



Functional Foods in Health and Disease

## Effects of the consumption of proanthocyanidins derived from acacia bark on blood pressure in healthy Japanese adults: A randomized, double-blind, placebo-controlled, parallel-group comparison study

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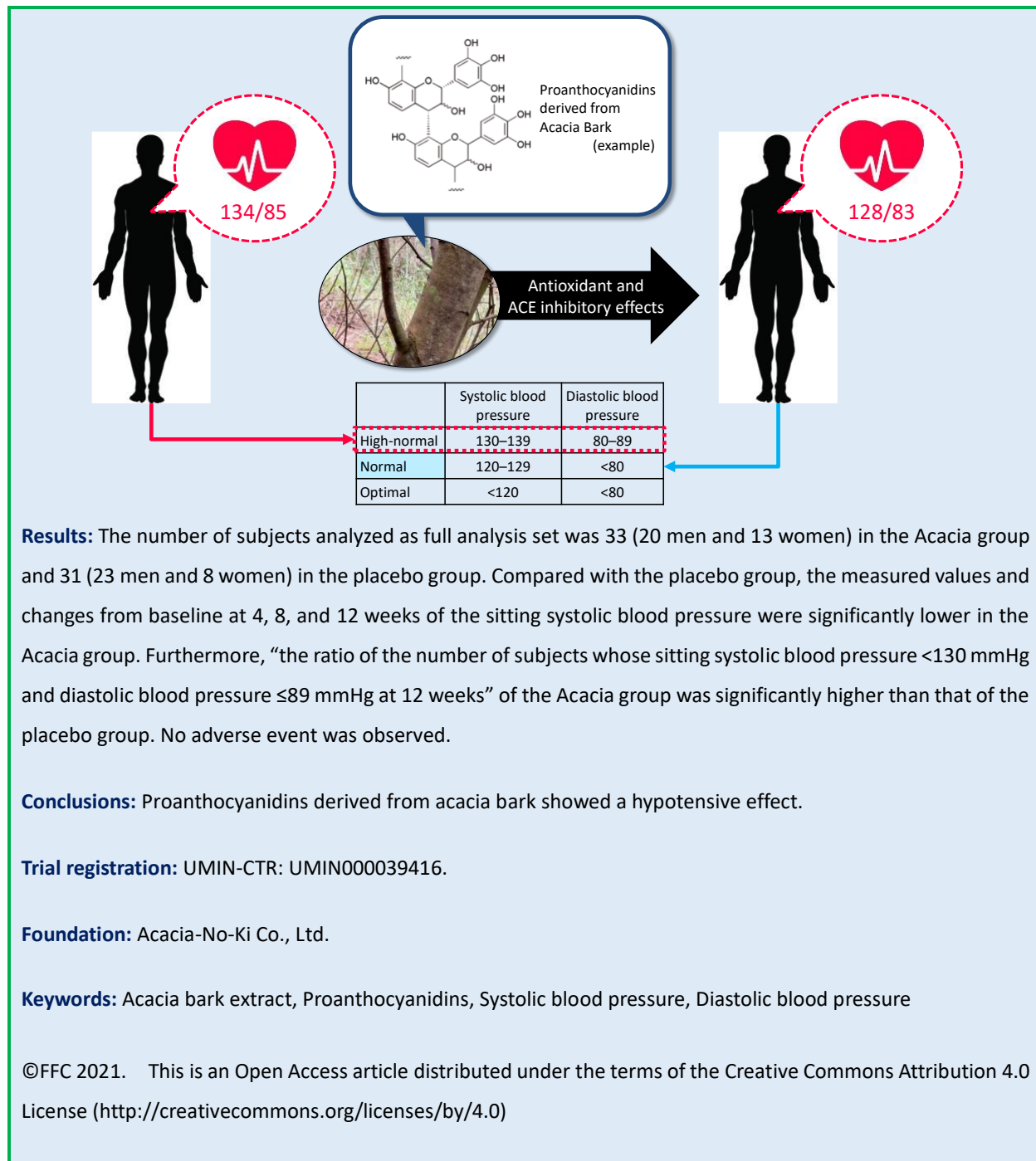
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### ABSTRACT

**Objective:** The aim of this study is to verify the effects of consuming proanthocyanidins derived from acacia bark on improving blood pressure and blood circulation in healthy Japanese adult subjects.

**Methods:** This was a randomized, double-blind, placebo-controlled, parallel-comparison study involving 66 healthy Japanese adults. Subjects were allocated into either acacia or placebo group (n = 33 each) using a random number generator. Subjects consumed six tablets/day of either acacia bark extract tablets or placebo for 12 weeks. The primary outcome was the measured value of sitting systolic blood pressure at 12 weeks, whereas the secondary outcomes were sitting systolic and diastolic blood pressures, superoxide dismutase activity in blood, and blood flow.



**INTRODUCTION**

According to the findings of the National Health and Nutrition Survey in Japan (2019), the mean systolic blood pressure (SBP) for all age groups over 20 years was 132.0 mmHg for men and 126.5 mmHg for women [1]. Furthermore, the percentage of people whose SBP was greater than 140 mmHg, which is one of the

diagnostic criteria for hypertension [2], was 29.9% for men and 24.9% for women. These data mark a significantly decreasing trend in the incidence observed in both men and women over the last 10 years [1]. Despite the declining rates of blood pressure in the Japanese population, hypertension remains the leading cause of death from cerebrovascular disease in Japan,

with an estimated 100,000 people dying from hypertension every year [3].

