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Differential effect of the 5-HTT gene-linked polymorphic region on emotional eating during stress exposure following tryptophan challenge☆

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Abstract

Stress and negative moods, which are thought to be partly mediated by reduced brain serotonin function, often increase emotional eating in dieting women (restrainers). Because the short (S) allele polymorphism in the serotonin transporter gene (5-HTTLPR) is associated with serotonin dysfunction, S allele compared to long (L) allele 5-HTTLPR genotypes may be more susceptible to stress-induced emotional eating. Consequently, serotonin challenge via tryptophan (TRP)-rich protein hydrolysate (TPH) may alleviate stress-induced emotional eating particularly in S/S allele carriers.

We tested whether acute stress affects emotional eating in women with high or low dietary restraints depending on their 5-HTTLPR genotype and TPH intake.

Nineteen female subjects who were homozygous for the short-allele 5-HTTLPR genotype ($S'/S'=S/L_G$, L_G/L_G: restrainers vs. nonrestrainers) and 23 female subjects who were homozygous for the long-allele 5-HTTLPR genotype ($L'/L'=L_A/L_A$: restrainers vs. nonrestrainers) were tested in a double-blind, placebo-controlled crossover study of stress-induced emotional eating following intake of TPH or a placebo.

TPH intake significantly increased the plasma TRP/large neutral amino acid ratio ($P<.0001$) in the L′/L′ group (70%) compared to the S′/S′ group (30%). TPH reduced food intake in both groups, but in the L′/L′ group, it also reduced stress-induced negative mood ($P=.037$) and the desire for sweet, high-fat foods ($P=.011$) regardless of dietary restraint. *Conclusions:* Since TPH caused a greater increase in the plasma TRP/large neutral amino acid ratio in the L′/L′ group compared to S′/S′ group, the exclusive beneficial effects of L′/L′ genotype may be due to enhanced brain 5-HT function.

Keywords: Serotonin; 5-HTTLPR; Stress; Emotional eating; Tryptophan

1. Introduction

Stress often deteriorates mood and affects food preference and intake [1]. Although stress can cause hypophagia [2,3], abundant studies have consistently revealed that mild to moderate acute stress and/or negative moods increase the preference for and intake of sweet (SW), high-fat foods [4-9]. This so-called 'emotional eating' in response to negative moods or distress has a clear female preponderance [10,11] because women try to restrain food intake to maintain or lose body weight more often than men [5,12].

Stress-induced emotional eating may be attributed to diminished serotonergic (5-HTergic) neurotransmission or function [13]. Reduced brain 5-HT function is associated with maladaptive stress coping and depressive moods [14-18] and with an increased appetite and intake of SW, high-fat foods [19,20]. 5-HT vulnerability or an enhanced sensitivity to dysregulation in the 5-HTergic system has a genetic basis [15,21]. Most genetic research has focused on a polymorphism in the promoter region of the 5-HT transporter (5-HTTLPR) gene, including a short (S) and a long (L) allele [22,23]. The S allele has lower transcriptional efficiency than the L allele [22,23], which is subsequently thought to promote 5-HT dysfunction [22-24]. Based on the notion that reduced 5-HT function is associated with reduced stress coping, negative moods and excessive energy intake, previous studies have found that the S allele of 5-HTTLPR is associated with stress-related depression [25,26], increased risk for obesity [27,28] and an increased effect of depression on emotional eating [29].

Based on the relationship between stress, 5-HT dysfunction and eating behavior, S-allele carriers of the 5-HTTLPR genotype may be particularly susceptible to emotional eating under acute stress exposure. This interaction between acute stress, 5-HTTLPR and emotional eating has not yet been experimentally investigated. Moreover, since S-allele 5-HTTLPR genotypes are thought to be 5-HT vulnerable for the negative effects of stress, brain 5-HT augmentation in these subjects may be beneficial in reducing stress-related emotional eating. Brain 5-HT can be augmented by increasing the availability of its precursor, tryptophan (TRP) [30]. A recent dietary method to increase TRP availability and 5-HT synthesis involves the administration of TRP-rich protein hydrolysate (TPH).

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TPH and less effective, intact TRP-rich proteins have previously been found to increase the ratio of plasma TRP to the sum of the other large neutral amino acids (the TRP/LNAA ratio) by 70%–220% [17,31-34] and to increase brain 5-HT synthesis and release [31,35]. Recently, TRP augmentation was found to improve mood, particularly in S-allele carriers [36].

The aim of the present study was twofold. The first objective was to examine the effects of acute stress on mood and emotional eating in female S/S- and L/L-allele carriers of the 5-HTTLPR genotype (both groups were divided into dietary restrainers and nonrestrainers). Second, a related goal was to explore the effects of 5-HT augmentation. The following was hypothesized: (a) only S/S allele carriers (particularly restrainers) would be susceptible to a debilitated mood and an increased desire for SW, high-fat foods after acute stress, and (b) administration of TPH would increase the plasma TRP/LNAA ratio and hence alleviate stress-induced emotional eating in S allele carriers.

2. Methods and materials

2.1. Participants

Undergraduate students at Maastricht University (N=550) completed a questionnaire screening package concerning general information (health, personal or family history of medical or psychiatric complaints, smoking and drinking habits, caffeine consumption, weight and height, use of psychoactive drugs) and several questionnaires concerning relevant symptoms, psychopathology and restrained eating styles. Participants were excluded from further evaluation if they reported chronic or current physical or psychiatric illness; family history of psychiatric illness; medication use; metabolic, hormonal or intestinal diseases; a body mass index >24 kg/m^2; irregular diets or deviant eating habits; excessive use of alcohol (>2 units a day), coffee (>10 units a day), cigarettes or other drugs; aversion for certain foods and pregnancy. Following this first selection, 400 participants attended a buccal sample extraction session to genotype for 5-HTTLPR (resulting in 26% S/S, 47% S/L and 27% L/L). Since brain 5-HT vulnerabilities for stress are mainly found in homozygotes S compared to homozygotes L allele carriers [25,37,38], only homozygous S allele (S/S, S/L_G, L_G/L_G; classified as S′/S′) and homozygous L allele (L_A/L_A; classified as L′/L′) (both divided into the highest vs. lowest restrained eaters) were then invited to participate in the experimental study. (After selection for restrained eaters and invitation, a large number of 88 S′/S′ and 85 L′/L′ subjects could not be included in the study due to their median scores on restrained eating styles, summer holiday or other nonspecified reasons.)

