Research Paper

Impact of acute tryptophan depletion on mood and eating-related urges in bulimic and nonbulimic women

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Background: Previous research has shown that many people experience a temporary worsening of mood following acute tryptophan depletion (ATD) and that concurrent use of serotonergic medications may influence such mood responses. We investigated mood and other consequences of ATD in women with bulimia nervosa who were or were not using concurrent serotonergic medications compared with women without bulimia. **Methods:** Women self-referred for treatment of bulimia who were either not currently using psychoactive medications (n = 26) or who were using serotonin reuptake inhibitor medications exclusively (n = 13), as well as medication-free normal-eater control women (n = 25) completed interviews and questionnaires assessing eating and comorbid psychopathology and then participated in an ATD procedure involving balanced and tryptophan-depleted conditions. **Results:** In the tryptophan-depleted condition, the groups displayed similar and significant decrements in plasma tryptophan levels and mood. Women with bulimia who were using serotonin reuptake inhibitors, but not the other groups, also reported an increased urge to binge eat in the tryptophan-depleted condition. **Limitations:** Application of medication in participants with bulimia was not random. **Conclusion:** Acute reductions in serotonin availability produced similar mood-reducing effects in bulimic and nonbulimic women. To the extent that ATD affected subjective experiences pertinent to eating (i.e., urge to binge eat), such effects appeared to depend upon ATD-induced competition with the therapeutic effects of serotonergic medications.

Introduction

Bulimia nervosa has been associated with alterations in central serotonin (5-HT) function,[1] thought to implicate constitutional (e.g., hereditary) influences[2–5] and state-related (e.g., dietary) factors.[2,6] Whereas pertinent findings associate bulimia with global alterations in the function of the serotonin system, most do not address the ways in which short-term alterations in serotonin activity may actually trigger bulimic symptoms (e.g., binge eating) and associated symptoms (e.g., depressed mood). In the present study, we examined such possible effects using an acute tryptophan depletion (ATD) paradigm.

Acute tryptophan depletion uses a dietary manipulation to produce transient reductions in tryptophan, the amino-acid precursor of serotonin.[7,8] In ATD, participants ingest a mixture of amino acids that contains no tryptophan, which induces protein synthesis.[9] As tryptophan is incorporated into proteins, its level in blood and tissues declines markedly; the result — observed in both animal[10] and human[11] brains — is a decline in central serotonin synthesis. Thus, if ATD produces increased urges to binge or actual binge episodes, a case can be made for a direct influence of serotonin variations in bulimic symptoms. There are currently 3 available reports using ATD in unmedicated, actively bulimic participants; the studies involve small and partially overlapping samples developed by the same team of investigators. Taken together, the reports suggest that ATD reliably lowers tryptophan levels and that the effects of ATD on mood and eating-related symptoms are somewhat variable.[12–14] Variable effects of ATD on mood and eating-related symptoms are also seen in unmedicated women remitted from bulimia: one report finding ATD to acutely worsen mood and eating-related urges[15] and another failing to find an effect of ATD on mood and eating-related urges.[16] Given the

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variability in the subjective effects of ATD, replication is warranted.

One hypothetical factor that influences the strength of ATD effects is utilization of serotonin reuptake inhibitor medications. Several studies conducted in nonbulimic populations report relatively more robust acute symptom recurrences following ATD among individuals who use such medications and who are remitted from depression[17–20] and anxiety disorders.[21,22] Given the preceding, and given the limited literature suggesting variable effects of ATD in bulimic and nonbulimic women, we opted to explore the biochemical, mood and eating-related effects of ATD in bulimic and nonbulimic women who were or were not using serotonin reuptake inhibitors. Our hypothesis was that, as in the literature on depression and anxiety disorders, individuals with bulimia who were using serotonergic medications would exhibit comparatively larger eating-related subjective responses to ATD.

Methods

Participants

Women with bulimia

We recruited women living in a large metropolitan area who met DSM-IV, text revised (DSM-IV-TR)[23] criteria for bulimia nervosa or for a bulimia-spectrum eating disorder not otherwise specified (defined as binge eating and engaging in purging or compensatory behaviours at least once per week); the women were recruited while in outpatient treatment at a specialized eating disorder program. We excluded participants if they met current criteria for anorexia nervosa or showed evidence of psychotic symptoms, and we established DSM-IV-TR criteria using responses from the Eating Disorder Examination (EDE, described in the Measures section).[24] The bulimic group comprised women who were not taking any psychotropic or other prescribed medications at the time of testing (i.e., minimum 4 weeks before ATD) and women who were using a prescribed serotonin reuptake inhibitor at the time of testing (but no other prescribed medications in the preceding 4 weeks).

