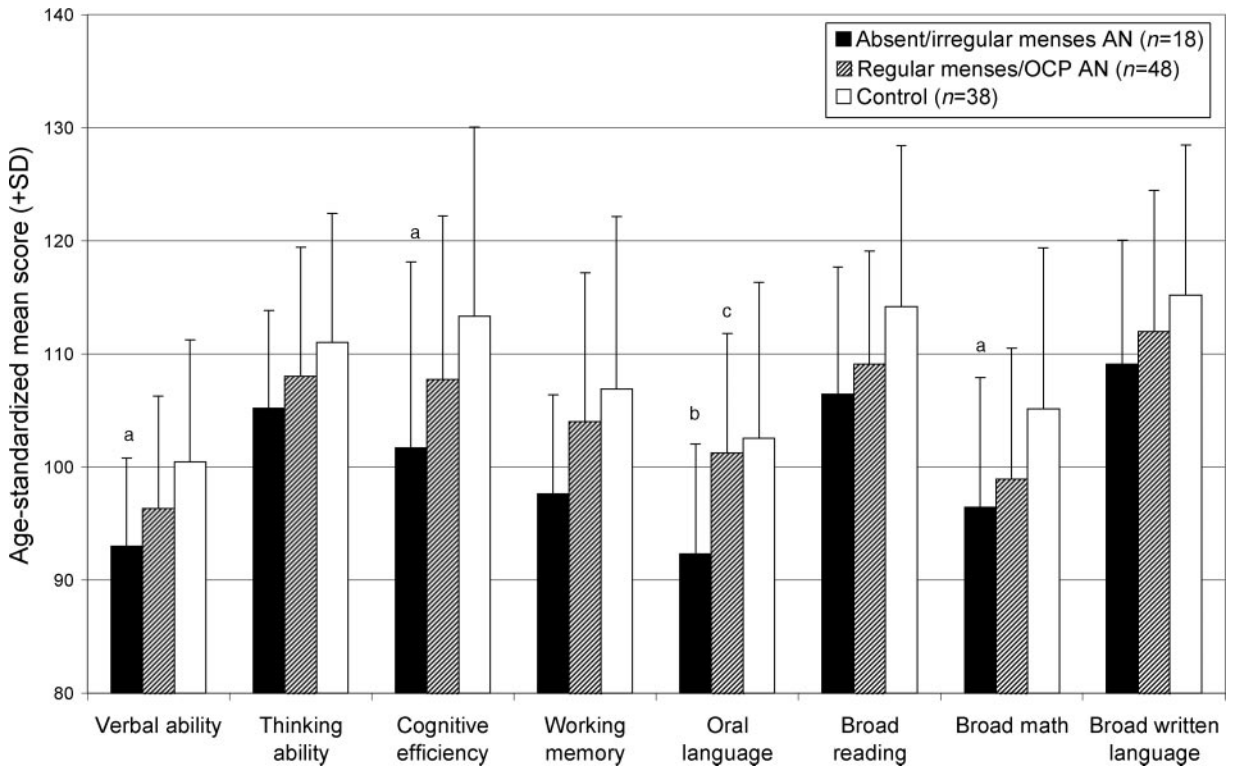

FIGURE 2

Performance on the WJ-III according to menstrual function. [a] $P < .05$ compared with control subjects; [b] $P < .01$ compared with control subjects; [c] $P < .05$ compared with irregular or absent menses AN.

ventricles compared with gross tissue compartments. Assuming that the ventricles expand to fill losses in brain tissue volume, the same absolute volume change represents different percentage changes depending on the size of the brain regions measured. Therefore, changes in small regions, such as the ventricles, may be a more sensitive indicator of structural brain abnormalities than changes in total tissue volume.