Nineteen female S′/S′ carriers (10 restrainers with the highest quartile scores of the "Three Factor Eating Questionnaire" or TFEQ [39]; 9 nonrestrainers with the lowest quartile TFEQ scores) and 23 female L′/L′ carriers (10 restrainers with the highest quartile TFEQ scores; 13 nonrestrainers with the lowest quartile TFEQ scores) were included in the study. Participants were between 18 and 25 years of age (M=19±2 years), and all were in the normal range of the body mass index (M=22±2 kg/m^2). Participants were matched for contraception and participated during their mid–late follicular phase (days 4–10) or when actually using the contraception pill. The study was approved by the Medical Ethics Committee of the Academic Hospital Maastricht (CTCM azM, Maastricht, the Netherlands), and the procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 1983. All participants gave their informed consent to participate and were paid for participation.

2.2. Design and procedure

The study was conducted in accordance to a double-blind, placebo-controlled crossover design. During two experimental sessions, participants visited the laboratory to monitor their mood, food liking and food intake before and after acute stress exposure and following either TPH or placebo protein (PLC) intake. The order of the dietary condition was counterbalanced over the two test days with a washout period of at least 1 week.

Before each test day, participants were instructed to refrain from alcohol for at least 36 h and to fast 12 h before the sessions; only water or caffeine-free tea without sugar was permitted. Fig. 1 shows a schematic diagram of the experimental protocol.

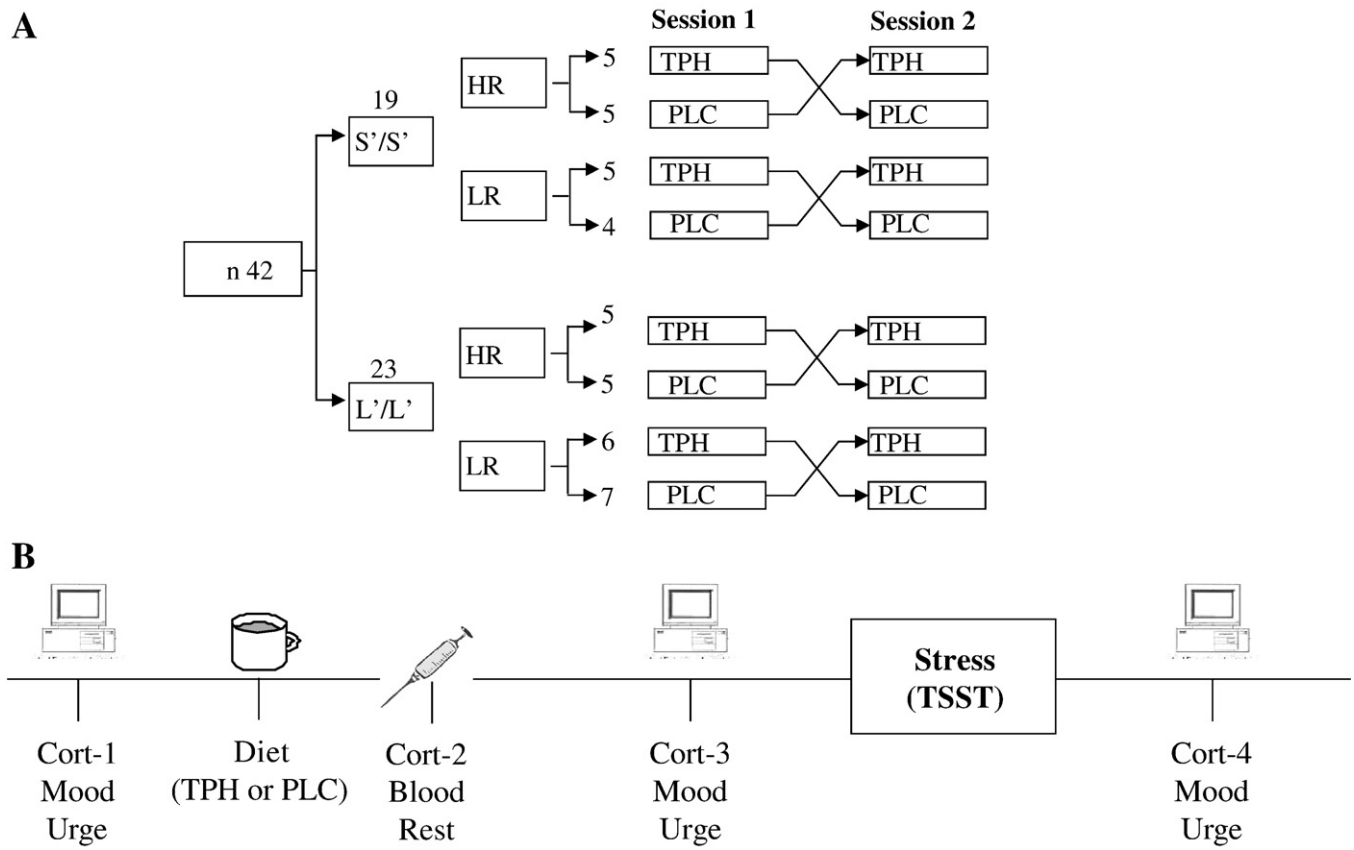

Fig. 1. General design (A) and schedule (B) of the experiment. (A) During two experimental acute stress sessions, healthy female S′/S′ and L′/L′ 5-HTTLPR genotypes with high (HR) or low (LR) restrained eating patterns were monitored for mood and urge for food before and after stress following intake of TPH and PLC. Order of treatment was balanced between groups. (B) During two experimental sessions, cortisol (cort), mood and urge for food were measured before and after acute stress (TSST) following TPH or PLC. Before and after stress induction, also actual food intake was measured. Blood drawing for plasma amino acid measure was conducted 90 min after TPH or PLC intake.

