International Journal of Medicinal Mushrooms, 17(9): 799–817 (2015)

Open-Label Study of the Influence of Food Containing the Royal Sun Mushroom, *Agaricus brasiliensis* KA21 (Higher Basidiomycetes), on the Quality of Life of Healthy Human Volunteers

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ABSTRACT: We conducted an open-label study in which food containing *Agaricus brasiliensis* KA21 was consumed continuously for 12 weeks. A questionnaire for subjective evaluation of the efficacy of this food (hereafter, subjective evaluation questionnaire) revealed significant improvements compared with before its intake; there were improvements in the scores of the amounts of hair loss and gray hair, fatigue and general malaise, eye strain, shoulder stiffness, coldness of extremities, difficulty staying awake during the day, and ease of getting out of bed. These findings suggest that intake of food containing *A. brasiliensis* KA21 results in the above-mentioned subjectively evaluated improvements, and the possibility that *A. brasiliensis* KA21 improves the body's immunity. Moreover, no issues regarding the safety of the test food were found.

KEY WORDS: medicinal mushrooms, *Agaricus brasiliensis*, *Agaricus blazei*, sleep quality, clinical study, enhancement of immunity, evaluation of safety

ABBREVIATIONS: CRO, contract research organization; **ERB**, ethical review board; **KA21**, King Agaricus 21; **sIgA**, salivary immunoglobulin A.

I. INTRODUCTION

The royal sun medicinal mushroom, or the himematsutake mushroom, *Agaricus brasiliensis* S. Wasser et al. (=*A. blazei* Murrill sensu Heinem.; Agaricaceae, higher Basidiomycetes),[1–3] is a health food material that was first successfully cultivated in Japan by artificial means in 1975, and its stable supply as a raw material was realized in the early 1990s. Starting with epidemiological studies based on food experiences in Brazil, followed by trial studies in Japan and elsewhere, it has become established over 20 years as a health food material that "contributes to the improvement of quality of life by increasing the natural healing power and immunity that humans possess innately."[4]

A. brasiliensis is said to "vary in properties and components depending on the strain, cultivation condition, and origin."[5] We have been conducting research and developing "King Agaricus (KA) 21," a strain of *A. brasiliensis* (deposited at the National Institute of Technology and Evaluation, Japan, with deposition no. FERM P-17695) cultivated outdoors under natural conditions in Brazil.

To date, studies of the safety and efficacy of *A. brasiliensis* KA21, including human clinical studies, have been conducted at various institutes, such as the Research Center for Food Safety of the University of Tokyo, the Laboratory for Immunopharmacology of Microbial Products at Tokyo University of Pharmacy and Life Science, Nagoya City University Graduate School, Kinki University Faculty of Medicine, and Juntendo University Medical School. The results of these studies have been published in journals such as *Evidence-based Complementary and Alternative Medicine*.[4] Recent studies have confirmed

that, compared with greenhouse-cultivated *A. brasiliensis*, *A. brasiliensis* KA21 cultivated outdoors under natural conditions in Brazil contains not only 1.5 times more β-glucan,[5] which is suggested to have immunopotentiative actions,[6,7] but also a large amount of vitamin D,[8] which is scarcely contained in products grown by greenhouse cultivation under dark conditions. Moreover, studies have revealed that warm (in the low-temperature region) air-drying performed during the processing of *A. brasiliensis* products does not inactivate heat-sensitive antioxidant enzymes such as peroxidase and polyphenol oxidase.[5,9] The effects of these enzymes on biological functions also are attracting attention. The results of previous clinical studies demonstrate that intake of *A. brasiliensis* KA21 activates systemic immune function in humans (natural killer cell activation).[4] However, no findings have been obtained with respect to subjective evaluation of the efficacy of *A. brasiliensis* KA21.

Therefore, we conducted an open-label study wherein food containing *A. brasiliensis* KA21 was continuously consumed for 12 weeks with the aim of specifically performing a subjective evaluation of the efficacy of food containing *A. brasiliensis* KA21.

II. MATERIALS AND METHODS

A. Study Administrative Structure

This study was conducted with the following administrative structure.

1. Study center: This study, including the clinical tests and the management of subjects, was performed at Nihonbashi Cardiology Clinic (Tokyo, Japan).

2. Contract research organization (CRO): This study was properly conducted and managed in accordance with the study protocol and in close cooperation with all departments under the administrative structure. Assistance in subject selection and management, verification of various test values, and tabulation and statistical analysis of various test values was provided by Total Technological Consultant, Co., Ltd. (Tokyo, Japan).

3. Site management organization: Under the guidance of the CRO and according to the study protocol, recruitment of subjects, contacting of subjects, management of the study's progress, assistance to the principal investigator, and distribution of the test food were performed by e-Kencom Co., Ltd. (Tokyo, Japan).

4. Clinical laboratories: Clinical laboratory tests were performed by Kishimoto Clinical Laboratory, Inc. (Hokkaido, Japan), and SRL, Inc. (Tokyo, Japan).

5. Ethical review board (ERB): On the basis of the study protocol and materials regarding the test food, the ERB at Nihonbashi Cardiology Clinic reviewed the study from ethical and scientific viewpoints in compliance with the Ethical Guidelines for Epidemiological Research.

B. Ethical Considerations

1. Compliance with the Declaration of Helsinki and Ethical Guidelines for Epidemiological Research: This study was conducted in accordance with the tenets of the Declaration of Helsinki, while always bearing in mind the protection of the human rights of subjects, and in compliance with the Ethical Guidelines for Epidemiological Research (Ordinances of the Ministry of Education, Culture, Sports, Science and Technology, and the Ministry of Health, Labor and Welfare).

2. Review and approval by the ERB: This study was reviewed by the study board of Nihonbashi Cardiology Clinic and conducted after receiving its approval on May 24, 2011.

3. Protection of privacy and personal information: The principal investigator (M.M.) anonymized adverse events and other data related to the study results and did not use the names of the subjects or numbers that may disclose the subjects' identity, address, and other information. In addition, those who were involved in this study ensured the protection of the personal information of the subjects when conducting the study.

