Effects of Acute Tryptophan Depletion on Mood in Bulimia Nervosa

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Background: The present study investigated the role of serotonin in the pathophysiology of bulimia nervosa (BN) by studying the affective and appetitive responses of women ill with BN to an acute tryptophan depletion (ATD) paradigm.

Methods: Twenty-two women with BN and 16 healthy control women (CW) were studied on 2 separate days during the follicular stage of the menstrual cycle. Participants drank a control mix of essential amino acids (100 g + 4.6 g tryptophan) on one day and a tryptophan deficient (100 g − 4.6 g tryptophan) mixture (ATD) on the other in a double-blind fashion. Mood/appetite ratings and blood samples were taken at baseline and at intervals up to 420 minutes. Participants were then presented with an array of foods and were allowed to binge and vomit if they desired.

Results: CW and BN women had a similar and significant reduction in plasma tryptophan levels and the tryptophan:LNAA ratio after ATD. After ATD, the BN women had a significantly greater increase in peak (minus baseline) depression, mood lability, sadness and desire to binge compared to the CW. BN subjects and CW had similar peak changes in mood after the control amino acid mixture. BN subjects and CW consumed similar amounts of food after the two amino acid treatments.

Conclusions: Women with BN seem more vulnerable to the mood lowering effects of ATD, suggesting they have altered modulation of central 5-HT neuronal systems.

Bulimia nervosa (BN) is a disorder that most commonly occurs in adolescent women who are of normal body weight (American Psychiatric Association 1994). BN is characterized by binge eating, purging, and a distorted body image, and is often associated with neuroendocrine abnormalities (Gwirtsman et al 1983) as well as comorbid depression, anxiety and obsessions (Lilenfeld et al 1998).

The pathophysiology of BN is poorly understood. One hypothesis postulated that bingeing behavior is precipitated by a reduction in serotonin (5-HT) activity in the brain (Jimerson et al 1990; Kaye et al 1988). This notion is based on several types of experimental evidence. First, in general, reducing transmission across brain 5-HT synapses by pharmacologic means is known to stimulate food intake (Blundell 1984). Second, several studies support the possibility that ill BN women have reduced 5-HT activity as suggested by blunting of prolactin response after drugs with serotonergic actions (Brewerton et al 1992; Goldbloom et al 1990; Jimerson et al 1997; Levitan et al 1997). Whereas one study found BN subjects had normal levels of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid (Kaye et al 1990), another study found this metabolite was low in subjects who binged more than twice daily (Jimerson et al 1992). Third, numerous double-blind, placebo controlled studies have found that a wide range of antidepressant medications are effective in reducing binge and purge frequency (Mitchell et al 1993; Walsh 1991). It is important to note that antidepressants with noradrenergic activity also are efficacious in BN suggesting that other neurotransmitter systems may also be involved.

The development of the acute tryptophan depletion (ATD) paradigm is of interest because this method has been reputed to transiently reduce brain 5-HT synthesis and release in humans (Delgado et al 1990; Young et al 1985). The synthesis of 5-HT depends on the availability of its amino acid precursor, tryptophan. Tryptophan is transported across the blood brain barrier by a competitive transport mechanism and is converted to 5-HT by trypto-
phen hydroxylase. Because tryptophan hydroxylase is normally only half saturated with its substrate, manipulations that lower the supply of tryptophan to the brain reliably reduce 5-HT synthesis (Biggio et al 1974; Fernstrom and Wurtman 1971; Moja et al 1989; Young et al 1989). The ATD paradigm has been used to manipulate 5-HT production through the ingestion of a tryptophan deficient amino acid mixture or diet (Young 1991). This technique relies on the occurrence of protein synthesis; normally when a protein meal is ingested, a large proportion of the digested large neutral amino acids (LNAA) are synthesized into labile protein stores in the muscle and liver. When a tryptophan deficient protein mixture is ingested, tryptophan is drawn from the circulation, enabling synthesis of labile protein stores to continue with the net result being a reduced plasma tryptophan:LNAA ratio. The tryptophan depletion technique can lower plasma tryptophan by 70% to 80% after 5 hours (Young et al 1985). It has been estimated that such a reduction in plasma tryptophan can reduce central 5-HT levels by 60% to 70% (Moja et al 1989). The behavioral effects of ATD include the instigation of depressive symptoms in normal (Smith et al 1997; Young et al 1985), remitted depressed (Delgado et al 1990), and obsessive-compulsive (Barr et al 1994) individuals. In addition, recent findings suggest that women may be more susceptible to the mood lowering effects of ATD than men (Smith et al 1997). Fewer studies have measured the effects of ATD on appetite; no changes were found in healthy women in a study by Oldman et al (1994), whereas Young et al (1985) found a decline in protein intake after ATD.

