Anxiolytic Effects of Acute Tryptophan Depletion in Anorexia Nervosa

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Abstract: **Objective:** *Recent studies have raised the question as to whether a dysregulation of the neurotransmitter serotonin may contribute to the alterations in mood seen in anorexia nervosa (AN). People with AN tend to be anxious, obsessional, perfectionistic, and harm avoidant. These traits are premorbid and persist after recovery. It has been suggested that increased activity of brain serotonin systems could contribute to this pathologic condition. Dieting in AN, which serves to reduce plasma levels of tryptophan (TRP), may serve to reduce symptoms of dysphoric mood.* **Method:** *Fourteen women currently symptomatic with AN (ILL AN), 14 women recovered from AN (REC AN), and 15 healthy control women (CW) underwent acute tryptophan depletion (ATD). Measures of psychological state were self-assessed at baseline and hourly after ATD to determine whether ATD would reduce negative mood.* **Results:** *ILL AN and REC AN had significantly higher mean baseline TRP/LNAA (tryptophan/large neutral amino acids) ratios compared with CW. In contrast to placebo, the ATD challenge demonstrated a significantly greater reduction in the TRP/LNAA ratio for ILL AN (−95%) and REC AN (−84%) compared with CW (−70%). Both the ILL AN and REC AN had a significant reduction in anxiety on the ATD day compared with the placebo day.* **Discussion:** *These data demonstrate that a dietary-induced reduction of TRP, the precursor of serotonin, is associated with decreased anxiety in people with AN. Restricting dietary intake may represent a mechanism through which individuals with AN modulate a dysphoric mood.* © *2003 by Wiley Periodicals,* Inc. Int J Eat Disord 33: 257–267, 2003.

Key words: *anorexia nervosa; tryptophan depletion; eating disorders; serotonin; mood*

INTRODUCTION

Anorexia nervosa (AN) is a disorder of unknown etiology that most commonly occurs in adolescent girls (American Psychiatric Association, 1994). AN is characterized by aberrant patterns of feeding behavior and weight regulation and disturbances in attitudes toward weight and the perception of body shape. People with AN have an

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inexplicable fear of eating and weight gain and an unrelenting obsession with fatness, even in the face of increasing cachexia. These symptoms result in profound weight loss, considerable morbidity, and the highest death rate of any psychiatric disorder (Sullivan, 1995).

Recent studies have raised the question as to whether a disturbance of the serotonin (5-HT) neurotransmitter system could contribute to behavioral alterations in AN. It is well known that brain 5-HT systems contribute to the modulation of appetite (Blundell, 1984; Leibowitz & Alexander, 1998; Wurtman & Wurtman, 1979). An increase of intrasynaptic 5-HT tends to reduce food consumption. Thus, increased brain 5-HT activity could play a role in enhanced satiety in AN. In addition, people with AN tend to be anxious, obsessional, perfectionistic, and harm avoidant. Such traits seem to be *premorbid* and persist after recovery (Bulik, Sullivan, Joyce, & Carter, 1995; Deep, Nagy, Weltzin, Rao, & Kaye, 1995; Kaye, Weltzin, Hsu, Bulik, McConaha, & Sobkiewicz, 1992; Srinivasagam, Kaye, Plotnicov, Greeno, Weltzin, & Rao, 1995; Strober, 1980), suggesting that such behaviors are not just secondary to malnutrition. Increased activity of brain 5-HT systems could contribute to anxious, harm-avoidant, obsessional symptoms (Charney, Woods, Krystal, & Heninger, 1990; Cloninger, 1987; Soubrie, 1986).

