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Effects of AHCC® on Immune and Stress Responses in Healthy Individuals

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Abstract

AHCC® is a functional food from the basidiomycete *Lentinula edodes*. We evaluated the effects of AHCC® on subjects under different kinds of stress and at rest. Physical stress was imposed using an active standing test, known as Schellong's test. Sympathetic nervous activity in the standing position was significantly greater in AHCC®-treated subjects than in a placebo group. In contrast, AHCC® significantly increased parasympathetic nervous activity at rest. Under mental stress, AHCC® increased sympathetic nervous activity, with no difference in the parasympathetic nervous system. In subjects with chronic mental stress, self-reported "initiation and maintenance of sleep" was significantly greater in the AHCC®-intake period than in the placebo intake period, and natural killer cell activity also increased after AHCC® intake, suggesting a possible mechanism of action of AHCC®. Our findings indicate that AHCC® is potentially effective in stress management and may be useful in the treatment of depression.

Keywords

AHCC®, immune function, stress response

The term *mibyo* in oriental medicine means a healthy human condition, but one prone to physical or mental illness.[1] Of the variety of factors that may cause "mibyo" subjects to exhibit illness, a major factor is believed to be stress.[2] Humans commonly suffer from a range of stresses, which can be generally grouped into mental stresses (e.g., pressure, sadness, and distress) and physical stresses (e.g., insufficient sleep and fatigue). Excess and/or chronic mental stress can manifest itself in many different ways: disturbed sleep patterns, fatigue, disruption of autonomic nervous system (ANS) activity, decreased capacity to learn and work, and immunosuppression.[3-6]

The ANS, in conjunction with the endocrine and immune systems, plays a key role in physiological homeostasis, and they also respond in coordination to stress stimuli.[7] The ANS mediates stress response via 2 pathways, the sympathetic nervous adrenal medullary (SAM) and the hypothalamic pituitary adrenal cortex.[8-10] In the SAM pathway, stress stimuli are transmitted via the cerebral cortex to the hypothalamus, which modulates the sympathetic nervous system. Consequently, noradrenaline and adrenaline are released from the adrenal medulla, elevating blood catecholamine levels to increase blood pressure and boost heart rate (HR).[8,9] Activation of sympathetic nervous function is necessary to mediate physiological responses to physical and mental stresses, ranging from immediate changes in blood pressure caused by standing up, to office work that requires focused concentration. Under healthy conditions, sympathetic nervous activity is normalized by homeostatic response, when mental or physical stressors are removed. However, under excessive or chronic stress, the sympathetic nervous system is continuously activated, leading to reduced quality of sleep and subsequent anxiety and depressive symptoms.[11-13]

Many neuroimmunological studies report that excessive and chronic stress alters the immune system.[7] It is known that mental disorders are related to the activity of natural killer (NK) cells in the innate immune system.[14,15] NK cell function is impaired in patients with chronic stress–associated depression, and is a measure of mental stress, in addition to their canonical

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role in the innate immune response to viral and bacterial infection. Maintaining normal NK cell activity may play a fundamental role both in the maintenance of a healthy immune system and in the body's response to mental stress.

In recent years, numerous studies on natural anti-stress products, including natural medicines and functional foods, have been carried out in the field of complementary and alternative medicine.[16] From the viewpoint of preventive medicine, safeguarding stress-prone "mibyo" subjects from progressing to illness may be achieved via daily stress management, combined with a balanced diet that involves functional foods.

AHCC® (Amino Up Chemical Co, Ltd, Sapporo, Japan) is a standardized extract of cultured *Lentinula edodes* mycelia. It has been reported that AHCC® increases the number of dendritic cells and enhances NK cell activity in the innate immune system of humans and other animals.[17-19] Furthermore, AHCC® has been shown to stabilize circulating levels of norepinephrine, epinephrine, dopamine, and glucose in rats subjected to immobilized stress, and has also been shown to relieve severe anxiety in patients with unresectable prostate cancer.[20,21] Collectively, these studies suggest that AHCC® might comprehensively improve the activity of the ANS, endocrine system, and immune system in humans, and be an effective treatment for stress. The present study was conducted to investigate the effects of AHCC® in healthy volunteers under temporary physical and mental stress condition as well as at rest. Furthermore, the effect of AHCC® on NK lymphocyte activation in subjects suffering from chronic stress was also investigated.