We hypothesized that the ATD experimental treatment should precipitate or facilitate bingeing and dysphoric mood in BN women. Previous studies from our group found that ATD increased irritability, labile mood, retarded affect, and caloric intake in BN women (Weltzin et al 1994a, 1995). By contrast, Oldman et al (1995) found no significant effects of tryptophan depletion on mood, appetite, or food intake in women with BN who were abstinent from binge eating and purging. The purpose of this study was to replicate and extend our initial findings in a larger sample of BN and healthy control women.

**Methods and Materials**

**Participants**

Twenty-two women who met criteria for DSM-IV (American Psychiatric Association 1994) BN were recruited from the eating disorders unit of Western Psychiatric Institute and Clinic for this study. For comparison purposes, 16 healthy control women (CW) matched to the BN women for age and weight, were recruited from the staff of Western Psychiatric Institute and Clinic and through advertisements. These subjects were a combination of those previously reported and new subjects. Results from 10 BN and 10 CW have been previously reported (Weltzin et al 1995). This study reports on an additional 12 BN and 6 CW subjects. All participants provided written informed consent before commencement of the study, and the study obtained approval from the University Institutional Review Board on the Use of Humans as Experimental Subjects. Depression was measured using the Beck Depression Inventory (Beck et al 1961). BN women were medication free for at least 4 weeks before the study.

**ATD Procedure**

Participants were studied within the first 10 days after the onset of their most recent menstrual period. Each subject was studied during a sequential two-day period on 2 separate occasions. On the first day, subjects ate standardized meals consisting of 1500 calories (kcal) (27 g protein and 164 mg tryptophan). No food was consumed after 9:00 PM. On the second day, an indwelling venous catheter was inserted into the antecubital vein at 6:30 AM. At 8:00 AM, participants drank an amino acid (AA) mixture within 5 minutes. Blood samples were drawn at 7:30 AM (baseline) and 1, 2, 4, and 7 hours after ingestion of the AA mixture. The subjects were allowed water but no food until 3:00 PM.

**Psychological Testing**

Psychological state was assessed at baseline and 2, 4, and 7 hours after ingestion of the AA mixtures using ratings adapted from those previously described (Asberg et al 1978; Murphy et al 1982; Overall et al 1962). Ratings were conducted by two Psychiatrists (TEW and WHK) who were blind to the treatment condition. At each time-point, using a 6-point scale, a number of psychological variables were assessed including: anxiety, depression, mood lability, obsessionality, irritability, and desire to overeat and purge. Peak changes in mood ratings (peak change from baseline at 4 or 7 hours after AA ingestion) were compared for each individual mood rating.

**Assessment of Feeding**

At 1500 hours, subjects were presented with food choices to assess the effects of ATD on feeding behavior. The original subjects (10 BN and 10 CW) previously reported by Weltzin et al (1995) selected their own food from a vending apparatus in the Human Feeding Laboratory (HFL) (Weltzin et al 1991). The vending apparatus in the HFL contained 38 different food and drink items. The food items represented typical binge and non binge foods and are described in detail elsewhere (Kaye et al 1992). Food items were weighed before dispensing so that the amount of calories and macronutrients consumed could be calculated from the uneaten food. The vending machine could be restocked so that a virtually unlimited amount of food was available. The participants were instructed to select as much of any of the foods they wanted and were allowed to binge and vomit if they so desired.