Several lines of evidence suggest that individuals who are ill with AN have modulatory defects in brain 5-HT neurotransmission (Brewerton, Brandt, Lessem, Murphy, & Jimerson, 1990; Hadigan, Walsh, Buttinger, & Hollander, 1995; Kaye, Gwirtsman, George, Jimerson, & Ebert, 1988; Monteleone, Brambilla, Bortolotti, La Rocca, & Maj, 1998). Disturbances of 5-HT transmission persist after recovery from AN in some studies (Kaye, Gwirtsman, George, & Ebert, 1991; O'Dwyer, Lucey, & Russell, 1996; Ward, Brown, Lightman, Campbell, & Treasure, 1998), and there is some evidence that people with AN may have altered frequency of gene polymorphisms for 5-HT receptors (Collier, Arranz, Li, Mupita, Brown, & Treasure, 1997; Enoch, Kaye, Rotondo, Greenberg, Murphy, & Goldman, 1998). More recently, our studies, using PET imaging with 5-HT specific radioligands, have shown that recovered AN patients have changes in 5-HT_{1A} and 5-HT_{2A} receptor activity consistent with increased 5-HT activity (Frank, Kaye, Meltzer, Price, Greer, McConaha, & Skovira, 2002; Kaye, G.K., Meltzer, Price, Drevets, & Mathis, Submitted).

Nutritional state also affects serotonin in AN. Underweight, malnourished AN patients have a significant reduction of basal cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5-HIAA; the major 5-HT metabolite) compared with healthy controls (Kaye, Ebert, Raleigh, & Lake, 1984). In contrast, once people are long-term recovered from AN, they have elevations of CSF 5-HIAA (Kaye et al., 1991), suggesting an increase in 5-HT activity persists after recovery. Importantly, these data raise the question of whether starvation produces a reduction of extracellular 5-HT concentrations in AN.

Self-starvation is not conducive to homeostatic adaptation and survival, and, in most people, food restriction is not an inherently reinforcing behavior. However, persistent dieting to the point of starvation in AN raises the speculation that food restriction might have some benefit for people with AN. We hypothesize that people with AN have a trait-related *increase* in 5-HT neuronal transmission that occurs in the premorbid state and persists after recovery. Increased 5-HT neurotransmission in turn contributes to uncomfortable core symptoms such as obsessionality, perfectionism, harm avoidance, and anxiety. We hypothesize that people with AN starve themselves to reduce 5-HT neuronal activity and thus reduce a dysphoric behavioral state.

It is well known that diet can influence brain 5-HT neurotransmission. Tryptophan (TRP), an essential amino acid only available in the diet, is the precursor of 5-HT. Meal consumption, depending on the proportion of carbohydrate and protein, can enhance brain 5-HT release (Fernstrom & Wurtman, 1971; Fernstrom & Wurtman, 1972), thereby

affecting appetite regulation. Carbohydrate consumption causes an insulin-mediated fall in plasma levels of the large neutral amino acids (LNAA; tyrosine; phenylalanine; valine; leucine; isoleucine) that compete with TRP for uptake into the brain. This elevates the plasma tryptophan to large neutral amino acid ratio (TRP/LNAA), and thus brain TRP, which rapidly accelerates brain 5-HT synthesis and release. Dietary proteins tend to block these effects by contributing large amounts of LNAA to the bloodstream. Considerable amounts of evidence from animal and healthy human studies (Anderson, Parry-Billings, Newsholme, Fairburn, & Cowen, 1990; Biggio, Fadda, Fanni, Tagliamonte, & Gessa, 1974; Fernstrom & Wurtman, 1971; Fernstrom & Wurtman, 1972; Gibbons, Barr, Bridger, & Leibowitz, 1979; Messing, Fisher, Phebus, & Lytle, 1976; Young & Gauthier, 1981) show that a restricted diet significantly lowers plasma TRP, resulting in a decreased plasma ratio of TRP to neutral amino acids, and, in turn, a reduction in the availability of TRP to the brain. Thus, restricted diet (and experimentially reduced TRP) decreases brain 5-HT synthesis, down-regulates the density of 5-HT transporters (Huether, Zhou, & Ruther, 1997), and produces a compensatory supersensitivity of postsynaptic receptors in response to reduced 5-HT turnover (Goodwin, Fairburn, & Cowen, 1987). Limited data show that malnourished and emaciated AN women have a reduction of plasma TRP availability (Schweiger, Warnhoff, Pahl, & Pirke, 1986). In addition, these alterations in postmeal amino acid metabolism are only partly reversed by nutritional rehabilitation (Schweiger et al., 1986).