In general, SBP reduction is linearly correlated with the risk of developing cardiovascular diseases and death, with the lowest risk at approximately 120–124 mmHg [4]. Moreover, reducing SBP to less than 130 mmHg has been shown to contribute to a reduced risk of cardiovascular disease events [5]. The “Guidelines for the management of hypertension, 2019” published by The Japanese Society of Hypertension (2019) sets the target blood pressure goal for adults under 75 years at SBP <130 mmHg and diastolic blood pressure (DBP) <80 mmHg. These values were based on the results of clinical trial meta-analyses in which the risk of coronary artery disease and death was significantly lower in groups with SBP lower than 130 mmHg compared to groups with SBP between 130 and 139 mmHg. The National Health and Nutrition Survey in Japan (2019) has reported that the percentage of people with high-normal blood pressure ( $130 \text{ mmHg} \leq \text{SBP} \leq 139 \text{ mmHg}$  and/or  $85 \text{ mmHg} \leq \text{DBP} \leq 89 \text{ mmHg}$ ) is approximately 15.4% among all Japanese adults who do not receive blood pressure-lowering medication [1]. In addition, the number of people with high-normal blood pressure is estimated to be approximately 13.5 million. There is a positive correlation between blood pressure level and the risk of developing cerebral cardiovascular disease, and the proportion of deaths from cerebral cardiovascular disease that are attributed to hypertension at each level (population attributable fraction) is higher in people with mild to moderate hypertension [2]. Thus, preventing the worsening of blood pressure in prehypertensive individuals is considered an effective means of hindering cardiovascular disease. In addition, “Health Japan 21,” a basic policy of the Ministry of Health, Labour and Welfare (MHLW) to promote the overall health of the

Japanese population, includes the “improvement of hypertension” as one of its indicators. In particular, the emphasis is on decreasing SBP levels, which is the most useful predictor of the onset of cardiovascular disease, because hypertension is a major population attributable fraction for the incidence and death from cardiovascular diseases in Japan [6]. Therefore, it is important to maintain normal blood pressure on a daily basis in order to reduce the risk of developing cardiovascular diseases.

According to a previous study that used spontaneously hypertensive (SHR; a model of hypertension) and Wistar Kyoto rats (control) under a 4-week diet containing 1% or 3% acacia bark extract with proanthocyanidins, findings showed a significant blood pressure attenuation in SHR rats [7]. In addition, the superoxide dismutase (SOD) activity in the blood of SHR rats, whose diet contained acacia bark extract, was significantly higher than that in SHR rats that did not follow that diet [7]. This is presumably due to the antioxidant effect of proanthocyanidins derived from acacia bark, which increased SOD activity in blood and suppressed the increase in blood pressure [7]. However, it is still unknown whether proanthocyanidins derived from acacia bark can have the same effect in humans. Therefore, the purpose of this study is to evaluate the effects of a 12-week diet based on the consumption of proanthocyanidins derived from acacia bark on blood pressure and blood circulation in subjects with high-normal blood pressure [2]: DBP of less than 89 mmHg and SBP of 130 to 139 mmHg.

## METHOD

**Study design:** This was a randomized, double-blind, placebo-controlled comparison study, and the allocation was based on a 1:1 ratio. This study was approved by the Ethics Committee of Takara Clinic

(Medical Corporation Seishinkai, Tokyo, Japan; Approval ID: 2001-2001-AK02-01-TC; January 24, 2020) and registered at the University Hospital Medical Information Network Clinical Trial Registry (Registry no. UMIN000039416). This study was conducted in accordance with the principles of the Declaration of Helsinki (2013) and the ethical guidelines for medical and health research involving human subjects in Japan, with complete consideration of medical ethics.

**Subjects:** The inclusion criteria of this study included healthy Japanese adults with DBP of less than 89 mmHg and SBP of 130 to 139 mmHg at screening, who have relatively high levels of blood pressure or adults concerned about their blood pressure. The exclusion criteria were defined as follows: (1) a medical history of current treatment for malignancy, heart failure, or myocardial infarction; (2) use of a pacemaker or an implantable cardioverter defibrillator; (3) current treatment for cardiac arrhythmia; hepatic, renal, or cerebrovascular disease; rheumatism; diabetes mellitus; hyperlipidemia; hypertension; or other chronic diseases; (4) severe anemia; (5) secondary hypertension; (6) alcoholic beverage consumption or smoking cigars immoderately; (7) working late-night shift or having irregular lifestyles; (8) vigorous exercise habits; (9) daily consumption of “foods for specified health uses,” “foods with function claims,” or other functional foods or beverages; (10) regular use of medications, including herbal medicines or supplements; (11) allergic reaction to medications or products that contain the study components; (12) pregnancy, lactation, or planning to become pregnant; (13) enrollment in other clinical trials within the previous three months before agreeing to participate in this trial or planning to participate in another trial during this trial; and (14) ineligibility to participate in

the study based on the evaluation of the principal physician. The subjects were recruited from members of the volunteer bank operated by ORTHOMEDICO Inc. (Tokyo, Japan) online via a member’s website (<https://www.go106.jp/>). The study protocols were comprehensively explained to all subjects at the office of ORTHOMEDICO Inc. Furthermore, all subjects provided informed consent before their participation in the study. No subject was part of the sponsors or funding companies. The study was conducted at Medical Corporation Seishinkai, Takara Clinic.

**Determination of sample size:** To date, no studies that used acacia bark extract tablets to measure sitting SBP (SSBP) in humans for a period of 12 weeks have been conducted. Thus, the sample size was calculated by assuming that the difference in the measured values of SSBP between the Acacia (acacia bark extract) and placebo groups at 12 weeks is large. As suggested by Cohen (1992), the sample size was calculated with an assumed effect size ( $d$ ) of 0.80, significance level ( $\alpha$ ) of 0.05, and statistical power ( $1-\beta$ ) of 0.80, leading to 52 subjects per group (26 subjects in each group). In order to maximize the statistical power as much as possible, the target number of patients was set at 60 (30 subjects in each group), which resulted in a statistical power of 0.86. In addition, the number of patients was set at 66 (33 subjects in each group) to allow for dropouts and noncompliance with the protocol during the study period.