On each experimental test day, three pairs of participants arrived at the laboratory at respectively 0900, 1000 and 1100 AM. After arrival, a first saliva sample was taken, immediately followed by consumption of the TPH or PLC drink. Then, participants were enabled to rest (reading or watching TV) for 90 min. Next, 1½ h after consumption, a second saliva sample was taken followed by a blood sample, to measure the effect of TPH compared to PLC on the plasma TRP/LNAA ratio. Following a 15-min relaxation period, participants provided a third saliva sample and were brought into their own separated laboratory room to conduct a test battery assessing mood and food liking. Directly after this test battery, still in their separated room, each participant was instructed to prepare for an interview to an unknown University staff-member panel. During this 5-min preparation phase, participants had free access to preweighed portions of snack foods and were instructed to eat as much as they wish. After the preparation phase, each pair of participants was brought together in a larger experimental room to conduct a 15-min stress-inducing interview (see "Stress manipulation"). After completion of the stress-inducing interview procedure, participants provided a final saliva sample and completed a second version of the mood and food liking test battery and had 5 min of free access to preweighed portions of snack foods.

2.3. Stress manipulation

The experimental stress procedure consisted of an adapted version of the Trier Social Stress Test (TSST) [40]. The TSST is an often used method to induce acute, psychological stress under laboratory conditions. The current task primarily consists of two phases: an individual preparation phase and a public speaking task including a competitive interview task performed in front of an audience.

2.4. Preparation phase

During a brief standardized written introduction, participants were separately informed that they must prepare for an interview–presentation about their personal strong points concerning academic career and personal life in front of a trained audience for 15 min. To increase stressfulness in the current adjusted version of the TSST, participants were required to give their interview–presentation in English and in competition with a peer participant. Furthermore, participants were led to believe that the participant with the highest score, as decided by the trained audience, would obtain an additional financial compensation of 75 Euro. Participants were additionally informed that during the interview also, their mathematical fluency would be tested by occasional serial subtraction tasks and that their performance would be video- and audio-taped in order to construct a profile of their personality and to relate this to their general performance.

2.5. Public speaking phase

After the preparation phase, both participants were joined in a large experimental room and physically separated (using a mobile wall-screen between participants) in front of a panel of three senior university staff members (two males and one female), and a video camera and voice recorder. The panel was introduced as being experts in the evaluation of nonverbal behavior, and participants were again told that their performance would be video- and audio-taped. For a total period of 15 min, participants were interviewed about their positive and negative personal characteristics and academic skills. The panel gave verbal and nonverbal indications of frustration over the quality of the interview. They displayed nonverbal signs of boredom and exchanged glances with each other that communicated mutual negative assessments. During interviewing, at four different unpredictable 120±30-s time interval occasions, participants were instructed to count backwards out loud by seven as quickly and accurately as possible starting from a certain number (403, 425, 530 or 840; counterbalanced within-subjects). Participants were urged by the experimenter to go faster, and when participants made a mistake, they were interrupted and had to start over.

2.6. Dietary manipulation

During both experimental sessions, a 200-ml drink was consumed containing different TRP or LNAA concentrations from TRP-rich protein hydrolysate (TPH; DSM Delft, the Netherlands) or placebo casein protein hydrolysate (PLC; DSM Delft). Key characteristics of the product are given in Table 1. All drinks were prepared and encoded by the supplier (DSM Delft) and contained 0.10 g sweetener (acesulfame) to improve palatability. A research assistant blind to the dietary and experimental conditions conducted the administration of the drinks.

2.7. Measurements

2.7.1. Mood

Changes in mood were measured using the Dutch shortened version of the Profile of Mood States (POMS) questionnaire [41], offered as a 100-unit visual analogue scale ranging from 'not at all' to 'extremely.' The POMS comprises five different subscales for mood, ranging from anger, tension, depression and fatigue that refer to a negative mood state, to vigor concerning a positive mood.

Table 1
Composition of the standard PLC and TPH condition

Source	Product	TRP (mg)	TRP/LNAA (mol/mol)
PLC	4 g/200 ml	32	0.02
THP	4 g/200 ml	235	0.21
Amino acid profile (g)		TPH	PLC
Isoleucine		143	152
Leucine		231	300
Phenylalanine		105	148
Tyrosine		137	176
Valine		131	204
TRP		235	32

2.7.2. Food liking

Food liking was defined as the anticipated pleasure derived from tasting a food and was assessed by a computer task [42]. Sixteen photographic food stimuli were presented on a computer screen. The foods were arranged into separate categories of SW, savory (SA), high fat (HF) and low fat (LF), or they could be combined to form high-fat sweet (HFSW), low-fat SW (LFSW), high-fat SA (HFSA) and low-fat SA (LFSA) categories. Each food picture was presented one at a time and rated according to a 100-unit visual analogue scale anchored at each end with 'not at all' to 'extremely' combined with the statement 'How pleasant would it be to taste this food now?' Participants were asked to move a centered cursor along the line to indicate their response. Mean liking ratings for each food category were calculated.

2.7.3. Food intake

To measure snacking behavior, participants were presented with a food tray before (preparation) and after stress exposure, containing preweighed portions of snack foods (minicandy bars, pretzels and nuts). Each time after preparation and after completion of the public speaking (interview) task, the food container was weighted to determine the total amount of food intake.

2.8. Biochemical analysis

2.8.1. Genotyping

Buccal cell samples for measuring triallelic variants of the 5-HTT-linked polymorphic region (5-HTTLPR) were obtained using sterile swabs (Omni Swabs; Whatman's Hertogenbosch, the Netherlands). Genomic DNA was isolated from buccal swabs using QIamp DNA Mini Kits from Qiagen (Westburg, Leusden, the Netherlands) for determination of the 5-HTTLPR genotype. Genotyping was performed using the polymerase chain reaction protocol according to Glatz et al. [43]. In compliance with previous work [36,43-45], triallelic variants were reclassified into a biallelic model as follows: S/S, S/L_G and L_G/L_G were classified as S′/S′ and L_A/L_A as L′/L′.

2.8.2. Plasma amino acids

Blood samples were collected using 5-ml Vacutainer tubes (half-filled) containing sodium heparin for amino acids and in a tube containing gel and clot activator for glucose analysis. The sodium–heparin tube was centrifuged at 5000 rpm for 5 min at 4°C. Subsequently, the supernatants were stored at −80°C until analysis. Before storage, the supernatant (2× 100 μl) was mixed with 4 mg sulfosalicylic acid. Plasma amino acid analysis was conducted with high-performance liquid chromatography (HPLC), making use of a 2- to 3-μm Bischof Spherisorb ODS II column. The plasma TRP ratio was ultimately calculated by dividing the plasma TRP concentration by the sum of the other large neutral amino acids, that is, valine, isoleucine, leucine, tyrosine and phenylalanine.