Control group

We recruited participants for the control group from newspapers and university classrooms in the same metropolitan area. These women had no identifiable lifetime eating disorder according to responses on the EDE and denied a history of psychological treatment. They also reported not taking psychoactive medications at the time of testing (i.e., minimum 4 weeks before ATD).

Measures

We assessed symptoms (e.g., binge eating and purging frequencies) and DSM-IV-TR diagnoses using the EDE.[24] Reliability exceeds 0.90 on all but 3 of 62 EDE items, and internal consistency and discriminant validity figures are excellent. In addition, we assessed lifetime histories of bipolar disorder, major depressive disorder and anxiety disorders with the Structured Clinical Interview for DSM-IV Axis I (SCID-I), outpatient version,[25] which has well-established psychometric properties.[26] We also characterized overall severity of depression symptoms using the 20-item Centre for Epidemiological Studies Depression scale[27] and assessed impulsive behaviours using the Barratt Impulsiveness Scale (version 11) total score.[28] For biological measures, we obtained plasma tryptophan and the ratio (from plasma) of tryptophan to large neutral amino acids from blood samples (see the Procedures section). We assessed mood responses using the bipolar Profile of Mood States,[29] a 72-item scale designed to measure moment-to-moment fluctuations in mood with 6 subscales: composed–anxious, agreeable–hostile, elated–depressed, confident–unsure, energetic–tired and clearheaded–confused. Using a numbered Likert-type scale (0 = minimum, 10 = maximum), we measured urges to binge eat and purge by vomiting.

Given a bilingual sample, we developed French translations of the EDE, SCID-I, Center for Epidemiological Studies for Depression Scale, Barratt Impulsiveness Scale, Profile of Mood States scale and the urge measures. Previous evaluations of psychometric equivalence of English and French language versions, to which we refer in earlier publications,[4,5] suggest absence of language-based effects. Using Student *t* tests in the present data set, we detected no significant differences in mean scores on these measures among participants who completed questionnaires in English or in French (data not shown).

Procedure

The Douglas Institute Research Ethics Board approved our protocol, which involved voluntary participation after signed informed consent. Upon arrival at the laboratory, participants completed the EDE, SCID-I Mood Disorders and Anxiety Disorders Modules and questionnaires in 1–2 sessions totalling roughly 4 hours' duration.

Participants were involved in 2 separate test days, presented double-blind and randomly ordered in blocks. On one test day, participants ingested a nutritionally balanced (B) amino acid mixture; on the other test day, participants ingested an amino acid mixture devoid of tryptophan.[7,8] The tryptophan deficient (T–) mixture temporarily reduced serotonin availability, synthesis and turnover, with maximal reductions roughly 5–7 hours following administration.[7,30,31] The base of both the B and T– mixtures was 100 mL of water and 1 tablespoon of chocolate syrup. In the T– mixture, we added 4.58 g of L-alanine, 2.97 g of glycine, 2.67 g of L-histidine, 6.67 g of L-isoleucine, 11.25 g of L-leucine, 9.17 g of L-lysine monohydrochloride, 4.75 g of L-phenylalanine, 10.17 g of L-proline, 5.75 g of L-serine, 5.42 g of L-threonine, 5.75 g of L-tyrosine and 7.42 g of L-valine. Supplemental to this, 4.08 g of L-arginine, 2.25 g of L-cysteine and 2.5 g of L-methionine were given to participants in capsule form since their smell and taste is unpleasant. In the B mixture, we included 1.92 g of L-tryptophan along with the other ingredients in the T– mixture. The mixtures were based on the 100-g mixtures of Young and colleagues,[8] but we reduced all components by 18% to allow for the lower average body weight of women relative to men.

Test days occurred in the follicular phase of menses (defined here as days 2–14 of the menstrual cycle). On the day before a test day, participants followed a low-protein meal plan.[32] On test days, participants presented to the laboratory at 8:30 am, not having eaten since the previous day and not having binged or purged (by self-report) in the preceding 24 hours. On arrival, participants provided a breath alcohol (breathalyser) sample and a urine sample for a TOX/See multidrug urine screen panel (Bio-Rad Laboratories), which can detect amphetamines, methamphetamine, opiates, cocaine, phencyclidine, 11-nor-delta-9-tetrahydrocannabinol-9-COOH, benzodiazepines, barbiturates, methadone and 3,4-methylenedioxymethampetamine (ecstasy). Participants who passed both tests were then seated in a comfortable reclining chair and received a butterfly catheter in the nondominant arm for blood draws. Participants provided blood samples in the morning and afternoon on both test days for measurement of plasma tryptophan and other large neutral amino acids (phenylalanine, tyrosine, leucine, isoleucine, valine, histidine, methionine and threonine). As in other recent investigations,[32] we measured total plasma tryptophan by isocratic high-performance liquid chromatography (HPLC) without derivatization and fluorescence detection. We measured levels of the other amino acids that compete with tryptophan for entry into brain by gradients HPLC using o-phthaladehyde (OPA) pre–column derivatization and a Beckman System Gold HPLC with fluorescence detection.