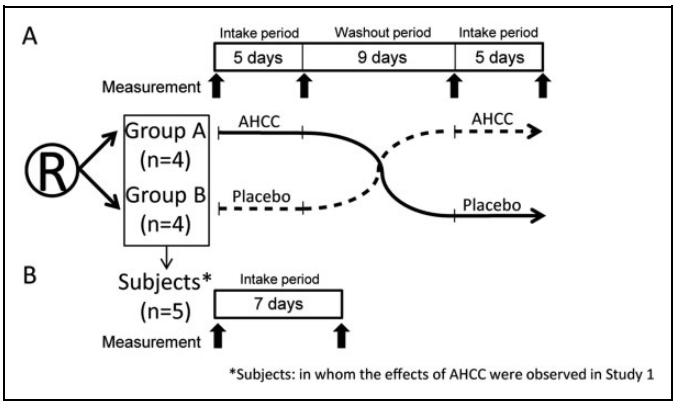


Figure 1. Design of study 1 (A) and study 2 (B). Study 1: 8 healthy volunteers were randomly assigned to 1 of 2 groups. The study consists of two 5-day treatment arms, separated by a 9-day washout period. Before and after treatments, Schellong's test was carried out to assess the variation of autonomic nervous activities under physical stress (A). Study 2: An open-label study was conducted to evaluate the effects of AHCC® at rest on 5 subjects who responded to AHCC® intake in study 1. Before and after treatments, daily habitats and mood state were evaluated by questionnaires, and autonomic nervous activity was also measured (B).

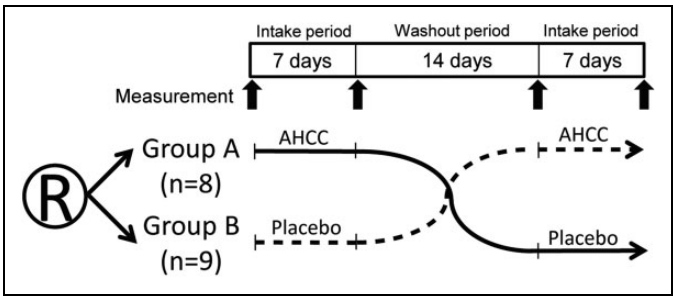


Figure 2. Design of Study 3. Seventeen healthy volunteers were randomly assigned to 1 of 2 groups. The study consists of two 7-day treatment arms, separated by a 14-day washout period. Before and after treatments, Uchida-Kraepelin test was carried out to assess the variation of autonomic nervous activities under mental stress.

Materials and Methods

Study Product

AHCC® is trademarked and manufactured by Amino Up Chemical Co, Ltd (Sapporo, Japan). *Lentinula edodes* mycelia were cultured in liquid medium. The culture was separated, concentrated, sterilized, and freeze-dried to produce the fine granular powder of AHCC®. AHCC® consists of carbohydrates, amino acids, lipids, minerals, and oligosaccharides, which are assumed to confer the biological activities of AHCC®. AHCC® contains approximately 20% (w/w) α-glucan, which is one of its major constituents.[22] This manufacturing process was carried out in accordance with Good Manufacturing Practice for dietary supplements, ISO9001:2008, and ISO22000:2005.

Study Design

We evaluated the effects of AHCC® on subjects under physical stress, at rest, under mental stress, and under chronic stress. To recruit subjects for participation, flyers were posted on bulletin boards across Teikyo Heisei University (Tokyo, Japan) campus. Written informed consent was obtained from all subjects. To ensure only healthy volunteers participated in this study, subject candidates were first screened by a self-administered questionnaire and a medical interview. Volunteers with underlying diseases treated with drugs or at hospitals, or allergies to mushrooms were excluded. Four studies were conducted (Figures 1–3). Each was approved by the Ethics Committee of Teikyo Heisei University (approval #24-049) and was conducted in accordance with the Declaration of Helsinki and the International Ethical Guidelines. The 4 studies were as follows.

Study 1: Effect of AHCC® on the Autonomic Nervous System Under Physical Stress. A placebo-controlled, double-blind, crossover study was conducted to investigate the effects of AHCC® on physical stress in 8 healthy subjects (males and females, mean age: 20.6 ± 0.9 years). An outline of the study is shown in Figure 1A. Eight subjects were randomly and equally divided into 2 groups, group A and group B. In group A, subjects took 3 g of AHCC® (9 capsules) a day for 5 days, and after a 9-day washout period, took 3 g of placebo (9 capsules) a day for 5 days. In the same manner, subjects in group B first took placebo capsules for 5 days, followed by a 9-day washout period, and then took AHCC® capsules for 5 days. A capsule of AHCC® contained 333 mg of AHCC®, and 3 capsules were taken each before

