ELSEVIER

Mood Response to Acute Tryptophan Depletion in Healthy Volunteers: Sex Differences and Temporal Stability

Mark A. Ellenbogen, B.A., Simon N. Young, Ph.D., Peggy Dean, R.N., Roberta M. Palmour, Ph.D., and Chawki Benkelfat, M.D.

We investigated (1) the mood response of normal women, without a family history of major affective disorder, to acute tryptophan depletion, and (2) the temporal stability of the mood change, within subjects, when rechallenged at least 1 month later. To deplete tryptophan, a tryptophan deficient amino acid mixture was ingested. The control treatment was a nutritionally balanced amino acid mixture containing tryptophan. A marked lowering of plasma tryptophan (80% to 90%) was achieved by both depletions. Compared to the balanced condition, the women exhibited a significant lowering of mood after the first tryptophan depletion on the elation-depression ($p < .05$), energetic-tired ($p < .005$), confident-unsure ($p < .01$), and clearheaded-confused ($p < .01$) scales of the bipolar profile of mood states. Whereas a lowering of mood was not found in a comparable sample of males studied earlier, these results were similar to those obtained in healthy males at genetic risk for major affective disorder (MAD). Inasmuch as a family history of MAD and female sex are predisposing factors to depression, these results suggest that a mood-lowering response to acute tryptophan depletion may occur preferentially in subjects with a susceptibility to lowered mood. However, the mood response to tryptophan depletion exhibited poor temporal stability in individual subjects. **© 1996 American College of Neuropsychopharmacology**
[Neuropsychopharmacology 15:465–474, 1996]

KEY WORDS: *Mood; Depression; Women; Tryptophan depletion; Serotonin*

Acute tryptophan depletion (ATD) is a research strategy that reduces the availability of the serotonin precursor tryptophan and thus provides a tool for studying the behavioral consequences of low brain serotonin in humans. To accomplish this end, subjects are given a mixture of amino acids devoid of tryptophan. Protein synthesis is enhanced, and the resulting incorporation of tryptophan into protein leads to a rapid (5 hours) and substantial (80% to 90%) lowering of tryptophan in plasma and tissues (Moja et al. 1984; Young et al. 1985; Delgado et al. 1990; Benkelfat et al. 1994). As tryptophan hydroxylase, the rate-limiting enzyme for serotonin synthesis, is normally about half-saturated in human brain (Young and Gauthier 1981), this decline in tryptophan availability is thought to reduce the rate of serotonin synthesis in the brain. The advantage of this technique is that any resulting change in mood or behavior is likely to be a consequence of the effects of the tryptophan-deficient amino acid mixture, and thereby believed to result from a lowering of central nervous system (CNS) serotonin. The disadvantages are that the effects of ATD on human brain serotonin synthesis have not yet been demonstrated and that the behavioral effects of ATD may be related in part to alterations in the

From the Department of Psychiatry (MAE, SNY, PD, RMP, CB), the School of Dietetics and Human Nutrition (SNY), and the Departments of Biology and Human Genetics (RMP), McGill University, Montréal, Québec, Canada.

Address correspondence to: Simon N. Young, Ph.D., Department of Psychiatry, McGill University, 1033 Pine Avenue West, Montréal, Québec H3A 1A1, Canada.

Received August 9, 1995; revised January 19, 1996; accepted February 12, 1996.

Published by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/96/$15.00
PII S0893-133X(96)00056-2

brain of the levels of other potentially psychoactive metabolites of tryptophan, such as tryptamine, melatonin, quinolinic acid, kynurenic acid, or even to alterations in brain protein synthesis.

In studies of normal male volunteers, ATD results in a significant lowering of mood in some subject groups (Young et al. 1985; Smith et al. 1987; Cleare and Bond 1995), but not in others (Danjou et al. 1990; Abbott et al. 1992; Benkelfat et al. 1994). This discrepancy may be due to differences in the baseline mood state between studies; earlier studies finding a mood-lowering effect of tryptophan depletion used subjects with mean baseline depression scores in the upper end of the normal range (Young et al. 1985; Smith et al. 1987; Cleare and Bond, 1995), whereas the recent negative findings, characterized by more rigorous screening, were obtained in fully euthymic subjects (Danjou et al. 1990; Abbott et al. 1992; Benkelfat et al. 1994). Recently, we showed that ATD significantly lowered mood in normal male subjects who had an extensive family history of MAD (Benkelfat et al. 1994).

In the general population, major depressive episodes are significantly more prevalent in women than men, with a female-male ratio close to 2:1 on average (Weissman and Klerman 1977; Myers et al. 1984; Regier et al. 1984; Perugi et al. 1990; Leon et al. 1993; Weissman et al. 1993; Blazer et al. 1994), suggesting that females are statistically at a greater risk for major depression than males. The effects of ATD in normal women have been examined in only two studies. Weltzin et al. (1994) reported a significant increase in ratings of depressed mood after ATD, whereas Oldman et al. (1994) did not. However, these findings were obtained in small samples, and neither family histories nor premenstrual mood change were formally assessed. Both of these factors are believed to affect the mood response to ATD (Benkelfat et al. 1994; Menkes et al. 1994). In the Oldman et al. (1994) study, the use of a 50-g amino load and no pre-experiment low-protein diet resulted in a lesser depletion of plasma tryptophan than in studies using a 100-g amino acid mixture and a 1-day low protein diet the day before the depletion (Delgado et al. 1990; Benkelfat et al. 1994). In one study, ATD caused a significant aggravation of premenstrual symptoms, particularly irritability, in women with premenstrual syndrome (Menkes et al. 1994).