Although weight recovery may be associated with the reversal of structural brain abnormalities in adolescent-onset AN, the mechanism through which this effect is achieved remains unclear. In this regard, nutrition may not be the sole factor, because current BMI was not correlated with volumetric brain measures in clinical participants. Clinical participants and control subjects also had comparable blood pressures, serum albumin, and total protein levels, which suggests that dehydration and altered oncotic pressure are unlikely mechanisms for structural brain abnormalities observed in our participants with AN.[8,13] Alternatively, high cortisol levels have been associated with structural brain abnormalities during the acute stages of AN.[8] Indeed, in the current follow-up study, cortisol levels were negatively correlated with hippocampal volumes and positively correlated with temporal horn volumes in clinical participants. Sensitivity of the hippocampus to cortisol because of the dense population of corticosteroid receptors[62] and proximity of the temporal horn to the hippocampus may explain why significant correlations were detected in these regions. The relationship between cortisol levels and structural brain abnormalities seems to persist into the recovery stages of AN.

Although clinical participants had higher cortisol levels than control subjects, their mean level was within the reference range. Consistent with previous research, this result suggests that cortisol levels normalize after weight recovery in AN.[35] Correction of hypercortisolemia has also been associated with reversal of structural brain abnormalities in Cushing syndrome.[63–65] Notably, children and adolescents with Cushing syndrome may experience especially rapid and complete reversal of structural brain abnormalities compared with adult patients.[64] These observations may explain why normal hippocampal volumes were found in participants with adolescent-onset AN in this study, whereas sustained hippocampal shrinkage was seen in patients with primarily adult-onset AN.[59] In addition, amygdala volumes may not increase with corrected hypercortisolemia, even in children with Cushing syndrome.[64] Inclusion of the amygdala in the previous hippocampal analysis[59] may also explain differences in findings. The parallels between adolescent-onset AN and Cushing syndrome in terms of structural brain abnormalities during periods of hypercortisolemia, reversal of anomalies after decline of cortisol levels, and perhaps better potential for younger patients to achieve such reversals[64] underscore the possibility that cortisol levels may contribute to the development of structural brain changes in adolescent-onset AN.

Cognitive Functioning

Participants with a history of adolescent-onset AN showed deficits in cognitive functioning over a broad range of neuropsychological domains compared with healthy control subjects. In terms of clinical correlates, we replicated previous reports of the lack of association between cognitive performance and weight recovery,[16,18] cortisol levels,[25] and severity of comorbid psychiatric symptoms.[23,24] In contrast, clinical participants who remained amenorrheic or had irregular menses had the lowest scores on all of the tested domains, followed by those who had resumed menses or who were on OCP, and was highest among healthy control subjects. Importantly, effect sizes between participants with absent or irregular menses and control subjects were large, whereas participants with regular menses had similar scores to those on OCP and did not differ from control subjects. Amenorrhea or menstrual irregularity may, thus, be associated with cognitive impairments in adolescent-onset AN.

The influence of estrogen and menstrual function on cognitive performance is an active area of research in several female conditions, including menopause,[66] surgically induced menopause,[67] premature ovarian failure,[68] and Turner's syndrome.[68] Abnormal menstrual function and estrogen depletion are generally associated with the presence of cognitive deficiencies. Notably, these effects may be more pronounced in younger than older female subjects,[68] making menstrual function especially relevant in the study of cognitive functioning in adolescent-onset AN.

The mechanisms through which menstrual function influences cognitive function in adolescent-onset AN are unclear. One possibility is by modification of brain structure. Reduced circulating estrogen after ovariectomy led to a loss of dendritic spines of the CA1 hippocampal pyramidal cells[69] and impaired working memory in rodents.[70] Although the microscopic examination of dendritic spines is not feasible in live humans, we could not detect greater volumetric abnormalities among participants with absent or irregular menses in this study. A logistic regression conducted retrospectively additionally demonstrated the lack of contribution of menstrual function to structural brain volumes, in contrast to cognitive function. Using menstrual status as the dependent variable (amenorrhea or irregular menses versus regular menses or OCP) and volumes of the temporal horn and hippocampus (both block 1) and oral language scores (block 2) as the independent variables, oral language scores in clinical participants were found to contribute significant variance (Wald: 7.84; $P = .005$) and to menstrual function beyond volumes of the temporal horns (Wald: 1.56; $P = .21$) or hippocampi (Wald: 0.68; $P = .41$). The extent to which estrogen influences cognition in adolescent-onset AN via changes in brain morphology remains to be established.