After the morning blood draws, participants completed the Profile of Mood States and the items measuring subjective urges to binge and purge (randomly ordered). Participants then consumed either the B or T– mixture and subsequently remained seated in the test room except for bathroom breaks and refrained from eating, drinking, exercising, sleeping and smoking. They were permitted to read or watch DVDs (from a selection of *Amadeus, Amelie, Fantasia, Forrest Gump, The Majestic* and *Seven Years in Tibet*). Five hours after drinking the amino acid mixture, participants repeated the Profile of Mood States and the items measuring urges to binge and purge. They then provided another blood sample used to measure amino acid levels. When testing was completed, participants were offered a light lunch and a tablet of tryptophan (0.5 g) to help bring plasma tryptophan levels back to normal in the depleted group and to maintain the blind in the control group.

Statistical analyses

We compared groups on age, body mass index (BMI), the Centre for Epidimiological Studies Depression Scale and the Barratt Impulsiveness Scale using univariate analyses of variance (ANOVAs) and Newman–Keuls post hoc tests for pairwise comparisons. Dimensional EDE measures (e.g., binge and purge frequencies) and the measure of eating disorder chronicity yielded zero values in the control group, so on these measures we applied ANOVAs to examine pairwise differences between bulimic groups only (medicated v. unmedicated). We used χ^2 tests to explore differences between the bulimic groups on categorical measures (e.g., EDE diagnoses, SCID-I Axis I Disorders). For amino acid responses and for urges to binge and purge, we used repeated-measures ANOVAs comparing the effects of condition (B v. T–) × time (preadministration baseline v. 5-h postadministration) × group (control v. unmedicated bulimia v. medicated bulimia), where condition and time were within-subject factors and group was a between-subject factor. We isolated pairwise (within, between) group effects from 3-way interactions using Bonferroni-adjusted (within level) least squares difference post hoc tests based on estimated marginal means. We further examined 2-way (condition × time) interactions by inspection of simple effects. For the Profile of Mood States analysis, we used a repeated-measures ANOVA examining condition (B, T–) × time (preadministration baseline, 5-h postadministration) × group (control, unmedicated bulimia, medicated bulimia) × subscale (composed–anxious, agreeable–hostile, elated–depressed, confident–unsure, energetic–tired and clearheaded–confused), where condition, time and subscale were within-subject factors and group was a between-subject factor. We further examined 2-way (condition × time) interactions by inspection of simple effects.

Results

The women with bulimia (n = 39) were aged 18–41 years and had BMIs of 17.3–28.8 kg/m^2. Of these participants, 26 were not taking any psychotropic or other prescribed medications and 13 were using a prescribed serotonin reuptake inhibitor at the time of testing. The medications used were citalopram (n = 6), fluoxetine (n = 2), venlafaxine (n = 2), paroxetine (n = 1), escitalopram (n = 1) and combined mirtazepine and buproprion (n = 1). We found no differences according to χ^2 between the unmedicated and medicated groups in subtype of EDE diagnoses. Respective frequencies of bulimia purging subtype, bulimia nonpurging subtype and eating disorder not otherwise specified with binge eating and purging/compensation were 19, 1 and 6 for the unmedicated group and 11, 1 and 1 for the medicated group. The control group (n = 25) comprised normal-eating women aged 18–33 years with BMIs of 18.0–27.2 kg/m^2.

Table 1 shows group means, standard deviations and statistics for dimensional measures of interest; ANOVA comparisons between the control, unmedicated bulimia and medicated bulimic groups were significant for all measures shown. Post hoc testing and inspection of the group means revealed that the medicated bulimic group was older and had a higher mean BMI than did the control group; no other significant pairwise differences were found for age or BMI. Post hoc comparisons and inspection of the group means in Table 1 also demonstrated that both bulimic groups had higher mean Center for Epidemiological Studies Depression scores and Barratt Impulsiveness Scale scores than did the control group; however, there were no differences between unmedicated and medicated bulimic groups. The 2 bulimic groups did not differ significantly on binge eating and purging episodes per month or on eating disorder chronicity (for self-induced vomiting, the table shows raw scores, but we performed the ANOVA on square root–transformed scores to

help correct for heterogeneity of error variance.). Taken together, the results demonstrate that both bulimic groups, compared with the control group, had greater depression severity and impulsiveness, that there were no significant differences between medicated and unmedicated bulimic participants on eating or comorbid symptom indices and that there were differences between medicated bulimic participants and controls on age and BMI.