2.8.3. Saliva cortisol

Cortisol samples were obtained by using the Salivette sampling device (Sarstedt, Etten-Leur, the Netherlands). With this procedure, saliva was collected in small polyester swabs and stored (−25°C) immediately upon collection until centrifugation. Saliva samples were centrifuged at $2650g_{max}$ for 3 min at 20°C. Salivary-free cortisol levels were determined in duplicate by direct radioimmunoassay (University of Liège, Belgium), including a competition reaction between ^{125}iodohistamine–cortisol and anticortisol serum made against the 3-carboxymethyl-oxime-bovine serum albumin (3-CMO-BSA) conjugate. After overnight incubation at 4°C of 100 μl of saliva, separation of free and antibody-bound ^{125}iodohistamine–cortisol was performed via a conventional 'second antibody' method. In order to reduce sources of variability, all samples were analyzed in the same assay.

2.9. Statistical analyses

Data were first examined for accuracy of data entry, missing values and normal distributions. Hardy–Weinberg equilibrium was determined on the original 5-HTTLPR database (N=400) using χ^2 tests, revealing that the genotype frequencies of S′/S′ (n=104, or 26%), S/L (n=188, or 47%) and L′/L′ (n=108, or 27%) did not depart significantly from Hardy–Weinberg equilibrium ($\chi^2_{(1)}$=1.56, P>.21).

The main research questions were analyzed by means of repeated-measures multivariate and univariate analyses of variance (MANOVA and ANOVA) by using the General Linear Model (GLM; SPSS 12.0 for Windows) with "genotype" (S′/S′ vs. L′/L′) and *Restrained Eating Style* (high vs. low) as between-subjects factor and "treatment" (TPH vs. PLC) and "stress" (prestress vs. poststress) as within-subjects factors on the several dependent measures (mood, food liking, food intake and cortisol). For measuring effects on the five different levels of the POMS, MANOVAs were performed. Significant results revealed by these procedures were further examined by univariate tests. Huynh–Feldt or Greenhouse–Geisser corrected *P* values, their corresponding epsilons and the original, that is, uncorrected, degrees of freedom are reported when the sphericity assumption was not met. The study, including validation of the group size, was designed to detect a large effect size (μ^2=0.20) for a power of 0.80 at α=.05. All statistics are evaluated at a significance level of 5% (two-tailed). Data are reported as means±S.D.

3. Results

3.1. Plasma TRP/LNAA

Repeated-measures ANOVA with *Genotype* (S′/S′ vs. L′/L′) and *Restrained Eating Style* (high vs. low) as between-subjects factor and *Treatment* (TPH vs. PLC) as within-subjects factor on the plasma TRP/LNAA ratio revealed a significant main effect of *Treatment* [F(1,35)=140; *P*<.0001] and a significant interaction effect of *Treatment*×*Genotype* [F(1,35)=12; *P*<.001]. As shown in Fig. 2, there was a significant increase in plasma TRP/LNAA after TPH (0.14±0.03) as compared with PLC (0.09±0.02), but this increase was significantly higher in L′/L′ (from 0.09±0.02 to 0.15±0.03) than in S′/S′ genotypes (from 0.10±0.01 to 0.13±0.02). No other effects where found.

3.2. Cortisol

Repeated-measures ANOVA with *Genotype* (S′/S′ vs. L′/L′) and *Restrained Eating Style* (high vs. low) as between-subjects factors and *Treatment* (TPH vs. PLC) and *Stress* (prestress vs. poststress) as within-subjects factors only revealed a significant main effect of *Stress* [F(1,40)=4.8; *P*=.03]. As shown in Fig. 3, there was a significant increase in cortisol concentrations after stress exposure without differences for genotype, restrained eating style or treatment condition.

3.3. Mood

A repeated-measures MANOVA with *Genotype* (S′/S′ vs. L′/L′) and *Restrained Eating Style* (high vs. low) as between-subjects factor and *Stress* (prestress vs. poststress) and *Treatment* (TPH vs. PLC) as within-subjects factors revealed a significant main effect of *Stress* [F(5,34)=13,78; *P*<.0001], a significant interaction of *Stress*×*Genotype* [F(5,34)=3.06; *P*=.022] and a significant interaction of *Treatment*×*Stress*×*Genotype* [F(5,34)=2.7; *P*=.037]. Further analysis revealed that this three-way interaction effect originated from significant changes in anger [F(1.38)=4.58; *P*=.039]. As indicated in Fig. 4, a stress-induced increase in feelings of anger following PLC was prevented following TPH only in the L′/L′ group, whereas in the S′/S′ group, no treatment differences were found.

Analysis also revealed a significant interactive effect of *Stress*×*Restrained Eating Style* [F(1.38)=4.58; *P*=.013] that originated from feelings of depression (*P*=.05), fatigue (*P*=.004) and vigor (*P*=.006). Subjects with high restrained eating styles were less affected by acute stress exposure than subjects with low restrained eating styles.

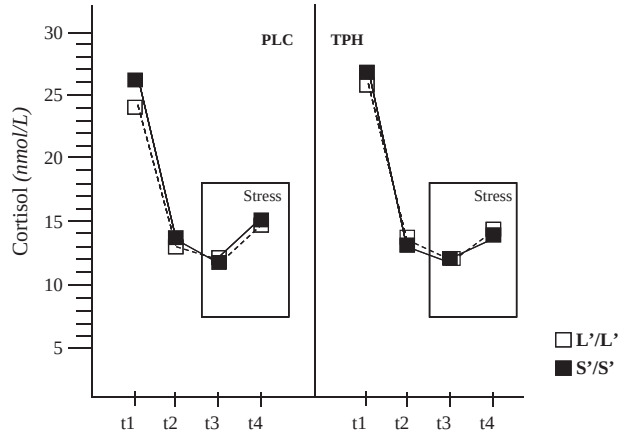

Fig. 3. Mean changes in salivary cortisol concentrations after intake of TPH and PLC in female S′/S′ (■) and L′/L′ (□) genotypes across morning baseline (1), treatment (2) and before (3) and after (4) stress exposure. Morning baseline cortisol significantly declined across time until prestress exposure (3), and then significantly increased poststress exposure (4) without any effect of dietary condition or 5-HTTLPR.