We report here on the mood response to ATD in healthy euthymic women devoid of any personal or familial history of psychiatric illness, who were tested on three separate occasions at least 1 month apart. All women underwent two ATD trials and one balanced control trial, randomized for order. Our hypotheses were that (1) normal females subjects, unlike the normal male subjects studied previously (Abbott et al. 1992; Benkelfat et al. 1994), would exhibit a significant mood lowering response to ATD, and (2) the mood response to ATD would be stable over time.

SUBJECTS AND METHODS

Selection of Subjects

Female subjects, aged 18 to 30 years, were recruited between June 1993 and November 1994 through local newspaper advertisements. Inclusion criteria for all participants included good physical health and a knowledge of the medical and psychiatric history of their biological relatives. Exclusion criteria included (1) having been adopted, (2) evidence of personal past or present Axis 1 DSM-III-R diagnosis, (3) evidence of MAD in any first- or second-degree relatives, or of any other DSM-III-R Axis-1 diagnosis in a first-degree relative, and (4) significant past medical illness.

Psychiatric evaluations were conducted for all subjects by a trained psychiatric research nurse (PD) in consultation with the project psychiatrist (CB), using the Structured Clinical Interview for DSM-III-R, non-patient version (SCID-NP) (Spitzer 1987). Family histories were determined by a graduate student (MAE), trained in genetic psychiatric interviews, using a family history assessment module (Nurnberger et al. 1994).

Premenstrual mood changes (PMC) were assessed prospectively for two complete cycles using a three-item visual analogue scale (depression, anxiety, and irritability), self-administered once a day in the morning (Rubinow et al. 1984). PMC was measured as the increase in ratings of negative mood in the week prior to menses (late luteal phase; day 7 through day 1) as compared with ratings obtained in the follicular phase (day 4 through day 10). Criteria for late luteal phase dysphoric disorder was a 30% increase in ratings of negative mood in the late luteal phase, compared with the follicular phase, in both menstrual cycles (Schnurr 1989).

Experimental Procedure

Overview. The experiment was a double-blind, placebo-controlled, cross-over study. The order of treatments was randomized in blocks of six. Subjects participated in two ATD experimental sessions ($T\text{-}_1$; $T\text{-}_2$) and one nutritionally balanced amino acid (B) control session, at least 1 month apart. All sessions were scheduled during the follicular phase of the menstrual cycle, with only one session per cycle. Each session consisted of two consecutive days. On day 1, each subject ate a low-protein diet. On day 2, subjects consumed either a tryptophan-free amino acid drink (T- session), or a similar amino acid drink which also contained the appropriate amount of L-tryptophan for a nutritionally balanced protein source (B session). Behavioral measurements and a venous blood sample were collected before and 5 hours after ingestion of the amino acid drink.

Details of procedure. Subjects were scheduled one to two per test day. When two subjects were tested on the same day, they remained in separate rooms for the en-

tire experimental procedure. For day 1, on which the subjects ate a low protein diet, prepacked, precooked meals were either collected by the subject or delivered to their home. These meals were the same as those used previously in the study of FH+ and FH− men (Benkelfat et al. 1994). Tryptophan (160 mg/24 hours), protein (22.6 g/24 hours), and caloric content (2212 kcal/24 hours) were similar to those found in the low protein diet used by Delgado et al. (1990). The same diet was provided before all experimental sessions to help maintain blindness for both subjects and investigators and to standardize baseline behavioral measurements. Subjects were instructed to eat at regular hours and were allowed ad libitum water and up to three cups of coffee or tea per day.

Subjects who had given informed consent were asked to arrive for the day 2 experimental sessions at 9:00 a.m., having fasted since midnight the previous night. Subjective rating scales of mood states were completed, and a 10-ml venous blood sample was taken for measurement of baseline total and free (non-albumin-bound) plasma tryptophan. Mood ratings were carried out blind to the nature of the amino acid mixture administered. Each subject was then given either a tryptophan-free amino acid drink (T- session), or a nutritionally balanced amino acid drink, containing 1.9 g L-tryptophan, as well as other amino acids (B session). For the next 5 hours, subjects stayed in a single room and were entertained by being shown one or two movies (Diner, Star Trek IV, The Right Stuff, and National Geographic documentaries). The movies were chosen to be relatively effectively neutral. Five hours after the ingestion of amino acids, mood ratings were again administered, and a second blood sample was drawn. Each subject was then given a high protein snack, and received a 1 g L-tryptophan tablet. Subjects in the T- session were administered tryptophan to restore their tryptophan levels, whereas subjects after the B session were administered tryptophan to maintain the double-blind status of the study. The tryptophan preparation used is available on prescription in Canada and has not been associated with any cases of eosinophilia myalgia syndrome (Wilkins 1990). Clinical supervision was offered for at least 1 hour after completion of the study session, with contact maintained by phone when necessary in the evening and during the day after the experimental session. All subjects resumed their normal diet between the two experimental sessions.

Amino acid drinks. The tryptophan deficient (T-) amino acid mixture ingested by subjects was based on the mixture used by Young et al. (1985) and consisted of 15 amino acids weighing 85.8 g. Because women weigh, on average, 16.7% less than men (Health and Welfare Canada 1990), the 103-g amino acid mixture used previously in male samples was reduced accordingly. The content of the T- drink was as follows: L-alanine 4.6 g, L-arginine 4.1 g, L-cyseine 2.3 g, glycine 2.7 g, L-histidine 2.7 g, L-isoluecine 6.7 g, L-leucine 11.3 g, L-lysine monohydrochloride 9.2 g, L-methionine 2.5 g, L-phenylalanine 4.8 g, L-proline 10.2 g, L-serine 5.8 g, L-threonine 5.4 g, L-tyrosine 5.8 g, L-valine 7.4 g. The balanced amino acid mixture contained the same amino acids plus 1.9 g L-tryptophan. The amino acids were given in the same proportion as they occur in human milk, which is presumably close to the optimum amino acid composition for humans, except that glutamate and aspartate were excluded because of concerns about their toxicity. This would not affect the ability of the T- mixture to lower tryptophan availability because glutamate and aspartate are not essential amino acids. Because of the unpleasant taste of methionine, cysteine, and arginine, these amino acids were encapsulated and administered separately from the other amino acids. The amino acid mixture was prepared within a few minutes of oral administration by mixing the powdered amino acids with 135 ml water, 45 ml chocolate syrup, and 0.6 g of sodium cyclamate. A second mixture was used in some cases, due to the development of a taste aversion after amino acid mixture ingestion. This mixture consisted of the powdered amino acids, 180 ml orange juice from concentrate, and 1.1 g sodium cyclamate.