Axis I disorders

No participant met lifetime criteria for bipolar disorder or for specific phobia, and none of the control participants met criteria for another anxiety disorder. Frequencies of lifetime major depressive disorder were: 8 (32.0%) for the control group, 13 (52.0%) for the unmedicated bulimic group and 8 (61.5%) for the medicated bulimic group. Among bulimic participants, frequencies of lifetime generalized anxiety disorder, social phobia, panic disorder and obsessive–compulsive disorder were as follows: 2 (8.0%), 1 (4.0%), 1 (4.0%) and 1 (4.0%) in the unmedicated group and 2 (16.7%), 2 (16.7%), 0 (0.0%) and 0 (0.0%) in the medicated group. Finally, χ^2 analyses (or Fisher exact test, as appropriate) revealed no differences in lifetime comorbid mood or anxiety disorders among the groups. In sum, there were no significant group differences on lifetime mood or anxiety disorders.

Responses to ATD

Table 2 shows group means, standard deviations and statistics for amino acid and subjective responses during the ATD procedure. As can be seen, there were a few missing data points owing to the complexity of the protocol and the nature of our participants; sample sizes are indicated for each of the measures.

Amino acid responses

Table 2 shows that, for plasma tryptophan levels, we observed a significant condition (B v. T–) × time (preadministration v. 5-h postadministration) interaction but no group effects. Within-condition simple effects and inspection of (total) mean scores in Table 2 indicate that tryptophan levels increased significantly from pre- to postadministration across groups in the B condition and decreased significantly from pre- to postadministration across groups in the T– condition. There was a mean 87.4% reduction across groups (from pre- to postadministration) in the T– condition. For the ratio of plasma tryptophan to large neutral amino acids, there was similarly a condition × time interaction and no group effects. Inspection of within-condition simple effects and of the (overall) mean scores in Table 2 showed that the ratio of tryptophan to large neutral amino acids decreased significantly from pre- to postadministration across groups in the B condition but decreased even further from pre- to postadministration across groups in the T– condition. There was a mean 95.6% reduction across groups (from pre- to postadministration) in the T– condition. Taken together, the results indicate that tryptophan levels and the ratio of tryptophan to large neutral amino acids decreased significantly in the T– condition (v. T– preadministration and v. postadministration in the B condition), indicating that the ATD procedure produced similar and large reductions in plasma tryptophan levels across groups; further, there were no group differences in baseline tryptophan levels, indicating similar tryptophan availability at preadministration.

Subjective responses

Table 2 indicates that for the total Profile of Mood States score, there was a significant condition × time interaction and no group effects; inspection of the condition × time interaction (see total scores in Table 2, which are collapsed across group) showed that mood was significantly lower in the T– condition postadministration (v. T– condition preadministration and v. B condition postadministration). Such results indicate that tryptophan depletion produced similar reductions in mood across groups. We also found a significant condition × time × group interaction for the subjective urge to binge (Table 2). Post hoc analyses (Bonferroni-corrected within level) and inspection of the mean scores indicated that the medicated bulimic group experienced an increased urge to binge in the T– condition at postadministration (v. T– preadministration v. B condition postadministration); the other groups did not experience any significant response effects on subjective urge to binge. The finding indicates that

Table 1: Group means and standard deviations on dimensional variables of interest among bulimic and nonbulimic women and healthy controls

Variable	Participant group; mean (SD)				
	Control	Unmedicated bulimic	Medicated bulimic	*F*	*p* value
Age, yr	22.4 (3.4)	25.4 (6.6)	26.5* (3.6)	$F_{2,63} = 3.63$	0.032
Body mass index, kg/m^2	21.3 (2.2)	22.4 (3.1)	23.8* (2.8)	$F_{2,63} = 3.59$	0.034
Center of Epidemiological Studies Depression score	9.3 (5.4)	25.1* (12.0)	30.8* (13.4)	$F_{2,59} = 23.4$	0.001
Barratt Impulsiveness Scale score	63.8 (9.6)	77.2* (9.3)	75.0* (9.0)	$F_{2,59} = 13.5$	0.001
Binge eating episodes, no./mo†	—	24.2 (21.3)	21.9 (11.4)	$F_{1,37} = 0.13$	0.72
Vomiting episodes, no./mo†	—	20.6 (20.0)	42.3 (76.3)	$F_{1,37} = 1.50$	0.23
Duration of eating disorder, mo	—	97.7 (92.3)	106.4 (54.3)	$F_{1,32} = 0.09$	0.76

SD = standard deviation.
*Significant pairwise difference versus control by Newman–Keuls test.
†Determined using the eating disorders examination.[24]

the medicated bulimic group (but not the control or the unmedicated bulimic group) experienced increased urges to binge following ATD. We found no significant response effects for urge to purge, indicating that ATD had no observable effect on this urge. The subjective responses to ATD suggest that mood worsened across groups and that the medicated bulimic group experienced an increased urge to binge.