**Selection, randomization, and blinding:** Of 180 subjects who signed informed consent forms, 66 eligible subjects were considered appropriate for the purposes of this study and were thus selected by the physician. Inclusion criteria were defined as follows: (a) eligible to participate in the study based on the evaluation of the principal physician, (b)  $DBP \leq 89$

mmHg at screening (Scr; before test-food consumption), and (c) SBP of 130 to 139 mmHg at Scr. Test foods were provided to the contract research organization by the sponsor. After the test foods were confirmed to be indistinguishable and after entering and verifying the data at Scr, an individual in charge of shipping, who is a member of the contract research organization, provided the code of the test foods to the allocation controller who was not directly involved in the studies. Allocation was performed by the allocation controller according to a computer-generated randomization list, and the allocation adjustment factors included sex, age, and SBP at Scr. Subjects were equally, but randomly, assigned to either the active group or the placebo group (n = 33 per group) by the allocation controller. The allocation table with the coded test foods generated by the allocation controller was provided only to the person in charge of shipping, who sent the test foods to each subject according to the table. The sponsors, principal investigator, subinvestigators, entire contract research organization staff (i.e., director of the trial, director of trial conduction, individual in charge of monitoring, director and staff of statistical analysis, and individual in charge of shipping), medical institution staff, institutional review board members, contract laboratory, and others who were related to this study were not aware of the group assignments. The

allocation controller locked the allocation table until the key opening day.

**Intervention:** The composition of the test foods in each tablet is shown in Table 1. The acacia bark extract used in this study was obtained through hot water extraction from the bark of *Acacia mearnsii* De Wild., a member of genus *Acacia*. This extract is a mixture of monomer, dimer, trimer, and polymer compounds with structures based on flavan-3-ol, such as gallicocatechin and robinetinidol [8,9], and approximately 80% of its compounds involve polyphenols [10,11]. Compared to proanthocyanidins with 5,7-dihydroxyflavan-3-ol units of grape seeds [12] and pine bark [13], proanthocyanidins derived from acacia bark have a complex mixture of proanthocyanidins that are mainly composed of 5-deoxycatechin units [14,15].

All subjects were administered acacia bark extract tablets or placebo (six tablets per day) to be taken with water before meals and without chewing for a period of 12 weeks. Six tablets of the acacia bark extract food contained 245 mg of proanthocyanidins derived from acacia bark, and placebo tablets were free from proanthocyanidins. In addition, both test foods did not contain any other effective polyphenols for blood pressure. The ethics committee declared that both tablets were indistinguishable in appearance.

**Table 1.** Nutritional composition of test foods

		Acacia bark extract tablets	Placebo tablets
<b>Calorie</b>	kcal	7.182	7.2
<b>Protein</b>	g	0.054	0.0432
<b>Fat</b>	g	0.0522	0.0522
<b>Carbohydrate</b>	g	1.6236	1.6398
<b>Salt equivalent</b>	g	0.001179	0.000522

The content per 6 tablets (1800 mg) is shown.

**Outcomes:** The schedule of this study is shown in Table 2. SOD and blood flow assessments were conducted only at Scr and at 12 weeks following initial intake (12w). Additional efficacy and safety assessments were conducted at Scr and before test-food consumption (0w), and at 4 weeks (4w), 8 weeks (8w), and 12w.

**(1) Primary outcome:** The measured value of SSBP at 12w. SSBP was evaluated using a HEM-6022 Blood Pressure Monitor (OMRON HEALTHCARE Co., Ltd., Kyoto, Japan).

## **(2) Secondary outcomes**

**1) The ratio of the number of subjects:** The ratio of the number of subjects whose SSBP < 130 mmHg and sitting DBP (SDBP)  $\leq$  89 mmHg at 12w was evaluated.

**2) SSBP and SDBP:** Except for the primary outcome, the measured values of SSBP at Scr, 4w, and 8w; the amount of changes of SSBP at Scr, 4w, 8w, and 12w; and the measured values and changes of SDBP at Scr, 4w, 8w, and 12w were also evaluated. As with the primary outcome, SSBP and SDBP were evaluated using a HEM-6022 Blood Pressure Monitor (OMRON HEALTHCARE Co., Ltd., Kyoto, Japan).

**3) SOD activity in blood:** For the evaluation of SOD activity in blood, a cold-water load test was conducted by cooling the palm of the dominant hand with cold water at 15°C for 1 min after 60 min from test-food intake. The measured values and changes of SOD before and after 30 min of the cold-water load at 0w and 12w and the changes in the respective values (12w–0w) were assessed.

Approximately 6 mL of venous blood was collected before the intervention and 30 min after cold-water loading. SOD activity in blood was measured by Kamakura Techno-Science, Inc. (Kanagawa, Japan).

**4) Blood flow:** For the evaluation of blood flow, the measurement was conducted simultaneously with blood SOD activity measurement. Blood flow was measured using the 2D laser blood flow imager OMEGAZONE (Omegawave Inc., Tokyo, Japan).

**(3) Safety evaluation:** Safety evaluation was accomplished by means of physical examination, urinalysis, and blood analysis.

Physical examination items included the subjects' weight, body mass index, body fat percentage, pulse rate, and body temperature. Height was only measured to calculate the respective body mass index.

In urinalysis, urine samples were collected to evaluate the levels of protein, glucose, urobilinogen, bilirubin, ketone bodies, pH, and occult blood. The collected samples were entrusted to LSI Medience Corporation (Tokyo, Japan), and each item was assessed in accordance with global standards.