Measurement of Mood Changes

Mood changes were determined using two subjective mood rating scales: the bipolar profile of mood states (POMS) (Lorr et al. 1982; McNair et al. 1988) and the visual analogue mood scale (VAMS) (Bond and Lader 1974). The bipolar form of the POMS, the main measure used in this study, is composed of six bipolar scales: agreeable-hostile, composed-anxious, elated-depressed, confident-unsure, energetic-tired, and clearheaded-confused, and is highly sensitive to nonclinical changes in mood states. The VAMS consists of 16 100-mm horizontal lines, each representing a bipolar dimension of a mood state, on which the subject is instructed to place a perpendicular mark that best describes her mood state.

Determination of Plasma Tryptophan Concentrations

Plasma tryptophan was measured in all blood samples as an index of the extent of ATD. The free (non-albumin-bound) plasma tryptophan concentration was assumed to be equivalent to the concentration of tryptophan found in an ultrafiltrate of plasma prepared at 25°C under an atmosphere containing 5% carbon dioxide by centrifugal ultrafiltration (MPS-1, Amicon Inc, Beverly, MA) through YMT membranes (Millipore Waters, Bedford, MA). Tryptophan in the ultrafiltrate and in deproteinized plasma was measured by high perfor-

mance liquid chromatography on a Waters μBondapak C_{18} (Millipore Waters) reverse phase column with fluorometric detection.

Data Analysis

In order to control for intraindividual variability in baseline measures over a period of months, all behavioral and biochemical results were converted to change scores (△ scores): the baseline measure on day 2 was subtracted from the post-treatment measure taken 5 hours after the ingestion of the amino acid mixture. All behavioral and blood data were analyzed using two-way mixed design ANOVAs (order × treatment). Order (B, $T\text{-}_1$, $T\text{-}_2$; $T\text{-}_1$, B, $T\text{-}_2$; $T\text{-}_1$, $T\text{-}_2$, B) was an independent factor, whereas treatment was a within-subject, repeated measures factor. Tests of simple effects were performed for all statistically significant interactions. In order to assess the mood response to ATD in women, only data from the B and first T- treatment were included in the analysis, so that the results would be comparable with the design used in an earlier study of a FH- males (Benkelfat et al. 1994).

The relationship between the first and second T- treatments was assessed by Pearson product moment correlations between the two T- treatments and ANOVAs comparing the two T- treatments and the B and second T- treatments.

The relationship between PMC and mood changes after ATD was assessed using Pearson product moment correlations.

Ethics

All subjects who participated in the study gave written informed consent. The study was approved by the Research Ethics Board of the Department of Psychiatry, McGill University. Subjects were compensated Can $170 for loss of time due to their participation in the study.

RESULTS

Subjects

In total, 110 potential subjects were evaluated in person or over the telephone for participation in the study. Subjects were excluded for the following reasons: 23 had a personal DSM-III-R Axis 1 diagnosis; 23 subjects were excluded on the basis of the presence of a DSM-III-R Axis 1 disorder in a first-degree relative or multiple second and/or third degree relatives, or due to insufficient information or uncertainty about one's family history; six were not physically healthy and two had insufficient knowledge of English or French. Twenty-nine subjects dropped out either before coming in for the initial interview or before completing an amino acid session. Four subjects dropped out after one session and three after two sessions. Reasons for failure to complete the study included: prolonged symptoms of nausea and/or fatigue ($n = 3$), medical reasons unrelated to this study ($n = 1$), or moved away during study ($n = 3$). Subjects completing only one trial were not included in the data analysis. One subject completing one B and one T- session was included in the analysis of mood changes after ATD, whereas two other subjects completing two T- trials and no B trial were excluded. These latter two subjects were included in the analysis of the stability over time of the mood response to ATD. The final sample consisted of 20 subjects completing all three trials (one B and two T- trials), one subject completing one B and one T- trial, and two subjects completing two T- trials. The mean age of the sample was 23.7 ± 3.5 years (range 18 to 30), mean weight was 61.1 ± 7.4 kg (range 44.5 to 74.8), and mean height was 1.67 ± 0.07 m (range 1.55 to 1.83).

Baseline Measures

Baseline measures of plasma tryptophan did not differ significantly between treatments. With the exception of higher ratings of energy at baseline on the VAMS, but not on the POMS, in the second T- treatment compared with the B treatment ($F_{1,17} = 4.4$, $p < .05$), baseline ratings of mood state did not significantly differ among the three treatments. All of the following analyses will use △ scores.

Plasma Tryptophan Concentrations

Due to technical difficulties, the number of subjects with plasma tryptophan measurements differ among the three conditions. Blood measurements in both the B and first T- treatment were completed for 19 subjects, in both the B and second T- treatment for 15 subjects, and in both T- conditions for 18 subjects. In order to include the maximum number of subjects in each condition, pairwise comparisons were performed (B, 1st T-; B, 2nd T-; 1st T-, 2nd T-), rather than analyzing all three conditions simultaneously. Overall, the tryptophan-deficient amino acid mixture resulted in a substantial decline in plasma total and free tryptophan concentrations in both T- treatments, whereas the balanced mixture caused increase in plasma tryptophan (Table 1). Treatment × order ANOVAs of plasma tryptophan concentrations demonstrated that both the first (total tryptophan: $F_{1,12} = 58.7$, $p < .0001$; free tryptophan: $F_{1,12} = 111.0$, $p < .0001$) T- conditions were significantly different from the B treatment and that there was no significant difference in the depletion of plasma tryptophan between the two T- trials.

