Effect of Enzyme-Treated Asparagus Extract (ETAS) on Psychological Stress in Healthy Individuals

Jun TAKANARI1,*, Jun NAKAHIGASHI1, Atsuya SATO1, Hideaki WAKI2, Shogo MIYAZAKI2,3,4, Kazuo UEBABA2,3,4 and Tatsuya HISAJIMA2,3,4

1 Amino Up Chemical Co., Ltd., 363–32 Shin-ei, Kiyota, Sapporo 004–0839, Japan
2 Graduate School of Health Science, 3 Faculty of Health Care, and 4 Research Institute of Oriental Medicine, Teikyo Heisei University, 2–51–4 Higashi Ikebukuro, Toshima-ku, Tokyo 170–8445, Japan

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Summary The aim of this study was to examine the effectiveness of Enzyme-Treated Asparagus Extract (ETAS) on improving stress response. A randomized, double-blind, placebo-controlled cross-over trial was undertaken in healthy volunteers. ETAS (150 mg/d) or a placebo was consumed for 28 d, with a washout period. Psychological parameters were examined using a self-report scale questionnaire and psychological stress was applied using the Uchida-Kraepelin (U-K) test. During the stress load, autonomic nervous function was analyzed. After the stress load, a profile of mood states (POMS) psychological rating was performed, and serum cortisol, plasma catecholamine, salivary secretory immunoglobulin A (sIgA), and salivary cortisol were analyzed. ETAS intake improved the self-reported rating for the items “Feel tired,” “Hard to get up,” and “Feel heavy” in the psychological questionnaire; ameliorated the self-reported rating for the items “Depression-Dejection” and “Fatigue” in the POMS questionnaire; and increased salivary sIgA levels after the U-K test. In contrast, serum and salivary cortisol levels, and plasma catecholamine did not change. During the U-K test, ETAS significantly upregulated the sympathetic nerve activity. Furthermore, ETAS intake significantly increased the number of answers and the number of correct answers in the U-K test, suggesting that it might improve office work performance with swiftness and accuracy under stressful conditions. In conclusion, ETAS supplementation reduced feelings of dysphoria and fatigue, ameliorated quality of sleep, and enhanced stress-load performance as well as promoted stress response by increasing salivary sIgA levels. These data suggest ETAS intake may exert beneficial effects, resulting from well-controlled stress management, in healthy individuals.

Key Words psychological stress, physical and mental fatigue, psychometric scales, secretory immunoglobulin A, asparagus

Exposure to stress factors, such as prolonged work, unemployment, or loneliness, is a common societal problem; and stress-related disorders place an increasing burden on the health care system (1, 2). Under healthy amounts of stress, the body maintains homeostasis by relaying the coordinated responses of the autonomic nervous system, the immune system, and the endocrine system. Stress response is mediated by two pathways, the sympathetic nervous adrenal medullary (SAM) system and the hypothalamic pituitary adrenal cortex (HPA) system (3–5). Activation of the SAM system is associated with stimulation of the autonomic nervous system centers in the hypothalamus. Sympathetic predominance induces the secretion of noradrenaline and adrenaline from the adrenal medulla, resulting in higher blood pressure and a faster heart rate (3, 4). Activation of the HPA system is involved in the hypothalamus to stimulate the adrenal cortex, leading to cortisol secretion (5).

Another component of the stress response is secretory immunoglobulin A (sIgA). Conventionally, sIgA functions as an important antibody in mucosal immune defense mechanisms (6). While mucosal sIgA is normally associated with immune defense against upper respiratory tract infection and oral disease, the sIgA level in saliva is affected by sympathetic nerve activity and is used as an immunological marker for acute stress (7–10). Since salivary sIgA can be estimated easily and non-invasively, it has widely been used as a stress indicator in various psychological studies.

Excessive or chronic stress, including psychological stress, disrupts the healthy stress response and can lead to the development of mental diseases such as depression and anxiety (11). Physical symptoms (e.g. insomnia and dizziness) and psychological symptoms (e.g. sense of unease and feeling of tension) accompanying chronic stress result in impaired concentration, reduced memory, and lower work capacity, heavily affecting performance and quality of life in healthy individuals (12–14). Recently, there has been an increase in research on natural anti-stress ingredients, such as traditional Chinese herbal extracts and nutraceuticals,

E-mail: takanari@aminoup.co.jp
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MATERIALS AND METHODS

Study product. Enzyme-Treated Asparagus Extract (ETAS®) was filed for trademark registration and industrially manufactured by Amino Up Chemical Co., Ltd., Sapporo, Japan. ETAS® was produced from freshly harvested unused stems of asparagus (Asparagus officinalis L.) grown regionally.