4. Acquisition of informed consent: Before study implementation, the principal investigator provided

an explanation form that described to subjects the items listed below, explained thoroughly the intent and content of the study, and obtained written informed consent from subjects based on their free will: (l) The subject fully understands the study purpose and procedures and can make a decision to participate in this study on a voluntary basis. (2) The subject will not receive a penalty even when he/she does not agree to participate in the study. (3) Even after agreeing to participate in the study, the subject can freely withdraw his/her informed consent and discontinue study participation at any time. Furthermore, this shall not cause the subject any disadvantage. (4) The subject can ask for clarification should they be uncertain or worried about any part of the explanation provided by the physician or the content of this explanation form. (5) The subject shall not tell others about his/her participation in this study, or the details of this study. (6) The objectives and methods by which the study will be implemented. (7) The content of the study (including participants' conditions). (8) The method of study implementation. (9) Points that must be observed. (10) Study period. (11) Expected advantages and disadvantages that may arise. (12) Management of the situation in the event that the subject's health is adversely affected during the study. (13) Provision of new information and changes in the study plan. (14) Discontinuation of study participation, interruption of study continuation. (15) Protection of privacy and personal information upon the publication of various data and results, among others. (16) Storage and disposal of materials. (17) Fair and impartial planning, implementation, and reporting of this study (conflict of interest). (18) Cost burden, cost of cooperation. (19) This medical institution. (20) Principal investigator. (21) ERB. (22) Contact information (consultation service, etc.).

C. Study Procedure

1. Study design: Open-label study
2. Number of registered subjects: 24 subjects
3. Study period: From May to November 2011
4. Subject inclusion and exclusion criteria: Subjects who met all of the following inclusion criteria and did not meet any of the exclusion criteria were registered.

4-1. Inclusion criteria: (1) Male or female ≥20 years and <60 years. (2) Individuals with a relatively low salivary immunoglobulin A (sIgA) secretion rate were selected.

4-2. Exclusion criteria: (1) Those who consume a food item that contains the active ingredient (β-glucan) of the test food in abundance, or a health food that has high levels of β-glucan. (2) Those with a history of allergic disease (e.g., seasonal allergic rhinitis [hay fever], perennial allergic rhinitis, asthma, atopic dermatitis, and allergic conjunctivitis). (3) Those undergoing treatment that might affect the study. (4) Those who undertake shift work or who are engaged in physical labor, such as the transportation of heavy objects. (5) Those who regularly perform strenuous activities, such as marathon running. (6) Those who are unable to follow the procedures of various examinations conducted during the study period as specified (e.g., collection of saliva). (7) Those who have a disease under treatment or for whom treatment is deemed necessary. (8) Those with a history of critical illness, such as diabetes, liver disease, kidney disease, hypertension, ischemic heart disease, and impaired glucose tolerance. (9) Those suspected of developing allergic reactions to the test food. (10) Those deemed to be unsuitable as subjects on the basis of laboratory and physical test results obtained beforehand. (11) Those who wish to become pregnant during the study period or who are currently pregnant or breastfeeding. (12) Those participating in another clinical study at the time when informed consent to participate in this study is obtained. (13) Those deemed by the principal investigator to be inappropriate as subjects.

5. Discontinuation and Dropout

5-1. Discontinuance criteria: Subjects meeting the following criteria, as deemed relevant by the principal investigator, were allowed to discontinue the study and were treated as discontinuation cases: (1) When it is judged that there may be a risk of compromising the safety of the subject. (2) When

continuation of the study is difficult owing to serious clinical abnormality or the occurrence of an accident. (3) When serious or continuous noncompliance of the subject with the study protocol is found. (4) When the principal investigator deems it appropriate to discontinue the study.

5-2. Dropout criteria: When a subject withdraws from the study for personal reasons after having provided his/her informed consent to participate in the study, the principal investigator discontinues the subject's study and regards the case as a dropout.

6. Instructions to Subjects and Restrictions

The principal investigator and the person responsible for assisting the study instructed the subjects to observe the following restrictions while leading their lives as usual during the study period: (1) During the study period, lifestyle habits (e.g., meals, drinking, exercise, and sleeping) from before study participation should be left unchanged as much as possible. (2) Excessive exercise, dieting, or overeating that deviates greatly from the usual range should be avoided. (3) During the study period, a new form of exercise should not be started, and exercise habits from before study participation should not be changed. (4) Use of health food during the study period is prohibited. (5) If any medication is used, the product name and dosage should be recorded in the diary. (6) The specified amount of test food should be consumed every day. The time and amount of the test food consumed should be recorded in the diary. (7) The diary should be kept every day. (8) Drinking alcohol is prohibited the day before the examination, and the use of medication should be avoided as much as possible. (9) Strenuous exercise is prohibited the day before and the day of the examination. (10) The day before the examination, eating and drinking should cease at around 10 p.m., and nothing other than water should be consumed thereafter. (11) The day before the examination, the subject should go to bed before midnight and get enough sleep. (12) The day of the examination, the subject should fast until the examination is completed (water can be consumed). (13) The day of the examination, smoking is not allowed after brushing teeth until the examination is completed.

7. Test food Containing *A. brasiliensis* KA21

7-1. Composition of the test food, daily intake, and intake period: The composition of the test food used in this study, the amounts of active ingredients, and other relevant information are shown in Table 1. The test food was consumed continuously for 12 weeks at a daily intake of 3 tablets (900 mg) or 5 tablets (1500 mg). The time of intake was specified as "after breakfast"; when the subject was unable to do so, he/she was instructed to consume the food later in the day. However, the day of the examination, subjects were not allowed to take the tablets until the end of the examination. The intake period was set to 12 weeks (84 days).

7-2. Rationale for setting the intake amount of active ingredient: A previous questionnaire demonstrated that intake of 800 mg of the test food improved the symptoms of athlete's foot. Moreover, in an overdose test that we conducted previously, intake of up to 30 tablets (9 g) a day was confirmed to be safe. On the basis of these findings, the intake amount in this study was set.

7-3. Management of test food: (1) The test food was supplied to the contract research organization, and this organization supplied the test food to the site management organization. (2) The site management organization sent the necessary amounts of test food to the subjects. (3) After completion of the study, the remaining test food (including that given to the subjects) was collected by the site management organization and returned to the CRO and, from there, back to our group.

