JOURNAL OF MEDICINAL FOOD
J Med Food 17 (3) 2014, 295–301
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DOI: 10.1089/jmf.2013.2934

Agaricus brasiliensis KA21 Improves Circulatory Functions in Spontaneously Hypertensive Rats

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ABSTRACT The present study aimed to clarify the effects of *Agaricus brasiliensis* KA21 (*i.e.*, *Agaricus blazei*) mushroom on circulatory function. Spontaneously hypertensive rats (SHRs) were fed 10% *A. blazei*-containing pellets (agaricus group) or normal pellets (control group) for 5 weeks from 6 to 11 weeks of age. For Experiment 1, tail blood pressure and heart rate were measured in the conscious SHRs. For Experiment 2, echocardiographic and blood biochemical measurements were performed in the anesthetized SHRs. In Experiment 1, blood pressure and heart rate were significantly lower in the agaricus group compared with the control group throughout the observation period. In Experiment 2, the agaricus group also showed a significant decrease in cardiac output accompanied by a decrease in heart rate and an increase in early and late ventricular filling velocity (E/A ratio). Moreover, levels of escape enzymes such as creatine kinase (CK), CK-BB, CK-MB, asparate aminotransferase, lactate dehydrogenase, and aldolase were significantly lower than in the control group. We concluded that the ingestion of feed containing *A. brasiliensis* KA21 can improve hypertensive cardiovascular hemodynamics by decreasing the working load of the heart, presumably by lowering the sympathetic nervous tone in SHRs.

KEY WORDS: • ***blood pressure*** • ***cardiovascular*** • ***heart rate*** • ***hypertension*** • ***supplements***

INTRODUCTION

A RELATIVELY LARGE number of reports have been published on the health effects of Agaricus mushrooms, represented by *Agaricus blazei*. Those reports have focused on immune system effects such as increases in immune activity or immunological modification,[1–7] anticancer activity,[4,8–12] toxicological effects, including the presence or absence of mutagenic or carcinogenic effects,[13–17] increases in antioxidant effects,[18,19] decreases in blood glucose levels,[20] and anti-inflammatory effects.[21] However, the effects of *A. blazei* on the cardiovascular system have not been well studied, even though cardiovascular function is thought to be the most important representative physiological and pathophysiological parameter. It is of particular interest to evaluate the effects of *A. blazei* as a natural food and supplement for high-risk patients with cardiovascular disorders such as hypertension.

The present study aims to clarify the cardiovascular effects of diets containing *A. blazei* in spontaneously hypertensive rats (SHRs) in which SHRs were fed pellets containing 10% *A. blazei* or normal pellets for 5 weeks.

Manuscript received 7 May 2013. Revision accepted 12 October 2013.

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MATERIALS AND METHODS

Animals

SHRs (SHR/NCrl) that were purchased from Charles River Laboratories Japan (Yokohama, Japan). Twelve SHRs were used for observation of blood pressure and heart rate by oscillometric measurement using a noninvasive tail-cuff method (Experiment 1), and 10 SHRs were used for the echocardiographic observation of the heart and peripheral vessels (Experiment 2). In both Experiments 1 and 2, these rats were randomly divided into two equal groups: the agaricus group and the control group.

Agaricus blazei

Powders of *A. brasiliensis* KA21 (Toei Shinyaku Co., Ltd., Tokyo, Japan), a mushroom fruit body cultivated outdoors in Brazil, were used in this study. *A. brasiliensis* KA21 was dried by air ventilation at a temperature lower than 60°C; this temperature condition has been confirmed to maintain the enzyme activities. The composition of *A. brasiliensis* KA21 has been described in a previous

report,[5] which found high protein (38.5%), fat (2.6%), carbohydrate (27.7%), and fiber content (20.6%), including β-glucan (12.4%) as well as high levels of vitamin B1, B2, B6, and D, niacin, panthothenic acid, folic acid, biotin, and many other minerals. The energy per 100 g (dry weight) of KA21 is 288 kcal.