Hematological tests were conducted to assess the following: leukocyte count, erythrocyte count, hemoglobin, hematocrit, platelet count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and white blood cell differential count (i.e., numbers of neutrophils, lymphocytes, monocytes, eosinophils, and basophils). For biochemical tests, we evaluated the following parameters: aspartate aminotransferase, alanine aminotransferase,  $\gamma$ -glutamyl transpeptidase,

alkaline phosphatase, lactate dehydrogenase, leucine aminopeptidase, total bilirubin, direct bilirubin, indirect bilirubin, cholinesterase, total protein, urea nitrogen, creatinine, uric acid, creatine kinase, calcium, serum amylase, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, glycoalbumin, serum iron, sodium, potassium, chloride, inorganic phosphorus, glucose, and nonspecific immunoglobulin E. In addition, nonspecific immunoglobulin E was only measured at Scr. The level of each parameter was measured by LSI Medience Corporation.

All subjects were asked to complete a medical questionnaire to allow for understanding of their health conditions at each examination. In addition, subjects were asked to keep a daily record of the consumption of the test food, health conditions, use of medications, and lifestyle.

**Statistical analysis:** All statistical analyses in this study were two-sided, and the significance level was set at 5%, with no adjustment for multiple comparisons. Data analyses were performed using Windows SPSS version 23.0 (IBM Japan, Ltd., Tokyo, Japan).

The subjects' background data were demographically aggregated by enrolled and analyzed subjects. The subjects' age, height, and nonspecific immunoglobulin E for both groups were compared using Student's *t*-test.

The primary and secondary outcomes (except for the ratio of the number of subject) and safety evaluation items were presented as mean  $\pm$  standard deviation. The value before cold-water loading for blood SOD activity and blood flow, which are the

secondary outcomes, and the data at Scr for all additional items were set as the baseline. The baseline and the changes in the value of the safety evaluation items were compared between groups using the Student's *t*-test. The measured values and changed values from baseline of the primary and secondary outcomes (except for the blood flow, the SOD activity in blood, and the ratio of the number of subjects) at 4w, 8w, and 12w were analyzed using the following methods: the measured values using post-hoc comparisons of the linear mixed model, with the baseline values utilized as covariates and with time, groups, group–time interaction, and subjects as factors, and the changes in the values were analyzed using post-hoc comparisons of the linear mixed model, with time, groups, group–time interaction, and subjects as factors. In addition, we analyzed the data of the secondary outcomes, namely, the blood flow and the SOD activity in blood, using analysis of covariance (ANCOVA), with the baseline values as covariates and groups as factors. To compare the ratio of the number of subjects, logistic regression analysis with factors of group, sex, age, and SSBP at baseline was performed to evaluate the odds ratio (OR), 95% confidence interval, and *P* value to the placebo group. Furthermore, urinalysis data were assigned codes, wherein "1" was identified as within the normal range and "0" was identified as outside the normal range. For the safety assessment items, the principal investigator evaluated and checked the data case-by-case to confirm that there were no medical problems associated with the consumption of the test food.





## RESULTS

**Analysis set:** Figure 1 shows the flowchart of this study. We recruited subjects from February 10, 2020, to April 25, 2020, and the test period lasted from February 10, 2020, to September 5, 2020. At the case review meeting after the intervention, three subjects were excluded from the subsequent analysis because of inappropriate follow-up (two patients who never received the test-food intervention after allocation and did not come to the clinic since 4w, and one subject who did not come to the clinic at 12w). In addition, with respect to safety evaluation, the analysis excluded two subjects who did not receive the test-food intervention after allocation. According to the key opening, three subjects who did not come to the clinic after 4w and 12w, respectively, were from the placebo group. The final efficacy analysis subjects were the full analysis set (FAS). The analysis excluding the two subjects who did not come to the clinic since 4w was designated as FAS1, and the analysis excluding the three subjects who did not come to the clinic after 4w and 12w was designated as FAS2. In other words, FAS1 represented the analysis population that excluded subjects that had never received the test-food intervention after allocation, and FAS2 represented the analysis population that excluded subjects that had never received the test-food intervention after allocation plus cases with any missing data. Therefore, the number of FAS1 subjects was 33 (20 men and 13 women) in the Acacia group and 31 (23 men and 8 women) in the placebo group. By contrast, the number of FAS2 subjects was 33 (20 men and 13 women) in the Acacia group and 30 (22 men and 8 women) in the placebo group. FAS1 was used for the analysis of measured values and changes of blood pressure, and FAS2 was used for other types of analyses. The subjects of the safety analysis were the safety analysis population (SAF), which was the same as that in FAS1.

The subjects' background and age distribution are shown in Tables 3-1 and 3-2. There were no significant differences between the background factors of both groups.

**Blood pressure:** Figures 2 and 3 show the blood pressure results.

There was a significant group–time interaction in the measured value of SSBP ( $P < 0.001$ ). The measured value of SSBP at 4w in the Acacia group was significantly lower than the SSBP value in the placebo group (Acacia group,  $128.6 \pm 5.7$  mmHg; placebo group,  $134.0 \pm 5.1$  mmHg;  $P = 0.001$ ). Furthermore, the measured value of SSBP at 8w in the Acacia group was significantly lower than the value of SSBP in the placebo group (Acacia group,  $129.2 \pm 8.2$  mmHg; placebo group,  $133.7 \pm 6.3$  mmHg;  $P = 0.006$ ). The measured value of SSBP at 12w in the Acacia group was significantly lower than the value of SSBP in the placebo group (Acacia group,  $128.6 \pm 8.9$  mmHg; placebo group,  $133.8 \pm 6.4$  mmHg;  $P < 0.001$ ).

There was a significant group–time interaction in the changes in SSBP ( $P < 0.001$ ). Changes in SSBP at 4w–Scr in the Acacia group were significantly lower than those observed in the placebo group (Acacia group,  $-5.5 \pm 5.0$  mmHg; placebo group,  $-0.3 \pm 5.6$  mmHg;  $P = 0.002$ ). SSBP changes at 8w–Scr in the Acacia group were significantly lower than the SSBP changes in the placebo group (Acacia group,  $-4.9 \pm 7.7$  mmHg; placebo group,  $-0.6 \pm 6.8$  mmHg;  $P = 0.008$ ). Finally, SSBP changes at 12w–Scr in the Acacia group were significantly lower than the changes observed in the placebo group (Acacia group,  $-5.9 \pm 8.4$  mmHg; placebo group,  $-0.3 \pm 7.2$  mmHg;  $P < 0.001$ ).