The new 12 BN and 6 CW subjects were given similar
instructions to those above, but did not use the HFL procedure. Rather, they were presented with a selection of packaged foods, on a tray, consisting of a wide range of normal meal foods (chicken, beef, cottage cheese, yogurt, bread, vegetables, fruit, beverages) and items that are typically preferred during binge eating episodes such as cookies, chocolate, chips, and ice cream (Hadigan et al 1989). Again, food items were weighed before being given to subjects so that the amount of calories and macronutrients consumed could be calculated from the uneaten food. The total energy content of the food provided on the trays was set at 3705 kilocalories.

**AA Mixtures**

Using a counterbalanced, double-blind design, two AA mixtures were administered. Both AA mixtures were formulated in our laboratory using purified AA (Ajinomoto USA, Teaneck NJ) by a method reported in detail elsewhere (Weltzin et al 1994a). Each mixture contained a total of 100 g of the following essential and nonessential AA: L-alanine; L-arginine; L-cysteine; L-glycine; L-histidine; L-isoleucine; L-leucine; lysine; L-methionine; L-phenylalanine; L-proline; L-serine; L-threonine; L-tyrosine; and L-valine. The ATD mixture did not contain tryptophan (the 0 g tryptophan mixture or “active” mixture), whereas the control mixture did contain tryptophan (the 4.6 g tryptophan mixture or “control mixture”). The addition of 4.6 g of tryptophan has previously been shown to prevent a reduction in the plasma tryptophan:LNAA ratio (Weltzin et al 1994a).

**Plasma AA Measurements**

Total plasma tryptophan concentrations were quantified fluorometrically (Denckla et al 1967). The plasma levels of other LNAAs (phenylalanine, tyrosine, leucine, isoleucine and valine) were measured using a Beckman Model 63000 AA analyzer, equipped with a fluorometer to detect O-phthalaldehyde-derivatized AA. Plasma samples were precipitated with sulfosalicylic acid (50 mg/ml plasma), and the supernatant passed through 0.22 mm centrifuge filters (Microfilteruge Tubes, Rainin Instruments, Woburn, MA). Aminooethyl cysteine was added as an internal standard before sample precipitation (Fernstrom et al 1987). Mean plasma tryptophan:LNAA ratio responses after the control and the deficient amino acid drinks were calculated from the sum of measurements taken at 1, 2, 4, and 7 hours. The change in the tryptophan:LNAA ratio was calculated by subtracting the baseline ratio from the calculated mean plasma tryptophan:LNAA response.

**Data Analysis and Statistics**

Analyses were conducted both within and between groups. Group comparisons for age and weight were done using group t-tests. Plasma tryptophan levels and the ratio of tryptophan to the sum of LNAA (Tryptophan: LNAA ratio) were analyzed by repeated measures ANOVA and Tukey’s post hoc t tests. Comparisons of plasma tryptophan and tryptophan:LNAA ratios, after AA ingestion, to those at baseline were done using Newman–Keuls (NK) multiple comparison procedure. Because of a lack of normal distributions, feeding and mood data were analyzed using nonparametric procedures for paired (Wilcoxon signed ranks test) and group comparisons (Mann–Whitney U test). Regression analyses were undertaken to determine if changes in mood and appetite were associated with BECK scores. Correlational analyses were used to examine any relationships between changes in tryptophan:LNAA ratios and changes in mood variables. All the data are presented as mean ± standard deviation.

**Results**

**Participant Characteristics**

There were no significant differences between the two groups for most demographic variables (Table 1). The BN women were slightly, but not significantly, younger than the CW women, and there was a trend for the BN group to have a greater percent high lifetime average weight. The BN women had significantly elevated levels of depression compared to the control women.

**Amino Acid Patterns and Mood and Behavior Ratings**

Baseline levels of tryptophan and the tryptophan:LNAA ratios were similar in CW and BN subjects (see Table 2).
Similarly, amino acid levels after both interventions were similar for the CW and BN groups. In both CW and BN groups, ATD produced a significant decline in plasma tryptophan concentrations (58% and 55%, respectively) and in the plasma tryptophan:LNAA ratio compared with the ingestion of a control amino acid mixture (85% and 92%, respectively).