Feeding protocol and measurements of cardiovascular functions

Rats in the control groups were fed a normal pellet type of diet (CE-2; CLEA Japan, Inc., Tokyo, Japan), and rats in the agaricus group were given the same type of diet mixed with 10% *A. brasiliensis* KA21 in the CE-2 base. The nutritional composition of CE-2 and energy are as follows: crude protein (24.9%), crude fat (4.6%), crude fiber (4.1%), crude ash (6.6%) with a standard level of various minerals and vitamins, and 344.9 kcal per 100 g of feed.

All the rats were given free access to their respective diets beginning at 6 weeks of age and continued on the diets for 5 weeks.

In Experiment 1, blood pressure and heart rate measurements were recorded in conscious rats. Each rat was placed in a cylindrical container with an interior warmed to 37°C, and the tail blood pressure was measured by an oscillometric method using tail-cuff and noninvasive blood pressure-measuring instruments (BP-98A; Softron, Tokyo, Japan). The measurement was performed three or five times in each rat, and the median value was chosen for the data analysis.

In Experiment 2, all the rats were anesthetized with 3% isoflurane and subjected to echocardiographic examinations of cardiac function such as cardiac output, stroke volume, early (E) and late (A) ventricular filling velocity (E/A ratio), heart rate, and hemodynamics such as blood flow velocity, caliber of vessels, pulsatility index (PI), and resistive index (RI) in the femoral and common carotid arteries using the echo color Doppler technique (Vevo2100; VisualSonics, Toronto, Canada).

All of the measurements in Experiments 1 and 2 were performed on the day before beginning the experimental diets (prefeeding test) and every week thereafter. In the final stage of Experiment 2, one day after the last echocardiographic observation, blood samples from the carotid artery were collected for biochemical examinations[22] and evaluation of oxidative stress parameters.[9]

All experiments were conducted in accordance with the Animal Experimentation Guidelines of the University of Tokyo and approved by the institutional Animal Care and Use Committee of the Graduate School of Agricultural and Life Sciences at the University of Tokyo.

Data analysis

All data are expressed by mean ± standard deviation (SD). Statistically significant differences between the control and experimental groups were determined by two-way repeated measures analysis of variance (ANOVA), and the Tukey's test was applied for comparisons between both the groups at each observation time if no significant interaction was present between the feed and time. Blood biochemical determinations, body weight, and feed intake were statistically compared by a Mann–Whitney U-test. A difference was considered to be significant if the P value was $< .05$.

RESULTS

Experiment 1

Systolic blood pressure. The mean ± SD systolic blood pressure in the prefeeding test (1 day before ingestion of normal or experimental feed) was 154.3 ± 5.8 mmHg in the control and 156.6 ± 7.0 mmHg in the agaricus group (Fig. 1A). There was no significant difference between the groups. The systolic blood pressure tended to elevate progressively in both groups. During the 5 weeks of observation, there was a significant difference between the control and agaricus groups ($P < .001$, two-way repeated measures ANOVA) without any interaction between the feeding and time, where the agaricus group showed a lower systolic blood pressure than the control group. The mean systolic pressure was 209.3 ± 9.9 mmHg and 196.7 ± 9.2 mmHg in the control and agaricus groups, respectively, at the 4th week of observation (Fig. 1A).

Diastolic blood pressure. The mean ± SD diastolic blood pressure in the prefeeding test was 110.3 ± 16.8 mmHg and 113.6 ± 12.8 mmHg in the control and agaricus groups, respectively (Fig. 1B). There was no significant difference between the groups. The diastolic blood pressure also tended to elevate progressively during the observation period in both groups. During the 5 weeks of observation, there was a significant difference between the control and agaricus groups ($P < .005$, two-way repeated measures ANOVA), where the agaricus group showed a lower diastolic blood pressure than the control group without any interaction between the time and feeding. The mean diastolic pressure was 171.6 ± 7.5 mmHg and 150.3 ± 2.4 mmHg in the control and agaricus groups, respectively, at the 4th week of observation when the difference was the largest (Fig. 1B).

