Effect of Tryptophan, Vitamin B₆, and Nicotinamide-Containing Supplement Loading between Meals on Mood and Autonomic Nervous System Activity in Young Adults with Subclinical Depression: A Randomized, Double-Blind, and Placebo-Controlled Study

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Summary Tryptophan (TRP), a precursor of the mood-enhancing neurotransmitter serotonin and decreased plasma TRP levels was found in depressed patients (1, 2). Data from previous studies provide strong evidence that acute TRP depletion negatively affected mood (3, 4), while inconsistent effects of TRP loading on mood have been reported in both healthy and depressed populations (5, 6). These inconsistencies may arise from differences in TRP dose or study design. Especially with regard to dose, a large dose may induce side effects, such as nausea, headache, and drowsiness (7, 8). It was reported that 1 g of TRP for pregnant women influenced fetal breathing activity (9). Therefore, dose should be as low as possible for safe and practical use.

The pathway for brain uptake of TRP is shared by other large neutral amino acids (LNAA)s, which compete with TRP for transport sites across the blood–brain barrier (BBB) (10). TRP is a limiting amino acid in most dietary proteins (11), thus the normal dietary intake of TRP from protein is lower (<1 g/d) than that of other LNAA_s (12). Thus, protein-rich foods increase LNAA, aside from TRP, leading to lowering of the TRP/LNAA ratio and increasing competition for uptake at the BBB. Therefore, it is desirable to exclusively supplement TRP. Previous studies reported that high protein meals decrease the ratio to 2–3 h after meals (12, 13). Therefore, it is considered that between meals represents the best time for when the influence of other LNAA_s is minimal and TRP is efficiently converted to serotonin. Accordingly, by taking TRP as a supplement between meals, it may be possible to quickly improve mood at a low dose.

Vitamin B₆ acts as a coenzyme in the TRP-serotonin
metabolic pathway (14). Nicotinamide reduces peripheral breakdown of TRP and showed antidepressant effect combined with TRP (15). Therefore these can boost the effect of TRP.

Autonomic nervous system (ANS) activity may also be associated with depressive symptoms. Increased sympathetic activity and/or decreased parasympathetic activity have been observed in patients with depression (16, 17). It has been reported that TRP causes drowsiness and sedation (8, 18), suggesting that parasympathetic activity is activated. Our heart rate variability (HRV) power spectral analysis is a well-accepted, useful, and non-invasive method, which we have previously used to obtain comprehensive quantitative and qualitative evaluation of neuroautonomic function under various physiological conditions (19–22). For instance, we have shown that capsaicin (23) and mixed food intake (24) modulate ANS activity using HRV analysis. However, to our knowledge, no study has investigated TRP supplements’ influence on ANS activity in depressive subjects.

The aim of this study was to investigate the effects of TRP, vitamin B₆, and nicotinamide-containing supplements loading between meals on mood and ANS activity in depressive young adults.

MATERIALS AND METHODS

Subjects. Depressive symptoms of 150 students from Kyoto University were assessed by the Center for Epidemiologic Studies Depression Scale (CES-D). Thirty students aged 18–22 y with subclinical depression (14 male, 16 female) served as subjects. All were in good physical health and had no personal or family histories of hypertension, cardiovascular disease, diabetes mellitus, or other endocrine diseases. None of the subjects smoked, heavily drank, and took any medications or nutritional supplements. Written informed consent was obtained from all subjects after they received a full explanation of the study. All procedures including preliminary experiments described below were conducted in accordance with the tenets of the Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects by the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labor and Welfare, Japan. The study was approved on May 6th, 2016 by the Ethics Committee of the Graduate School of Human and Environmental Studies at Kyoto University (28-H-7). All experiments were conducted between October and December, 2016. This study was registered as a clinical trial to UMIN-CTR (UMIN000024035).

Assessment of depressive symptoms. Depressive symptoms were assessed using the Japanese version of the CES-D (25), an instrument widely used to evaluate depressive state in the general population (26). The instrument includes 20 questions related to emotions, such as loneliness and sadness. Subjects were required to indicate how often they had experienced such emotions in the past week. Each question was assigned a score from 0 to 3 points, for a maximum possible total score of 60. A score ≥16 indicated subclinical depression. In analysis, supplement groups were further classified into two subgroups according to CES-D score (mild to moderate vs. severe depressive symptoms). Severe subclinical depression was defined as a CES-D score ≥26 based on Cho et al. (27). The basic demographic and physical characteristics of the subjects are summarized in Table 1.