It has been speculated (Vitousek & Manke, 1994; Strober, 1995) that there is an anxiety-reducing character to dietary restraint in people with AN. This study tested the hypothesis that the anxiolytic effects of dieting were relate to a reduction in 5-HT neurotransmission. On one day, subjects were administered an acute tryptophan depletion (ATD), which reduces plasma tryptophan availability (Young, Smith, Pihl, & Ervin, 1985) and decreases brain 5-HT concentrations (Moja, Cipolla, Castoldi, & Tofanetti, 1989). We hypothesized that the ATD challenge would reduce negative mood in AN individuals. On the control day, subjects were given an amino acid mixture that contained TRP. We compared malnourished AN, long-term weight restored AN, and control women.

METHODS

Participants

Fourteen women who were symptomatic with AN (ILL AN) as defined by the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) (American Psychiatric Association, 1994) were recruited from the inpatient eating disorders program of the University of Pittsburgh Medical Center, Western Psychiatric Institute and Clinic for this study. Eight subjects had a history of restricting type AN, four had a history of restricting and vomiting, and two had binge-eating/purging-type AN. Subjects must not have used psychoactive medication such as antidepressants within 4 weeks of the study.

Fourteen women were recruited who had recovered from AN (REC AN). Of this group, 10 subjects had a history of restricting-type AN and four of binge-eating/purging-type AN. Subjects were previously treated in the eating disorders program at the University of Pittsburgh Medical Center, Western Psychiatric Institute and Clinic, or were recruited through advertisements. To be considered ''recovered,'' subjects had to (1) maintain a weight above 85% average body weight (ABW, Metropolitan Life Insurance Company, 1959), (2) have regular menstrual cycles, (3) have not binged, purged, or

engaged in significant restrictive eating patterns for 1 or more years before the study. In addition, subjects must not have used psychoactive medication such as antidepressants and not met criteria for alcohol or drug abuse or dependence, major depressive disorder, or a severe anxiety disorder within 3 months of the study.

Fifteen healthy control women (CW) matched for age were recruited from within the health center and local universities and through advertisements. The CW had no history of an eating disorder or any psychiatric, medical, or neurological illness. They had no first-degree relative with an eating disorder. They had normal menstrual cycles and had been within a normal weight range since menarche. All participants provided written informed consent before commencement of the study, and the study had approval of the University of Pittsburgh's Institutional Review Board.

Procedure

Participants with normal menses were studied within the first 10 days after the onset of their most recent menstrual period. Each subject was studied during a sequential 2-day period on two 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 2100 hours. On the second day, an indwelling venous catheter was inserted into the antecubital vein at 0630. At 0800, participants were orally administered either the control mixture or the ATD mixture within 5 minutes. Blood samples were drawn at 0730 (baseline) and 1, 2, 4, and 7 hours after administration of the test mixtures. The subjects were allowed no food and only water until 1500 hours.

Test Mixtures

Using a randomized double-blind design, two test mixtures were administered. Each test mixture was formulated in our laboratory using purified amino acids (Ajinomoto USA, Teaneck, NJ) by a method reported in detail elsewhere (Weltzin, Fernstrom, McConaha, & Kaye, 1994) and contained a mix of essential and nonessential amino acids. The ATD condition involved administration of 100 g of an amino acid mixture with no added TRP. The control condition involved administration of 100 g of an amino acid mixture that included 4.6 g TRP. The addition of 4.6 g of TRP has previously been shown to prevent a reduction in the plasma TRP/LNAA ratio (Weltzin et al., 1994).

Psychological Testing

Psychological state was self-assessed by subjects at baseline and hourly up to 6 hours after ingestion of the test mixtures using ratings adapted from the National Institute of Mental Health global rating scale (Murphy, Pickar, & Alterman, 1982) for measures of anxiety, depression, irritability, restlessness, and sadness.

Data Analysis and Statistics

Baseline clinical variables and self-assessment mood ratings (Table 1) were compared across the three groups by ANOVA for the 2 days of the study. Because values were similar (data not included), a mean baseline value was calculated and is shown in Table 1. Missing data were estimated for subjects by extrapolation of neighboring points. Subjects who had more than 2% missing data were dropped from analysis.