Mean blood pressure. The mean blood pressure in the prefeeding test was 124.8 ± 10.4 mmHg and 127.9 ± 10.2 mmHg in the control and agaricus groups, respectively (Fig. 1C). There was no significant difference between the groups. The mean blood pressure tended to increase progressively during the observation period in both the groups. During the 5 weeks of observation, there was a significant difference between the groups ($P < .001$, two-way repeated measures ANOVA), where the agaricus group showed a lower mean blood pressure than the control group without any interaction between the time and feeding. The mean blood pressure was 184.2 ± 7.3 mmHg and 165.8 ± 4.4 mmHg in the control and agaricus groups, respectively, in the 4th week of observation (Fig. 1C).

Heart rate. The mean heart rates (± SD) in the prefeeding test was 423.8 ± 26.3 bpm and 402.1 ± 11.1 bpm in the control and agaricus groups, respectively, with no

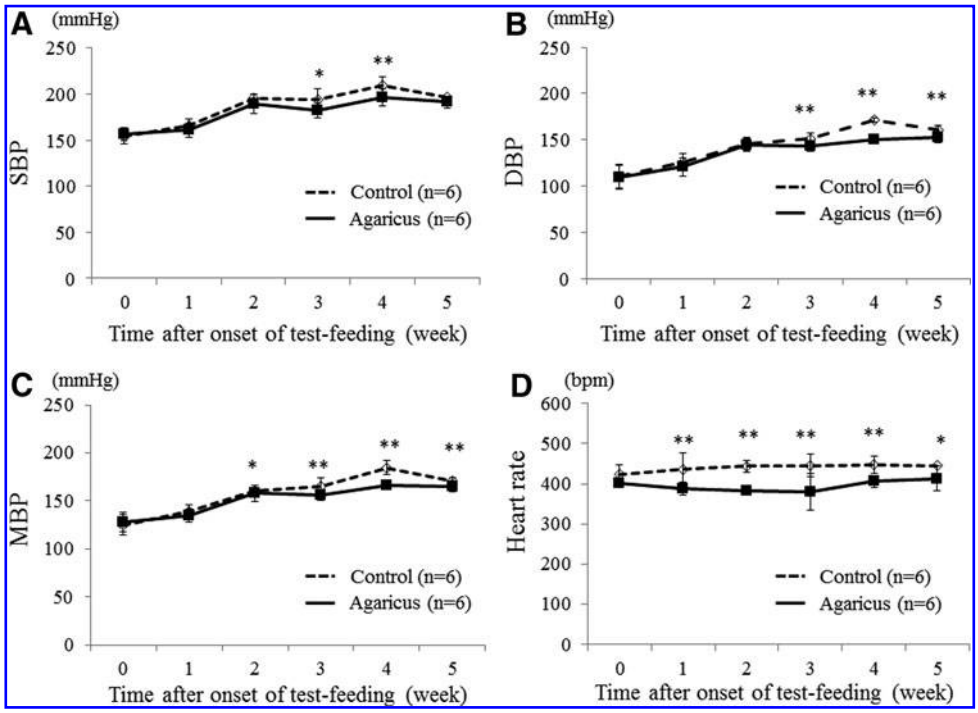

FIG. 1. Changes in systolic blood pressure **(A)**, diastolic blood pressure **(B)**, mean blood pressure **(C)**, and heart rate **(D)** in the control and agaricus groups. The asterisks show significant differences (*$P < .05$ and **$P < .01$) between the control and agaricus groups at the corresponding time. The data in each plot are expressed as the mean ± SD. SBP, systolic blood presure; DBP, diastolic blood pressure; MBP, mean blood pressure; bpm, beats per minute.

significant difference found between the groups (Fig. 1D). The heart rate in the control group increased gradually until the 3rd week of the feeding test (9 weeks old) and thereafter, it remained at a plateau. In the agaricus group, it gradually decreased until the 3rd week of the feeding test after which it marginally increased. The heart rate in the control and agaricus groups showed a significant difference ($P < .0001$, two-way repeated measures ANOVA) without any interaction between the feed and feeding time. Throughout the observation period, a lower heart rate was observed in the agaricus group compared with that in the control group (Fig. 1D).