Table 1. Changes in Total and Free Plasma Tryptophan Levels after Amino Acid Ingestion[a]

	Balanced (n = 19)	1st trp depletion (n = 23)	2nd trp depletion (n = 18)
Total plasma tryptophan			
Pretreatment, μg/ml	11.9 ± 0.6	11.8 ± 0.4	11.6 ± 0.6
5 hr post-ingestion, mg/μl	18.2 ± 1.6	1.4 ± 0.1	1.9 ± 0.2
Percentage change	52.8	−88.5	−83.5
Free plasma tryptophan			
Pretreatment, μg/ml	1.47 ± 0.17	1.32 ± 0.09	1.29 ± 0.08
5 hr post-ingestion, mg/μl	2.24 ± 0.21	0.19 ± 0.02	0.25 ± 0.02
Percentage change	52.4	−85.6	−80.6[b]

[a]Values expressed as mean ± SEM.
[b]n = 17.

Mood Response to ATD

In contrast to normal male subjects (Benkelfat et al. 1994), female subjects exhibited a significant lowering of mood on four of the six POMS scales after ATD. These results are presented in the bar graph on the left of Figure 1; results of a comparable male sample, taken from our previous study (Benkelfat et al. 1994), are presented on the right. ANOVA revealed significant main effects of treatment on the POMS elation-depression ($F_{1,18}$ = 5.3, $p < .05$), energetic-tired ($F_{1,18}$ = 10.5, $p < .005$), confident-unsure ($F_{1,18}$ = 9.1, $p < .01$), and clear-headed-confused scales ($F_{1,18}$ = 8.5, $p < .01$); neither order nor interaction effects were found. No significant differences were found between treatments on either the relaxed anxious or the agreeable-hostile scales.

Similar results were obtained with the VAMS. Com-

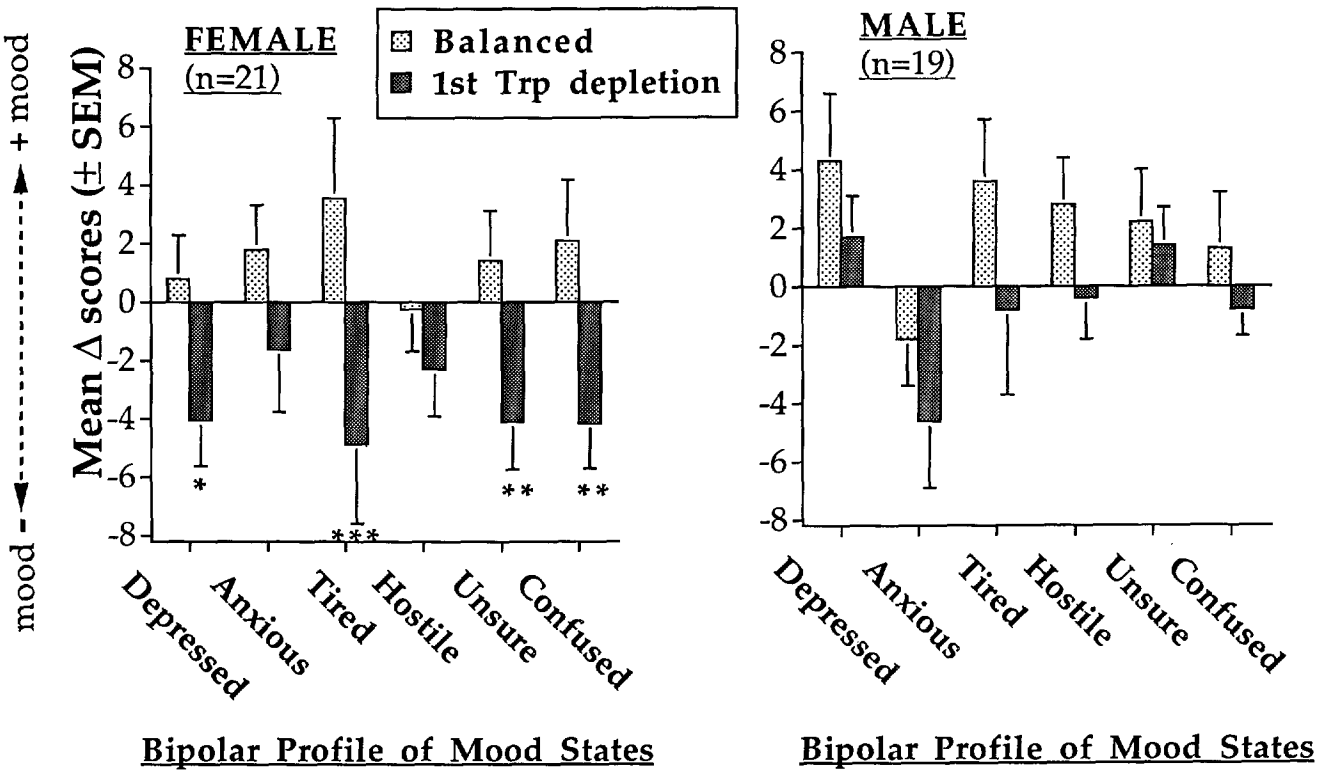

Figure 1. Mood change in females (*left*) and male (*right*) controls after the first tryptophan depletion and ingestion of a balanced amino acid mixture. In contrast to male controls, females exhibited a significant mood-lowering response to tryptophan depletion compared with the balanced control treatment. The bar graph of male controls is derived from data by Benkelfat et al. (1994).