The safety of ETAS was proven by negative toxicity results in the Ames test, bone marrow micronucleus test in mice, and acute and subacute oral toxicity tests in rats (17). Previously, we showed that oral administration of ETAS suppressed the stress-induced increase in blood corticosterone and lipid peroxides (an oxidative stress marker) and also reduced the loss of hair in sleep-deprived/stress-loaded mice (18). In the human study of healthy subjects with a bit of anxiety about sleep, ETAS administration showed the tendency to improve autonomic nerve balance, decreased levels of salivary chromogranin A (a psychological stress marker), decreased the incidence of early-morning awakening, and improved dreams (19). To date, however, the efficacy of ETAS in reducing psychological stress on long-term administration has not been investigated.

This study had two objectives. First, we examined the effects of long-term ETAS administration on autonomic nervous function and on stress biomarkers that fluctuate with mental arithmetic stress. Secondly, we verified the efficacy of long-term intake of ETAS on decreasing psychological stress parameters in healthy individuals.

Study design. Twenty-five healthy volunteers were randomly assigned to one of two groups. The study consists of two 4-wk treatment arms, separated by a 2-wk washout period. R: random assignment of healthy volunteers.
B, first consumed placebo capsules for 4 wk, followed by referring to previous clinical trial testing (19). Compliance with this intake regimen was 100% in both Group A and B. No side-effects were reported during the study.

**Psychological evaluation and stress testing.** Before and after each dietary intervention, subjects were asked to come to the trial center for psychological evaluation, stress testing, and sample collection. Stress testing was conducted at the Teikyo University Ikekuburo Clinic (Tokyo, Japan) and overseen by experienced researchers and trained clinical personnel.

An overview of the measurement design is given in Fig. 2. Upon arrival in the trial center, subjects answered a brief questionnaire to survey their psychological condition. The subjects then rested in the sitting position for 10 min. They were then subjected to mental stress by mental arithmetic testing (Uchida-Kraepelin (U-K) test) for 30 min. During the test period and rest period, autonomic nervous function was measured by heart rate variability analysis (HRV). After the U-K test, blood and saliva were collected, and then subjects answered the profile of mood states (POMS) questionnaire.

Uchida-Kraepelin (U-K) test. The U-K test is a serial addition test, which requires subjects to perform calculations as fast and accurately as possible within 30 min. A standardized test form, containing 10 lines of random, single-digit, horizontally aligned numbers, was distributed to each subject. Subjects were instructed to add up the numbers of each line and to write the result on the bottom of the line. For each minute of the test, the subject was instructed to begin calculating a new line regardless of his/her position on the current line. Each line contained an excess of numbers such that the subjects were not able to finish calculating any line for a particular minute before being prompted to move on to the next line. This test was performed twice for a continuous 15-min session each, with a 5-min rest cycle between. The total number of answers was calculated by summing the results of all subjects in the same treatment arm. The number of correct answers was calculated by summing only the correct results of all subjects in the same treatment arm.

Assessment of autonomic nervous system (ANS). Autonomic nervous function was measured at rest and during mental arithmetic by real-time heart rate variability (HRV) analysis using HRV wave-analysis software (Crosswell Co., Ltd., Yokohama, Japan). Sympathetic and parasympathetic activities were evaluated by spectral analysis of HRV. In this analysis, low frequency (LF: 0.04–0.15 Hz) is associated with both sympathetic and parasympathetic activities, while high frequency (HF: 0.15–0.4 Hz) is associated with parasympathetic activity. We measured the LF and HF components of HRV. The ratio of LF/HF was assumed to represent sympathetic nervous activity, while HF alone was assumed to represent parasympathetic nervous activity. After confirming in preliminary experiments that the U-K test increases the LF/HF components and decreases the HF components in autonomic nerve activities, we used the U-K test as a mental stress load in this study.