Body weight. The mean body weight (±SD) in the prefeeding test was 177.5 ± 5.6 g and 174.1 ± 5.1 g for the control and agaricus groups, respectively, with no significant difference between the groups (Fig. 2). However, the body weight during the feeding test was significantly different between the groups ($P < .0001$, two-way repeated measures ANOVA), without any interaction between the feed and time, where the body weight in the agaricus group was lower than that in the control group. At the 5th week of feeding, the body weight was 305.9 ± 10.5 g and 287.3 ± 8.3 g in the control and agaricus groups, respectively, showing a 6.1% lower body weight in the agaricus group compared with that in the control group (Fig. 2).

Experiment 2

Cardiac output. The mean ± SD cardiac output in the prefeeding test was 37.5 ± 3.2 mL in the control group and 43.1 ± 7.0 mL in the agaricus group, with no significant difference between the groups (Fig. 3A). A significant decrease ($P < .05$; two-way repeated measures ANOVA) was observed in the agaricus group compared with the control group, and a significant interaction ($P = .02$) was observed between the feed and time. At the 5th week of the feeding, the cardiac output was found to be 56.1 ± 5.9 mL in the control group and 45.3 ± 9.5 mL in the agaricus group (Fig. 3A).

Stroke volume. At the 5th week of feeding, the stroke volume was 157.4 ± 10.8 μL in the control group and 134.8 ± 27.7 mL in the agaricus group (Fig. 3B). A lower stroke volume, but no significant difference ($P = .17$) was found between the groups during the observation period (Fig. 3B).

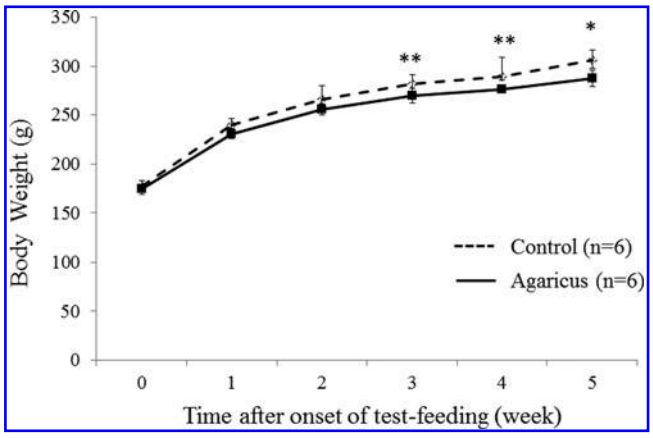

FIG. 2. Changes in body weight in the control and agaricus groups. The asterisks show significant differences (*$P < .05$ and **$P < .01$) between the control and agaricus groups at the corresponding time. The data in each plot are expressed as the mean ± SD.