Table 2. Changes on the Visual Analogue Mood Scale after the First Tryptophan Depletion and Ingestion of a Balanced Amino Acid Load in Female Controls[a]

VAMS Item	Balanced	1st trp depletion	F (1,18)[b]	*p*
Happy-Sad	−3.9 ± 2.9	5.8 ± 3.2	5.3	<0.05
Content-Discontent	−3.8 ± 3.1	8.8 ± 4.0	6.9	<0.05
Gregarious-Withdrawn	−5.3 ± 3.8	9.8 ± 5.4	5.3	<0.05
Tranquil-Troubled	−1.5 ± 1.8	0.7 ± 2.2	0.6	NS
Relaxed-Tense	0.8 ± 3.2	−0.9 ± 3.6	0.01	NS
Clearheaded-Muzzy	−9.0 ± 5.2	13.5 ± 6.9	10.8	<0.005
Quick-witted-Mentally slow	−2.0 ± 5.1	8.1 ± 5.4	2.4	NS
Proficient-Incompetent	−0.7 ± 3.2	6.2 ± 2.7	3.4	Tr (<0.09)
Amicable-Antagonistic	−3.1 ± 2.9	0.9 ± 3.1	0.8	NS
Energetic-Lethargic	−7.6 ± 5.2	10.1 ± 6.2	5.4	<0.05
Alert-Drowsy	−12.3 ± 7.0	10.9 ± 7.2	7.5	<0.05
Interested-Bored	5.2 ± 4.4	16.3 ± 5.7	2.2	NS

[a]All values are mean Δ scores ± SEM, $n = 21$. Positive Δscores represent a lowering of mood (i.e. toward the sad or discontent end of the dimension), whereas negative Δscores reflect a heightening of mood. NS: nonsignificant; Tr: trend for significance

[b]Order x condition repeated measures ANOVA; no effect of order.

pared with the B treatment, female subjects demonstrated a significant lowering of mood on the happy-sad, content-discontent, gregarious-withdrawn, clearheaded-muzzy, energetic-lethargic, and alert-drowsy items of the VAMS (Table 2). A trend toward significance was found on the proficient-incompetent item of the VAMS. No effect of order was found on any of the VAMS items.

Mood Response to ATD: Stability over Time

Overall, the mood responses on both the POMS and VAMS during the first and second T- treatments demonstrated no temporal stability in individual subjects ($n = 22$; Table 3). Some positive correlations were found on the VAMS, but these were more closely related to arousal and alertness (energetic-lethargic, clearheaded-muzzy, alert-drowsy, interested-bored) than to mood.

The mean mood response after a second T- treatment was either attenuated or completely abolished, as measured by either the POMS or the VAMS (Table 3). ANOVAs ($n = 20$) revealed that there was no significant difference in mood change between the B and second T- treatment on all POMS and VAMS scales. Furthermore, significant differences were found between the two T- treatments ($n = 22$) on the energetic-lethargic ($F_{1,19} = 5.5$, $p < .05$ and alert-drowsy ($F_{1,19} = 8.9$, $p < .01$) items of the VAMS.

Due to a smaller (although not significantly so) decrease in plasma tryptophan after the second T- treatment compared with the first (see Table 3), POMS elation depression data was reanalyzed after excluding five subjects with a depletion of total tryptophan less than 80% in the second T- treatment and four subjects in which plasma tryptophan samples were not obtained. In the remaining 13 controls, plasma total tryptophan in the first and second T- treatments was reduced by 88.7% and 87.8%, respectively. This gave similar results, as the mean mood-lowering response (±SE) after the first T- treatment (−6.1 ± 1.8) was significantly attenuated after the second T- treatment (0.9 ± 2.0; $F_{1,10} = 7.4$, $p < .5$), and the correlation between T- treatments was poor ($r = -.038$, NS).

Relationship Among the Mood Response to ATD, Premenstrual Mood Changes, and Alterations in Plasma Tryptophan Levels

Prospective visual analogue ratings over two menstrual cycles were obtained in 15 subjects. No subject fulfilled the criteria for late luteal phase dysphoric disorder. Measures of PMC were obtained for depression (PMC-D), anxiety (PMC-A), irritability (PMC-I), and the sum total of these three measures (PMC-T). For each item, PMC was calculated by subtracting the sum total of follicular ratings (baseline) from the sum total of late luteal ratings for each menstrual cycle (△ scores). Then the △ score for each cycle was averaged to yield one measure of PMC. Pearson correlations were performed between the logarithm transformed measures of PMC and the △ scores on the elation-depression, energetic-tired, confident-unsure, and clearheaded-confused scales of the POMS, all of which demonstrated significant change after the first T- treatment. PMC was unrelated to changes in mood after the first T- treatment. No significant correlations were found between PMC-T ($r = 0.121$, NS), PMC-D ($r = 0.110$, NS), PMC-I ($r = 0.345$, NS0) and PMC-A ($r = 0.240$, NS) and the POMS elation-depression change scores after the first T- treatment. With the exception of a significant correlation between PMC-I and the POMS-U ($r = 0.578$, $p < .05$), no other significant correlations were found.