There was no significant difference in the group–time interaction and the value of SDBP between the groups.

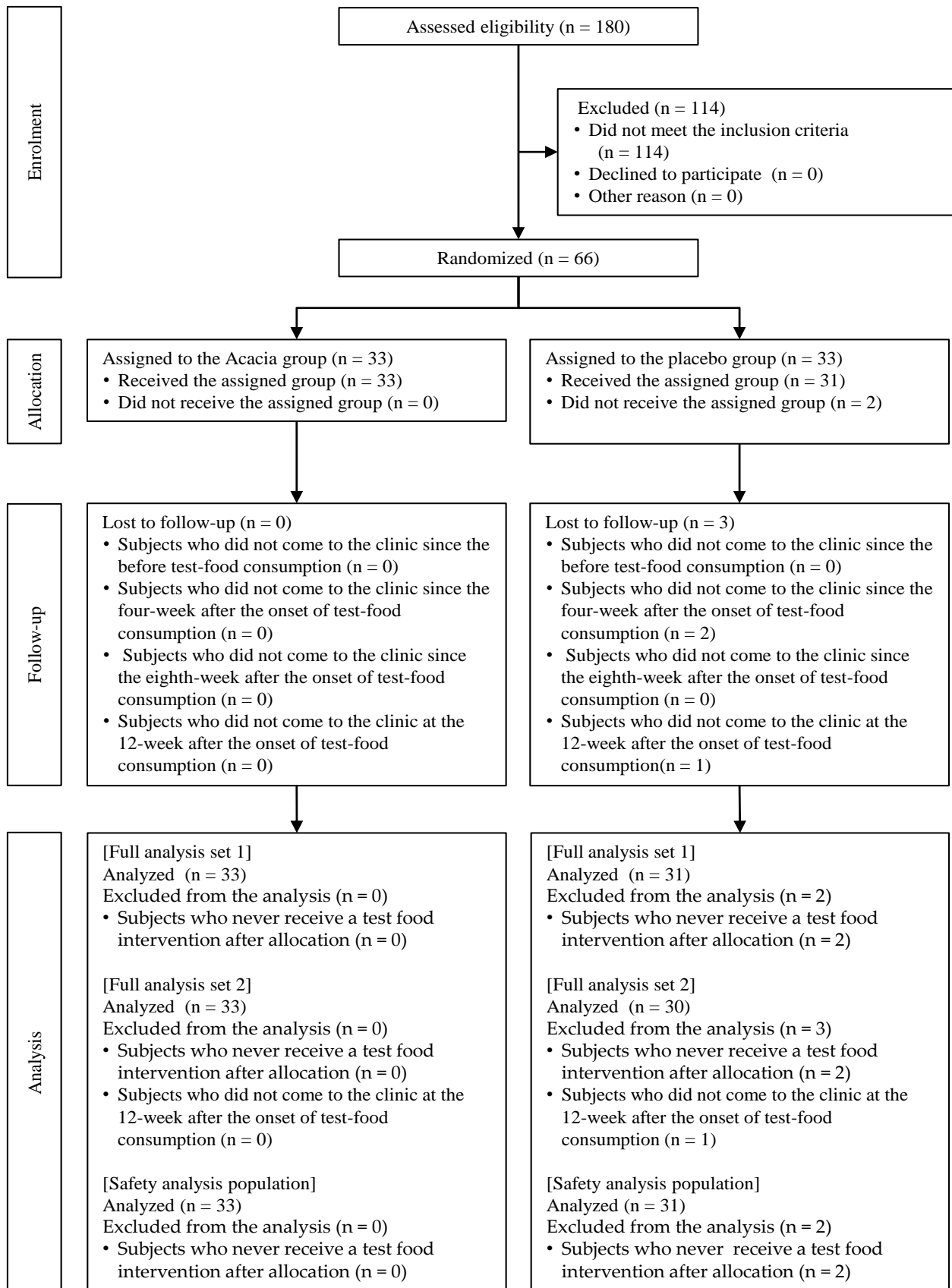


Figure 1. Flowchart of subjects in this study

**Table 3-1.** Subjects' background information (Gender, Age)

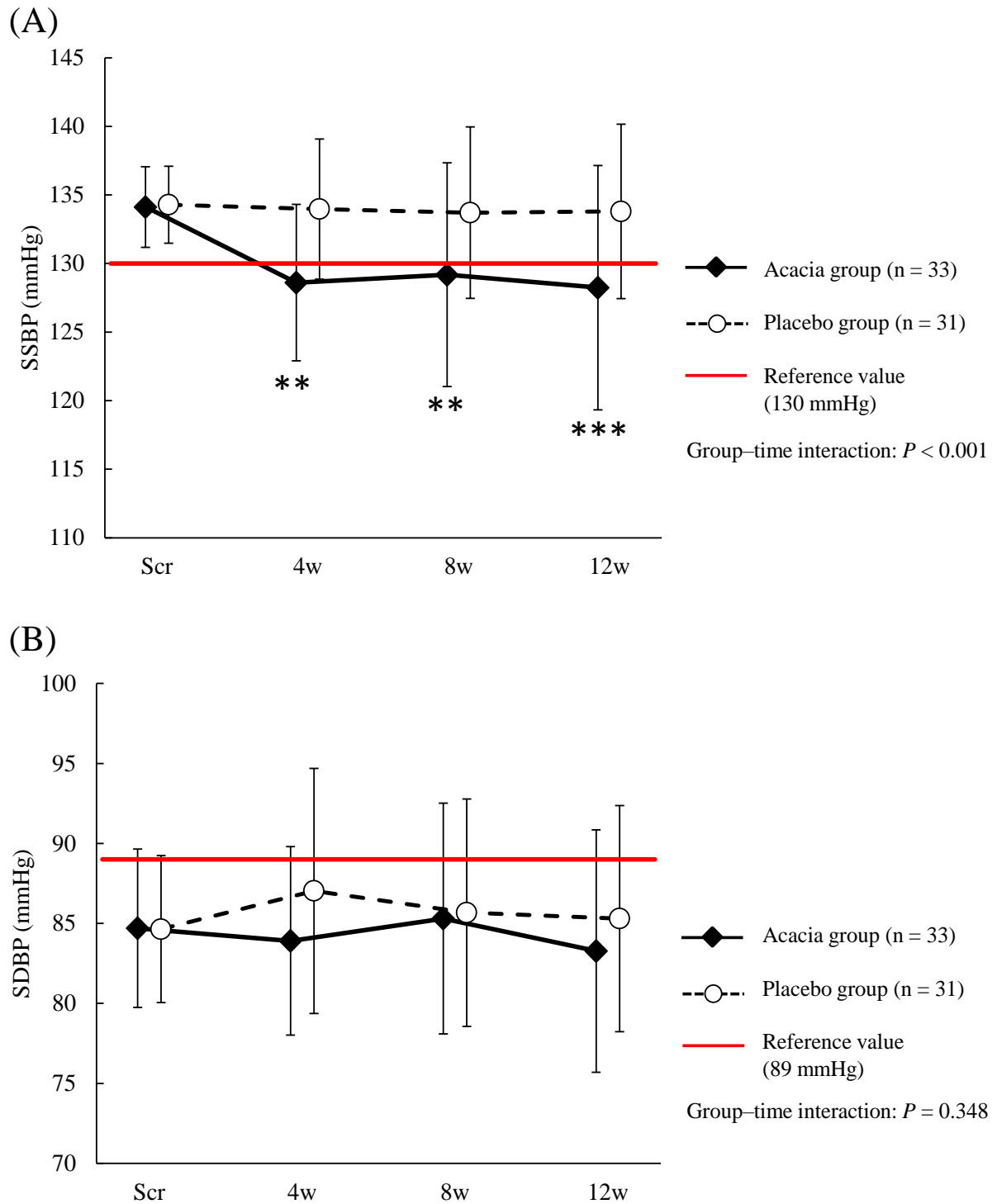
Item	Acacia group		Placebo group		P value
	Men	Women	Men	Women	
<b>Allocated subjects (Acacia group n = 33, Placebo group n = 33)</b>					
<b>Gender</b>	20 (60.6%)	13 (39.4%)	23 (69.7%)	10 (30.3%)	0.606
<b>Age (years)</b>					
<b>20-29</b>	3 (9.1%)	0 (0%)	1 (3%)	0 (0%)	-
<b>30-39</b>	2 (6.1%)	0 (0%)	3 (9.1%)	0 (0%)	-
<b>40-49</b>	6 (18.2%)	3 (9.1%)	7 (21.2%)	4 (12.1%)	-
<b>50-59</b>	6 (18.2%)	7 (21.2%)	9 (27.3%)	4 (12.1%)	-
<b>60-69</b>	2 (6.1%)	3 (9.1%)	3 (9.1%)	1 (3%)	-
<b>70-79</b>	1 (3%)	0 (0%)	0 (0%)	1 (3%)	-
<b>Full analysis set 1; FAS 1 and Safety analysis population; SAF (Acacia group n = 33, Placebo group n = 31)</b>					