7-4. Study schedule: Forty-nine candidate subjects who provided informed consent underwent examinations before intake, including the completion of a lifestyle habit questionnaire, a physical condition checkup, physical measurements, physical examination, general laboratory blood examination, saliva examination, special examinations, and a subjective evaluation questionnaire. On the basis of the results of these examinations, 24 subjects who had low sIgA secretion rates and fit the study purpose were selected and assigned into the low-

TABLE 1: Test Food Composition, Ingredients, and Related Information

	Test Food
Raw material	*Agaricus brasiliensis* KA21
Form	Tablets, press-through packaging
Active ingredient	β-glucan
Date of manufacture	April 22, 2011
Expiration date	April 22, 2013
Storage method	Store at room temperature; avoid direct sunlight, high temperature, and high humidity
Test food provider	Terra Forte Co., Ltd.
Nutrient composition (per 100 g)	
β-glucan* (g)	12.40
Energy (kcal)	179
Protein† (g)	39.8
Lipid‡ (g)	2.9
Carbohydrate§ (g)	43.1
Sodium¶ (mg)	3.4

*Assessed using the enzymatic method.
†Assessed using the Kjeldahl method (total nitrogen × 6.25).
‡Assessed using the acid hydrolysis method.
§Assessed using the following formula: 100 − (moisture + protein + lipid + ash).
¶Assessed by atomic absorption spectrophotometry.

dose intake group (3 tablets daily) or the high-dose intake group (5 tablets daily). The test food and a diary to keep during the intake period were distributed to each subject, and intake of the test food and recording in the diary were initiated. The subjects were instructed to record the time and amount of test food they consumed throughout the course of the study. They were instructed to visit the hospital 6 weeks (on the 43rd day) and 12 weeks (on the 85th day) after the initiation of intake for examinations, during which they underwent a physical condition checkup, physical measurements, physical examination, saliva examination, and special examinations, as well as completed the subjective evaluation questionnaire. In addition to the above-mentioned examinations, a general laboratory blood examination was performed during the examinations at the 12th week. However, in the event that the subjects developed cold symptoms (e.g., fever, sore throat, cough, and runny nose) or infectious diarrhea symptoms within 7 days before the scheduled date of examination, the examination date was changed to 7 days or more after the day subjective symptoms disappeared. The day before the scheduled examinations, drinking alcohol was prohibited, and subjects were asked to finish eating and drinking by around 10 p.m.; drinking/eating thereafter was prohibited, except for water, until the end of the examination. The subjects were instructed to go to bed by around midnight to get enough sleep. In the morning of the day scheduled for the examination, the subjects were instructed to brush their teeth at home, after which eating and drinking (other than water), as well as smoking, were prohibited. The subjects thus visited the hospital in a fasted state.

8. Subjective Evaluation Questionnaire

The subjective evaluation questionnaire comprised 34 items asking about the conditions of the skin, stool, hair, and body, as well as sleeping habits.

The subjects scored their skin, stool, hair, and physical conditions on a 5-point scale, from very bad (1 point) to very good (5 points). Regarding sleeping habits, the subjects were asked to complete the Pittsburgh Sleep Questionnaire (Japanese version) and to score their ease of getting out of bed on a 4-point scale, from very good (0 points) to very bad (3 points).[10–12] For the items in the Pittsburgh Sleep Questionnaire (Japanese version), the total score and subscale scores for the quality of sleep, time required for falling asleep, sleep duration, sleep efficiency, difficulty in sleeping, use of sleeping pills, and difficulty in staying awake during the day were calculated using the conventional method for the Pittsburgh Sleep Questionnaire (Japanese version) (Table 2).

9. Adverse Events

Definition of adverse events: Any unfavorable medical events that had occurred or worsened in subjects after intake of the test food (subjective symptoms, objective findings, and abnormal changes in test results) were considered as adverse events. Adverse events were assessed regardless of whether they were related to the test food.

Assessment of adverse events: Adverse events reported as subjective symptoms or objective findings were assessed by the principal investigator. Regarding the test results of individual subjects, adverse events were assessed by the principal investigator according to the reference ranges specified by the study center.

10. Collection and Recording of Adverse Events

For a subject in whom an adverse event occurred, appropriate treatment was provided as necessary. The following were described and recorded on a case report form: symptoms and diagnosis, the severity of the adverse event, whether it is considered a serious case, relationship with the test food, date of occurrence, date of recovery, whether treatment was provided (details of treatment), and outcomes. Adverse events were assessed according to the following criteria:

10-1. Severity: The severity of all adverse events that occurred was determined according to the following three criteria: (1) Mild: daily living is not particularly compromised. (2) Moderate: daily living is compromised. (3) Severe: daily living is almost impossible.

10-2. Seriousness: When the adverse events that occurred fell into any of the following categories, they were considered serious: (1) Fatal. (2) Events that could lead to death (life-threatening events). (3). Events that require hospitalization or a prolonged hospital stay for treatment. (4) Disability (results in functional impairment to the extent that one's daily living is compromised). (5). Events that may lead to disability. (6) Events that are serious according to the disability described above. (7) Congenital disease or anomaly in later generations.

10-3. Relationship with the test food: All adverse events that occurred were assessed according to the following 4 criteria in terms of their relationship with the test food: (1) Unrelated: the adverse event is caused by etiologies other than the test food (e.g., primary disease, underlying disease or complications, and concomitant medication), and its relationship with the test food can almost certainly or completely be ruled out. (2) Probably unrelated: the adverse event is considered non–late onset, and there is no sufficient temporal relationship between the intake of the test food and the occurrence of the adverse event. (3) Probably related: etiologies other than the test food (e.g., primary disease, underlying disease or complications, and concomitant medication) have been thoroughly investigated, but the relationship is not certain. (4) Definitely related: the occurrence of the adverse event has a reasonable temporal relationship with the intake of the test food and can be explained as a known reaction or pharmacological action of the test food or of similar compounds.

10-4. Outcomes: The course of all adverse events that occurred was assessed according to the following five items: (1) Disappearance or recovery: disappearance of symptoms or recovery; normalization of test results or recovery to levels before intake. (2) Remission: improvement in severity by 1 stage or more, disappearance of almost all symptoms or findings in mild cases, or recovery of laboratory test results to near-normal levels or

TABLE 2: Items in the Subjective Evaluation Questionnaire (Japanese version)

Items	Questions
Skin condition	The suppleness and resilience of the skin in a recent week, skin troubles in a recent week, and the condition of athlete's foot in a recent week
Stool condition	Bowel movement in a recent week, and stool condition and form in a recent week
Hair condition	The stiffness of hair in a recent week, the amount of hair loss in a recent week, and the amount of gray hair in a recent week
Physical condition	Tiredness of the body in a recent week (fatigue, general malaise), fatigue of the eyes (eyestrain) in a recent week, shoulder stiffness in a recent week, stress in a recent week, coldness of extremities in a recent week, dry eye condition in a recent week, and physical condition in a recent week (e.g., cold)
Sleeping habits	Bedtime during the past month, time required for falling asleep during the past month, wake-up time during the past month, sleep duration during the past month, frequency of and reasons for difficulty sleeping during the past month, quality of sleep during the past month, frequency of sleeping pill use during the past month Frequency of difficulty staying awake during social activities in the past month, and maintenance of motivation during the past month Ease of getting out of bed during the past month

near levels before intake. (3) Unchanged: almost no change in symptoms or abnormal levels. (4) Aggravation: exacerbation of symptoms and abnormal levels. (5) Unknown: impossible to follow-up despite the effort to follow up symptoms and abnormal levels (owing to adverse circumstances, including death).