No significant correlations were found between the

Table 3. Stability over Time: A Comparison of the Mood Response to the First and Second Tryptophan Depletion Trials in Female Controls[a]

	1st T- mean Δscore ± SEM	2nd T- mean Δscore ± SEM	r[b]	p
POMS scales:				
Elated-Depressed	−3.9 ± 1.5	−0.9 ± 1.5	0.001	NS
Relaxed-Anxious	−2.5 ± 2.1	−0.6 ± 1.0	0.026	NS
Energetic-Tired	−4.6 ± 2.9	1.6 ± 2.3	0.120	NS
Aggreeable-Hostile	−2.7 ± 1.6	1.8 ± 1.3	−0.372	Tr (<.09)
Confident-Unsure	−3.4 ± 1.7	0.2 ± 1.2[e]	−0.105	NS
Clearheaded-Confused	−4.3 ± 1.6	−1.0 ± 1.0[e]	0.125	NS
VAMS item:				
Happy-Sad	6.9 ± 3.2	2.2 ± 2.9	0.071	NS
Content-Discontent	8.5 ± 3.9	0.4 ± 3.7	0.033	NS
Gregarious-Withdrawn	10.0 ± 5.6	1.3 ± 4.6	0.315	NS
Tranquil-Troubled	1.1 ± 2.1	−4.9 ± 3.7	0.161	NS
Relaxed-Tense	0.5 ± 3.7	−0.4 ± 2.8	0.011	NS
Clearheaded-Muzzy	12.9 ± 6.9	0.1 ± 5.0[e]	0.472	<.05
Quick-witted-Mentally slow	8.1 ± 5.5	−0.5 ± 4.7	0.328	NS
Proficient-Incompetent	5.8 ± 2.9	1.8 ± 3.1	0.107	NS
Amicable-Antagonistic	1.2 ± 3.0	0.2 ± 3.2	−0.034	NS
Energetic-Lethargic	9.6 ± 6.2	−5.0 ± 5.3[c]	0.527	<.05
Alert-Drowsy	11.0 ± 7.6	−5.7 ± 6.1[d]	0.606	<.005
Interested-Bored	17.9 ± 5.7	7.8 ± 4.6	0.532	<.05

[a]All values are mean Δ scores ± SEM, $n = 22$. On the POMS, positive Δ scores represent a heightening of mood, while negative Δ scores reflect a lowering of mood. On the VAMS, it is the opposite. NS: nonsignificant; Tr: trend for significance.

[b]Pearson product moment correlation coefficients.

[c]ANOVA, 1st T- vs 2nd T-, $p < .05$.

[d]$p < .01$.

[e]Trend for significance, $p < .10$.

POMS-D Δ scores and changes in plasma total and free tryptophan after either the first or second tryptophan depletions.

DISCUSSION

The first objective of this study was to examine the effect of ATD in women with no family history of MAD. Healthy euthymic women, unlike a comparable sample of men, showed a lowering of mood in response to ATD. In addition to changes in mood, the various scales of the POMS and VAMS demonstrated significant decreases in energy, gregariousness, confidence, clearheadedness, and alertness. The lowering of mood in the women appears to be largely independent of the degree of premenstrual mood changes. However, premenstrual mood changes were relatively mild. The second objective of this study was to examine the temporal stability of the affective response to ATD. The results were unequivocal; in women with no family history of affective disorder, the response of individuals to ATD was not stable over a period of 1 or 2 months.

A few potential limitations should be considered in evaluating these results. First, the male comparison sample was studied approximately 2 years earlier. Although comparisons of different studies must be made with caution, the two studies employed similar designs and were carried out by the same research team. Second, baseline POMS depression scores of the women were higher than those in the previous study on males. Although both groups had baseline depression scores within the normal range, slight differences in baseline mood may account for the increased susceptibility of normal women to ATD (Young 1992). Third, as women are believed to report more affective symptoms than men do (Young et al. 1990), we cannot rule out the possibility that women, compared with men, paid more attention to, and reported more fully, those negative symptoms that occurred after ATD.

In a previous study on healthy female controls, Oldman and colleagues (1994) found that ATD did not alter mood or appetite in a group of 12 volunteers. However, the amino acid mixture was only 50 g, yielding a 78% decrease in plasma total tryptophan, compared with the 84 g mixture and pre-test low-protein diet used in the present study, which lowered total tryptophan by 88%. The results of Weltzin and colleagues (1994) were more

consistent with those obtained in the present study; healthy women (whose family history was unknown) exhibited a significant increase in ratings of depression after ATD, but not after the administration of a balanced amino acid mixture. The aggravation of premenstrual symptoms seen by Menkes et al. (1994) was characterized particularly by an increase in irritability, but a lowering of mood was also seen. There was no increase in symptoms when the subjects were studied in the follicular phase instead of the premenstrual phase. However, the measurement of symptoms was done using a scale designed to measure premenstrual symptoms, which would not necessarily pick up the type of mood changes detected by the POMS and VAMS.

The response of the normal females in the present study was similar to that of FH+ males in our previous study (Benkelfat et al. 1994). This is consistent with the hypothesis that the mood-lowering response to ATD may be associated with a susceptibility to MAD, because both a positive family history and female sex are considered risk factors for depression.

Whereas the response to ATD may be associated with a susceptibility to MAD, the actual lowering of mood seen is not of clinical significance. Thus, in the previous study of FH+ males (Benkelfat et al. 1994), the lowering of mood on the POMS was similar to that seen in the present study. In the previous study we also looked at mood using the Hamilton Depression Rating Scale and the Beck Depression Inventory but saw no changes after ATD on either scale. Thus, the response we found is different from that seen by Delgado et al. (1990) in newly recovered depressed patients on antidepressants, some of whom exhibited a full clinical relapse.