Assessment of mood states. Transient mood states were measured by the Profile of Mood States (POMS) (28), which consists of 65 adjectives that are rated by subjects on a five-point scale. The subscales are tension–anxiety (TA), depression-dejection (D), anger-hostility (AH), vigor (V), fatigue (F), and confusion (C).

Blood TRP measurements. Blood samples to measure the total TRP levels were collected in vacuum tubes (Venoject II Vacuum Tube, Terumo Co, Tokyo, Japan) and centrifuged at 3,000 × g for 15 min at 4°C. The plasma samples were stored at −20°C until the assay. Total TRP levels were determined by the sandwich enzyme-linked immunosorbent assay method using a TRP Immunoassay Kit (Abnova, Taipei, Taiwan). All assays were performed in duplicates, as recommended
by the manufacturer. The intra- and inter-assay coefficients of variation were below 10%.

**Supplements and study design.** The effects of between-meal TRP, vitamin B₆, and nicotinamide-containing supplement loading on mood and ANS activity were examined using a randomized, double-blind, placebo-controlled study design. Subjects were divided randomly into two groups, a TRP, vitamin B₆, and nicotinamide-containing supplement group and a placebo control group. The TRP, vitamin B₆, and nicotinamide-containing supplement contained 100 mg of TRP, 4 mg of vitamin B₆, and 4 mg of nicotinamide as active components, with 117 mg of lactate and 5 mg of calcium stearate as additives. The vitamin B₆ in the supplement was in the form of pyridoxine hydrochloride. The placebo supplement contained 270 mg of lactate. The difference of weight was because of filling for the same volume. Both samples were sealed into identical gelatin capsules and were same in appearance, taste, and packaging. Both supplements were obtained from Nitto Pharmaceutical Industries, Ltd., Kyoto, Japan. Both groups took one capsule of the assigned supplement between breakfast and lunch and another between lunch and supper for 7 consecutive days. The meal time differs from individuals because of classes or part-time jobs; therefore, the accurate intake time could not be designated. One given example was 10:30 am and 4:30 pm.

The 200 mg daily dose of TRP was chosen based on the upper limit (220 mg/d) prescribed by the French Food Sanitation and Safety Agency (http://www.fsc.go.jp/fsciis/foodSafetyMaterial/show/syu02950750188). This amount is less than that contained in 100 g of cheese or beef round. In order to investigate the effect of TRP 200 mg dose, we conducted preliminary study. Students aged 18–21 y with subclinical depression (5 male, 2 female) took TRP, vitamin B₆, and nicotinamide-containing and placebo supplements separated by a 1-wk period. The results revealed that there was a significant time×supplements interaction in POMS D scores (p<0.01), indicating that TRP, vitamin B₆, and nicotinamide-containing supplement can significantly improve depressive mood, while placebo supplement did not show such effect. Therefore, it was confirmed that the dose of 200 mg of TRP were enough to improve mood.

A daily 8-mg dose of vitamin B₆ was selected after referring to the Council for Responsible Nutrition “upper levels for supplements.” The U.S. Institute of Medicine (IOM) Food and Nutrition Board “tolerable upper intake level,” the European Commission Scientific Committee on Food UL, and the U.K. Expert Group on Vitamins and Minerals (UK EVM) “guidance level” and safe upper level (https://www.crnusa.org/sites/default/files/files/resources/CRN-SafetyBook-3rdEdition-2014-fullbook.pdf). We considered that the minimum dose among these guidelines was 10 mg (UK EVM), and the daily vitamin B₆ dose was set to 8 mg/d. This dose was several-fold higher than the daily requirements; however, vitamin B₆ is water soluble and not stored in the body (14). Similar to vitamin B₆, a daily 8-mg dose of nicotinamide was selected to be below 35 mg, by referring to the US IOM (29).

**ANS activity measurements.** The interval between heartbeats (the R-R interval) fluctuates beat by beat. HRV is a measure of the amount of this fluctuation around the mean heart rate, which reflects cardiorespiratory control by sympathetic and parasympathetic activities as well as the sympathovagal balance (30). The electrocardiogram (ECG) R-R interval is determined by the net effect of sympathetic and parasympathetic inputs. The HRV can be evaluated by analyzing R-R intervals using a computer system, providing an indication of ANS activity. Generally, the high-frequency component of HRV power spectrum (HF, >0.15 Hz) is almost entirely associated with vagal nerve activity, whereas the low frequency component (LF, <0.15 Hz) appears to depend on both vagal and sympathetic activity (31). Total power (Total, <0.5 Hz) is thought to reflect the overall ANS activity. Absolute basal spectral values differ greatly among individuals, so the spectral powers were logarithmically transformed for statistical testing.