<b>Gender</b>	20 (60.6%)	13 (39.4%)	23 (74.2%)	8 (25.8%)	0.294
<b>Age (years)</b>					
<b>20-29</b>	3 (9.1%)	0 (0%)	1 (3.2%)	0 (0%)	-
<b>30-39</b>	2 (6.1%)	0 (0%)	3 (9.7%)	0 (0%)	-
<b>40-49</b>	6 (18.2%)	3 (9.1%)	7 (22.6%)	3 (9.7%)	-
<b>50-59</b>	6 (18.2%)	7 (21.2%)	9 (29%)	4 (12.9%)	-
<b>60-69</b>	2 (6.1%)	3 (9.1%)	3 (9.7%)	0 (0%)	-
<b>70-79</b>	1 (3%)	0 (0%)	0 (0%)	1 (3.2%)	-
<b>Full analysis set 2; FAS 2 (Acacia group n = 33, Placebo group n = 30)</b>					
<b>Gender</b>	20 (60.6%)	13 (39.4%)	22 (73.3%)	8 (26.7%)	0.423
<b>Age (years)</b>					
<b>20-29</b>	3 (9.1%)	0 (0.0%)	1 (3.3%)	0 (0.0%)	-
<b>30-39</b>	2 (6.1%)	0 (0.0%)	3 (10.0%)	0 (0.0%)	-
<b>40-49</b>	6 (18.2%)	3 (9.1%)	7 (23.3%)	3 (10.0%)	-
<b>50-59</b>	6 (18.2%)	7 (21.2%)	9 (30.0%)	4 (13.3%)	-
<b>60-69</b>	2 (6.1%)	3 (9.1%)	2 (6.7%)	0 (0.0%)	-
<b>70-79</b>	1 (3.0%)	0 (0.0%)	0 (0.0%)	1 (3.3%)	-

The data are presented as the number of subjects and as a percentage of the each group.