10-5. Side effects: Among adverse events, those that were determined to be definitely related and probably related to the test food were considered side effects.

11. Efficacy Analysis

Data from subjects who completed all of the scheduled examinations were included in the efficacy analysis, excluding those who met the following exclusion criteria for analysis.

11-1. Exclusion criteria for analysis: (1) When diary entries were missing or actions that could compromise the reliability of test results, such as noncompliance with specimen collection procedures, were notable. (2) When it becomes apparent after entry into the study that the subject met exclusion criteria or was unable to comply with restrictions.

11-2. Safety analysis: (1) Adverse events: all subjects who consumed the test food even once were included. (2) Measurements: the analysis of test results included the same subjects who were included in the efficacy analysis.

11-3. Evaluation methods: (1) Efficacy endpoint: subjective evaluation questionnaire. (2) Efficacy evaluation method: with regard to the subjective evaluation questionnaire, differences in the scores of all items between after intake and before intake were evaluated using the Wilcoxon signed-rank test. (3) Safety endpoint: adverse events (subjective/objective symptoms, laboratory test results, physical measurements, physical test results). (4) Safety evaluation method: with regard to adverse events, the principal investigator assessed whether there was a causal relationship or association of such events with the test food. The principal investigator evaluated the numerical data from laboratory tests and measurements of the subjects with respect to the reference ranges specified by the study center (or, in the case of laboratory test results, the reference ranges of the testing agency). Furthermore, for each test item, the levels before intake and those of examinations at the 12th week were evaluated using a 1-sample *t* test. (5) Expression and significance level: results are expressed as mean ± standard deviation, and a 5% significance level (2-sided) was used for all tests.

TABLE 3: Background Features of Subjects in the Efficacy Analysis

Item	Low-Dose Group	High-Dose Group
Subjects (n)	12	10
Sex (n)		
Male	7	5
Female	5	5
Age (years)	42.8 ± 9.9	43.2 ± 10.7
Salivary immunoglobulin A secretion rate (μg/min)	36.57 ± 10.53	36.02 ± 9.41

Data are mean ± standard deviation unless otherwise indicated.

III. RESULTS

In the following, subjects who consumed 3 tablets of the test food daily are referred to as the "low-dose (L) group." Those who consumed 5 tablets of the test food daily are referred to as the "high-dose (H) group."

A. Selection of Subjects

A total of 24 individuals (12 male and 12 female) were selected and registered as subjects, and 12 subjects each (6 male and 6 female) were assigned to the L and H groups. The subjects then started test food intake as prescribed. Note that, for some subjects, some of the physical/laboratory test items showed levels outside the reference range before intake was initiated; those subjects were enrolled in the study after it the principal investigator verified that there should be no problem with them participating in the study.

B. Breakdown of Subjects

After the initiation of intake, 2 subjects withdrew from the study of their own volition (ID no. 808, female, 43rd day, H group; ID no. 840, female, 45th day, L group). In addition, ID no. 812 was initially assigned to the H group but was taking only 3 tablets a day; accordingly, this subject was treated as belonging to the L group. No other subjects deviated from the study protocol or consumed prohibited food. The rates of test food intake were 91.7% for ID no. 803, 91.8% for ID no. 836, 98.8% for ID no. 812, and 100% for the remaining 19 subjects. Consequently, 22 subjects were considered eligible for efficacy analysis. ID no. 849 presented with cold symptoms 78 to 85 days after the initiation of intake, so the examinations at the 12th week were postponed for 7 days and performed on the 92nd day.

C. Background Features of Analyzed Subjects

Table 3 shows the background features of subjects in the efficacy analysis, including age, sex, and sIgA secretion rate at the time of examination before intake.

D. Efficacy Evaluation

Tables 4 and 5 show changes in subjective evaluation questionnaire scores. Scores for the amount of hair loss significantly increased in the H group at both the 6th and the 12th weeks, from 2.7 ± 0.5 before intake to 3.2 ± 0.4 and 3.2 ± 0.6, respectively. Scores for the amount of gray hair significantly increased in the H group at both the 6th and the 12th weeks, from 2.4 ± 1.1 before intake to 3.2 ± 0.7 and 3.2 ± 1.0, respectively. Scores for fatigue and general malaise significantly increased in the L group, from 2.1 ± 0.9 before intake to 2.9 ± 0.8 at the 12th week. In the H group as well, a significant increase was observed at the 12th week, from 2.1 ± 1.1 before intake to 3.0 ± 0.9. Scores for eye strain significantly increased in the H group, from 2.4 ± 0.8 before intake to 3.3 ± 0.8 at the 12th week.