**Experimental procedures.** Each subject was instructed to avoid any food or beverage containing alcohol or caffeine after 10:00 pm on the day preceding the study. The subjects arrived at the laboratory at 8:00 am before and after the supplement intervention period for physiological examination. The room was temperature-controlled (25˚C), quiet, and comfortable, with minimal arousal stimuli. Body mass and percentage body fat were determined using a bioelectrical impedance analyzer (Model BC118-D, Tanita Corp., Tokyo, Japan). The subjects were then fitted with ECG electrodes, and they rested for at least 15 min before HRV recording. After the resting period, the ECG signals were continuously recorded for 5 min while each subject remained seated in a chair. During ECG measurements, the subject breathed in synchrony with a metronome at 15 times/min (0.25 Hz) to ensure that respiratory-linked variations in heart rate did not overlap with LF heart rate fluctuations (<0.15 Hz) from other sources (31). Finally, a 5-ml venous blood sample was obtained by a doctor to measure TRP levels in plasma. During waiting periods, subjects completed the CES-D and POMS. At the first test day, they received bottle with 14 capsules and after intervention period; the bottle was collected and confirmed that all capsules were taken.

**Statistical analysis.** Data are presented as mean±SD. Initial baseline values of each physiological variable were compared among the four subgroups using one-way ANOVA. A three-way repeated-measure ANOVA with two between-subjects factors, supplement (TRP vs. placebo) and subclinical depression severity (mild to moderate vs. severe depressive symptoms), and one within-subjects factor, time (pre- vs. post-intervention), was used to compare mood changes between the TRP and placebo groups. To determine whether mood changes differed within supplement groups, a two-way repeated-measures ANOVA was performed with depression severity (mild to moderate vs. severe depressive
symptoms) as a between-subjects factor and time (pre- vs. post-intervention) as a within-subjects factor. Where necessary, we interpreted significant interaction effects using simple main effects analyses. Similar analyses were performed for the placebo group and subgroups. Bonferroni post hoc corrections were carried out to allow a type I error by multiple testing and correlated dependent variables. All statistical analyses were performed using SPSS version 21.0 (IBM Corp., Armonk, NY, USA). A $p$ value, $0.05$ was considered significant.

**RESULTS**

**Physical characteristics**

The demographic and physical characteristics of the four subgroups defined by treatment (TRP or placebo) and subclinical depression severity (mild to moderate or severe depressive symptoms) are summarized in Table 1. Mean age, body mass, BMI, percentage of body fat, and resting heart rate did not differ among subgroups. No one caused side effect as previously reported, and all completed the study.

**CES-D**

Figure 1 presents CES-D scores for the TRP and placebo groups. Three-way repeated-measures ANOVA demonstrated significant main effects of depression severity ($p<0.001$) and time ($p<0.001$), as well as a significant depression$\times$time interaction ($p<0.01$). The two-way repeated-measures ANOVA for the combined severe subclinical depression subgroup demonstrated a significant main effect of time ($p<0.001$), while there was no main effect in the combined mild to moderate subclinical depression subgroup. Simple main effects tests revealed significantly improved CES-D scores in both severe subgroups (both $p<0.05$), indicating that severe depression was alleviated by both TRP and placebo treatment.

**POMS**

Figure 2 shows the POMS D scores before and after 7 d of treatment for all four subgroups. There were significant main effects of subclinical depression severity ($p<0.05$) and time ($p<0.001$), as well as a significant depression$\times$time$\times$supplement interaction ($p<0.05$). Two-way repeated-measures ANOVA for the combined severe and combined mild to moderate subclinical depression subgroups showed a significant main effect of time (severe: $p<0.001$ and mild to moderate: $p<0.05$). Two-way repeated-measures ANOVA for the TRP supplement group revealed a significant main effect of time ($p<0.01$) and depression$\times$time interaction ($p<0.05$), while the placebo group exhibited only a significant main effect of time ($p<0.001$). Finally, simple main effects tests revealed that TRP had a significant effect on the combined severe depression subgroup ($p<0.05$) but not the mild to moderate subgroup. Neither severity subgroup showed a significant change in the placebo group. Thus, TRP was more effective than placebo for severe subclinical depression according to POMS score reduction.