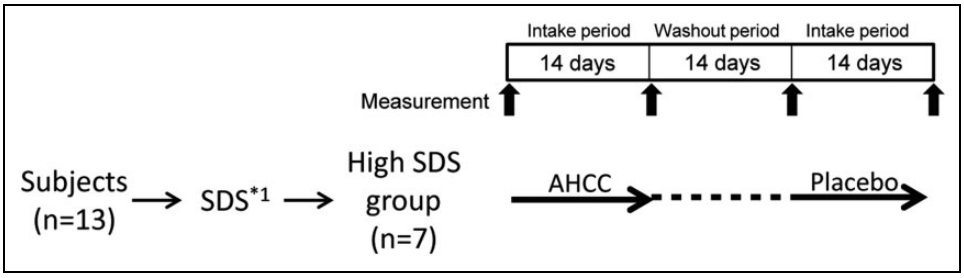


Figure 3. Design of study 4. To select subjects with mild depression, Self-rating Depression Scale (SDS) was conducted on 13 healthy volunteers. Seven subjects with an SDS score of 40 to 49 points were considered to be mild depression and were referred to as the "high SDS" group. First, high SDS group took AHCC® for 14 days, and after a 14-day washout period, proceeded to take the placebo for 14 days. Before and after treatments, OSA-MA (Oguri-Shirakawa-Azumi Sleep Inventory Middle Age version), Uchida-Krapelin test, and blood drawing were carried out. Blood samples were used for measurement of natural killer (NK) cell activity.

breakfast, lunch, and dinner. A capsule of placebo contained 170 mg of malt extract, 93 mg of dextrin (Pinedex; Matsunami Chemical Industry Co, Ltd, Itami, Japan) and 70 mg of crystalized cellulose (CEOLUS; Asahi Kasei Chemicals Co, Ltd, Osaka, Japan). The active standing test, also known as Schellong's test, was administered as a physical stress; standing up from a sitting position causes blood pressure to rapidly decrease. To normalize the blood pressure immediately, the ANS reacts via sympathetic nervous system activation and parasympathetic nervous system suppression. The outcome is an increase in HR and cardiac contraction force, and constriction of resistance vessels to maintain blood pressure. In our study, subjects were asked to actively stand up from a seated rest position and maintain the upright position for 1 minute.[23] In Schellong's test, the activity of the ANS and HR were measured using a memory electrocardiograph (LRR-03; GMS Co, Ltd, Tokyo, Japan). The frequency measured from heart rate variability (HRV) was analyzed using HRV analysis software (Crosswell Co, Inc, Tokyo, Japan). For ANS activity, the low frequency (LF) (0.05-0.15 Hz) and high frequency (HF) (0.15-0.40 Hz) components were extracted. The ratio obtained by dividing the LF component by the HF component (LF/HF) was used as an index of sympathetic nervous activity, and the HF component was used as an index of parasympathetic nervous activity. The sympathetic and parasympathetic nervous activities were expressed as ratios of the value obtained in the standing position to that obtained at rest.

Study 2: Effect of AHCC® Intake on the Autonomic Nervous System and Psychological State at Rest. An open-label study was conducted to investigate the effects of AHCC® on daily habits, mood state, and the ANS at rest in 5 subjects, in whom the effects of AHCC® were observed in study 1 (males and females, mean age: 20.4 $\pm$ 0.5 years) (Figure 1B). Subjects took AHCC® at a daily dosage of 3 g for 7 days, as in study 1. To assess ANS activity, the frequency measured from HRV was analyzed before the start of intake and after 7 days of intake, using the same method as in study 1. Additionally, in this study, questionnaires assessing psychological condition and mood were administered before the start of intake and after 7 days of intake, as described below.

Psychological condition survey. Before and after administration of AHCC®, subjects were asked to fill out a questionnaire containing 17 items: "Feel tired," "Hard to get up," "Feel heavy," "Feel low," "Hard to sleep," "Awake during the night," "Have an appetite," "Feel good," "Have a headache," "Feel queasiness in the stomach," "Have gastric pain," "Have constipation," "Have diarrhea," "Have a stomachache," "Have a sore throat," "Have a runny nose," and "Have a cough." For each item, subjects answered the questionnaire by specifying their level of agreement or disagreement on a symmetric agree-disagree scale with 5 grades (1 = strongly disagree to 5 = strongly agree).

Profile of Mood States survey. A shortened Japanese version of Profile of Mood States (POMS) survey, adapted from the original POMS standard version, was used in this trial.[24] POMS is a self-report measure that allows for the quick assessment of transient, fluctuating feelings and enduring emotional 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 as the standardized score using the following formula: T-score = 50 + 10 $\times$ [base score − average score]/standard deviation.

Study 3: Effects of AHCC® on the Autonomic Nervous System Under Mental Stress. A placebo-controlled, double-blind, crossover study was conducted using 17 healthy volunteers (males and females; mean age: 20.6 $\pm$ 0.9 years) (Figure 2). The subjects were randomly divided into 2 groups, group A (n = 8) and group B (n = 9). In group A, subjects took 3 g of AHCC® a day for 7 days, and after a 14-day washout period, took 3 g of placebo a day for 7 days. In the same manner, subjects in group B first took 3 g of placebo for 7 days, followed by a 14-day washout period, and then took 3 g of AHCC® for 7 days. ANS activity was measured after administration of the Uchida-Kraepelin (U-K) test, a mental arithmetic test, which was employed as a mental stress task.[25]