**Table 3-2.** Subjects' background information (Age, Height, BMI, and Non-specific IgE)

	Unit	Acacia group		Placebo group		P value
		Mean	SD	Mean	SD	
<b>Allocated subjects (Acacia group n = 33, Placebo group n = 33)</b>						
<b>Age</b>	years	50.8	11.3	49.8	9.6	0.682
<b>Height</b>	cm	165.6	8.1	166.0	7.4	0.862
<b>BMI</b>	kg/m <sup>2</sup>	24.2	3.4	24.3	3.9	0.913
<b>Non-specific IgE</b>	IU/mL	211.3	451.5	141.8	201.1	0.422
<b>Full analysis set 1; FAS 1 and Safety analysis population; SAF (Acacia group n = 33, Placebo group n = 31)</b>						
<b>Age</b>	years	50.8	11.3	49.5	9.6	0.606
<b>Height</b>	cm	165.6	8.1	166.7	7.1	0.591
<b>BMI</b>	kg/m <sup>2</sup>	24.2	3.4	24.3	4.0	0.902
<b>Non-specific IgE</b>	IU/mL	211.3	451.5	140.3	207.3	0.427
<b>Full analysis set 2; FAS 2 (Acacia group n = 33, Placebo group n = 30)</b>						
<b>Age</b>	years	50.8	11.3	49.1	9.5	0.503
<b>Height</b>	cm	165.6	8.1	167.0	7.1	0.499
<b>BMI</b>	kg/m <sup>2</sup>	24.2	3.4	24.4	4.0	0.825
<b>Non-specific IgE</b>	IU/mL	211.3	451.5	139.3	210.8	0.428

The data are presented as the number of subjects, or the mean and standard deviation (SD).



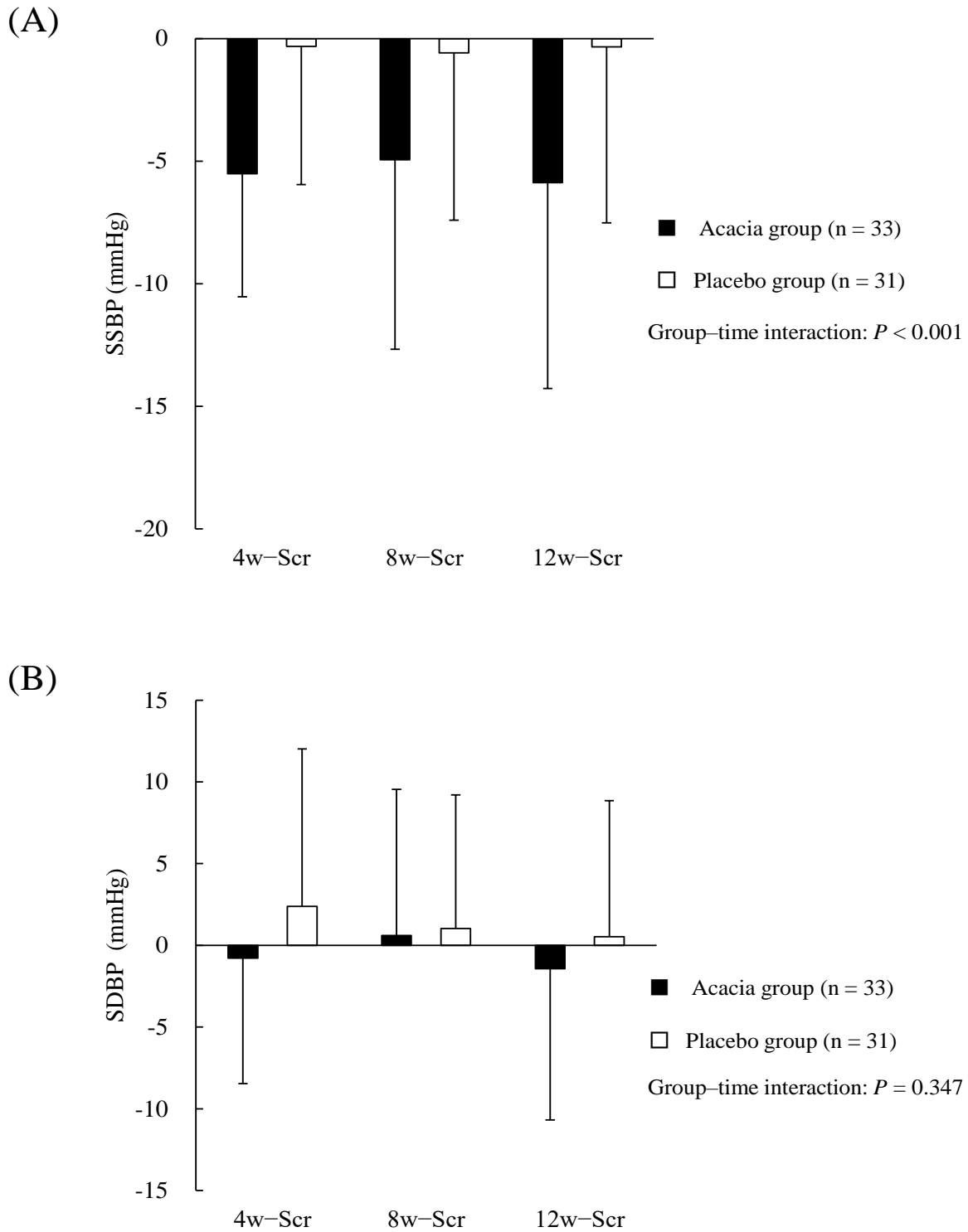
**Figure 2.** Blood pressure (Measured values)

(A) SSBP, (B) SDBP.

The data are presented as the number of subjects, or the mean and standard deviation (SD).

\*\* $: P < 0.01$ , \*\*\* $: P < 0.001$  vs the placebo group.

SSBP, sitting systolic blood pressure; SDBP, sitting diastolic blood pressure



**Figure 3.** Blood pressure (Change values)

(A) SSBP, (B) SDBP.

The data are presented as the number of subjects, or the mean and standard deviation (SD).