At baseline, the BN women had significantly higher ratings of anxiety, depression, and obsessionality than the controls (Table 2). For mood levels after both interventions, baseline values were subtracted from peak values (change score). Compared to their response to the control amino acid mixture, BN women had significantly higher levels of mood lability, irritability, and desire to purge after ATD. By contrast, CW only had a significant increase in irritability after ATD as compared to the control amino acid mixture.

After ATD, the BN women showed significantly elevated changes in depression, mood lability, and desire to overeat and purge compared to the CW women. There were no significant differences in mood changes between BN and CW women after the control amino acid mixture.

Regression analyses indicated that changes in mood and appetite ratings after ATD were not associated with the higher Beck Depression Inventory scores in the BN women compared to the CW women (F = 0.24, p = .63). Correlational analyses also failed to reveal significant relationships between peak changes in tryptophan:LNAA ratios and peak changes in any of the mood variables in the CW or in the BN groups.

**Test Meal Intake**

There were no significant differences in the intake of calories or the intake of any macro nutrients between the two amino acid treatments for either group.

**Discussion**

This paper confirms and expands previous reports (Weltzin et al 1994b, 1995) that found that ATD increased negative mood states in BN women compared to healthy controls. Specifically, we found increased susceptibility to depression, mood liability, and desire to overeat and purge after ATD in women with BN compared to controls. These data suggest that women with BN are vulnerable to the mood lowering effects of tryptophan depletion. This corroborates results from other studies that have shown that individuals predisposed to other, possibly serotonergically-mediated psychiatric disorders are particularly susceptible to the depressant effects of tryptophan depletion (Benkelfat et al 1994; Moreno et al 1995; Young et al 1985).

We found that ATD produced mild irritability in healthy CW. Other investigators have also found that the ATD paradigm can have mild mood-lowering effects in healthy individuals who do not have psychiatric diagnoses (Barr et al 1997; Smith et al 1987, 1997; Young et al 1985). Studies that have not found this have used a relatively low dose of amino acids (Delgado et al 1989; Moreno et al 1995; Oldman et al 1994; Saloman et al 1997) or demonstrated differential effects in males and females (Ellenbogan et al 1996). ATD was associated with an increased desire to overeat and purge in the BN women. Despite these self reports, food intake was not increased in the BN women after ATD.

Differences in the test meal presentation may explain discrepant findings between this current study and our previous report (Weltzin et al 1995), that did find increased food intake after ATD. In the current investigation, presentation of a set selection of foods on a tray may have been more inhibiting than the previously used HFL procedure that permitted access to a virtually unlimited

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**Table 2. Plasma Amino Acids and Mood Ratings: Absolute Mean Baseline Measurements and Peak Changes after a Control Amino Acid Mixture and after Acute Tryptophan Depletion (ATD)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control women (n = 16)</th>
<th>Women with bulimia nervosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Control</td>
</tr>
<tr>
<td><strong>Plasma amino acids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tryptophan (nmol/ml)</td>
<td>60 ± 13</td>
<td>134 ± 62</td>
</tr>
<tr>
<td>Tryptophan:LNAA ratio</td>
<td>0.13 ± 0.03</td>
<td>+0.03 ± 0.05</td>
</tr>
<tr>
<td><strong>Mood states</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxious</td>
<td>0.5 ± 0.7</td>
<td>−0.5 ± 0.8</td>
</tr>
<tr>
<td>Depressed</td>
<td>0.1 ± 0.5</td>
<td>+0.3 ± 0.9</td>
</tr>
<tr>
<td>Mood liability</td>
<td>0 ± 0</td>
<td>+0.1 ± 0.5</td>
</tr>
<tr>
<td>Obsessional</td>
<td>0 ± 0</td>
<td>+0.2 ± 0.5</td>
</tr>
<tr>
<td>Irritable</td>
<td>0.3 ± 0.6</td>
<td>−0.06 ± 1.1</td>
</tr>
<tr>
<td>Desire to overeat</td>
<td>0 ± 0</td>
<td>+0.3 ± 0.7</td>
</tr>
<tr>
<td>Desire to purge</td>
<td>11.2 ± 24.2</td>
<td>+7.2 ± 11.8</td>
</tr>
</tbody>
</table>