**Profile of mood states (POMS) questionnaire.** A shortened Japanese version of POMS, adapted from the original POMS standard version, was used in this trial (21, 22). POMS is a self-report measure that allows for the quick assessment of transient, fluctuating feelings and enduring affect states. The questionnaire consists of 6 factors: Tension-Anxiety (T-A), Depression-Dejection (D-D), Anger-Hostility (A-H), Vigor (V), Fatigue (F), and Confusion (C) in numerical values, and consists of 30 items. Subjects were asked to rate each item by choosing an answer on a scale from 1 to 5, namely 1) “not at all,” 2) “a little,” 3) “moderately,” 4) “quite a bit,” and 5) “extremely.” We evaluated each factor at the standardized score using the following equation: T-score = 50 + 10×[base score – average score]/standard deviation.

Blood sample collection and measurement of cortisol and catecholamine. Blood was collected by a clinician from an arm vein. Serum and plasma were collected accord-
ing to standard blood separation procedures and stored at −80°C until further use. Serum cortisol and plasma catecholamine (adrenaline, noradrenaline, and dopamine) were measured by a laboratory testing service (BML Inc., Tokyo, Japan).

Collection of saliva samples and measurement of salivary sIgA concentrations. An absorbent cotton swab contained in the Salivette (Sarstedt, Inc., Numbrecht, Germany) was chewed by subjects for 2 min, and placed back into the Salivette. The Salivette was then centrifuged at 3,500 rpm for 10 min. Saliva samples were kept at −20°C until analysis.

Salivary sIgA was quantitated by ELISA using the Human sIgA ELISA Quantitation Set (Bethyl Laboratories, Inc., Montgomery, TX) according to the manufacturer’s instructions. Briefly, 100 µL of primary antibody solution (goat anti-human sIgA-affinity purified) was dispensed to each well of a Nunc-Immuno plate (Nunc, Roskilde, Denmark) and incubated at 4°C overnight. After the primary antibody solution was collected, the plate was washed 3 times by dispensing 380 µL of 0.05% Tween20 in phosphate-buffered saline (PBST) to each well. For blocking, 380 µL of 1% bovine serum albumin (BSA) in phosphate-buffered saline was dispensed into each well and incubated at room temperature for 120 min. The plate was then washed again 3 times with PBST. For the standard curve, 100 µL each of a series of sIgA standard solutions, containing 500, 250, 125, 62.5, 31.25, 15.6, and 7.8 ng/mL sIgA diluted in sterile saline, was dispensed into duplicate wells. For the samples, each subject’s saliva was diluted 2,000-fold in sterile saline, and dispensed into duplicate wells. The plate was incubated at room temperature for 60 min and then washed 4 times with PBST. An aliquot of 100 µL secondary antibody solution (goat anti-human sIgA-HRP conjugate) was dispensed into each well and allowed to stand at 37°C for 60 min; then the plate was washed 4 times with PBST. For color reaction, 100 µL of TMB solution (KPL, Inc., Gaithersburg, MD) was dispensed to each well and allowed to stand at room temperature for 5–10 min. To terminate the color reaction, 100 µL of 1 M phosphoric acid aqueous solution was dispensed to each well, and absorbance was measured with a spectrophotometer (microplate reader model: iMark; Bio Rad, Inc.) at 450 nm. The level of salivary cortisol was expressed as nanogram cortisol per milligram total salivary protein.

Statistical analysis. All data were expressed as mean±standard deviation (SD). Changes between Day 0 (pre-intake) and Day 28 (post-intake) values for the two groups were analyzed using the two-tailed paired Student’s t-test. Comparisons among groups were analyzed using the unpaired t-test. A p-value less than 0.05 was considered significant and a p-value less than 0.10 but larger than 0.05 was considered to indicate a tendency. For autonomous nervous system assessment, two levels of significance, a p-value of less than 0.01 and a p-value of less than 0.05, were used. SPSS software (version 19, IBM Corp., New York, NY) was used to perform statistical analysis.

RESULTS

Questionnaire survey of condition

Among the 17 items in the questionnaire used to survey the psychological condition of subjects on Day 0 and Day 28 of the study, significant differences were observed in the ETAS group on 3 items. ETAS subjects felt “less tired” (Fig. 3A), “less hard to get up” (Fig. 3B), and “less heavy” (Fig. 3C) on Day 28 than on Day 0. In contrast, the placebo group showed no significant change for any of the items.