Uchida-Kraepelin test. The U-K test is a serial addition test that requires subjects to perform calculations as fast and accurately as possible within 15 minutes. A standardized test form, containing 17 lines of random, single-digit, horizontally aligned numbers, was given to each subject. Subjects were instructed to add up pairs of single digit numbers on each line, and to write the results on the bottom of the line. For example, if given the line "3 4 8 9 4 6," subjects would calculate 3 + 4, 4 + 8, 8 + 9, 9 + 4, 4 + 6 and answer: 7, 2, 7, 3, 0. For each minute of the test, the subject was instructed to begin calculating a new line regardless of his or her position on the current line. Each line contained an excess of numbers, such that the subjects were unable to finish calculating any line within a minute before being prompted to move on to the next line. The total number of answers was calculated

Table 1. Changes in Autonomic Nervous Activities Under Physical Stress (n =8).[a]

			Before Intake (Mean ± SD)	After Intake (Mean ± SD)
Standing up/Sitting at rest	Sympathetic nervous activity	Placebo	7.95 ± 6.08	2.63 ± 1.65*
		AHCC	2.97 ± 1.97	5.62 ± 3.50
	Parasympathetic nervous activity	Placebo	0.83 ± 0.50	0.82 ± 0.39
		AHCC	0.77 ± 0.24	0.87 ± 0.44

Abbreviation: SD, standard deviation.
[a]The activity of autonomic nervous system was measured by heart rate variability (HRV) analysis. Sympathetic and parasympathetic nervous activities were evaluated by spectral analysis of HRV. The ratios of low frequency (LF)/high frequency (HF) and HF were assumed to present sympathetic nervous activity and parasympathetic nervous activity, respectively. Schellong's test was used as a physical stress loading. In this test, the sympathetic and parasympathetic activities were expressed as ratios of the values obtained in the standing position to that obtained at sitting.
*$P < .05$ versus placebo.

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.

Study 4: Effects of AHCC® in Volunteers Under Chronic Stress. To identify subjects suffering from chronic stress, a depression test was performed in 13 healthy volunteers (males and females, mean age: 24.1 ± 3.8 years) using the Self-rating Depression Scale (SDS) questionnaire, as described below.[26] Seven volunteers who were considered to have mild depression ("mibyo") on the basis of their SDS scores were selected and referred to as a high SDS group. The effects of AHCC® on their sleep, task performance (U-K test), and changes in NK cell activities were investigated. Subjects in high SDS group took AHCC® for 14 days at a daily dosage of 3 g, and after a 14-day washout period, proceeded to take the placebo for 14 days (Figure 3). Measurements were performed before and after each administration. On the measurement day, first, the quality of sleep was investigated using the Oguri-Shirakawa-Azumi Sleep Inventory for Middle Age version (OSA-MA).[27] After a 5-minute rest, the U-K test was administered for 15 minutes to evaluate the effects of AHCC® on task performance. Finally, a blood sample was taken to measure NK cell activity.

Self-rating Depression Scale. The SDS is used to quantitatively determine the severity of depression and comprises 20 items that are based on the results of a factor analysis of depression.[26] If the SDS score is less than 40 points, depression is unlikely. Depression is determined to be mild at an SDS score of 40 to 49 points, and moderate to severe at an SDS score of 50 points or more. In the present study, subjects with an SDS score between 40 and 49 points were referred to as the "high-SDS" group.[28,29]

Oguri-Shirakawa-Azumi Sleep Inventory MA version. The OSA-MA is a self-reported questionnaire composed of 16 items with a 4-point scale, which are consolidated into "sleepiness on rising," "initiation and maintenance of sleep," "frequent dreaming," "refreshment," and "sleep length" subcategories.[27]

Measurement of NK cell activity. Blood samples were collected using heparin sodium blood collection tubes, allowed to stand at room temperature, and sent to BML, Inc (Tokyo, Japan) for measurement of NK cell activity using the chromium-51 (^{51}Cr) release assay, which measures radioactivity released from ^{51}Cr-loaded K562 target cells.

Statistical Analysis

All data were expressed as mean ± standard deviations (mean ± SD). Changes between pre- and postintake values for the 2 groups were analyzed using the 2-tailed paired Student's t test. Comparisons among groups were analyzed using the unpaired t test. A P value less than .05 was considered significant and a P value less than .10 but larger than .05 was considered to indicate a tendency. SPSS software (version 19, IBM Corp, Armonk, NY) was used to perform all statistical analyses.

Results

Study 1: Effect of AHCC® on the Autonomic Nervous System Under Physical Stress

The effects of AHCC® on the ANS under physical stress were investigated. Schellong's test was administered as a physical stress; subjects were asked to stand up from the sitting position and remain standing for 1 minute. ANS activity was measured at sitting, and during the 1-minute standing period before the start of AHCC® intake and after 5 days of intake. Sympathetic and parasympathetic nervous activities were calculated as the ratio of the standing to the sitting position. Results showed that after 5 days of AHCC® intake, the sympathetic nervous activities were greater in the AHCC® intake period than in the placebo intake period (5.62 ± 3.50 AHCC®, 2.63 ± 1.65 placebo, $P < .05$, n = 8) (Table 1). However, there were no differences in parasympathetic nervous activity. As described in detail in the Methods section, standing up from a sitting position induces a change in blood pressure, in which the ANS normally acts to enhance sympathetic nervous activity and to suppress the parasympathetic nervous system, thereby normalizing blood pressure. We conclude that AHCC® acts either directly or indirectly to enhance sympathetic nervous activity.