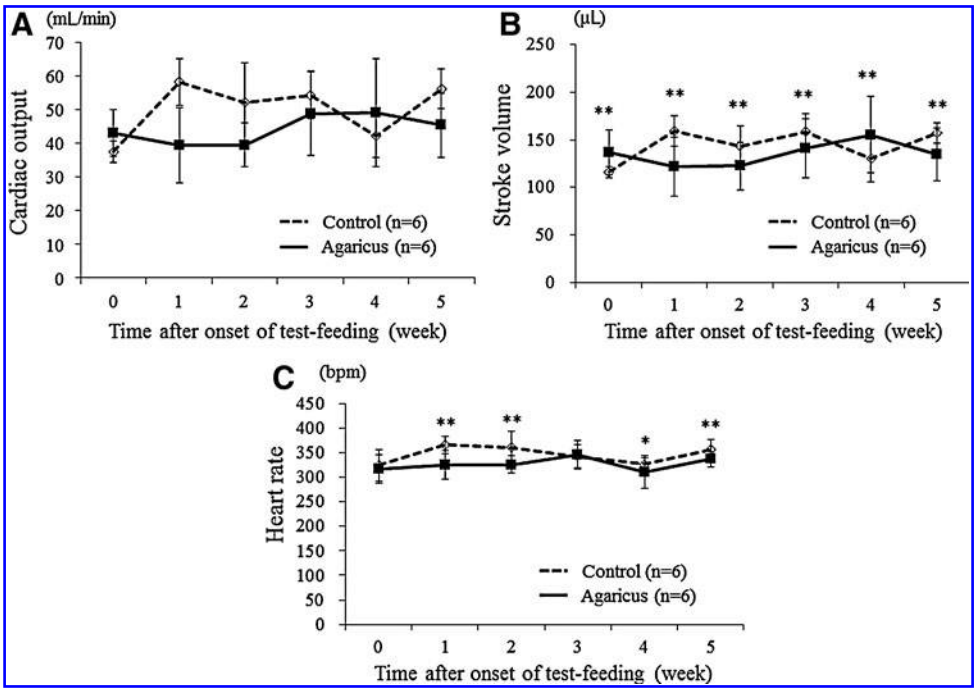

FIG. 3. Changes in cardiac output **(A)**, stroke volume **(B)**, and heart rate **(C)** in the control and agaricus groups. The asterisks show significant differences (*$P < .05$ and **$P < .01$) between the control and agaricus groups at the corresponding time. The data in each plot are expressed as the mean ± SD.

Heart rate. The electrocardiogram (ECG) was simultaneously monitored in isoflurane anesthetized rats during the echocardiographic examination, and the heart rate was automatically calculated from the ECG. In the prefeeding test, the control and agaricus group showed heart rates of 323.9 ± 32.8 bpm and 316.5 ± 29.0 bpm, respectively, with no significant difference (Fig. 3C). However, a significant difference was observed between the groups over the observation period, with a lower heart rate in the agaricus group than in the control group ($P < .005$, two-way repeated measures ANOVA) without any interaction between the feed and time (Fig. 3C).

PI of the left femoral artery and common carotid artery. PI calculated from the following formula (peak systolic velocity–end diastolic velocity)/mean velocity was measured at the left femoral artery and left common carotid artery (Fig. 4A, B). For the left femoral artery, there was a significant increase ($P < .005$, two-way repeated measures ANOVA) of PI in the agaricus group compared with the