Statistical controls on subjective urge to binge

To address potential problems related to extraneous influences

Table 2: Responses to acute tryptophan depletion among bulimic and nonbulimic women and healthy controls

Response; participant group	Condition; before or after administration of amino acid mixture; mean (SD)				Effect	*F* value	*p* value
	Balanced (B)		Tryptophan depletion (T–)				
	Before	After	Before	After			
Plasma tryptophan level, ug/mL							
Control (*n* = 25)	9.23 (1.53)	18.8 (6.1)	9.12 (1.49)	1.13 (0.77)			
Unmedicated bulimic (*n* = 23)	9.64 (1.19)	20.5 (6.6)	9.45 (1.91)	1.17 (0.42)			
Medicated bulimic (*n* = 13)	9.97 (1.56)	23.3 (7.2)	9.62 (1.15)	1.24 (0.47)			
Total (*n* = 61)	9.54 (1.42)	20.4* (6.7)	9.35 (1.59)	1.17* (0.59)	C × T	$F_{1–58}$ = 476.8	< 0.001
Ratio of trytophan to large neutral amino acids, %							
Control (*n* = 24)	2.37 (0.50)	1.57 (2.01)	2.31 (0.43)	0.10 (0.12)			
Unmedicated bulimic (*n* = 23)	2.46 (0.41)	1.29 (0.36)	2.35 (0.43)	0.10 (0.09)			
Medicated bulimic (*n* = 13)	2.49 (0.40)	1.35 (0.35)	2.43 (0.31)	0.09 (0.05)			
Total (*n* = 60)	2.43 (0.44)	1.42* (1.29)	2.35 (0.40)	0.10* (0.09)	C × T	$F_{1–57}$ = 51.5	< 0.001
Profile of Mood States							
Composed–anxious							
Control (*n* = 25)	26.6 (6.9)	29.1 (6.1)	27.5 (5.6)	27.1 (6.3)			
Unmedicated bulimic (*n* = 25)	22.8 (8.3)	24.9 (6.9)	23.7 (8.8)	23.6 (6.9)			
Medicated bulimic (*n* = 12)	25.7 (6.2)	26.0 (6.2)	25.0 (6.1)	21.8 (7.1)			
Elated–depressed							
Control (*n* = 25)	23.8 (4.8)	23.2 (5.5)	24.1 (5.1)	21.6 (4.8)			
Unmedicated bulimic (*n* = 25)	17.5 (7.6)	18.1 (6.9)	19.6 (7.7)	18.4 (7.2)			
Medicated bulimic (*n* = 12)	17.5 (6.0)	17.7 (7.3)	16.4 (7.0)	14.5 (5.7)			
Energetic–tired							
Control (*n* = 25)	17.8 (6.3)	15.5 (8.8)	17.2 (8.3)	13.7 (8.8)			
Unmedicated bulimic (*n* = 25)	14.2 (8.50)	13.2 (8.3)	13.6 (7.9)	13.6 (8.6)			
Medicated bulimic (*n* = 12)	9.5 (5.1)	11.6 (8.8)	12.5 (7.1)	8.8 (5.5)			
Agreeable–hostile							
Control (*n* = 25)	28.2 (4.69)	27.7 (4.8)	28.3 (5.4)	26.5 (5.0)			
Unmedicated bulimic (*n* = 25)	25.0 (5.58)	24.3 (5.9)	25.8 (6.4)	23.0 (6.9)			
Medicated bulimic (*n* = 12)	25.2 (5.08)	24.8 (7.3)	24.8 (5.7)	21.1 (6.6)			
Confident–unsure							
Control (*n* = 25)	22.5 (5.17)	22.6 (5.5)	23.1 (5.5)	21.3 (6.1)			
Unmedicated bulimic (*n* = 25)	17.2 (7.35)	16.8 (7.6)	16.7 (8.3)	16.8 (7.3)			
Medicated bulimic (*n* = 12)	13.8 (6.06)	14.8 (7.8)	14.1 (5.2)	11.6 (6.2)			
Clearheaded–confused							
Control (*n* = 25)	26.4 (6.40)	24.8 (6.1)	27.5 (5.0)	24.0 (6.9)			
Unmedicated bulimic (*n* = 25)	23.2 (6.26)	21.0 (8.0)	23.7 (7.2)	21.5 (6.9)			
Medicated bulimic (*n* = 12)	19.8 (7.15)	20.1 (6.2)	21.8 (7.1)	17.3 (7.3)			
Total (*n* = 62)	20.9 (5.48)	20.9 (5.9)	21.4 (5.9)	19.2*† (6.1)	C × T	$F_{1–59}$ = 4.00	0.05
Urge to binge							
Control (*n* = 25)	0.24 (0.72)	0.76 (1.83)	0.32 (0.80)	0.32 (1.14)			
Unmedicated bulimic (*n* = 26)	3.23‡ (3.28)	2.77‡ (3.39)	2.85‡ (3.23)	2.62‡ (3.21)			
Medicated bulimic (*n* = 12)	3.00‡ (3.44)	1.67 (2.06)	1.83 (2.48)	3.58*†‡ (4.06)	C × T × G	$F_{2–60}$ = 4.59	0.014
Urge to purge by vomiting							
Control (*n* = 24)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.04 (0.20)			
Unmedicated bulimic (*n* = 26)	1.35 (2.58)	0.77 (2.29)	1.27 (2.63)	0.65 (1.67)			
Medicated bulimic (*n* = 13)	0.54 (1.94)	0.08 (0.28)	0.38 (1.39)	0.38 (1.39)	C × T × G	$F_{2–60}$ = 0.30	0.74