The sex difference in the mood-lowering response to ATD in healthy controls is of interest given the evidence suggesting that there are sex differences in serotonin metabolism. Mean levels of CSF 5-HIAA (Sjöström and Roos 1972; Åsberg et al. 1973; Young et al. 1980; Bowden et al. 1981; Ågren et al. 1986; von Knorring et al. 1986), CSF tryptophan (Young et al. 1980), as well as the prolactin response to a variety of serotonergic challenges, including fenfluramine (McBride et al. 1990), tryptophan (Delgado et al. 1989; Deakin et al. 1990), m-cholorophenylpiperazine (Kahn et al. 1991), and buspirone (Meltzer and Maes 1994) are greater in women than in men. Sex differences in serotonin metabolism could possibly underlie the difference in mood response to ATD between males and females, with females being more sensitive to acute changes in CNS serotonin. In response to repeated stress, female rats were characterized by marked behavioral deficits and a lowering of brain 5-HIAA levels, whereas males, who were acutely affected at first, appeared to rapidly adapt to chronic stress and suffered no subsequent decline in brain 5-HIAA (Kennett et al. 1986). These results indicate that female rats, once exposed repeatedly to an uncontrollable stressor, are more susceptible than male rats to an animal model of depression and to subsequent changes in brain serotonergic neurotransmission.

The lack of temporal stability of the affective response to ATD does not support its use as phenotypic marker of vulnerability to depression in normal women characterized by the absence of any familial antecedents of MAD. Whether this conclusion also applies to patients or to women with a multigenerational family history of affective disorder is not known.

The diminished response to the second ATD is of interest, even if the reason for this is unknown. Acclimatization to the laboratory environment on the second T- trial is unlikely as the effect was seen in women who had a T- mixture, as well as those who had a B amino acid mixture, on their first visit. Still, it is possible that some other form of learning or habituation may have confounded the results of the second T- treatment. Alternately, the attenuated mood response in the second T- trial may reflect an adaptive change in serotonergic neurotransmission resulting from the previous challenge. Whatever the reason for this result, it enhances the idea that ATD will have no long lasting adverse effect, and this finding has relevance to the ethics of ATD studies.

In conclusion, the mood response to ATD and its stability over time were investigated in healthy female controls free of any personal or familial history of psychiatric disorder. Women were more susceptible to the mood-lowering effects of tryptophan depletion than were male controls, consistent with the hypothesis that the mood-lowering response to ATD could indicate a vulnerability to depression. Whereas its poor temporal stability precludes its use as a potential marker of susceptibility to depression in control populations, a more pertinent evaluation of this query awaits further study in individuals with personal and familial antecedents of MAD.

ACKNOWLEDGMENTS

This work was supported by grants from the Medical Research Council of Canada (to SNY and to RMP) and the Fonds de la Recherche en Santé du Québec (to CB, SNY, and RMP). CB is the recipient of a joint Pharmaceutical Manufacturer's Association of Canada and Medical Research Council of Canada Scholarship (PMAC/MRC career award in medicine). We thank Dr. Pierre Blier for medical consultations and Franceen Lenoff for technical assistance.

REFERENCES

Abbott FV, Etienne P, Franklin KBJ, Morgan MJ, Sewitch MJ, Young SN (1992): Acute tryptophan depletion blocks morphine analgesia in the cold-pressor test in humans. Psychopharmacology 108:60–66

Ågren H, Mefford IN, Rudorfer MV, Linnoila M, Potter WZ (1986): Interacting neurotransmitter systems. A non-experimental approach to the 5-HIAA-HVA correlation in human CSF. J Psychiatr Res 20:175–193

Åsberg M, Bertilsson L, Tuck D, Cronholm B, Sjöquist F (1973): Indoleamine metabolites in the cerebrospinal fluid of depressed patients before and during treatment with nortriptyline. Clin Pharmacol Ther 14:277–286

Benkelfat C, Ellenbogen MA, Dean P, Palmour RM, Young SN (1994): Mood-lowering effect of tryptophan depletion: Enhanced susceptibility in young men at genetic risk for major affective disorders. Arch Gen Psychiatry 51:687–697

Blazer DG, Kessler RC, McGonagle KA, Swartz MS (1994): The prevalence and distribution of major depression in a national community sample: The national comorbidity survey. Am J Psychiatry 151:979–986

Bond A, Lader M (1974): The use of analogue scales in rating subjective feelings. Br J Med Psychol 47:211–218

Bowden CL, Redmond E, Swann A, Maas JW (1981): Pretreatment amine neurotransmitter system relationships in depression. Psychopharmacol Bull 17:70–72

Cleare AJ, Bond A (1995): The effect of tryptophan depletion and enhancement on subjective and behavioral aggression in normal male subjects. Psychopharmacology 118:72–81

Danjou P, Hamon M, Lacomblez L, Warot D, Kecskemeti S, Puech AJ (1990): Psychomotor, subjective and neuroendocrine effects of acute tryptophan depletion in the healthy volunteer. Psychiatr Psychobiol 5:31–38

Deakin JFW, Pennell I, Upadhyaya AJ, Lofthouse R (1990): A neuroendocrine study of 5-HT function in depression: Evidence for biological mechanisms of endogenous and pyschosocial causation. Psychopharmacology 101:85–92

Delgado PL, Charney DS, Price LH, Landis H, Heninger GR (1989): Neuroendocrine and behavioral effects of dietary tryptophan restriction in healthy subjects. Life Sci 45:2323–2332

Delgado PL, Charney DS, Price LH, Aghajanian GK, Landis H, Heninger GR (1990): Serotonin function and the mechanism of antidepressant action: Reversal of antidepressant-induced remission by rapid depletion of plasma tryptophan. Arch Gen Psychiatry 47:411–418

Health and Welfare Canada (1990): Nutrition Recommendations: The Report of the Scientific Review Committee. Ottawa, Minister of Supply and Services Canada

Kahn RS, Wetzier S, Asnis GM, Kling MA, Suckow RF, van Praag HM (1991): Pituitary hormone response to metachlorophenylpiperazine in panic disorder and healthy control subjects. Psychiatry Res 37:25–34

Kennett GA, Chaouloff F, Marcou M, Curzon G (1986): Female rats are more vulnerable than males in an animal model of depression: The possible role of serotonin. Brain Res 382:416–421