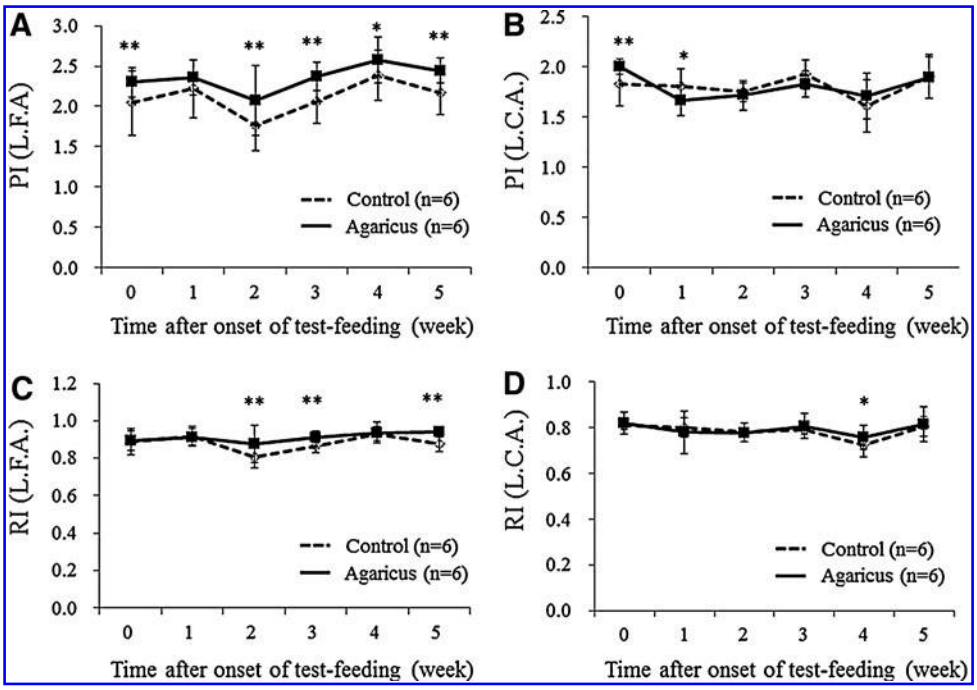

FIG. 4. Changes in pulsatility index **(A, B)** and resistive index **(C, D)** in the control and agaricus groups. The asterisks show significant differences (*$P < .05$ and **$P < .01$) between the control and agaricus groups at the corresponding time. L.F.A., left femoral artery; L.C.A., left carotid artery; PI, pulsatility index; RI, resistive index. The data in each plot are expressed as the mean ± SD.

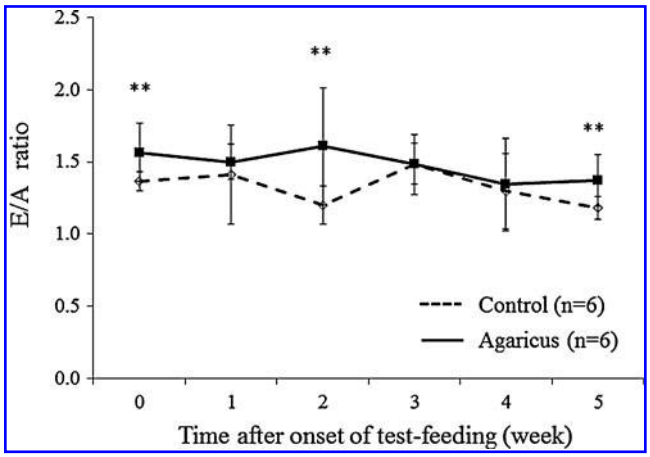

FIG. 5. Changes in the E/A ratio in the control and agaricus groups. The asterisks show significant differences (**$P < .01$) between the control and agaricus groups at the corresponding time. The data in each plot are expressed as the mean ± SD.

control group; however, the PI was already higher in the agaricus group than in the control group at the onset on the feeding test. No significant difference was found between the agaricus and control groups in PI at the left common carotid artery.

RI of the left femoral artery and common carotid artery. RI, calculated from the following formula (peak systolic velocity–end diastolic velocity)/peak systolic velocity was measured at the left femoral artery and left common carotid artery (Fig. 4C, D). The RI in the left femoral artery showed a significant increase ($P = .03$, two-way repeated measures ANOVA) in the agaricus group, whereas no significant difference was found in the left common carotid artery.

E/A ratio. The ratio of E wave versus A wave, in which the ventricular filling velocity was measured across the mitral valve, was calculated (Fig. 5). There was a significant increase in the E/A ratio, without any interaction between the feed and time, in the agaricus group ($P < .05$, two-way repeated measures ANOVA) when compared with the control group.

Biochemical profiles of the blood. Creatine kinase (CK) and its isozymes showed a tendency to be lower in the agaricus group than in the control group (Table 1), with significant differences in CK ($P < .05$), CK-BB ($P < .05$), and CK-MB ($P < .05$), and no significant difference, but a lower value, in CK-MM ($P = .22$).

Aspartate aminotransferase, lactate dehydrogenase, aldolase, and triglyceride levels were significantly lower in the control than in the agaricus group (Table 1). There were no significant differences between the groups for the values of alanine aminotransferase, alkaline phosphatase, choline esterase, leucine aminopeptidase, and total cholesterol (Table 1).