The change in mood was calculated by subtracting baseline values from each hourly interval measurement for the 2 study days. Within-group mood changes were analyzed

Table 1. Comparison of mean (of the 2 days of the study) baseline clinical variables and self-assessed mood ratings

	ILL AN	REC AN	CW	*F*	*df*	*p*
Number	14	14	15			
Age (yr)	24.7 ± 6.0	23.4 ± 3.7	22.5 ± 3.1	0.89	2,40	0.42
% ABW	69.6 ± 7.8[a,b]	101.5 ± 12.9[a]	105.0 ± 8.1[b]	52.69	2,39	0.001
Age onset	16.8 ± 4.2	15.7 ± 2.0	—	0.74	1,23	0.40
Months of recovery	—	40.9 ± 23.5	—			
BHBA	46.0 ± 57.5	88.7 ± 150.5	44.4 ± 16.0	0.93	2,36	0.41
Estradiol	8.0 ± 5.0[b]	39.8 ± 52.6	57.7 ± 48.9[b]	3.77	2,35	0.03
GSC anxiety	2.2 ± 1.5[a,b]	0.7 ± 0.6[a]	0.3 ± 0.4[b]	15.95	2,40	0.001
GSC depression	1.7 ± 1.4[a,b]	0.3 ± 0.4[a]	0.0 ± 0.1[b]	16.91	2,40	0.001

Same letters denote significant ($p < 0.05$) group differences; data expressed as mean ± SD.

by repeated measures ANOVA (drug × time). All the data are presented as mean ± standard deviation.

Similarly, baseline TRP levels were meaned across study days for comparisons across groups. Tryptophan levels were adjusted for baseline values on each study day for between-group ANOVAs and within-group paired *t* tests.

RESULTS

Participant Characteristics

The CW, REC AN, and ILL AN groups were similar in age (Table 1). ILL AN weighed significantly less than the other groups. The REC AN and ILL AN groups had a similar age of onset of illness. The REC AN group was recovered for a mean of 40.9 ± 23.5 months. All three groups had similar values for plasma *B*-HBA concentrations, a ketone body that is a measure of acute malnutrition. The ILL AN had significantly lower plasma estradiol concentrations than the CW.

ILL AN women had higher baseline self-ratings for anxiety, depression, irritability, and restlessness compared with the REC AN and CW. The REC AN and CW had similar values for baseline behavioral measures.

Plasma Levels of Tryptophan Ratios (e.g., TRP/LNAA) and Plasma TRP

ILL AN and REC AN had significantly higher mean baseline TRP/LNAA ratios compared with the CW (Table 2). However, baseline TRP/LNAA were similar for ILL AN and REC AN. Baseline TRP/LNAA ratios were subtracted from the TRP/LNAA ratios at 360 minutes on the ATD or control day. On the control day, there was no change in TRP/LNAA ratios at 360 minutes for any group, when compared with each other or to baseline. Thus, on the control day, the TRP/LNAA ratio showed a reduction of a range of −0.7% to −7.2% for the three groups. On the ATD day, paired *t* tests for each group showed a highly significant reduction in TRP/LNAA ratios at 360 minutes compared with baseline (ILL AN: $t = 15.845$; $df = 12$; $p < 0.000$; REC AN: $t = 11.02$; $df = 11$; $p > 0.000$; CW: $t = 12.109$; $df = 12$; $p > 0.000$). On the ATD day, the decrease in the TRP/LNAA ratio was greater for the ILL AN (−95%) and REC AN (−84%) compared with the CW (−70%). The reduction in the TRP/LNAA ratio on the ATD day was similar for ILL AN and REC AN.