[Study 2] Effect of AHCC® on the Autonomic Nervous System and Psychological State at Rest

The results of Schellong's test suggest that AHCC® enhances sympathetic nervous activation under physical stress. Study 2 was conducted to investigate the effects of AHCC® on the ANS in a resting (stress-free) position. In addition, the effects of AHCC® on the daily activities and frame of mind of subjects

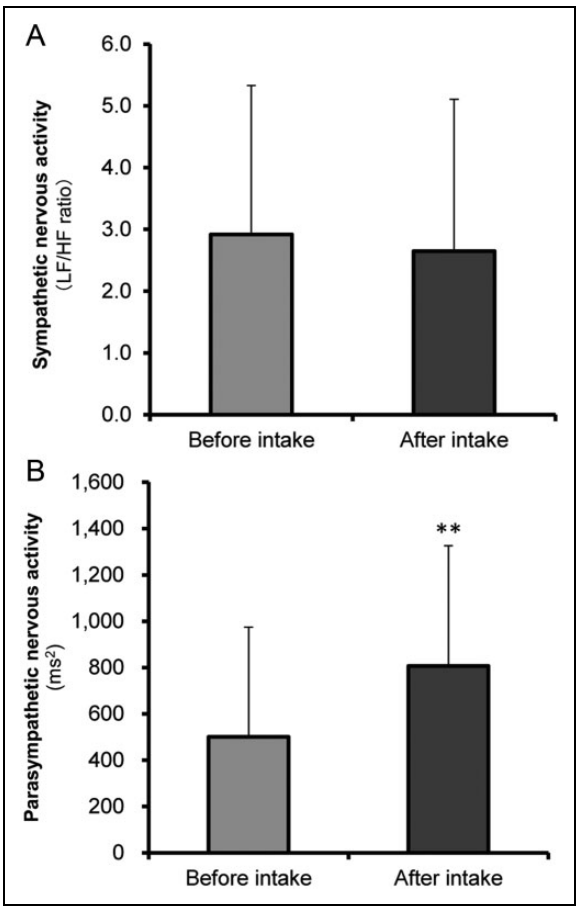


Figure 4. Changes in sympathetic (A) and parasympathetic (B) nervous activities at rest by AHCC® administration. The activity of autonomic nervous system was measured by heart rate variability (HRV) analysis. Sympathetic and parasympathetic nervous activities were evaluated by spectral analysis of HRV. The ratios of low frequency (LF)/high frequency (HF) and HF were assumed to present sympathetic nervous activity and parasympathetic nervous activity, respectively. The value represents mean ± standard deviation (SD) (n = 5). $^{**}P < .01$ versus before intake.