Leon AC, Klerman GR, Wickramaratne P (1993): Continuing female predominance in depressive illness. Am J Public Health 83:754–756

Lorr M, McNair DM, Fisher S (1982): Evidence for bipolar mood states. J Pers Assess 46:432–436

McBride PA, Tierney H, DeMeo M, Chen J-S, Mann JJ (1990): Effects of age and gender on CNS serotonergic responsitivity in normal adults. Biol Psychiatry 27:1143–1155

McNair DM, Lorr M, Droppleman LF (1988): Manual for the Profile of Mood States. San Diego, CA, Educational and Industrial Testing Service

Meltzer HY, Maes M (1994): Effects of buspirone on plasma prolactin and cortisol levels in major depressed and normal subjects. Biol Psychiatry 35:316–323

Menkes DB, Coates DC, Fawcett JP (1994): Acute tryptophan depletion aggravates premenstrual syndrome. J Affect Disord 32:37–44

Moja EA, Antinoro E, Cesa-Bianchi M, Gessa GL (1984): Increases in state 4 sleep after ingestion of a tryptophan-free diet in humans. Pharmacol Res Commun 16:909–914

Myers JK, Weissman MM, Tischler GL, Holzer CE, Leaf PJ, Orvaschel H, Anthony JE, Boyd JH, Burke JD, Kramer M, Stoltzman R (1984): Six-month prevalence of psychiatric disorders in three communities. Arch Gen Psychiatry 41:959–967

Nurnberger JI, Blehar MC, Kaufmann CA, York-Cooler C, Simpson SG, Harkavy-Friedman J, Severe JB, Malaspina D, Reich T, Miller M, Bowman ES, Depaulo JR, Cloninger CR, Robinson G, Modlin S, Gershon ES, Maxwell E, Guroff JJ, Kirch D, Wynne D, Berg K, Tsuang MT, Faraone SV, Pepple JR, Ritz AL (1994): Diagnostic interview for genetic studies: Rationale, unique features, and training. Arch Gen Psychiatry 51:849–859

Oldman AD, Walsh AES, Salkovskis P, Laver DA, Cowen PJ (1994): Effect of acute tryptophan depletion on mood and appetite in healthy female volunteers. J. Psychopharmacol 8:8–13

Perugi G, Musetti L, Simonini E, Piagentini F, Cassano GB, Akiskal HS (1990): Gender mediated clinical features of depressive illness: The importance of temperamental differences. Br J Psychiatry 157:835–841

Regier DA, Myers JF, Kramer JK, Robins LN, Blazer DG, Hough RL, Eaton WW, Locke BZ (1984): The NIMH epidemiologic catchment area program: Historical context, major objectives, and study population characteristics. Arch Gen Psychiatry 41:934–967

Rubinow DR, Roy-Byrne P, Hoban MC, Gold PH, Post RM (1984): Prospective assessment of menstrually related mood disorders. Am J Psychiatry 141:684–686

Schnurr PP (1989): Measuring the amount of symptom change in the diagnosis of premenstrual syndrome. Psychol Assess 1:277–283

Sjöstrom R, Roos B-E (1972): 5-Hydroxyindoleacetic acid and homovanillic acid in cerebrospinal fluid in manic-depressive psychosis. Eur J Clin Pharmacol 4:170–176

Smith SE, Pihl RO, Young SN, Ervin FR (1987): A test of possible cognitive and environmental influences on the mood lowering effect of tryptophan depletion in normal males. Psychopharmacology 91:451–457

Spitzer RL (1987): Structured Clinical Interview for DSM-IIIR. New York, Biometrics Research Department, New York State Psychiatric Institute

von Knorring L, Oreland L, Haggendal J, Magnusson T, Almay B, Johanssson F (1986): Relationship between platelet MAO activity and concentrations of 5-HIAA and HVA in cerebrospinal fluid in chronic pain patients. J Neural Transm 66:37–46

Weissman MM, Klerman GR (1977): Sex differences in the epidemiology of depression. Arch Gen Psychiatry 34:98–111

Weissman MM, Bland RC, Joyce PR, Newman S, Wells JE, Wittchen H-U (1993): Sex differences in rates of depression: Cross-national perspectives. J Affect Disord 29:77–84

Weltzin TE, Fernstrom JD, McConaha C, Kaye WH (1994): Acute tryptophan depletion in bulimia; Effects on large neutral amino acids. Biol Psychiatry 35:388–397

Wilkins K (1990): Eosinophilia-myalgia syndrome. Can Med Assoc J 142:1265–1266

Young MA, Fogg LF, Scheftner WA, Keller MB, Fawcett JA (1990): Sex differences in the lifetime prevalence of depression: Does varying the diagnostic criteria reduce the female/male ratio? J Affect Disord 18:187–192

Young SN, Gauthier S, Anderson GM, Purdy WC (1980): Tryptophan, 5-hydroxyindoleacetic acid and indoleacetic acid in human cerebrospinal fluid: Interrelations and the influence of age, sex, epilepsy, and anticovulsant drugs. J Neurol Neurosurg Psychiatry 43:438–445

Young SN, Gauthier S (1981): Effect of tryptophan administration on tryptophan, 5-hydroxyindoleacetic acid and indoleacetic acid in human lumbar and cisternal cerebrospinal fluid. J Neurol Neurosurg Psychiatry 44:323–328

Young SN, Smith SE, Pihl RO, Ervin FR (1985): Tryptophan depletion causes a rapid lowering of mood in normal males. Psychopharmacology 87:173–177

Young SN (1992): The effect of increasing and decreasing tryptophan availability on mood and behavior in humans. In Advances in Tryptophan Research, Toyoake, Fujita Health University Press, pp 49–54