Table 2. Comparison of mean (of the 2 days of the study) reductions in TRP and the TRP/LNAA ratio

	ILL AN	REC AN	CW	*F*	*df*	*p*
Baseline TRP/LNAA ratio	0.143 (0.02)[a]	0.139 (0.03)[b]	0.115 (0.02)[a,b]	5.81	2,38	0.007
ATD day						
TRP/LNAA ratio reduction at 360 min	−0.136 (0.03)[a]	−0.116 (0.03)[b]	−0.080 (0.02)[a,b]	11.15	2,37	0.000
Percent change at 360 min compared with baseline	−95%	−84%	−70%			
Control mixture day						
TRP/LNAA ratio reduction at 360 min	−0.007 (0.04)	−0.01 (0.04)	−0.0008 (0.06)	0.252	2,40	0.78
Percent change at 360 min compared with baseline	−5%	−7%	−1%			
Baseline TRP	49.3 (8.1)[a]	60.9 (10.9)[a]	52.4 (8.3)	5.48	2,38	0.008
ATD day						
TRP reduction at 360 min	−36.2 (9.5)	−41.5 (11.8)	−32.2 (11.3)	2.26	2,37	0.12
Percent change at 360 min compared with baseline	−74%	−69%	−62%			
Control mixture day						
TRP reduction at 360 min	60.6 (35.5)[a,b]	27.5 (21.4)[b]	17.8 (35.5)[a]	7.49	2,40	0.002
Percent change at 360 min compared with baseline	125%	46%	35%			

Same letters denote significant ($p < 0.05$) group differences; data expressed as mean ± SD.

In terms of baseline TRP concentrations, ILL AN had significantly lower levels compared with REC AN (Table 2). However, baseline TRP levels were similar for ILL AN and CW. Baseline TRP levels were subtracted from the TRP levels at 360 minutes on the ATD or control day. On the control day, when 4.6 g of TRP was administered with 100 g of neutral amino acids, each group had a significant increase in plasma TRP levels compared with their baseline. The increase of plasma TRP was significantly greater in the ILL AN compared with the other two groups. On the ATD day, paired *t* tests for each group showed a highly significant reduction in plasma TRP levels at 360 minutes compared with baseline. On the ATD day, the decrease in plasma TRP levels were similar for the ILL AN (−74%), REC AN (−69%), and CW (−62%).

Self-Reported Anxiety, Depression, or Irritability

ILL AN had a significant reduction in anxiety on the ATD day compared with the control day (−236 ± 303 vs. 39 ± 277; F = 11.1; $df = 1,13$; $p = 0.005$) (Figure 1). A significant reduction in anxiety on the ATD day vs. the control day was also found in REC AN (−159 ± 295 vs. 49 ± 142; F = 4.8; $df = 1,13$; $p = 0.04$). CW had a trend toward a difference (−102 ± 208 vs. 4 ± 106; $F = 3.8$; $df = 1,14$; $p = 0.07$). An ANOVA did not show a significant drug × group effect when ATD and control day response were compared between subject groups.

ILL AN showed a significant relationship on the ATD day between the reduction in the TRP/LNAA ratio (360 minute time point minus baseline) and change in anxiety ($r = -0.749$; $p = 0.003$; $n = 13$). However, on the ATD day, no relationship was found between the reduction in the TRP/LNAA ratio (360 minute time point minus baseline) and change in anxiety for the REC AN ($r = -0.195$; $p = 0.54$; $n = 12$) or CW ($r = 0.349$; $p = 0.24$; $n = 13$). When the reduction in the TRP/LNAA ratio (360 minute time point minus baseline) was covaried with anxiety, ILL AN continued to show a significant reduction in

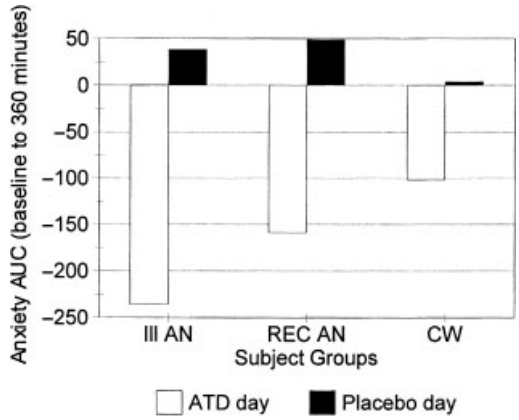

Figure 1. Comparison of mean (of the 2 days of the study) changes in anxiety ratings.

anxiety on the ATD day compared with the control day ($F = 5.16$; $df = 1,10$; $p = 0.04$). However, after covarying the TRP/LNAA ratio, there was no difference on the ATD and control day for REC AN ($F = 1.05$; $df = 1,7$; $p = 0.4$) or CW ($F = 0.02$; $df = 1,10$; $p = 0.88$).