Values of diacron-reactive oxygen metabolites (d-ROMs), biological antioxidant potential (BAP), and BAP/d-ROMs ratio were not significantly different between the control and agaricus groups (Table 2).

Body weight and feed intake. Mean body weights (± SD) at the last stage of experiments (11 weeks of age) were 287.8 ± 12.3 g in the control group and significantly lower at 266.4 ± 7.4 g in the agaricus group (Table 2). The total amount of feed intake per rat was 612.0 ± 32.5 g and 614.0 ± 33.3 g and the mean daily intake per rat was 18.5 ± 1.0 g and 18.6 ± 1.0 g in the control and agaricus groups, respectively. There was no difference in those values between the two groups.

DISCUSSION

Past studies have not elucidated the cardiovascular effects of *A. blazei*, including its influence on hypertensive dysfunction in patients and experimental animals.

Through experiments with SHRs, the present study found that *A. brasiliensis* KA21 can improve certain cardiovascular functions; the blood pressure level, heart rate, and cardiac output in the agaricus group were significantly lower than in the control group during the test period. A gradual and progressive increase in blood pressure was found during the observation period from 6 to 11 weeks of age in both the groups. This age-related increase in blood pressure and heart rate levels are consistent with the data reported by Charles River Laboratories International, Inc.,[22] although

Table 1. A Comparison of Blood Biochemical Findings Between the Control and Agaricus Groups

	CK (IU/L)	*CK-BB (IU/L)*	*CK-MB (IU/L)*	*CK-MM (IU/L)*	*AST (IU/L)*	*ALT (IU/L)*	*LDH (IU/L)*	*ALP (IU/L)*	*ALD (IU/L)*	*AChE (IU/L)*	*LAP (IU/L)*	*TG (mg/dL)*	*Tot Chol (mg/dL)*
Control													
Mean	3313.0[a]	899.8[b]	221.3[c]	2191.9	170.6[d]	46.8	2257.4[e]	961.6	98.7[f]	4.8	74.0	65.8[g]	51.8
SD	1156.0	240.2	72.1	1216.9	20.6	2.4	559.3	129.0	21.5	3.3	3.0	14.6	3.3
Agaricus													
Mean	1861.0[a]	485.7[b]	133.3[c]	1242.1	123.8[d]	46.8	1213.3[e]	845.75	65.8[f]	2.2	75.3	40.5[g]	51.5
SD	373.0	127.4	43.5	246.8	21.6	20.3	389.5	98.2	11.6	1.3	3.4	5.9	6.2

[abcdefg] Indicate a significant difference ($P < .05$) between items with the same letter.

CK, creatine kinase; AST, aspartate amino transferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; ALD, aldolase; AChE, choline esterase; LAP, leucine aminopeptidase; TG, triglyceride; Tot Chol, total cholesterol; SD, standard deviation.

Table 2. Comparisons of Blood Oxidative and Antioxidative Parameters, Body Weight, and Food Intake Between the Control and Agaricus Groups

	d-ROMs (U.CARR)	*BAP (μmol/L)*	*BAP/d-ROMs*	*BW (g)*	*Feeding (g/day)*
Control					
Mean	352.4	2590.6	7.35	287.8*	18.5
SD	15.7	180.2	0.66	12.3	1.0
Agaricus					
Mean	382.4	2697.6	7.05	266.4*	18.6
SD	24.9	242.4	0.58	7.4	1.0

*Asterisks indicate a significant difference between groups.

d-ROMs, diacron reactive oxygen metabolites; BAP, biological antioxidant potential; BW, body weight; Feeding, food intake.

the indirect measurement of blood pressure tends to show a higher value than that by the direct measurement.

The blood pressures in both the control and agaricus groups were already high, with the systolic blood pressure measuring more than 150 mmHg at the prefeeding test (6 weeks of age). The blood pressure in both the groups increased further during the 5-week observation period; however, the extent of increase was significantly lower in the agaricus group than in the control group over the course of the observation period. Moreover, the heart rate level was significantly lower in the agaricus group than in the control group during the same period. Because the blood pressure and heart rate values were similar for the two groups in the prefeeding test, the differences after the onset of feeding tests are thought to be attributable to the intake of *A. brasiliensis* KA21.