3.4. HFSW or LFSW food liking

Two separate repeated-measures ANOVAs with *Genotype* (S′/S′ vs. L′/L′) and *Restrained Eating Style* (high vs. low) as between-subjects factors and *Treatment* (TPH vs. PLC) and *Stress* (prestress vs. poststress) as within-subjects factors were conducted on liking for HFSW foods and for LFSW foods. Analyses revealed a significant effect of *Stress* [F(1,34)=5.17; *P*=.029] and a *Treatment*×*Stress*×*Genotype*

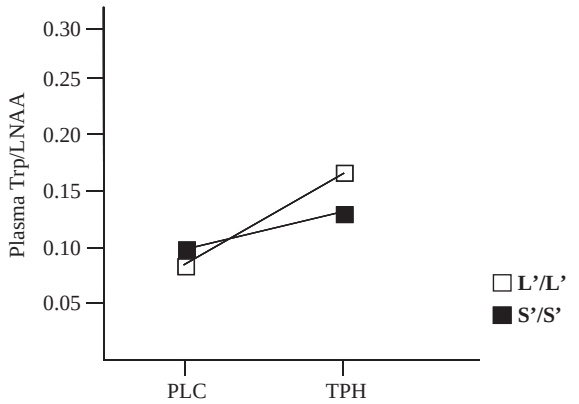

Fig. 2. Mean changes in plasma TRP as compared with the other LNAAs after intake of TPH and PLC in female S′/S′ (■) and L′/L′ (□) 5-HTTLPR genotypes. There was a significant increase in plasma TRP/LNAA after TPH as compared to PLC, but this increase was significantly higher in L′/L′ (70%) than in S′/S′ genotypes (30%).

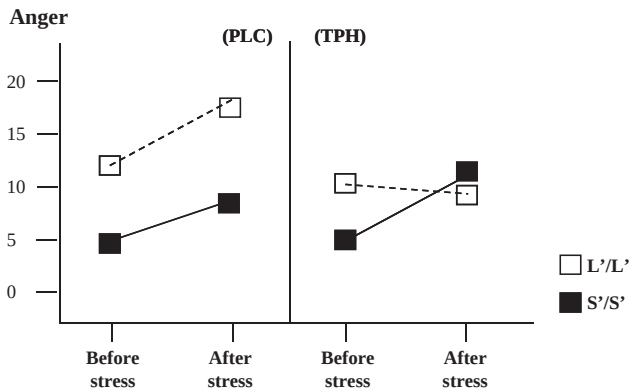

Fig. 4. Acute stress increased negative mood following PLC in female S′/S′ (■) and L′/L′ (□) 5-HTTLPR genotypes. This stress-induced negative mood only in S′/S′ was prevented by intake of TPH.

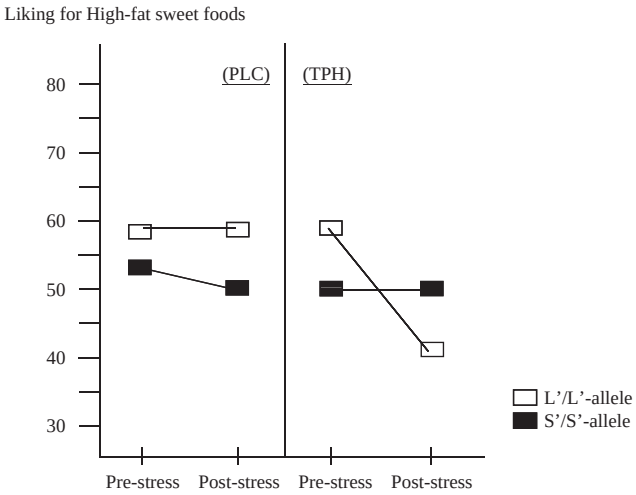

Fig. 5. In female L′/L′ 5-HTTLPR genotypes (□), TPH compared to PLC significantly reduced liking for HFSW foods after acute stress exposure, which was not found in S′/S′ genotypes (■).

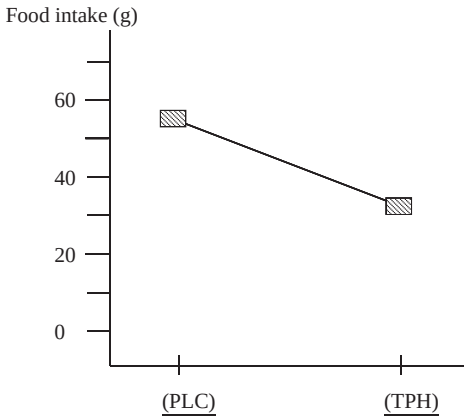

Fig. 6. General food intake significantly reduced after TPH compared to PLC intake, regardless of stress or 5-HTTLPR (data pooled).

interaction [F(1,34)=7.96; *P*=.008] on liking for HFSW foods indicating that treatment differently affected liking for HFSW foods after stress depending on genotype. As indicated in Fig. 5, only in L′/L′-allele genotypes TPH compared to PLC significantly reduced liking for HFSW foods after stress, which was not found in S′/S′-allele genotypes. No other effects were found.

3.5. High- or low-fat SA food liking

Two separate ANOVAs with *Genotype* (S′/S′ vs. L′/L′) and *Restrained Eating Style* (high vs. low) as between-subjects factors and *Treatment* (TPH vs. PLC) and *Stress* (prestress vs. poststress) as within-subjects factors were conducted on liking for high-fat protein foods and low-fat protein foods. Analyses did not reveal any main or interaction effects on liking for HF or LF protein foods.

3.6. Food intake

Two separate ANOVAs with *Genotype* (S′/S′ vs. L′/L′) and *Restrained Eating Style* (high vs. low) as between-subjects factors and *Treatment* (TPH vs. PLC) and *Stress* (prestress vs. poststress) as within-subjects factors on food intake did not reveal any main or interaction effects. To explore whether effects might have particularly appeared during stress preparation, two additional analyses were conducted with *Genotype* (S′/S′ vs. L′/L′) and *Restrained Eating Style* (high vs. low) as between-subjects factors and *Treatment* (TPH vs. PLC) as within-subjects factor on total food intake during preparation. Analyses revealed a main *Treatment* effect [F(1,35)=4.73; *P*=.037], indicating a total reduction of food intake of 21g after the TPH (34±27 g) compared to PLC (55±50 g) (see Fig. 6). No other effects were found.