There was no significant difference in depression or irritability when a within-group analysis (ATD day vs. control day) was done for each cohort or a between-group analysis (ATD day minus control day) was considered.

DISCUSSION

These data show that a dietary-induced reduction of TRP, the precursor of 5-HT, is associated with decreased anxiety in people with AN. We hypothesize that people with AN have increased 5-HT signal transmission that does not respond to normal modulatory regulation. Instead, they use diet-induced reductions in TRP to reduce dysphoric mood states.

To our knowledge, ATD has not been administered to people with AN. However, studies have shown that ATD increases dysphoric mood in ILL and REC people with bulimia nervosa (BN) (Kaye, Gendall, Fernstrom, Fernstrom, McConaha, & Weltzin, 2000; Smith, Fairburn, & Cowen, 1999; Weltzin, Fernstrom, Fernstrom, Neuberger, & Kaye, 1995). Importantly, people with AN and BN have also been shown to have a different behavioral response to m-CPP, a relatively 5-HT–specific drug (Frank, Kaye, Weltzin, Perel, Moss, McConaha, & Pollice, 2001; Kaye, Greeno, Moss, Fernstrom, Fernstrom, Lilenfeld, Weltzin, & Mann, 1998). That is, m-CPP is associated with euphoria in AN and dysphoria in BN. Together these findings suggest that people with AN and BN have differences in 5-HT functional activity and support the clinical observation that dieting is rewarding for AN, but not for BN, so that only people with AN are able to lose large amounts of weight.

We hypothesize that a trait-related disturbance of 5-HT neuronal modulation predates the onset of AN. With normal dietary intake, this 5-HT disturbance results in increased 5-HT transmission (as reflected in elevated CSF 5-HIAA levels and altered brain 5-HT_{1A} and 5-HT_{2A} receptor activity), which in turn, creates a vulnerability for restricted eating, as well as dysphoric mood states. Restricted eating, in turn, reduces synaptic 5-HT concentrations (as reflected in reduced CSF 5-HIAA levels), perhaps through effects on TRP availability or even reduced gonadal steroids, which in turn briefly reduces dysphoric mood. The daily requirement of TRP is small, and TRP is present in almost all

proteins. Thus, it is difficult to select a diet that is low in TRP. Because TRP competes with other LNAA, it is not the absolute amount of TRP in the diet that modulates TRP entry into the brain. Rather, it is the effect of caloric intake on insulin secretion, which in turn drives the LNAA into tissue and spares TRP. During starvation insulin secretion is reduced in AN (Alderdice, Dinsmore, Buchanan, & Adams, 1985). We hypothesize that people with AN may discover that reduced dietary intake, by effects on plasma TRP, is a means by which they can crudely reduce brain 5-HT functional activity and anxious mood.

These data raise the interesting possibility that ATD has anxiolytic effects in AN, and this effect may be proportionate to the degree of reduction in the TRP/LNAA ratio. Considerable evidence has implicated disturbances in 5-HT neurotransmission in the pathology of anxiety (Charney et al., 1990; Longo, 1998). Up to 70% of people with AN have a lifetime anxiety disorder (Halmi, Eckert, Marchi, Sampugnaro, Apple, & Cohen, 1991; Lilenfeld, Kaye, Greeno, Merikangas, Plotnicov, Pollice, Rao, Strober, Bulik, & Nagy, 1998), and a preexisting anxiety disorder has been associated with a prolonged illness and poorer outcome (Toner, Garfinkel, & Garner, 1988). If trait-related elevations in 5-HT activity generates cognitions leading to anxious or constrained behavior in AN, then these individuals may find food restriction rewarding, because it provides a means of cognitive distraction from anxiety. Still, ATD has not been shown to reduce anxiety in people with primary anxiety disorders (Goddard, Sholomskas, Walton, Augeri, Charney, Heninger, Goodman, & Price, 1994; Kent, Coplan, Martinez, Karmally, Papp, & Gorman, 1996) or obsessions or compulsions in people with OCD (Barr, Goodman, McDougle, Delgado, Heninger, Charney, & Price, 1994; Huwig-Poppe, Voderholzer, Backhaus, Riemann, Konig, & Hohagen, 1999; Smeraldi, Diaferia, Erzegovesi, Lucca, Bellodi, & Moja, 1996). However, ATD has been found to increase depressed mood in people with primary depressive disorders (for review, see Anderson & Mortimore, 1999) and in their first-degree relatives (Benkelfat, Ellenbogen, Dean, Palmour, & Young, 1994; Klaassen, Riedel, van Someren, Deutz, Honig, & van Praag, 1999).