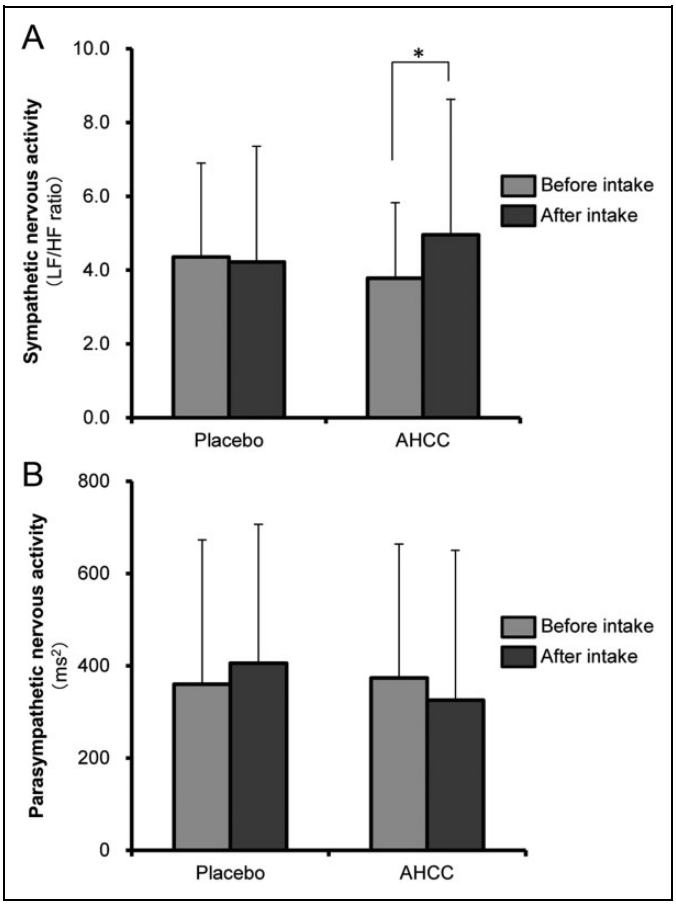


Figure 5. Changes in sympathetic (A) and parasympathetic (B) nervous activities by AHCC® administration under mental stress condition. The activity of autonomic nervous system was measured by heart rate variability (HRV) analysis. Sympathetic and parasympathetic nervous activities were evaluated by spectral analysis of HRV. The ratios of low frequency (LF)/high frequency (HF) and HF were assumed to present sympathetic nervous activity and parasympathetic nervous activity, respectively. Uchida-Kraepelin test, a mental arithmetic test, was employed as a mental stress task. The value represents mean ± standard deviation (SD) (n=17). $^{*}P < .05$ between before and after intake.

were investigated using questionnaires for psychological state and mood. The present study was conducted in the 5 subjects from study 1, who showed an AHCC®-dependent enhancement in sympathetic nervous activity in response to physical stress. When AHCC® was taken for 7 days at a daily dosage of 3 g, parasympathetic nervous activity became significantly higher (500.9 ± 473.8 preintake and 807.4 ± 518.8 postintake, $P < .01$, n = 5). However, no changes were seen in sympathetic nervous activity (Figure 4). These results indicate that AHCC® enhances sympathetic nervous activity under physical stress and parasympathetic nervous activity in the absence of physical stress. According to our survey for the effects of AHCC® on daily activities, the items "Hard to sleep" and "Feel tired" ($P = .080$ and .099, respectively) showed a trend toward improvement. According to the POMS questionnaire, feelings of anger/hostility improved significantly ($P < .05$). Considering these results, AHCC® appears to improve feelings of anger/hostility, and might be a promising functional food to improve sleep problems and feeling tired.

Study 3: Effects of AHCC® on the Autonomic Nervous System Under Mental Stress

The results of studies 1 and 2 suggest that AHCC® enhances sympathetic nervous activity under physical stress, while promoting parasympathetic nervous activity when in a resting position. In study 3, the effects of AHCC® on the ANS under mental stress were investigated after 7 days of AHCC® intake at a daily dosage of 3 g. The U-K test, a mental arithmetic test, was administered as a mental stress and ANS activity was measured. ANS activities under mental stress before AHCC® intake and after 7 days of intake are shown in Figure 5. The

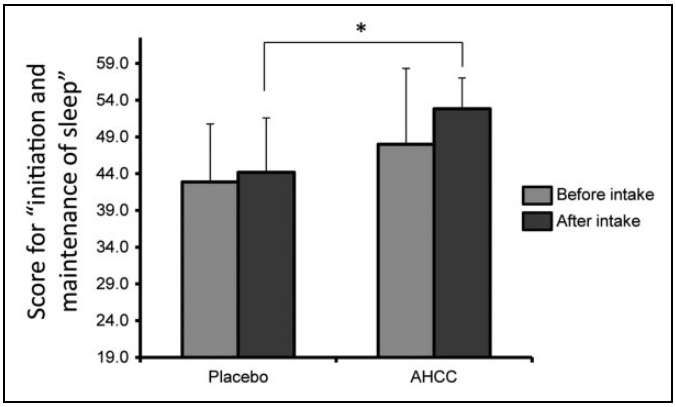


Figure 6. Ogri-Shirakawa-Azumi Sleep Inventory. Item "initiation and maintenance of sleep" is shown. The value represents mean ± standard deviation (SD) (n = 7). *$P < .05$ between intakes of AHCC® and placebo.

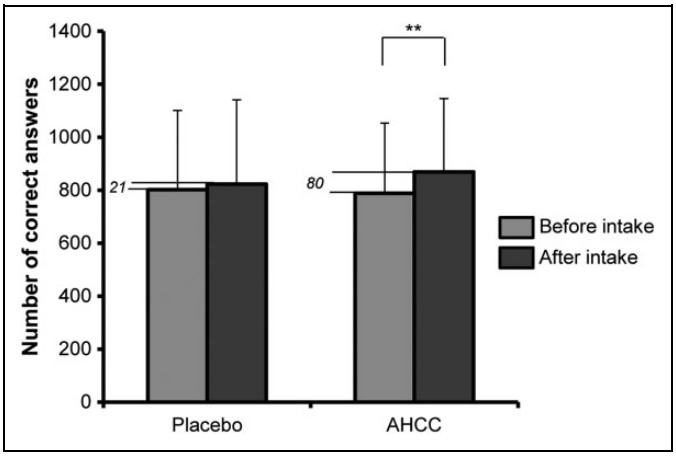


Figure 7. The total number of correct answers in Uchida-Kraepelin test conducted by subjects under chronic stress condition. The total number of answers was calculated by summing the results of all subjects in the same treatment arm. Subjects under chronic stress condition were selected based on sore of the Self-rating Depression Scale (SDS) questionnaire. Subjects with an SDS score of 40 to 49 points were considered as being under chronic stress condition. The italic value on the left side of the gray bar indicates difference between before intake and after intake. The value represents mean ± standard deviation (SD) (n = 7). **$P < .01$ between before and after intake.