Another possible mechanism involves the ability of estrogen to improve blood circulation. Increased cerebral blood flow has been documented in menopausal women receiving estrogen replacement therapy.[71] In particular, gains were most significant in the temporal and parietal

lobes. Similar increases in blood flow to brain regions such as the hippocampus may occur in female subjects with adolescent-onset AN after the resumption of regular menses, protecting the brain from metabolic compromise associated with hypoxia.

Estrogen may also modulate cognition by altering neurotransmission. In rats, ovariectomy has been shown to reduce serotonin 2A receptor density, which, in turn, increased after estradiol administration.[72] Postmenopausal women receiving estrogen also had increased serotonin 2A receptor binding.[73,74] In contrast, serotonin 1A receptor mRNA and binding decreased after estradiol treatment in ovariectomized rats.[75,76] Functionally, estrogen enhanced serotonin 1A-mediated acetylcholine release that is important in learning and memory[77] and may modulate serotonin 2A receptor-mediated working memory and executive function.[73] It is, therefore, conceivable that fluctuating estrogen levels influence cognition in AN by altering the dynamics of the serotonin system.

Reduced and increased binding of the serotonin 2A and serotonin 1A receptors, respectively, have been found in low-weight and weight-recovered participants with AN.[78–81] Although these differences may reflect preexisting anomalies,[82] low circulating estrogen levels associated with amenorrhea may sustain or exacerbate underlying serotonin abnormalities. Although no association was found between estradiol levels and serotonin 1A or 2A binding in 1 study,[80] a small sample size and restricted estradiol ranges could have precluded detection of significant findings. How serotonin transmission influences cognitive functioning in AN is not clear at present, but possible aggravation of existing serotonergic abnormalities by estrogen depletion underscores that the return of menstrual function and normal circulating estrogen levels may be key in reducing the magnitude of neural or cognitive abnormalities or both in adolescent-onset AN.

Limitations

There are several limitations in this study. The analysis was correlational. Therefore, causal relationships cannot be assumed between grouping variables and measures of brain structure and cognitive function. The cross-sectional design also prevents us from concluding whether abnormalities in brain structure and cognitive function predate or occur as a consequence of the illness. Although the functional significance of moderate cognitive deficits found in the clinical participants was not addressed in this study, it is clearly an important area for future research.

We did not control for the type, dose, and duration of OCP that the participants received. These variables may affect the availability of estrogen to the brain and its efficacy in modulating cognitive function.[30] However, the association found between menstrual function or hormone therapy and cognitive functioning despite such variability deserves additional investigation. Replication with larger samples is needed.

CONCLUSIONS

To our knowledge, this is the first study to report on the relationship between menstrual function and cognitive functioning in female subjects with adolescent-onset AN. The volumetric analysis of brain structure supports the reversibility of structural brain abnormalities after weight recovery[11–13,17,61] and correction of hypercortisolemia.[63–65] Moreover, cognitive function and brain structure were differentially associated with clinical variables, menstrual status and cortisol levels, respectively. Taken together, future research in adolescent-onset AN should focus on the interaction between sex hormones and neurotransmitter systems that may not manifest as detectable structural brain changes. Clinical investigation addressing the role of estrogen in mediating cognitive function in AN also requires additional study.

ACKNOWLEDGMENTS

This study was supported by the Ontario Mental Health Foundation, Ontario Graduate Scholarship, SickKid's Research Institute and Carol Mitchell and Richard Venn.

We thank Kim Gordon and Stacey Pollock for assistance with participant recruitment and data collection. We also thank Dr James Cantor for assistance with writing up the neuroimage-processing methods and Dr Arlene Young for the design of the neuropsychological testing protocol.

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Pediatrics 2008;122;e426-e437
DOI: 10.1542/peds.2008-0170