Three-way repeated-measures ANOVA for TA demonstrated a significant main effect of time ($p<0.01$) and a depression$\times$time interaction ($p<0.05$). Two-way repeated-measures ANOVA for combined severe and
mild to moderate depression subgroups showed a significant main effect of time only in the severe depression subgroup \( (p<0.01) \). Three-way repeated-measures ANOVA for AH, C, F, and V demonstrated significant main effects of subclinical depression severity (C, F, and V: \( p<0.05 \)) and time (AH: \( p<0.05 \), C, V: \( p<0.01 \) and F: \( p<0.001 \)).

**Plasma total TRP**

Table 2 presents the changes in plasma total TRP for each subgroup; however, these changes were not significant.

**ANS activity**

Figure 3 shows the frequency components analysis of HRV for the TRP and placebo groups. Although there were no significant changes in any variable, total power was lower in the severe subclinical depression subgroup within the TRP group following treatment, while total power increased in all other subgroups. Similarly, LF power also decreased, but only in the severe subclinical depression subgroup within the TRP group. Alternatively, the severe subclinical depression subgroup within the placebo group demonstrated increased HF following treatment, while all other subgroups showed a post-treatment decrease. Finally, the LF/HF ratio increased following treatment in all subgroups.

**DISCUSSION**

The novel finding of this study was that TRP, vitamin B₆, and nicotinamide-containing supplement loading between meals improved depressive mood in short term and quite low-dose in young adults with severe subclinical depression.

CES-D scores improved in both the placebo and TRP severe subclinical depression subgroups. The POMS D score was reduced, but only in the TRP severe subclinical depression subgroup. This difference may be explained by the time frame of each instrument. CES-D assesses moods over a week; therefore, it is suitable for measuring the effects of long-term intervention such as for 8 wk \( (32) \) or 12 wk \( (33) \). On the other hand, POMS measures current mood and is used to measure transient mood changes such as acute TRP supplementation \( (34) \) or TRP depletion \( (3) \). In this study, the term of intervention was only 7 d; therefore, CES-D may not have been able to detect the difference by intervention. Other measurements often used in measuring depressive symptoms are Beck Depression Inventory (BDI), the Hamilton Depression Rating Scale (HAM-D), and Montgomery–Asberg Depression Rating Scale (MADRS). BDI, however, assesses depressive symptoms experienced in the last 2 wk, and HAM-D and MADRS are used to determine a patient’s level of depression. Considering that subjects in this study had depressive symptoms but were not clinically depressed patients, it is believed that POMS is the most suitable to measure their mood change. Of the six POMS items, only D was significantly changed, suggesting that TRP supplementation can specifically and effectively improve subclinical severe depressive mood in young adults. Numerous studies have examined the mood-altering efficacy of TRP loading in healthy volunteers. Markus et al. found that 800 mg of pure TRP improved the POMS total score 1 h after a single dose \( (34) \). On the other hand, Greenwood et al. reported that 5 g of TRP did not
cause euphoria but induced severe nausea and headache (8). Similarly, Luciana et al. found that 10.3 g of TRP caused headache and mild dissociative symptoms but did not improve mood (35). Murphy et al. reported that 3 g of TRP for 14 d caused neither side effects nor mood improvement (36). These results indicate that in healthy subjects, TRP loading ≥ 3 g is not effective for improving mood, and large dose may cause side effects. Conversely, in the present study, the daily dose was only 200 mg (2×100 mg), and did not cause side effects, but still induced a detectable improvement in severe subclinical depressive symptoms within 7 d. To the best of our knowledge, the minimum dose of TRP in terms of the mood-altering efficacy of TRP loading is 800 mg (34). There have been no reports of mood improvement at doses of 200 mg or lower. Previous studies have reported that selective serotonin reuptake inhibitors need more than 4 wk to elicit clear effects (37, 38). In this study, the intervention period was only 1 wk. This efficacy may stem from the fact that TRP, vitamin B6, and nicotinamide-containing supplements were ingested between meals, when the influence of LNAAs by meals is expected to be minimal (10). Using this protocol, TRP would be expected to enter the brain easily and be quickly synthesized to serotonin faster than usual. In order to clarify the effect of supplement intake timing itself, we conducted an additional study. Seven students aged 18–22 (5 male, 2 female) served as subjects. Five of them were positive for subclinical depression by CES-D. They took TRP, vitamin B6, and nicotinamide-containing supplements immediately after breakfast and lunch for 7 d. A paired t-test was used to examine the change. CES-D and all POMS scores did not significantly change, suggesting that taking TRP, vitamin B6, and nicotinamide-containing supplements between meals, when the competitive influences of other LNAAs are small, may enhance TRP transport into the brain and promote serotonin synthesis, thus leading to quick improvements in depressive mood.