TABLE 4: Summary of the Subjective Evaluation Questionnaire

Item	Group	Before Intake	At Week 6	At Week 12
Skin suppleness and resilience	Low dose	2.6 ± 0.7	3.1 ± 0.8	2.9 ± 0.8
	High dose	2.9 ± 0.9	3.4 ± 0.5	3.1 ± 0.3
Skin problems	Low dose	2.8 ± 0.8	3.0 ± 0.7	2.9 ± 0.9
	High dose	3.7 ± 1.1	3.5 ± 0.8	3.8 ± 0.6
Athlete's foot*	Low dose	2.0 ± 0.0	3.0 ± 0.0	4.0 ± 0.0
	High dose	—	—	—
Bowel movement	Low dose	3.6 ± 1.4	3.3 ± 1.0	3.7 ± 1.0
	High dose	3.2 ± 0.6	3.5 ± 0.8	3.7 ± 0.7
Stool condition and form	Low dose	3.6 ± 0.9	3.3 ± 1.1	3.9 ± 0.9
	High dose	3.4 ± 0.7	3.5 ± 0.7	3.6 ± 0.8
Stiffness of hair	Low dose	2.5 ± 0.9	3.2 ± 0.8	3.1 ± 0.9
	High dose	2.8 ± 0.6	3.0 ± 0.5	3.2 ± 0.6
Amount of hair loss	Low dose	3.0 ± 1.0	3.0 ± 0.0	3.0 ± 0.7
	High dose	2.7 ± 0.5	3.2 ± 0.4†	3.2 ± 0.6†
Amount of gray hair‡	Low dose	2.8 ± 0.9	3.4 ± 0.9	3.3 ± 1.0
	High dose	2.4 ± 1.1	3.2 ± 0.7†	3.2 ± 1.0†
Fatigue and general malaise	Low dose	2.1 ± 0.9	2.7 ± 0.9	2.9 ± 0.8†
	High dose	2.1 ± 1.1	2.7 ± 0.8	3.0 ± 0.9†
Eyestrain	Low dose	2.6 ± 1.2	2.8 ± 0.8	3.3 ± 1.0
	High dose	2.4 ± 0.8	2.7 ± 0.9	3.3 ± 0.8†
Shoulder stiffness	Low dose	2.1 ± 1.0	2.8 ± 0.6†	3.1 ± 0.9†
	High dose	2.5 ± 1.1	3.1 ± 1.3	3.5 ± 1.1†
Stress	Low dose	2.4 ± 0.7	2.6 ± 0.9	3.1 ± 0.9
	High dose	2.6 ± 0.8	2.5 ± 1.0	3.1 ± 0.9
Coldness of extremities	Low dose	3.0 ± 0.9	3.2 ± 0.7	3.3 ± 0.6
	High dose	3.5 ± 1.0	3.9 ± 0.7†	3.6 ± 0.8
Dry eye condition§	Low dose	2.2 ± 0.4	3.2 ± 0.8	3.3 ± 0.5
	High dose	2.5 ± 1.7	3.3 ± 1.3	3.3 ± 1.3
Physical condition (e.g., cold)	Low dose	3.8 ± 1.1	3.8 ± 1.0	3.8 ± 0.9
	High dose	3.8 ± 0.6	3.9 ± 1.0	3.6 ± 1.0

Values are expressed as mean ± standard deviation.
Low-dose group: n = 12; high-dose group: n = 10.
*Low-dose group: n = 2; high-dose group: n = 0.
†$P < 0.05$ compared with before intake (Wilcoxon signed-rank test).
‡Low-dose group: n = 12; high-dose group: n = 9.
§Low-dose group: n = 5 (at week 6), n = 4 (at week 12); high-dose group: n = 4.

TABLE 5: Summary of the Pittsburgh Sleeping Questionnaire

Item	Group	Before Intake	At Week 6	At Week 12
Total score	Low dose	5.1 ± 2.6	4.0 ± 1.2	3.7 ± 1.4
	High dose	4.7 ± 1.5	4.5 ± 1.4	3.9 ± 1.1
C1: Quality of sleep	Low dose	1.2 ± 0.6	1.1 ± 0.3	0.9 ± 0.5
	High dose	1.4 ± 0.5	1.3 ± 0.5	1.1 ± 0.3
C2: Time required for falling asleep	Low dose	0.3 ± 0.7	0.1 ± 0.3	0.3 ± 0.5
	High dose	0.2 ± 0.4	0.4 ± 0.5	0.1 ± 0.3
C3: Sleep duration	Low dose	1.5 ± 0.7	1.4 ± 0.5	1.5 ± 0.7
	High dose	1.2 ± 0.8	1.3 ± 0.5	1.2 ± 0.6
C4: Sleep efficiency	Low dose	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	High dose	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
C5: Difficulty in sleeping	Low dose	0.8 ± 0.6	0.9 ± 0.5	0.6 ± 0.5
	High dose	0.9 ± 0.3	1.0 ± 0.0	1.0 ± 0.0
C6: Sleeping pill use	Low dose	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	High dose	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
C7: Difficulty in staying awake during the day	Low dose	1.3 ± 1.1	0.5 ± 0.5*	0.4 ± 0.5*
	High dose	1.0 ± 0.5	0.5 ± 0.7*	0.5 ± 0.7*
Q5: Ease in getting out of bed	Low dose	1.3 ± 0.5	1.2 ± 0.4	1.0 ± 0.0*
	High dose	1.4 ± 0.5	1.3 ± 0.5	1.1 ± 0.3

Values are expressed as mean ± standard deviation. Low-dose group: n = 12; high-dose group: n = 10.
*$P < 0.05$ compared with before intake (Wilcoxon signed-rank test).

Scores for shoulder stiffness significantly increased in the L group at both the 6th and the 12th weeks, from 2.1 ± 1.0 before intake to 2.8 ± 0.6 and 3.1 ± 0.9, respectively. In the H group, a significant decrease was observed at the 12th week, from 2.5 ± 1.1 before intake to 3.5 ± 1.1. Scores for coldness of the extremities significantly increased in the H group, from 3.5 ± 1.0 before intake to 3.9 ± 0.7 at the 6th week. Scores for difficulty staying awake during the day (C7) significantly decreased in the L group at both the 6th and the 12th weeks, from 1.3 ± 1.1 before intake to 0.5 ± 0.5 and 0.4 ± 0.5, respectively. In the H group, significant decreases were observed at the 6th and the 12th weeks, from 1.0 ± 0.5 before intake to 0.5 ± 0.7 and 0.5 ± 0.7, respectively. Scores for ease of getting out of bed significantly decreased in the L group, from 1.3 ± 0.5 before intake to 1.0 ± 0.0 at the 12th week. Regarding the scores for symptoms of athlete's foot, although no statistically significant difference was obtained because there were only two subjects with such symptoms in the L group, both of them showed improvements in symptoms.

E. Safety Evaluation

Changes in hematological test, biochemical blood test, and physical measurement results, as well as the number and a list of adverse events observed during the study period, are shown in Tables 6–10, respectively. Regarding adverse events, subjects complained of several subjective symptoms and the principal investigator observed several objective symptoms. The symptoms all were mild, and there were no serious adverse events. The principal investigator evaluated 2 incidents of a feeling of heaviness of the stomach (ID no. 808, H group) as probably related to the test food. Other than these,