C = condition; G = group; SD = standard deviation; T = time.
*Significant pairwise difference v. before administration of amino acid mixture.
†Significant pairwise difference v. after administration of amino acid mixture in balanced condition.
‡Significant pairwise difference within–column v. control.

owing to group differences in age and BMI, we reran the 3 group analyses on "urge to binge" using age and BMI as covariates; the covariate effects did not reach significance and the condition × time × group interaction remained significant. In addition, to address potential problems related to homogeneity of error variance (i.e., small variance in the control group), we reran the analysis on "urge to binge" using only the 2 bulimic groups (i.e., excluding the controls); the condition × time × group effect remained significant and, as in the preceding analysis, only the medicated bulimic group experienced an increased urge to binge in the T– condition. Finally, owing to the deviation from normality of the distributions for the urge to binge, we also conducted a nonparametric group comparison. To do so, we calculated the percentage of participants within each group whose urge to binge increased (v. remained unchanged or decreased) in the balanced condition and again in the tryptophan depletion condition. The numbers and percentages of participants whose urge to binge increased (from pre- to postadministration) in the balanced condition were 4 (16.0%) for the control group, 7 (26.9%) for the unmedicated bulimic group and 2 (of 12; 16.7%) for the medicated bulimic group. There were no significant differences among the 3 groups on percentage of individuals who experienced an increased urge to binge in the balanced condition ($\chi^2_2 = 1.07$, $p = 0.58$). In contrast, the numbers and percentages of participants whose urge to binge increased (from pre- to postadministration) in the tryptophan depletion condition were 2 (8.0%) for the control group, 7 (26.9%) for the unmedicated bulimic group and 7 (of 13; 53.8%) for the medicated bulimic group, indicating significantly different percentages of individuals among the 3 groups who experienced an increased urge to binge eat ($\chi^2_2 = 9.67$ $p = 0.008$) and a significant pairwise difference (v. control) for the medicated bulimic group ($\chi^2_1 = 9.95$, $p = 0.002$) but not for the unmedicated bulimic group ($\chi^2_1 = 0.90$, $p = 0.35$). We interpret such results as being generally consistent with the ANOVA results presented previously and as indicating that the medicated bulimic group (but not the unmedicated group) was associated with an increased urge to binge eat following acute tryptophan depletion.

Discussion

Bulimic and nonbulimic women reported on psychopathological symptoms and participated in an ATD procedure (in which we measured biochemical and subjective responses). A first set of findings was that, compared with normal-eating controls, individuals with bulimia reported more current depression symptoms and impulsiveness. Such results are consistent with previous reports that suggest stable, trait-linked alterations in mood and impulsiveness in individuals with bulimia.[1,4,5]

Second, we found that ATD significantly lowered tryptophan levels and reduced mood in bulimic and nonbulimic women alike (i.e., without any evidence of differences in sensitivity to the ATD procedure linked to diagnosis or medication). Such a finding is generally consistent with previous reports showing that ATD induces comparable biochemical and mood reductions in both healthy and bulimic individuals.[12–14] Other studies with ATD have also found a similar lowering of mood in healthy women.[33] An implication of the preceding may be that the role played by serotonin in regulating mood in women with bulimia does not differ markedly from the role it plays in healthy women. In other words, lowering serotonin availability worsened mood about equally in both groups. Further, the lowering of mood was not prevented by treatment with serotonin reuptake inhibitors in participants with bulimia. Such a result is consistent with the finding that formerly depressed individuals using serotonin reuptake inhibitors are not buffered from the mood-lowering effects of ATD.[19]