Uchida-Kraepelin (U-K) test

Regarding the total number of answers, irrespective of whether the answers were correct or not, ETAS subjects showed a significant increase in the number of answers on Day 28 as compared to Day 0 (Day 0: 1,410±446, Day 28: 1,527±412), whereas no significant change was observed in the placebo group (Day 0: 1,462±425, Day 28: 1,522±411) (Fig. 4A).

Regarding the number of correct answers, the ETAS group showed a significant increase on Day 28 as compared to Day 0 (Day 0: 1,402±448, Day 28: 1,519±415), whereas no significant change was observed in the placebo group (Day 0: 1,453±426, Day...
Assessment of autonomic nervous system (ANS)

With regard to the changes in autonomic nerve activity at rest and during the U-K test, ETAS subjects showed a significant difference in LF/HF ratio (sympathetic nerve activity) during the U-K test versus at rest (2.87 ± 1.31 versus 1.75 ± 1.08; *p* < 0.01) on Day 28, whereas placebo subjects showed no significant difference (3.20 ± 1.31 versus 2.66 ± 2.22) on this day (Table 1).

Profile of mood states (POMS) questionnaire

In the POMS questionnaire, ETAS subjects showed significant improvements in 2 of 6 items on Day 28 compared to Day 0. ETAS subjects were rated significantly lower on the items “Depression-Dejection” (Day 0: 44.81 ± 8.21, Day 28: 41.71 ± 5.61) (Fig. 5A) and “Fatigue” (Day 0: 45.36 ± 9.60, Day 28: 41.47 ± 9.39) (Fig. 5B), indicating an improvement in these two mood states after ETAS intervention. In the placebo group, no significant changes were observed in any of the items.

Plasma catecholamine

After the U-K test, a significant increase in plasma noradrenaline was observed in the placebo group on Day 28 compared to Day 0 (Day 0: 0.06 ± 0.03 ng/mL, Day 28: 0.17 ± 0.07 ng/mL). In contrast, no significant change in plasma noradrenalin was observed

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**Table 1. Changes in autonomic nervous system measurements.**

<table>
<thead>
<tr>
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<th>Placebo</th>
<th>ETAS</th>
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<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>U-K T</td>
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<tr>
<td>Sympathetic nerve activity (LF/HF)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>2.33 ± 1.85</td>
<td>3.15 ± 1.52</td>
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<tr>
<td>Day 28</td>
<td>2.66 ± 2.22</td>
<td>3.20 ± 1.31</td>
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<tr>
<td>Parasympathetic nerve activity (HF)</td>
<td></td>
<td></td>
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<tr>
<td>Day 0</td>
<td>370.5 ± 252.4</td>
<td>297.2 ± 176.2</td>
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<tr>
<td>Day 28</td>
<td>355.3 ± 188.1</td>
<td>318.2 ± 208.7</td>
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The value represents mean ± SD.

U-K T: Uchida-Kraepelin test.

n.s.: no significant difference.
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[198x788]Effects of Enzyme-Treated Asparagus Extract on Stress 203 [60x404]0.14

[80x392]0.05 ng/mL, Day 28: 0.17

[91x284]154.71), whereas no signifi cant change was

[127x260]127.00) (Fig. 6).

**DISCUSSION AND CONCLUSION**

In this study, we examined the effects of long-term ETAS intake on autonomic nervous function and on stress biomarkers that fluctuate with a mental arithmetic stress, and verifi ed the effi cacy of long-term intake of ETAS on lowering psychological stress parameters in healthy individuals.

Subjects who consumed ETAS for 4 wk showed a signifi cant improvement in 3 of 17 items on the psychological condition questionnaire. ETAS subjects felt “less tired,” “easier to get up,” and “less heavy” after ETAS intervention. In particular, improvement of the item “Hard to get up” suggests that ETAS intake might exert good quality sleep rather than extension of sleep length, because only subjects with suffi cient sleeping time were enrolled in the study. Similarly, in the state of moods

*Fig. 5. T-score of profi le of mood states (POMS) questionnaire. Two items “Depression-Dejection” (A) and “Fatigue” (B) are shown. Each factor was standardized using following equation: T-score = 50 + 10 × [base score – average score]/standard deviation, in which the range of T-score of “Depression-Dejection” (A) and “Fatigue” (B) is 39–85 and 33–75, respectively. The values represent mean ± SD. *p < 0.05 vs Day 0.