TABLE 6: Summary of the Hematology Test

Item	Group	Before Intake	At Week 12
WBC (×10^3 μL)	Low dose	4.55 ± 0.98	5.06 ± 1.30
	High dose	4.67 ± 0.60	5.29 ± 1.64
RBC (×10^4 μL)	Low dose	450.5 ± 44.6	445.8 ± 42.8
	High dose	450.3 ± 35.4	451.3 ± 42.7
Hemoglobin (g/dL)	Low dose	13.16 ± 1.48	13.43 ± 1.60
	High dose	13.42 ± 0.89	13.60 ± 1.46
Hematocrit (%)	Low dose	40.78 ± 4.17	41.56 ± 4.48
	High dose	41.30 ± 2.57	42.16 ± 4.02
PLT (×10^4 μL)	Low dose	23.13 ± 6.07	22.38 ± 5.65
	High dose	24.48 ± 3.10	24.79 ± 4.21
NEUT (%)	Low dose	58.5 ± 3.4	59.4 ± 5.4
	High dose	51.6 ± 9.1	52.2 ± 10.2
NEUT count (×10^3 μL)	Low dose	2.670 ± 0.654	3.030 ± 0.880
	High dose	2.411 ± 0.528	2.807 ± 1.246
EOS (%)	Low dose	1.8 ± 1.0	2.8 ± 1.3
	High dose	3.6 ± 3.1	4.3 ± 3.8
EOS count (×10^3 μL)	Low dose	0.080 ± 0.041	0.140 ± 0.062*
	High dose	0.166 ± 0.141	0.215 ± 0.166*
BAS (%)	Low dose	0.8 ± 0.5	0.6 ± 0.5
	High dose	0.8 ± 0.6	0.8 ± 0.6
BAS count (×10^3 μL)	Low dose	0.033 ± 0.021	0.027 ± 0.025
	High dose	0.036 ± 0.030	0.037 ± 0.030
MON (%)	Low dose	6.6 ± 1.4	6.8 ± 1.9
	High dose	5.7 ± 1.1	4.6 ± 0.8**
MON count (×10^3 μL)	Low dose	0.294 ± 0.061	0.331 ± 0.072
	High dose	0.266 ± 0.061	0.244 ± 0.093
LYM (%)	Low dose	32.3 ± 3.8	30.3 ± 4.7
	High dose	38.3 ± 6.7	38.1 ± 8.5
LYM count (×10^3 μL)	Low dose	1.472 ± 0.348	1.531 ± 0.474
	High dose	1.791 ± 0.378	1.987 ± 0.584

Values are expressed as mean ± standard deviation. Low-dose group: n = 12; high-dose group: n = 10.
*P < 0.05, **P < 0.01 compared with before intake (1-sample t test).
BAS, basophil; EOS, eosinophil; LYM, lymphocyte; MON, monocyte; NEUT, neutrophil; PLT, platelet; RBC, red blood cell; WBC, white blood cell.

however, all events were evaluated as unrelated or probably unrelated. This feeling of heaviness of the stomach in ID no. 808 occurred on the 18th day of intake. After the onset of this symptom, the subject decided on their own to stop taking the test food, and the symptom disappeared 5 days later. After a 3-day follow-up from the disappearance of symptoms, the subject restarted test food intake at

TABLE 7: Summary of the Biochemical Blood Test

Item	Group	Before Intake	At Week 12
AST (IU/L)	Low dose	20.7 ± 7.9	22.8 ± 11.0
	High dose	20.5 ± 4.9	19.4 ± 3.3
ALT (IU/L)	Low dose	15.5 ± 5.4	21.3 ± 17.2
	High dose	18.2 ± 7.2	19.1 ± 8.3
γ-GTP (IU/L)	Low dose	19.4 ± 12.6	28.5 ± 21.8
	High dose	19.8 ± 9.7	23.1 ± 13.7
T-Bil (mg/dL)	Low dose	0.72 ± 0.15	0.71 ± 0.24
	High dose	0.81 ± 0.38	0.71 ± 0.35
TP (mg/dL)	Low dose	6.68 ± 0.31	6.92 ± 0.34*
	High dose	6.89 ± 0.31	7.06 ± 0.32†
UN (mg/dL)	Low dose	10.80 ± 2.30	10.37 ± 2.73
	High dose	10.40 ± 1.68	10.33 ± 1.93
Creatinine (mg/dL)	Low dose	0.746 ± 0.125	0.747 ± 0.105
	High dose	0.668 ± 0.151	0.661 ± 0.160
TC (mg/dL)	Low dose	184.8 ± 20.3	188.7 ± 20.8
	High dose	196.9 ± 29.6	200.7 ± 32.2
HDL-C (mg/dL)	Low dose	65.8 ± 14.6	73.8 ± 14.9*
	High dose	62.9 ± 15.9	67.4 ± 16.5†
Triglyceride (mg/dL)	Low dose	69.8 ± 30.5	74.7 ± 38.3
	High dose	61.6 ± 43.4	78.1 ± 58.1†
Glucose (mg/dL)	Low dose	90.8 ± 10.1	90.8 ± 9.9
	High dose	91.7 ± 3.7	92.3 ± 5.2
IRI (μU/mL)	Low dose	6.20 ± 2.49	5.49 ± 2.26
	High dose	5.08 ± 2.35	5.00 ± 3.25
Sodium (meq/L)	Low dose	142.1 ± 1.4	140.9 ± 1.1†
	High dose	141.6 ± 1.3	141.2 ± 0.6
Potassium (meq/L)	Low dose	4.32 ± 0.39	4.24 ± 0.43
	High dose	4.32 ± 0.36	4.22 ± 0.20
Chlorine (meq/L)	Low dose	105.3 ± 1.8	104.6 ± 1.6
	High dose	105.3 ± 1.1	105.1 ± 1.5
Immunoglobulin G (mg/dL)	Low dose	1178.3 ± 241.8	1217.8 ± 246.2
	High dose	1259.0 ± 215.6	1278.2 ± 195.7
Hemoglobin A1c (%)	Low dose	4.84 ± 0.22	4.73 ± 0.20
	High dose	5.02 ± 0.34	4.97 ± 0.31

Values are expressed as mean ± standard deviation. Low-dose group: n = 12; high-dose group: n = 10.
*$P < 0.01$, †$P < 0.05$ compared with before intake (1-sample *t* test).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; GTP, glutamyltransferase; HDL-C, high-density lipoprotein cholesterol; IRI, immunoreactive insulin; T-Bil, total bilirubin; TC, total cholesterol; TP, total protein; UN, urea nitrogen.

a reduced amount of 2 tablets daily, as instructed by the principal investigator, upon confirming that there were no problems with the subject's physical condition. Because the symptom did not recur after 3 consecutive days, the principal investigator instructed the subject to go back to the specified amount (5 tablets daily). The subject took the test food accordingly, but a similar feeling of heaviness of the stomach recurred, leading to the discontinuation of further intake. The symptom disappeared