Values are mean ± SD. In Control and ATD columns, + indicates an increase compared to baseline and − indicates a decrease compared to baseline. For between group comparisons (CW vs. BN Women), group t comparing the same condition: *p < .05, †p < .01, ‡p < .001. For within group comparisons (control vs. ATD), paired t: •p < .05, •p < .001.
amount of food. It should also be noted that the small proportion of patients that completed the test-meal procedure may have limited the ability to detect changes in food intake, that tend to be modest after ATD (Oldman et al 1995; Young et al 1988). Alternatively, although reduced 5-HT activity may contribute to negative affect and desire to eat in BN women, it may not in itself be sufficient to precipitate binge eating. The presence of certain other mediating circumstances, such as a high degree of food deprivation, distorted cognitions or perceived negative experiences of the self or of interpersonal interactions (Heatherton 1991; Powell et al 1996), may be necessary to mediate binge eating in the context of reduced 5-HT activity. In addition, the laboratory setting may have been inhibiting for some subjects.

What mechanisms could explain the sensitivity of BN patients to tryptophan depletion? It is thought that aberrations in nutritional status (Laessle et al 1988) or dieting can reduce tryptophan availability (Anderson et al 1990; Goodwin et al 1990; Walsh et al 1995; Weltzin et al 1995; Wolfe et al 1997). Baseline plasma tryptophan levels and tryptophan ratios were similar between eating disordered women and controls. Thus, it is unlikely that reduced availability of tryptophan at baseline for conversion to 5-HT accounted for our findings. Still, it is possible that there were differences in bioavailable tryptophan stores that may not have been evident in plasma.

Several studies have shown that women with BN have an altered neuroendocrine response to 5-HT agents (Brewerton et al 1992; Goldbloom et al 1990; Halmi et al 1993; Jimerson et al 1997; Levitan et al 1997; McBride et al 1992). Our group (Kaye et al 1998) has found that 5-HT abnormalities persist after recovery from BN. In addition, a recent study showed that a dysphoric response to ATD persisted in remitted BN women (Smith et al 1999). Together these studies suggest that a disturbance of 5-HT neuronal function may be trait-related and contribute to the pathogenesis of BN. We hypothesize that the 5-HT neuronal system in individuals with BN is inherently unstable and poorly modulated. This instability results in the 5-HT system fluctuating erratically rather than having a more precise compensation that buffers the effects of diet or stress as is found in the control women. Dieting (or ATD) may then cause a reduction of 5-HT signal transmission in BN subjects that, in turn, may contribute to binge eating and dysphoric mood (Jimerson et al 1992; Kaye et al 1988). In line with our theory, Jimerson et al (1992) have proposed a model in which individuals with BN may binge eat as a means of self-modulating 5-HT activity. In brief, binge episodes may increase the tryptophan:LNAA ratio in plasma, that in turn increases tryptophan availability to the brain, resulting in increased 5-HT synthesis and release (Fernstrom et al 1971, 1972, 1978). Thus, individuals with BN may use dieting and binge eating to modulate an unstable 5-HT system. In summary, people with an inherent modulatory defect in 5-HT function may be prone to develop an eating disorder. Because of their modulatory 5-HT defect, they cannot respond appropriately and precisely to stress or stimuli, or modulate their affective states. They may learn that extremes of dietary intake, by effects on plasma TRP, are a means by which they can crudely modulate their brain 5-HT functional activity.

Overall, the present study adds to the expanding body of evidence suggesting that aberrations in serotonergic modulation exist in individuals with BN. The tryptophan depletion paradigm demonstrates that the mood of BN women is sensitive to acute changes in 5-HT activity. Further investigations using larger samples may be required to elucidate the definitive effects of tryptophan depletion on appetite and food intake in eating disordered women.

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References


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