*Fig. 6. Effect of ETAS on saliva sIgA level. Saliva sIgA was measured on Day 0 and Day 28. The level of saliva sIgA was expressed as microgram sIgA per milligram total salivary protein. The values represent mean ± SD. **p < 0.01 vs Day 0.

in the ETAS group (Day 0: 0.14 ± 0.05 ng/mL, Day 28: 0.17 ± 0.08 ng/mL). Adrenaline and dopamine were below the limits of detection.

**Serum and salivary cortisol levels**

Serum and salivary cortisol levels did not show any significant changes in either group.

**Salivary sIgA concentrations**

A signifi cant increase in salivary sIgA (μg/mg total protein) was observed in the ETAS group on Day 28 compared to Day 0 (Day 0: 208.11 ± 168.20, Day 28: 298.66 ± 154.71), whereas no signifi cant change was observed in the placebo group (Day 0: 217.14 ± 130.55, Day 28: 262.12 ± 127.00) (Fig. 6).

These results are in agreement with a previous study in humans, in which 7-d administration of ETAS exhibited an anti-stress effect and improved the quality of sleep by suppressing the increase of serum and salivary cortisol levels as compared to a placebo (19). Similarly, these fi ndings are consistent with the murine model experiment, in which 7-d oral administration of ETAS showed an anti-stress eff ect by decreasing corticosterone and the ratio of lipid peroxides during a sleep-deprivation stress period (18). A decrease in psychological
and physical stress is likely to improve sleep quality (19). In contrast to previous studies, administration of ETAS in the present study did not show any effect on serum or salivary cortisol levels. This might be because these effects are short-term, rather than long-term. However, it can be assumed that ETAS nevertheless had a positive effect on the quality of sleep in this study, based on the results of the psychological condition questionnaire.

Since the U-K test, with its speed and accuracy, can be used as a tool to evaluate the adaptability to learning activities and job performance skills, it is used not only as a mental stress load in research but also as an aptitude test by companies (25). ETAS intake increased both the number of answers and correct answers in this test. With regard to changes in autonomic nerve activity at rest and during the U-K test, the ETAS group showed an increase in LF/HF during the test compared with that at rest, whereas the placebo group showed no significant effect. An increase in LF/HF components during the stress period is interpretable as a stress response of the sympathetic nervous adrenal medullary (SAM) system, primarily as a strategy to maintain homeostasis (3, 4), although there was the controversial result that the ETAS group showed no increased plasma levels of catecholamines such as noradrenaline and adrenaline. It is therefore likely that ETAS subjects responded to the psychological stress load by activating the SAM system, which in turn produced the demonstrated positive effects on calculation speed and accuracy. The involvement of the hypothalamic pituitary adrenal cortex (HPA) system in ETAS subjects remains unknown because serum and salivary cortisol levels during the U-K test were not measured. These beneficial effects on calculation speed and accuracy during the test suggest that ETAS has the potential to be effective in enhancing performance during tasks involving psychological stress.

ETAS is the extract produced from asparagus, itself a natural product, so that it naturally consists of various ingredients. Thus far, Ito’s group has reported that ETAS contains 5-hydroxymethyl-2-furfural (HMF) and a novel HMF derivative named asfural, both of which enhance heat shock protein (HSP) 70 mRNA expression in human promyelocytic leukemia HL-60 cells (20). The content of these compounds in ETAS is remarkably increased by the enzyme treatment. Therefore, HMF and asfural might be some of the biological active ingredients in ETAS demonstrating HSP70 induction activity. HSP70 has a huge variety of actions, such as apoptosis-suppressive and anti-inflammatory activities, leading to cytoprotective function from various stresses (26–29). Active ingredients of ETAS contributing to beneficial effects shown in this study remain unknown; however, HMF and asfural might partly be involved in these effects through HSP70 enhancement.

In conclusion, the results of this study indicate that the long-term administration of ETAS can potentially improve the capacity to adapt to psychological stress loads encountered on a daily basis, and also help stabilize the quality of sleep through this anti-stress effect. In this respect, ETAS is seen as a promising functional food ingredient for the prevention of stress-induced performance loss and for the general improvement of the physical and psychological well-being of healthy individuals. It is also suggested that ETAS intake could enhance office work performance in terms of swiftness and accuracy under stressful condition. However, since the present study was conducted with a small sample size and subjects were relatively young, a larger trial recruiting a representative sample of the working population should be carried out to verify the results.

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REFERENCES

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