The causal factors producing such alterations in blood pressure are considered complex. However, the most important factor influencing the blood pressure is thought to be the cardiac output because this parameter directly reflects the blood pressure as well as the peripheral vascular resistance. In the present study, the cardiac output in the agaricus group was significantly lower compared with the control group. This decrease might be reflected by a significant decrease in the heart rate, although a statistically insignificant tendency for decrease in the stroke volume was also observed in the agaricus group. The decrease in heart rate in the agaricus group was commonly observed even if different experimental conditions were applied, that is, under the conscious condition in Experiment 1 (Fig. 1D) and the anesthetic condition in Experiment 2 (Fig. 3C). The mechanism leading to the decrease in the heart rate in the agaricus group was not elucidated in the present study, although a possibility of continuous and augmented activities from the baroreceptor in response to increased vascular resistance or a direct influence of *A. blazei* on the autonomic nervous system are assumed to be responsible. However, no substantial differences in the PI and RI that indicate peripheral vascular resistance may exclude the influence of the baroreceptor activity on the heart rate decrease in the agaricus group. The previous study reported by Fujisawa *et al.*[23] that the renin-angiotensin system (RAS) in the central nervous system (CNS) has an important role in regulation of cardiovascular hemodynamics in response to the activation of the afferent renal nerve. Accordingly, precise studies are necessary to clarify the participation of such renin-angiotensin and autonomic nervous mechanisms on the decreased blood pressure and heart rate that were found in the agaricus group in the present study. Moreover, the analysis of heart rate variability is assumed to be useful to clarify the changes in the autonomic nervous activity in the agaricus group.

Histological abnormalities such as the cardiovascular hypertrophy due to the RAS and other pathophysiological mechanisms are induced in SHRs.[24–27] Such histological or morphological changes in the cardiovascular system in SHRs are thought to be induction factors for the increase in escape enzymes. Interestingly, in the present study, a significant decrease in the activities of escape enzymes such as CK, CK-BB, CK-MB, aspartate aminotransferase, lactate dehydrogenase, and aldolase was found in the agaricus group when compared with the control group. These changes might suggest that the extent of tissue damage in the body, including the cardiovascular system, and/or the working load on the cardiovascular system was reduced in the agaricus group compared with the control group. This suggestion might be supported by the finding that cardiac output and heart rate in the agaricus group were lower than those exhibited in the control group. The significant increase in the E/A ratio measured at the mitral valve level of the agaricus group suggests that the cardiac pumping function in the agaricus group was maintained in good condition.

A significantly lower body weight, which was estimated at 6.1% (Experiment 1) to 7.4% (Experiment 2) lower compared with the control group, was observed in the agaricus group even though the amount of feed intake was not different between the groups. It is unclear how such a decrease in body weight affects the cardiovascular function, although it is a possible that such a change in the body weight may partially improve cardiovascular function in hypertensive individuals. Moreover, the significant decrease in triglyceride in the agaricus group might be involved in the observed improvement of cardiovascular functions. Similar effects of *A. blazei* on the body weight in human subjects was described in the previous report,[5] with a significant decrease in body weight accompanied by decreases in body and visceral fats and with a tendency for decrease in the blood glucose level.

We predicted that some oxidative stress in SHRs may be reduced by the ingestion of *A. blazei*. However, no significant differences in oxidative stress markers (d-ROMs) and antioxidative markers (BAP) in the blood were observed between the control and agaricus groups. Therefore, the findings of cardiovascular functional changes in the agaricus group are not likely a result of an alteration of oxidative stress involving the action of free radicals.

In conclusion, the present study demonstrates that the ingestion of food containing *A. brasiliensis* KA21 can modulate hypertensive hemodynamics and may protect the body tissues, including the cardiovascular system from damage derived from mechanical and/or biochemical stresses in hypertensive rats.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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