Vitamin B6 is involved in the metabolism of homocysteine. Indeed, deficiencies in vitamin B6 can lead to increased homocysteine concentrations, which are associated with depression (39). Previous studies have shown that low levels of vitamin B6 are associated with depressive symptoms (40, 41) and vitamin B6 enhances the treatment response to antidepressant (42, 43). These results indicate that vitamin B6 promotes TRP conversion to serotonin. In this study, nicotinamide was given to reduce peripheral breakdown of TRP by inhibiting liver TRP 2,3-dioxygenase activity (44). Nicotinamide plus TRP has been shown to be as effective as imipramine (45). In this study, the effects of vitamin B6 and nicotinamide alone were not investigated; however, the combination of vitamin B6 and nicotinamide with TRP may boost the effect of TRP and permit the use of lower TRP dose, while exerting a plausible effect on mood in a short period of time.

Previous studies of healthy subjects have reported increased plasma TRP after supplementation. Greenwood et al. reported an eight-fold increase 2 h after 5 g TRP supplementation (8) and Luciana et al. reported a 10-fold increase 5 h after 10.3 g TRP ingestion (35). Markus et al. also reported a 2.5-fold increase 1.5 h after 800 mg dose of TRP (34). Conversely, we found no significant change in plasma TRP. This may be because a single 100-mg dose and total 1.4-g dose were too small to induce a detectable increase in plasma. Nevertheless, depressive mood was significantly improved. One plausible explanation is the change of TRP/LNAA ratio. Previous studies reported that the ratio is correlated with mood (46), and 50–100% rise in plasma TRP/LNAA ratio will be enough to produce a change in brain 5-HT synthesis (47). In fact, 163% rise in TRP/LNAA was found when mood was improved 1 h after 800 mg of TRP (34). When high-carbohydrate low-protein drink and 300 mg TRP were consumed at breakfast (47), the ratio rose by 180%. When high-carbohydrate low-protein soup and 400 mg TRP were consumed at dinner, the ratio tripled (48). These results were because carbohydrate produces major insulin-mediated decrease in LNAAs (49). In the present study, consuming TRP, vitamin B6, and nicotinamide-containing supplements between meals is the same as consuming high-carbohydrate low-protein meals thus leading to low LNAA levels; therefore, only 200 mg dose can influence the ratio TRP/LNAA and brain TRP.

No differences in ANS function were observed among subgroups, possibly because all subjects had depressive symptoms, which may attenuate ANS reactivity. Indeed, a previous study reported blunted ANS activity in response to physiological stimuli in depressive subjects (50). Another possibility is that in young adults, especially university students, daily life activities may change drastically throughout the week (e.g., different classes, irregular part-time jobs, weekly club activities, etc.), which may cause greater ANS effects than TRP. Finally, 7 d may be too brief a dosing period to alter ANS activity, although it was sufficient to influence mood. In our previous study, it took 12 wk of exercise training to improve ANS activity in obese middle-aged men and women (51). Therefore, a longer intervention period is required to test for changes in ANS activity.

The present study had several limitations. First, acute effect of TRP, vitamin B6, and nicotinamide-containing supplements was not measured; hence, acute effects of this TRP dose on plasma TRP are unclear. Second, blood analysis was performed only for plasma TRP, and other LNAAs were not measured; therefore, it is not unclear whether the ratio TRP/LNAA and brain TRP was changed. However, subjects with severe subclinical depression significantly improved mood, and no such effect was found in taking the same supplements after meals, suggesting that the influence of LNAAs is expected to be minimal. Finally, because the TRP, vitamin B6, and nicotinamide-containing supplements had several components, further studies should be conducted to clarify the role of each component of the TRP, vitamin B6, and nicotinamide-containing supplements on mood. Especially for TRP, future studies are required as to whether only 200 mg TRP alone changes mood.
Tryprophan Supplementation and Depression


REFERENCES


together with TRP concentration.

In summary, our findings indicate that TRP, vitamin B₆, and nicotinamide-containing supplements loading between meals can quickly improve depressive mood in quite low dose in young adults with severe subclinical depression.

Disclosure of state of COI
No conflicts of interest to be declared.

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