A third finding was that the medicated bulimic group (but not the control or unmedicated bulimic group) experienced increased urges to binge eat following ATD. Thus, whereas medication did not affect ATD-induced lowering of mood, it did seem to alter ATD-induced alterations in the urge to binge eat among bulimic participants. The preceding effect suggests that the medicated bulimic group was more sensitive than were other groups to the effects of acute serotonin depletion on subjective urge to binge. The finding of increased urge to binge in currently medicated active bulimic participants mirrors findings from other areas of research showing greater subjective effects of ATD in remitted clinical participants taking serotonin reuptake inhibitors.[17–22] To our knowledge, this is the first report dealing specifically with a group of actively bulimic participants using serotonin reuptake inhibitors at the time of the ATD procedure. We offer 2 possible interpretations for the effect we observed in this group of patients. First, if the group that received antidepressants had a more severe disorder, this could account for both their treatment with antidepressants and the exacerbation of the urge to binge after ATD. Second, as the effect on urges to binge occurred more strongly in medicated than in unmedicated bulimic individuals, one could infer that the effect depended on ATD-induced competition with the therapeutic effects of serotonergic medications on a bulimia-relevant cognitive state — such that we observed a transient, ATD-induced cancellation of a reduction in the urge to binge reduced by serotonin reuptake inhibitors. The preceding speculation is based on the assumption that ATD antagonizes serotonin function[33] whereas serotonin reuptake inhibitors enhance serotonin function.[34] Although the preceding explanation is plausible, we offer it with some reserve, as the effect to which it refers occurs in only a small group of medicated bulimic patients (in whom medication was not randomly applied).

Limitations

We note some limitations of our study. We were unable to collect information on past medication use and on the length of time participants had been using their current medications. The effect of past medication use therefore cannot be eliminated as a potential confound. Further, we note the limited sample size, particularly of the medicated bulimic group, which may jeopardize the stability of our findings.

Conclusion

In summary, we replicate biochemical and mood responses

to ATD reported elsewhere in bulimic and nonbulimic women. We also observe an increased subjective urge to binge following ATD in bulimic participants using serotonergic medications. With reserve, we suggest that the latter effect may be attributable to greater disorder severity in the medicated group or an ATD-induced competition with the therapeutic effects of serotonergic medications on bulimia-relevant impulses or cognitions. Finally, the results may have some clinical management implications. For example, the effects of serotonergic medication on urges to binge may be influenced by the availability of tryptophan. Since tryptophan comes from dietary sources, bulimic individuals who have access to appropriate nutritional rehabilitation and psychotherapy support necessary to normalize eating behaviours may experience a larger effect of medication on reducing urges to binge.