Another issue raised by this study is the question of whether ATD reduces anxiety in healthy women. Studies of dieting and/or TRP metabolism suggest that gender-specific effects may occur in healthy subjects (Anderson et al., 1990; Goodwin, Fairburn, & Cowen, 1987; Walsh, Oldman, Franklin, Fairburn, & Cowen, 1995). In one study (Smith, Clifford, Hockney, Clark, & Cowen, 1997) ATD lowered mood in healthy women, but not healthy men. However, ATD has not been found to influence levels of anxiety in volunteer women (Ellenbogen, Young, Dean, Palmour, & Benkelfat, 1996; Oldman, Walsh, Salkovskis, Laver, & Cowen, 1994; Smith et al., 1997).

The TRP/LNAA ratios reported in this study are similar to values previously reported (Kaye et al., 1984). Favaro and colleagues (2000) found that the TRP/LNAA ratio was higher in malnourished AN with a more severe catabolic status and those who exercised excessively. Moreover, the TRP/LNAA ratio was inversely correlated with body mass index, body fat, muscle mass, daily energy intake, and daily TRP intake. It is possible that elevations of the TRP/LNAA ratio in severely underweight AN may be related to the effects of severe malnutrition, such as reduced protein intake or insufficient insulin secretion.

It is also possible that a reduction of extracellular 5-HT in ILL AN results in a compensatory upregulation of 5-HT postsynaptic receptor(s), such as 5-HT_{2A}. Postsynaptic upregulation may explain why ILL AN patients are so dysphoric when they eat and resistant to refeeding. In other words, the more AN individuals starve and reduce 5-HT release, the more compensatory postsynaptic upregulation occurs. Thus, at best, *food refusal produces a temporary and brief* respite from a dysphoric state. However, when people with AN are compelled to eat, the resulting secretion of insulin may increase extracellular 5-HT

and thus exaggerate dysphoric mood. It is not clear whether 5-HT_{2A} receptors upregulate, because this may not occur after serotonergic denervation. However, there is limited and mixed evidence that upregulation of 5-HT_{2A} receptors may occur in rodents in response to glucocorticoid administration (Kuroda, Mikuni, Ogawa, & Takahashi, 1992), which is of interest because hypercortisolemia invariably occurs in malnourished AN subjects. In addition, several stress paradigms decrease 5-HT metabolism and upregulate cortical 5-HT_{2A} receptors in rodents (Rilke, Freier, Jahkel, & Oehler, 1998; Stanford, 1996).

The limitations of this study include small cell sizes that had a mixture of AN subtypes in the ILL and REC groups. Although we did not find differences in response to ATD in purging and bulimic/purging AN, this study should be replicated in a larger sample with adequate numbers of AN subtypes.

In summary, the mechanism responsible for an alteration in 5-HT functional activity in AN remains obscure. One possibility is that 5-HT pathophysiology in eating disorders is a consequence of a disturbance within one or more of the regulatory feedback systems within the 5-HT neuronal system; in other words, an abnormal compensatory response to 5-HT synaptic release. Thus, the 5-HT neuronal systems in women with AN may not be able to sufficiently compensate and ''buffer'' the ATD-induced changes in 5-HT release. It is possible that people with an inherent modulatory defect in 5-HT function may be prone to developing an eating disorder, because they cannot respond appropriately and precisely to stress or stimuli or modulate their affective states. People with AN may discover that restricted eating, by its effects on availability of plasma TRP, is a means by which they can crudely reduce extracellular 5-HT concentrations, and thus briefly reduce a dysphoric state.

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