4. Discussion

The aim of the present study was to examine the effects of acute stress on emotional eating in healthy female S′/S′ and L′/L′ allele carriers of the 5-HTTLPR genotype and to investigate whether administration of TPH would alleviate stress-induced emotional eating in S′/S′ allele carriers; subjects were divided into restrained and nonrestrained eaters. TPH significantly reduced the negative effects of stress on mood and the desire for SW, high-fat foods in the L′/L′ allele carriers and lowered general food intake regardless of 5-HTTLPR genotype and restrained eating.

4.1. TRP/LNAA

Stress-induced emotional eating, particularly in S′/S′ allele carriers, was expected to be affected by TPH due to enhanced 5-HT vulnerability (see "Introduction"). Changes in plasma TRP/LNAA ratios were assessed to measure the effects of TPH on brain TRP availability. Previous data have shown that as little as 50%–70% variation in the plasma TRP/LNAA ratio is sufficient to cause meaningful changes in brain TRP and 5-HT levels [17,31,35,46,47], and >70% increases in plasma TRP/LNAA ratios have been observed in response to TPH or intact TRP-rich protein intake [31,34,35]. Surprisingly, TPH caused a meaningful (70%) increase in the plasma TRP/LNAA ratio in L′/L′ allele carriers, whereas in S′/S′, the increase was less significant (30%). Based on previously mentioned findings, this suggests that TPH was able to cause a meaningful increase in 5-HT only in individuals with the L′/L′ genotype. The intriguing question is why TPH causes a greater increase in the plasma TRP/LNAA ratio in L′/L′ than in S′/S′ genotypes. No other studies have yet examined changes in the plasma TRP/LNAA ratio following TRP augmentation, although data obtained from acute TRP depletion studies did not reveal 5-HTTLPR-related differences in the attenuating effects of this plasma amino acid ratio [45,48]. It may be suggested that the commonly assumed enhanced stress vulnerability of S′/S′ individuals is accompanied by faster TRP uptake or metabolism in peripheral tissue, which may reduce the availability of plasma TRP for uptake into the brain. One possible mechanism involves the kynurenine (KYN) pathway, which is initiated by the enzyme indoleamine 2,3-dioxygenase and that metabolizes most plasma TRP after dietary intake [49]. Immunological challenges, such as proinflammatory cytokines and stress experiences, have been found to increase peripheral TRP metabolism by inducing indoleamine 2,3-dioxygenase and increasing glucocorticoids [50]. Since S′/S′ allele genotypes are more prone to stress, this might be an involved mechanism. This then suggests that stress vulnerability in S allele genotypes is partly mediated by enhanced peripheral TRP metabolism (reducing its uptake into the brain), which is an interesting hypothesis that should be explored in future studies.

4.2. Acute stress induction

Acute stress exposure caused a significant cortisol stress response and negative mood regardless of 5-HTTLPR genotype and showed

successful acute stress induction by the current stress protocol. Previous studies investigating the mediating effects of 5-HTTLPR on stress-induced hormonal or affective changes revealed mixed results. Some studies reported acute stress-induced cortisol responses only in S′/S′ compared to L′/L′ subjects [18,51], whereas others could not replicate such interactions [36,52,53]. A likely explanation for these conflicting results might be the presence of an additional (cognitive) stress vulnerability factor that mediates the effect of 5-HTTLPR on stress vulnerability [54,55].

4.3. Emotional eating

Contrary to the negative effects on mood, stress exposure did not increase any signs of emotional eating regardless of 5-HTTLPR genotype or restraint eating styles. As indicated in "Introduction," previous studies have reported that mild to moderate stress increases emotional eating, particularly the desire and intake of SW, high-fat foods, especially in females with high scores on dietary restraint [4-9]. This absence of effect of acute stress exposure on emotional eating may be due to the inability of the current stress task to cause severe changes in depressive mood. In a previous study, 5-HTTLPR was found to mediate emotional eating only when it was associated with depressive feelings [29]. In addition, the absence of an effect of stress on emotional eating may also be due to the relative small group sizes of restrained and unrestrained S′/S′ and L′/L′ groups because restrained females appeared to be more vulnerable to the negative mood effects of stress exposure compared to restrained females [5,12].

As expected, administration of TPH significantly reduced negative moods and signs of stress-induced emotional eating depending on the 5-HTTLPR genotype. However, contrary to our expectations, the beneficial effects of TPH were exclusively found in L′/L′ individuals; this was demonstrated by a reduction in stress-induced feelings of anger and hence a reduced desire for SW, high-fat foods. This exclusive effect of TPH on mood improvement and reduced attention to carbohydrate-rich food cues in L′/L′ genotypes are likely caused by its greater enhancing effect on plasma TRP/LNAA ratios in L′/L′ individuals compared to S′/S′ individuals. As a 50%–70% increase in TRP/LNAA ratio has already been found to increase brain TRP and 5-HT [17,31,35], the current exclusive beneficial effects of TPH in L′/L′ individuals are in line with previously demonstrated findings of an enhanced preference for carbohydrate-rich SW foods as a consequence of reduced brain 5-HT function [56,57]. Contrary to the exclusive effects of TPH in L′/L′ individuals on mood and attention to SW, high-fat food cues, TPH did reduce general food intake (even though all subjects had been fasting before they began the study) regardless of 5-HTTLPR genotype. This apparent contradiction of TPH may be in line with the inadequate rise in plasma TRP/LNAA ratio in S′/S′ individuals; this explains the absence of a TPH effect on cerebral-related changes in mood and food preferences in S′/S′ individuals (by lower brain TRP uptake and 5-HT synthesis) and supports the role of TPH in reducing general food intake in L′/L′ and S′/S′ individuals (a minimal increase in peripheral TRP may have increased peripheral signals for satiation in both groups [58]).