sympathetic nervous activity showed a significant increase after 7 days of AHCC® treatment (3.78 ± 2.03 before intake and 4.96 ± 3.67 after intake, $P < .05$) (Figure 5A). There were no differences in the parasympathetic nervous system activity (Figure 5B). AHCC® thus enhanced sympathetic nervous activity in response to mental stress (as measured by the U-K test) as well as physical stress (as measured by Schellong's test in study 1 above).

Study 4: The Effects of AHCC® on People Under Chronic Stress

In patients with depression resulting from exposure to chronic stress, it is known that NK cell activity is decreased.[14,15] We investigated the possible mechanism by which AHCC® might enhance sympathetic nervous activity in response to stress. To investigate the effects of AHCC® on NK cell activity in subjects with chronic mental stress, subjects with mild depression were selected using the SDS questionnaire. The SDS score was in the range of 40 to 49 points in 7 subjects (mean: 45.0 ± 5.7 points). Because it was determined that these 7 subjects had mild depression and were suffering from chronic mental stress, they were designated a high-SDS group. The mean SDS score in the remaining 6 subjects was 35.4 ± 1.7 points, so these individuals were considered as subjects with less stressful condition. In the high SDS group, the effects of AHCC® on sleep (OSA-MA), office work performance (U-K test), and changes in NK cell activity were investigated. The score for the high SDS group's "initiation and maintenance of sleep" was significantly greater in AHCC® intake period than in the placebo intake period ($P < .05$); however, we found no significant difference between pre- and post-AHCC® intake (Figure 6). The score for "recovery from fatigue" increased during the AHCC® intake period ($P = .052$). AHCC® also increased the number of correct answers in the U-K test for this high SDS score group ($P < .01$) (Figure 7). After 14 days of AHCC® intake by the high SDS score group, NK cell activity changed by 9.68 ± 5.74 points ($P < .05$) (Table 2).

Table 2. Natural Killer Cell Activity Change in Subjects With Chronic Stress (n = 7).[a]

	Before Intake, % (Mean ± SD)	After Intake, % (Mean ± SD)	*P* Value of Before Intake vs After Intake	Amount of Variation
AHCC	28.16 ± 10.96	37.84 ± 15.92	.020	9.68 ± 5.74*
Placebo	32.15 ± 12.64	29.70 ± 10.73	.511	−2.45 ± 6.58

Abbreviations: SD, standard deviation.
[a] Natural killer cell activity was measured using the chromiun-51 release assay.
*$P < .05$, versus placebo intake.

Discussion

Many clinical studies of AHCC® have been conducted over the past dozen years.[17,18,30-32] In many cases, AHCC® was used at a daily dose of 3 g, and showed various beneficial effects such as immune modulation, an improvement of prognosis of hepatocellular carcinoma patients after surgery, and reduction of adverse event in patients with advanced cancer during chemotherapy, and so on. In addition, even 1 g of AHCC® ingestion for 4 weeks attenuated the seasonal reduction of the NK cell count compared with placebo.[19] Based on these results, we considered the daily dose used in the studies, 3 g of AHCC®, was reasonable and proper. Intervention and washout periods are different for each study. The adequacy of them should be considered in a further large-scale study. Considering results in our preliminary studies, however, we determined that at least a 5-day intervention and a 7-day washout were adequate. When

participants' baselines were measured at the beginning of the second intake period, that is, after washout period, no significant difference was observed between each group, therefore, the washout period was deemed to be sufficient.

In this study, we used a variety of approaches to test the efficacy of AHCC® in treating stress. We used Schellong's test to trigger physical stress.[23] AHCC® significantly increased sympathetic nervous activity under physical stress, compared with placebo (Table 1). This appears to suggest that in response to the temporary physical stress imposed by standing up, AHCC® enhances the response of the sympathetic nervous system to maintain blood pressure homeostasis, resulting in adaptation to physical stress. Therefore, we propose that AHCC® may alleviate or prevent lightheadedness and dizziness associated with impaired autonomic control that is a symptom of stress.[33,34] Furthermore, the effects of AHCC® on the activity of the ANS under temporary mental stress were also investigated. When the U-K test was administered as a mental stressor, sympathetic nervous activity did not change in the placebo group but increased significantly in the AHCC® group (Figure 5). It appears to suggest that AHCC® also enhances the activity of the sympathetic nervous system under temporary mental stress, and thus affects adaptation to stress stimuli, resulting in maintenance of homeostasis.