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References

1. Steiger H. Eating disorders and the serotonin connection: state, trait and developmental effects. *J Psychiatry Neurosci* 2004;29:20-9.
2. Kaye WH, Greeno CG, Moss H, et al. Alterations in serotonin activity and psychiatric symptoms after recovery from bulimia nervosa. *Arch Gen Psychiatry* 1998;55:927-35.
3. Kaye WH, Frank GK, Meltzer CC, et al. Altered serotonin 2A receptor activity in women who have recovered from bulimia nervosa. *Am J Psychiatry* 2001;158:1152-5.
4. Steiger H, Richardson J, Israel M, et al. Reduced density of platelet-binding sites for 3H-paroxetine in remitted bulimic women. *Neuropsychopharmacology* 2005;30:1028-32.
5. Steiger H, Gauvin L, Joober R, et al. Intrafamilial correspondences on platelet 3H-paroxetine-binding indices in bulimic probands and their unaffected first-degree relatives. *Neuropsychopharmacology* 2006;31:1785-92.
6. Wolfe BE, Metzger ED, Levine JM, et al. Serotonin function following remission from bulimia nervosa. *Neuropsychopharmacology* 2000;22:257-63.
7. Moore P, Landolt HP, Seifritz E, et al. Clinical and physiological consequences of rapid tryptophan depletion. *Neuropsychopharmacology* 2000;23:601-22.
8. Young SN, Smith SE, Pihl RO, et al. Tryptophan depletion causes a rapid lowering of mood in normal males. *Psychopharmacology (Berl)* 1985;87:173-7.
9. Moja EA, Restani P, Corsini E, et al. Cycloheximide blocks the fall of plasma and tissue tryptophan levels after tryptophan-free amino acid mixtures. *Life Sci* 1991;49:1121-8.
10. Gessa GL, Biggio G, Fadda F, et al. Effect of oral administration of tryptophan-free amino acid mixtures on serum tryptophan, brain tryptophan and serotonin metabolism. *J Neurochem* 1974;22:869-70.
11. Nishizawa S, Benkelfat C, Young SN, et al. Differences between males and females in rates of serotonin synthesis in human brain. *Proc Natl Acad Sci U S A* 1997;94:5308-13.
12. Kaye WH, Gendall KA, Fernstrom MH, et al. Effects of acute tryptophan depletion on mood in bulimia nervosa. *Biol Psychiatry* 2000;47:151-7.
13. Weltzin TE, Fernstrom JD, McConaha C, et al. Acute tryptophan depletion in bulimia: effects on large neutral amino acids. *Biol Psychiatry* 1994;35:388-97.
14. Weltzin TE, Fernstrom MH, Fernstrom JD, et al. Acute tryptophan depletion and increased food intake and irritability in bulimia nervosa. *Am J Psychiatry* 1995;152:1668-71.
15. Smith KA, Fairburn CG, Cowen PJ. Symptomatic relapse in bulimia nervosa following acute tryptophan depletion. *Arch Gen Psychiatry* 1999;56:171-6.
16. Oldman A, Walsh A, Salkovskis P, et al. Biochemical and behavioural effects of acute tryptophan depletion in abstinent bulimic subjects: a pilot study. *Psychol Med* 1995;25:995-1001.
17. Bell CJ, Hood SD, Nutt DJ. Acute tryptophan depletion. Part II: clinical effects and implications. *Aust N Z J Psychiatry* 2005;39:565-74.
18. Delgado PL, Charney DS, Price LH, et al. Serotonin function and the mechanism of antidepressant action: reversal of antidepressant-induced remission by rapid depletion of plasma tryptophan. *Arch Gen Psychiatry* 1990;47:411-8.
19. Delgado PL, Miller HL, Salomon RM, et al. Tryptophan-depletion challenge in depressed patients treated with desipramine or fluoxetine: implications for the role of serotonin in the mechanism of antidepressant action. *Biol Psychiatry* 1999;46:212-20.
20. Spillmann MK, Van der Does AJ, Rankin MA, et al. Tryptophan depletion in SSRI-recovered depressed outpatients. *Psychopharmacology (Berl)* 2001;155:123-7.
21. Argyropoulos SV, Hood SD, Adrover M, et al. Tryptophan depletion reverses the therapeutic effect of selective serotonin reuptake inhibitors in social anxiety disorder. *Biol Psychiatry* 2004;56:503-9.
22. Davies SJ, Hood SD, Argyropoulos SV, et al. Depleting serotonin enhances both cardiovascular and psychological stress reactivity in recovered patients with anxiety disorders. *J Clin Psychopharmacol* 2006;26:414-8.
23. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 4th ed., text revised. Washington: The Association; 2000.
24. Fairburn C, Cooper P. The eating disorders examination. 12th ed. In: Fairburn C, Wilson G, editors. *Binge eating: nature, assessment and treatment*. New York (NY): Guilford Press; 1993. p. 317-60.
25. First MB, Spitzer R, Gibbon M, et al. *Structured clinical interview for DSM-IV-TR axis I disorders, research version, patient edition*. New York (NY): Biometrics Research, New York State Psychiatric Institute; 1997.
26. Zanarini MC, Skodol AE, Bender D, et al. The Collaborative Longitudinal Personality Disorders Study: reliability of axis I and II diagnoses. *J Pers Disord* 2000;14:291-9.
27. Weissman MM, Sholomskas D, Pottenger M, et al. Assessing depressive symptoms in five psychiatric populations: a validation study. *Am J Epidemiol* 1977;106:203-14.
28. Patton JH, Stanford MS, Barratt ES. Factor structure of the Barratt Impulsiveness Scale. *J Clin Psychol* 1995;51:768-74.
29. McNair DM, Lorr M, Droppleman LF. Manual for the Profile of Mood States. San Diego (CA): Educational and Industrial Testing Servicel; 1988.
30. Carpenter LL, Anderson GM, Pelton GH, et al. Tryptophan depletion during continuous CSF sampling in healthy human subjects. *Neuropsychopharmacology* 1998;19:26-35.
31. Coccaro EF, Kavoussi RJ, Cooper TB, et al. Acute tryptophan depletion attenuates the prolactin response to D-fenfluramine challenge in healthy human subjects. *Psychopharmacology (Berl)* 1998;138:9-15.
32. Benkelfat C, Ellenbogen MA, Dean P, et al. Mood lowering effect of tryptophan depletion: enhanced susceptibility in young men at genetic risk for major affective disorders. *Arch Gen Psychiatry* 1994;51:687-97.
33. Young SN, Leyton M. The role of serotonin in human mood and social interaction: Insight from altered tryptophan levels. *Pharmacol Biochem Behav* 2002;71:857-65.
34. Anderson GM. Peripheral and central neurochemical effects of the selective serotonin reuptake inhibitors (SSRIs) in humans and nonhuman primates: assessing bioeffect and mechanisms of action. *Int J Dev Neurosci* 2004;22:397-404.