TABLE 8: Summary of the Physical Measurements

Item	Group	Pre-intake	At 6th week	At 12th week
Body height (cm)	Low dose	165.18 ± 9.62	—	—
	High dose	167.26 ± 9.15	—	—
Body weight (kg)	Low dose	59.52 ± 10.59	59.22 ± 11.17	59.70 ± 10.81
	High dose	62.68 ± 12.13	62.93 ± 12.30	63.06 ± 11.71
Body mass index (kg/m^2)	Low dose	21.68 ± 2.60	21.56 ± 2.81	21.74 ± 2.66
	High dose	22.23 ± 2.56	22.32 ± 2.72	22.39 ± 2.63
Systolic blood pressure (mmHg)	Low dose	107.1 ± 14.0	108.2 ± 12.9	109.8 ± 13.3
	High dose	119.1 ± 11.5	115.4 ± 12.6*	120.0 ± 12.4
Diastolic blood pressure (mmHg)	Low dose	66.4 ± 11.1	66.5 ± 8.8	68.8 ± 9.9
	High dose	73.7 ± 11.3	72.5 ± 9.5	74.9 ± 7.8
Pulse rate (beats/min)	Low dose	62.8 ± 4.2	65.6 ± 6.2	64.3 ± 4.9
	High dose	66.9 ± 5.1	67.1 ± 5.4	68.9 ± 7.5

Values are expressed as mean ± standard deviation. Low-dose group: n = 12; high-dose group: n = 10.
*P < 0.05 compared with before intake (1-sample t test).

5 days after intake discontinuation. In an interview, the subject reported that a similar symptom had occurred on many occasions in the past after the consumption of food containing a large amount of dietary fiber. The test food contains 56.7 mg of dietary fiber per tablet. Regarding this symptom, it cannot be ruled out that causes other than the test food were responsible. However, these events were evaluated by the principal investigator as being probably related to the intake of the test food because the possibility could not be ruled out that the symptoms occurred owing to the intake of the test food, which was rich in dietary fiber, in addition to the subject's constitution.

Compared with before the test food intake, blood tests at the 12th week revealed significant changes in eosinophil count and total protein, high-density lipoprotein cholesterol, and sodium levels in the L group, and in the percentages of eosinophils and monocytes, as well as the total protein, high-density lipoprotein cholesterol, and triglyceride levels in the H group; however, these were all minor changes, and amounts fell within the reference ranges. Accordingly, the principal investigator evaluated that there were no clinical problems associated with the test food.

Although the physical measurements revealed a significant decrease in systolic blood pressure in the H group at the 6th week compared with before intake of the test food, this was minor variation. Therefore, the principal investigator determined that there were no clinical problems associated with the test food.

IV. DISCUSSION AND CONCLUSIONS

We conducted an open-label study of 24 adult men and women who consumed food containing *A. brasiliensis* KA21 continuously for 12 weeks. The subjective evaluation questionnaire revealed significant improvements in the scores for the amount of hair loss, amount of gray hair, fatigue and general malaise, eye strain, shoulder stiffness, cold extremities, difficulty staying awake during the day, and ease of getting out of bed compared with the scores before intake (Tables 4 and 5). These results suggest the possibility that intake of food containing *A. brasiliensis* KA21 may improve subjectively evaluated scores of hair loss, gray hair, fatigue and general malaise, eye strain, shoulder stiffness, coldness of extremities, difficulty staying awake during the day, and ease of getting out of bed. With

TABLE 9: Number of Adverse Events

Item	Group	
	Low Dose	High Dose
Subjects analyzed (n)	13	11
Subjects with adverse events (n)	4	4
Frequency of incidence (%)	31	36
Total incidents (no. of cases)	5	11
Incidents by symptom (no. of cases) □	□	□
Itching in the back of the knees	1	0
Feeling of heaviness of stomach	0	2
Diarrhea	0	2
Stomachache	0	1
General malaise	0	1
Abdominal pain	0	2
Runny nose	1	0
Abdominal pain, abdominal discomfort	1	0
Diarrhea, abdominal pain	0	1
Loose stool	0	1
Cold symptoms (sore throat, fever, cough)	0	1
Eosinophilia	1	0
Increased AST, ALT, and γ-GTP levels	1	0

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GTP, glutamyltransferase.

regard to athlete's foot symptoms, no statistically significant differences were observed because there were only 2 subjects with this condition, although both subjects showed improvements. The improvements in hair, coldness of extremities, and subjective symptoms such as those of athlete's foot were suggested by clinical studies and questionnaire surveys previously conducted by our group (unpublished results), demonstrating the efficacy of *A. brasiliensis* KA21. This study provided results that confirm the reproducibility of previous findings.

The design of this study was limited in several aspects: The study group was small, and the conditions under which the study was performed were limited with respect to similarities in the age (middle age) of the study participants, limited study time during the year, and other uncontrollable circumstances. Therefore, the above-mentioned results do not provide a general consensus for the benefits of using this mushroom. However, this (middle-aged) population may be the major consumer of functional foods for the prevention of lifestyle-related diseases or for ensuring a longer, higher-quality life span. It could be speculated that these types of food may not be adopted by the younger and/or elderly population because of the low interest in health shown by a majority of youth and the use of (need for) pharmaceutical products by a majority of the elderly. Therefore, the results of this study could be used in the development of functional foods targeting the middle-aged population.

It has been revealed that the properties and components of *A. brasiliensis* largely vary depending on its origin, cultivation method, and processing.[5,8] The test food used in this study mainly contains powder, which was obtained from *A.*

TABLE 10: List of Adverse Events

Group	Subject ID No.	Sex	Age	Symptoms	Date of Onset	Outcome	Relationship with Test Food	Physician's Comments, Treatment Details
L	803	Male	45	Increased AST, ALT, and γ-GTP levels	10/8	Unchanged	Not likely related	It was observed during the examination at week 12 (October 8); therefore reexamination was performed 4 weeks later on November 5. The results showed almost no improvement, but no change was observed 4 weeks after completion of intake. The event thus was evaluated as unlikely to be related to the test food.
L	804	Male	52	Itching in the back of the knees (both legs)	7/21	Recovery/ disappearance	Unrelated	The condition was observed for only a short time on the 6th day of intake, and since it did not reappear afterward, the event was evaluated to be unrelated to the test food.
L	836	Female	37	Runny nose	7/26–7/28	Recovery/ disappearance	Unrelated	The subject reported changes in weather condition as the cause. The event was deemed to be unrelated to the test food.
L	840	Female	40	Abdominal pain, abdominal discomfort (feeling of heaviness of stomach)	8/20–9/5	Recovery/ disappearance	Not likely related	Since the condition appeared from the 36th day of intake, it was deemed unlikely to be related to the test food. The subject requested withdraw from the study on August 29, and hence was treated as a dropout. It was confirmed on September 5 that the symptoms had disappeared. Abdominal pain: August 20, 23 (menstrual pain); abdominal discomfort: August 21, 22, 24–31, September 1–5.Oral intake of 1 tablet of Buscopan A on August 20, 28; oral intake of 4 tablets of Eve A on August 23.
L	840	Female	40	Eosinophilia	8/29–9/17	Recovery/ disappearance	Not likely related	During the examination at week 6 (discontinuation), an increase in eosinophil count (19%) was observed. A decrease of this count was noted during reexamination 20 days later (4%; 2% during the examination before intake). This event was evaluated as unlikely to be related to the test food.