While appropriate activation of sympathetic nervous system against stress stimuli is necessary to maintain homeostasis, its chronic activation causes ANS imbalance, which causes psychosomatic disorders such as depression and autonomic ataxia.[35,36] Therefore, the activated sympathetic nervous system should be normalized via a negative feedback system under non–stress-exposed conditions. In a resting state, the effects of AHCC® on ANS activity were evaluated in subjects, who had higher sympathetic nervous activities against physical stress after AHCC® intake. At rest, AHCC® significantly enhanced parasympathetic nervous activity, while there were no changes in sympathetic nervous activity (Figure 4). This result suggests that AHCC® could enhance sympathetic nervous activity only against stress stimuli, and that the activation is not chronic. Furthermore, the increase in parasympathetic nervous activity at rest indicates stabilization of ANS activity. Subjects also showed improvement in sleep and fatigue based on our questionnaire that assessed their psychological condition, and also showed improvements in anger/hostility in our mood-profiling questionnaire. We therefore propose that AHCC® may be useful for stress management in daily life.

Various stressors can cause immunosuppression, along with diverse physical and mental symptoms including insomnia, dizziness, anxiety and nervousness that impair concentration, memory, and task performance.[3-6] Some mental illness symptoms are thought to be related to NK cell activity; in particular, it is known that depression is related to impaired NK cell function.[14,15] Based on these findings, the effects of AHCC® on immune function, task performance, and sleep were assessed in subjects with mild depression by NK cell activity, the number of correct answers in the U-K test, and OSA-MA, respectively. NK cell activity significantly increased only in the AHCC® intake period compared to the placebo (Table 2). It has been reported in animal experiments and clinical studies that AHCC® enhances the activity of dendritic cells, NK cells and other innate immunity cell types.[17-19,31] We therefore consider that AHCC® might improve stress-associated immunosuppression and mental symptoms in patients with depression. The number of correct answers in the U-K test significantly increased in the AHCC® intake period (Figure 7). In the U-K test, the speed and accuracy of answers are indicative of the capacity to learn and work, so the test is used to assess vocational aptitude as well as to administer mental stress tasks.[25] Depression reduces working memory capacity, and thereby impairs concentration, memory and task performance.[37] AHCC® increased the number of correct answers in the U-K test, suggesting that AHCC® may act in improving depression. The results of the U-K test and OSA-MA (improvement of "initiation and maintenance of sleep" and "recovery from fatigue") were not definitive; however, considering that AHCC® has shown antistress effects in animal experiments, we think that AHCC® will exhibit anti-stress effects in patients with depression, improving their concentration, sleep, and fatigue. It is reported that human herpes viruses are reactivated by physical and mental stress, and are considered an objective biomarker for stress and fatigue.[38] AHCC® significantly decreased the level of human herpes virus–6 in saliva of cancer patients receiving chemotherapy in a previous study.[32] In a further study, other immune parameters such as cytokines and secretory immunoglobulin A will be measured as stress markers.

It is reported that depressive disorder is accompanied with oxidative stress and is characterized by significantly lower plasma concentrations of various antioxidants.[39] In addition, it is suggested that oxidative stress is related to diverse psychiatric disorders.[40] Therefore, adequate supplementation of antioxidants might lead to alleviation of mental stress. AHCC® has antioxidant properties as well as immune modulation effects, and ameliorated endocrine disturbances induced by oxidative stress by acting as an antioxidant.[41,42] In the present study, the antioxidant properties of AHCC® may be involved in stress management. In a further study, for instance, 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 8-isoprostane will be used as oxidative stress markers.

Although the mechanism of action of AHCC® remains to be fully investigated, the current data support the view that AHCC® may be beneficial for the management of stress. These outcomes may be the result, in part, of modulation of ANS homeostasis and an enhancement in NK cell activity. However, the present studies were conducted in a small sample and for a short period of time, and the subjects were limited to young people. Therefore, large-scale, long-term trials should be carried out in various subjects in a proof-of-concept study.

Conclusion

Based on the results of the present and previous studies, AHCC® appears to enhance the activity of the sympathetic nervous system under various stress conditions and might improve immunity and mental illness symptoms in physically

healthy people with depression. No adverse drug reactions were seen in the present study, suggesting that AHCC® is safe. Therefore, we conclude that AHCC® is a potentially useful functional food to maintain and promote mental health in humans with depression, but further studies are needed.

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Authors Contributions

Jun Takanari, Atsuya Sato, and Hideaki Waki participated in the design of the study, contributed to data analysis, and drafted the article. Kazuo Uebaba and Shogo Miyazaki participated in the design of the study, and supervised the experiment. Tatsuya Hisajima participated in the design of the study, drafted the article, and supervised the experiment. All authors read and approved the final version of the article.

Declaration of Conflicting Interests

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Ethical Approval

This study was conducted in accordance with the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Review Board, Teikyo Heisei University as approval no. 24-049 (Tokyo, Japan). Valid informed written consents were obtained from all subjects.

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