4.4. Limitations

The results of the current study should be considered in the context of three methodological limitations. First, no measurements of differences in SW or SA food intake were included in the study. Because TPHs were found to reduce attention to and urges for SW, high-fat foods, it may be likely that it also reduced actual intake of such foods. This is in line with previous findings of an enhanced preference for carbohydrate-rich, SW foods as a consequence of reduced brain 5-HT function [56,57,59]. Second, because group sizes for restrained versus unrestrained eaters were rather small, findings (as well as nonfindings) related to restrained eating should be interpreted with caution. Third, because only female participants were examined, current findings should not be generalized to males. In the current study, only female participants were included because of the higher prevalence of stress-induced eating in females and to increase the homogeneity of the sample. However, to explore possible gender differences, further studies should include male participants.

5. Conclusion

The current findings reveal that hydrolyzed proteins rich in TRP may reduce nonspecific food intake regardless of 5-HTTLPR genotype, but may diminish the negative effects of stress on mood and preference for SW, high-fat foods in only L′/L′ individuals. Because TPH caused a meaningful rise in plasma TRP/LNAA ratios only in L′/L′ individuals, the exclusive beneficial effects of TPH on mood and preference for SW foods in L′/L′ individuals may be related to a more enhanced brain 5-HT function. Further investigation is needed to explore the effects of dietary TRP manipulation on stress and 5-HTTLPR-mediated actual intake of specific SW and SA foods in a larger population of restrained and unrestrained male and female subjects. In addition, it might also be interesting to explore the effects of TRP protein manipulation on food intake among obese emotional overeaters.

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References

[1] Greeno CG, Wing RR. Stress-induced eating. Psychol Bull 1994;115:444–64.
[2] Adam TC, Epel ES. Stress, eating and the reward system. Physiol Behav 2007;91:449–85.
[3] Pollard TM, Steptoe A, Canaan L, Davies GJ, Wardle J. Effects of academic examination stress on eating behavior and blood lipid levels. Int J Behav Med 1995;2:299–320.
[4] Epel E, Lapidus R, McEwen B, Brownell K. Stress may add bite to appetite in women: a laboratory study of stress-induced cortisol and eating behavior. Psychoneuroendocrinology 2001;26:37–49.
[5] Mitchell SL, Epstein LH. Changes in taste in dietary-restrained women following stress. Physiol Behav 1996:495–9.
[6] Newman E, O'Connor DB, Conner M. Daily hassles and eating behaviour: the role of cortisol reactivity status. Psychoneuroendocrinology 2007;32:125–32.
[7] Oliver G, Wardle J, Gibson EL. Stress and food choice: a laboratory study. Psychosom Med 2000;62:853–65.
[8] Rutters F, Nieuwenhuizen AG, Lemmens SGT, Born JM, Westerterp-Plantenga MS. Acute stress-related changes in eating in the absence of hunger. Obesity 2008;17:72–7.
[9] Zellner DA, Loaiza S, Gonzales Z. Food selection changes under stress. Physiol Behav 2006;87:789–93.
[10] Cyranowsky JM, Frank E, Young E, Shear MK. Adolescent onset of the gender difference in lifetime rates of major depression. Arch Gen Psychiatry 2000;57:2127.
[11] Wade TJ, Carney J, Pevalin DJ. Emergence of gender differences in depression during adolescence: national panel results from three countries. J Am Acad Child Adolesc Psychiatry 2002;31:190–8.
[12] Levine MD, Marcus MD. Eating behavior following stress in women with and without bulimic symptoms. Ann Behav Med 1997;19:132–8.
[13] Leibowitz SF, Alexander JT. Hypothalamic serotonin in control of eating behavior, meal size, and body weight. Biol Psychiatry 2000;44:851–64.
[14] McAllister-Williams RH, Ferrier IN, Young AH. Mood and neuropsychological function in depression: the role of corticosteroids and serotonin. Psychol Med 1998;28:573–84.