TABLE 10: List of Adverse Events *(continued)*

Group	Subject ID No.	Sex	Age	Symptoms	Date of Onset	Outcome	Relationship with Test Food	Physician's Comments, Treatment Details
H	808	Female	44	Feeling of heaviness of stomach	8/2–8/10	Recovery/ disappearance	Probably related	The symptom appeared from the 18th day of intake. The subject stopped taking the test food from August 2. Intake was restarted on August 4, but stopped again from August 5 because of diarrhea. The symptom disappeared on August 10. This event was evaluated to be probably related to the test food.
H	808	Female	44	Diarrhea	8/4	Recovery/ disappearance	Unlikely related	Intake had been stopped since August 2 because of the feeling of heaviness of the stomach. One hour after resuming intake on August 4, the subject experienced diarrhea, although it lasted for only a short time. No similar symptoms were observed after intake was resumed. This event was evaluated as unlikely to be related to the test food.
H	808	Female	44	Feeling of heaviness of stomach	8/20–8/26	Recovery/ disappearance	Probably related	Intake was resumed on August 12. The subject took 2 tablets on August 12–14, and 5 tablets on August 15 and thereafter, since there was no change in the physical condition. Feeling of heaviness of the stomach reappeared 9 days after resumption (August 20). Intake was interrupted on August 22, and the symptom disappeared on August 26. This event was evaluated to be probably related to the test food. The subject requested withdraw from the study on August 27 and hence was treated as a dropout. Oral intake of Tateyama Digestive Medicine A →1 package on August 22, 23; 3 packages on August 23.
H	826	Female	56	Stomachache	7/23–7/24	Recovery/ disappearance	Unrelated	The subject reported eating cold food in excess as the cause. The event was deemed to be unrelated to the test food.
H	826	Female	56	Malaise	8/15	Recovery/ disappearance	Unrelated	The subject reported tiredness as the cause. The event was deemed to be unrelated to the test food.
H	826	Female	56	Abdominal pain	8/22	Recovery/ disappearance	Unrelated	The subject reported eating cold food as the cause. The event was deemed to be unrelated to the test food.

TABLE 10: List of Adverse Events *(continued)*

Group	Subject ID No.	Sex	Age	Symptoms	Date of Onset	Outcome	Relationship with Test Food	Physician's Comments, Treatment Details
H	826	Female	56	Abdominal pain	9/25	Recovery/ disappearance	Unrelated	The subject reported changes in weather condition as the cause. The event was deemed to be unrelated to the test food.
H	841	Male	55	Diarrhea, abdominal pain	7/22	Recovery/ disappearance	Unrelated	The condition was observed only on the 7th day of intake, and was thus deemed to be unrelated to the test food.
H	849	Female	39	Loose stool	7/23	Recovery/ disappearance	Unrelated	The subject reported suffering from cold as the cause. The event was deemed to be unrelated to the test food.
H	849	Female	39	Diarrhea	8/8–8/12	Recovery/ disappearance	Unrelated	The subject reported catching gastroenteritis from her child. The condition was observed only from the 25th to the 29th day of intake, and not afterward. Thus the event was evaluated as being unrelated to the test food. August 9: oral intake of 1 package of BIO-THREE.
H	849	Female	39	Cold symptoms (sore throat, fever, cough)	9/30–10/7	Recovery/ disappearance	Unrelated	The symptoms were due to a cold and thus the event was deemed to be unrelated to the test food. Sore throat, September 30 to October 5; fever, October 1–2; cough, October 6. Oral drugs: Kakkonto, September 30 (1 package); new S-TAC granules, October 1 (2 packages); Colgen Kowa B, October 2 (6 tablets); October 3 (9 tablets), October 4 (6 tablets), October 5 (3 tablets); Rakunaru GX, October 2 (2 bottles), October 3 (1 bottle).

brasiliensis KA21 cultivated outdoors in Brazil by warm (in the low-temperature region) air-drying and has been shown to be rich in β-glucan, antioxidant enzymes—which have recently attracted much attention with regard to their involvement in biological functions—and vitamin D compared with other products derived from greenhouse-cultivated *A. brasiliensis*. Therefore, the efficacy demonstrated by the results of this study could be due to the characteristic ingredients that are abundant in *A. brasiliensis* KA21, in addition to the major active ingredient of *A. brasiliensis*, namely, β-glucan.

Initially, medicinal mushrooms were primarily used as immunotherapy for cancer and have commonly been applied as a complementary and alternative therapy.[13–17] Animal experiments studying cancer immunotherapy with various functional materials have been conducted, and many effective foods have been found.[13–17] The number of clinical studies is limited, however, and the clinical efficacy of many foods remains to be verified. In the 21st century, a large number of molecule-targeting drugs were identified and applied to cancer therapy, contributing to marked advances in treatment. The presence of the immunological surveillance mechanism proposed by Burnet and Thomas has been established.[18–20] However, a large clinical study to demonstrate a food-related improvement in the immune function of patients with cancer is not extensively studied yet because of the significantly high cost; it remains to be clarified. In the future, data from patients showing effects should be accumulated for a specific period.

On the other hand, the arrangement of social environments, marked advances in medical techniques, development of potent new drugs, advances in treatment protocols, and establishment of vaccination have markedly prolonged the life span of humans.[21–28] In such an era, the importance of complementary and alternative therapy has also been emphasized in preventive medicine.[29–31] The idea of disease prevention was proposed a long time ago in traditional Chinese herbal medicine. Recent studies have indicated that it is important to establish appropriate lifestyles for disease prevention, and that complementary and alternative therapy should be used to achieve this.[32–36] So, complementary and alternative medicine has commonly been applied to prevent lifestyle-related diseases.

Clinical trials involving patients have been conducted. However, it is necessary to register healthy adults in clinical studies of disease prevention. This is an important clinical study, involving healthy adults and designed based on such a viewpoint. The significance of this article may further increase with marked advances in research in this field.

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