[15] Jans LA, Riedel WJ, Markus CR, Blokland A. Serotonergic vulnerability and depression: assumptions, experimental evidence and implications. Mol Psychiatry 2007;12:522–43.
[16] Firk C, Markus CR. Review: Serotonin by stress interaction: a susceptibility factor for the development of depression? J Psychopharmacol 2007;21:538–44.
[17] Markus CR. Dietary amino acids and brain serotonin function; implications for stress-related affective changes. Neuromolecular Med 2008.
[18] Gotlib IH, Joormann J, Minor KL, Hallmayer J. HPA axis reactivity: a mechanism underlying the associations among 5-HTTLPR, stress, and depression. Biol Psychiatry 2008;63:847–51.
[19] Simansky KJ. Serotonergic control of the organization of feeding and satiety. Behav Brain Res 1996;73:37–42.
[20] Brewerton TD. Towards a unified theory of serotonin dysregulation in eating and related disorders. Psychoneuroendocrinology 1995;20:561–90.
[21] Kato M, Serretti A. Review and meta-analysis of antidepressant pharmacogenetic findings in major depressive disorder. Mol Psychiatry 2008;15:473–500.
[22] Heils A, Teufel A, Petri S, Stober G, Riederer P, Bengel D, et al. Allelic variation of human serotonin transporter gene expression. J Neurochem 1996;66:2621–4.
[23] Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 1996;274:1527–31.
[24] Hariri AR, Holmes A. The serotonin transporter and the genetics of affect regulation: the role of the serotonin transporter in neural function. Trends Cogn Sci 2006;10:182–91.
[25] Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, et al. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 2003;301:386–9.
[26] Uher RMP. The moderation by the serotonin transporter gene of environmental adversity in the aetiology of mental illness: review and methodological analysis. Mol Psychiatry 2008;13:131–46.
[27] Sookoian S, Gemma C, Garcia SI, et al. Short allele of serotonin transporter gene promotor is a risk factor for obesity in adolescents. Obesity 2007;15:271–6.
[28] Sookoian S, Gianotti TF, Gemma C, et al. Contribution of the functional 5-HTTLPR variant of the SLC6A4 gene to obesity risk in male adults. Obesity 2008;16:488–91.
[29] Van Strien T, van der Zwaluw CS, Engels RCME. Emotional eating in adolescents: a gene (SLC6A4/5-HTT) — depressive feelings interaction analysis. J Psychiatr Res 2010:1–8.
[30] Markus CR. Effects of carbohydrates on brain tryptophan availability and stress performance. Biol Psychol 2007.
[31] Markus CR, Olivier B, Panhuysen GE, Van Der Gugten J, Alles MS, Tuiten A, et al. The bovine protein alpha-lactalbumin increases the plasma ratio of tryptophan to the other large neutral amino acids, and in vulnerable subjects raises brain serotonin activity, reduces cortisol concentration, and improves mood under stress. Am J Clin Nutr 2000;71:1536–44.
[32] Markus CR, Jonkman LM, Lammers JH, Deutz NE, Messer MH, Rigtering N. Evening intake of alpha-lactalbumin increases plasma tryptophan availability and improves morning alertness and brain measures of attention. Am J Clin Nutr 2005;81:1026–33.
[33] Booij L, Merens W, Markus CR, Van der Does AJ. Diet rich in {alpha}-lactalbumin improves memory in unmedicated recovered depressed patients and matched controls. J Psychopharmacol 2006;20:526–35.
[34] Markus CR, Firk C, Gerhardt C, Kloek J, Smolders GF. Effect of different tryptophan sources on amino acids availability to the brain and mood in healthy volunteers. Psychopharmacology (Berl) 2008;201:107–14.
[35] Orosco M, Rouch C, Beslot F, Feurte S, Regnault A, Dauge V. Alpha-lactalbumin-enriched diets enhance serotonin release and induce anxiolytic and rewarding effects in the rat. Behav Brain Res 2004;148:1–10.
[36] Markus C, Firk C. Differential effects of tri-allelic 5-HTTLPR polymorphisms in healthy subjects on mood and stress performance after tryptophan challenge. Neuropsychopharmacology 2009;34:2667–74.
[37] Wilhelm K, Mitchell PB, Niven H, Finch A, Wedgwood L, Scimone A, et al. Life events, first depression onset and the serotonin transporter gene. Br J Psychiatry 2006;188:210–5.
[38] Zalsman G, Huang YY, Oquendo MA, Burke AK, Hu XZ, Brent DA, et al. Association of a triallelic serotonin transporter gene promoter region (5-HTTLPR) polymorphism with stressful life events and severity of depression. Am J Psychiatry 2006;163:1588–93.
[39] Stunkard A, Messick S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. J Psychosom Res 1985;29:71–83.
[40] Kirschbaum C, Pirke KM, Hellhammer DH. The 'Trier Social Stress Test' — a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 1993;28:76–81.
[41] Wald FDM, Mellenbergh GJ. De verkorte versie van de Nederlandse vertaling van de Profile of Mood States (POMS). Ned Tijdschr Psychol 1990;45:86–90.
[42] Finlayson G, King N, Blundell JE. Is it possible to dissociate 'liking' and 'wanting' for foods in humans? Physiol Behav 2007;90:36–42.
[43] Glatz K, Mossner R, Heils A, Lesch KP. Glucocorticoid-regulated human serotonin transporter (5-HTT) expression is modulated by the 5-HTT gene-promotor-linked polymorphic region. J Neurochem 2003;86:1072–8.
[44] Neumeister A, Hu XZ, Luckenbaugh DA, Schwarz M, Nugent AC, Bonne O, et al. Differential effects of 5-HTTLPR genotypes on the behavioral and neural responses to tryptophan depletion in patients with major depression and controls. Arch Gen Psychiatry 2006;63:978–86.
[45] Walderhaug E, Magnusson A, Neumeister A, Lappalainen J, Lunde H, Refsum H, et al. Interactive effects of sex and 5-HTTLPR on mood and impulsivity during tryptophan depletion in healthy people. Biol Psychiatry 2007.
[46] Carpenter LL, Anderson GM, Pelton GH, Gudin JA, Kirwin PD, Price LH, et al. Tryptophan depletion during continuous CSF sampling in healthy human subjects. Neuropsychopharmacology 1998;19:26–35.
[47] Fernstrom JD, Wurtman RJ. Brain serotonin content: physiological dependence on plasma tryptophan levels. Science 1971;173:149–52.
[48] Firk C, Markus CR. Differential effects of 5-HTTLPR genotypes on mood, memory and attention bias following acute tryptophan depletion and stress exposure. Psychopharmacology (Berl) 2009;203:805–18.
[49] Stone TW, Darlington LG. Endogenous kynurenines as targets for drug discovery and development. Nat Rev Drug Discov 2002;1:609–20.
[50] Miura H, Ozaki N, Sawada M, Isobe K, Ohta T, Nagatsu T. A link between stress and depression: shifts in the balance between the kynurenine and serotonin pathways of tryptophan metabolism and the etiology and pathophysiology of depression. Stress 2008;11:198–209.
[51] Baldwin MW, Taylor SE. The serotonin transporter promotor polymorphism is associated with cortisol response to psychosocial stress. Biol Psychiatry 2010;67:487–92.
[52] Alexander N, Kuepper Y, Schmitz A, Osinsky R, Kozyra E, Hennig J. Gene-environment interactions predict cortisol responses after acute stress: implications for the etiology of depression. Psychoneuroendocrinology 2009;34:1294–303.
[53] Wust S, Kumstra R, Treutlein J, Frank J, Entringer S, Schulze TG, et al. Sex-specific association between the 5-HTT gene-linked polymorphic region and basal cortisol secretion. Psychoneuroendocrinology 2009;34:972–82.
[54] Kendler KS, Kuhn J, Prescott CA. The interrelationship of neuroticism, sex, and stressful life events in the prediction of episodes of major depression. Am J Psychiatry 2004;161:631–6.
[55] Jacobs N, Kenis G, Peeters F, Derom C, Vlietinck R, van Os J. Stress-related negative affectivity and genetically altered serotonin transporter function: evidence of synergism in shaping risk of depression. Arch Gen Psychiatry 2006;63:989–96.
[56] Leibowitz SF, Weiss GF, Walsh UA, Viswanath D. Medial hypothalamic serotonin: role in circadian patterns of feeding and macronutrient selection. Brain Research 1989;27:132–40.
[57] Leibowitz SF, Shor-Posner G. Brain serotonin and eating behaviour. Appetite 1986;7:1–14.
[58] Backus RC, Howard KA, Qr R. The potency of dietary amino acids in elevating plasma cholecystokinin immunoreactivity in cats is related to amino acid hydrophobicity. Regul Pept 1997;26:31–40.
[59] Wurtman RJ, Wurtman JJ. Carbohydrate craving, obesity and brain serotonin. Appetite 1986;7:99.