\*\* $: P < 0.01$ , \*\*\* $: P < 0.001$  vs the placebo group.

SSBP, sitting systolic blood pressure; SDBP, sitting diastolic blood pressure

**The ratio of the number of subjects:** Table 4 shows the ratio of the number of subjects.

The ratio of the number of subjects who achieved the criteria was significantly higher in the Acacia group than in the placebo group ( $P = 0.006$ ).

**Blood flow and SOD activity in blood:** Table 5 shows the results of blood flow and SOD activity in blood.

The measured blood flow rate before cold-water loading at 0w was significantly higher in the Acacia group than in the placebo group (Acacia group,  $8.9 \pm 2.0$

mL/min/100 g; placebo group,  $7.3 \pm 1.7$  mL/min/100 g;  $P = 0.001$ ). In addition, the measured blood flow rate before cold-water loading at 12w was significantly higher in the Acacia group compared to the placebo group (Acacia group,  $11.3 \pm 2.9$  mL/min/100 g; placebo group,  $9.7 \pm 2.6$  mL/min/100 g;  $P = 0.025$ ). Following the test-food intervention, there were no items that showed significant differences among the groups.

There was no significant difference in blood SOD activity among the groups.

**Table 4.** The ratio of the number of subjects (FAS 2)

	Decision criterion	
	Acacia group (n = 33)	Placebo group (n = 30)
Number of subjects who achieved the criteria (percentage)	18 (54.5%)	6 (20%)
Odds ratio against placebo group	6.933	
[95% confidence interval]	[1.721, 27.928]	
<i>P</i> value	0.006**	

Logistic regression analysis was performed with group, sex, age, SSBP at Scr as factors and SDBP at Scr as factors, and odds ratios, their 95% confidence intervals, and significance probabilities are presented.

Decision criterion was defined as follows: subjects who are less than 130 mmHg in SSBP and no more than 89 mmHg in SDBP.

\*\* $: P < 0.01$  vs. the placebo group. SSBP, sitting systolic blood pressure; SDBP, sitting diastolic blood pressure.

**Safety assessment:** Supplemental Tables 1-1 to 1-3 exhibit the safety assessment results.

Under the conditions of this study, no side effects or adverse events were observed throughout the study period. Although significant differences in several test items were observed between the groups, we assumed that these differences were not clinically meaningful because the measured values of the relevant items

were within the reference values. In addition, these changes did not induce nor facilitate any medical emergencies or abnormalities. Therefore, no medically problematic changes were observed as a result of the continued ingestion of the test food.

**Table 5.** Blood flow, SOD activity in blood (Scr, 12w / FAS 2)

Unit		Before cold water load#				After cold water load				Before and after cold water load#				
		Mean	SD	Mean (95%CI)	P value	Mean	SD	EMM (95%CI)	P value	Mean	SD	Mean (95%CI)	P value	
<b>Blood flow</b>														
<b>0w</b>	mL/min/100g	Acacia group (n = 33)	8.9	2.0	1.6 (0.7, 2.5)	0.001**	8.4	2.1	-0.2 (-1.0, 0.7)	0.691	-0.4	1.7	-0.6 (-1.4, 0.2)	0.132
		Placebo group (n = 30)	7.3	1.7			7.4	1.9			0.2	1.5		
<b>12w</b>	mL/min/100g	Acacia group (n = 33)	11.3	2.9	1.6 (0.2, 3.0)	0.025*	9.1	3.3	-0.2 (-1.7, 1.2)	0.740	-2.2	2.9	-1.0 (-2.6, 0.5)	0.190
		Placebo group (n = 30)	9.7	2.6			8.5	2.8			-1.2	3.2		
<b>SOD activity in blood</b>														
<b>0w</b>	U/mL	Acacia group (n = 33)	3.60	2.88	0.6 (-0.8, 1.9)	0.401	3.58	2.69	0.1 (-0.1, 0.3)	0.337	-0.02	0.40	0.1 (-0.1, 0.2)	0.546
		Placebo group (n = 30)	3.03	2.33			2.96	2.33			-0.07	0.30		
<b>12w</b>	U/mL	Acacia group (n = 33)	3.29	2.32	0.1 (-1.1, 1.4)	0.842	3.24	2.22	-0.1 (-0.2, 0.1)	0.409	-0.05	0.34	-0.1 (-0.2, 0.1)	0.396
		Placebo group (n = 30)	3.17	2.59			3.18	2.66			0.01	0.24		

The data are presented as the number of subjects, or the mean and standard deviation (SD).

At other time points, intergroup differences (Acacia group-Placebo group) are presented as estimated marginal mean (EMM) and 95% confidence interval of EMM (95% CI-, 95% CI+).

#Scr presented the intergroup differences (Acacia group-Placebo group) as mean and 95% confidence interval of Mean (95% CI-, 95% CI+).

\*P < 0.05; \*\*P < 0.01 vs. the placebo group.



## DISCUSSION

Vasorelaxation is an effective approach to reduce blood pressure. Nitric oxide (NO), a vasorelaxant factor, diffuses into the vascular smooth muscle cells adjacent to endothelial cells and activates soluble guanylate cyclase, thus causing a vasorelaxant response [16]. When vascular endothelial cells are damaged by oxidative stress, NO production, which is involved in the regulation of vasorelaxation and vasoconstriction, is reduced or loses its bioactivity, which, in turn, facilitates vascular endothelium dysfunction [17]. However, vascular endothelium dysfunction may contribute to elevated blood pressure and persistent hypertension, which is particularly pronounced in systolic hypertension and in elderly patients [18].

In a study performed on SHR rats (a model of hypertension), blood SOD activity was significantly increased following acacia bark extract consumption that contained proanthocyanidins [7]. It is known that oxidative stress causes vascular endothelium dysfunction due to the decreased production or loss of bioactivity of NO [17]. Because reactive oxygen species (ROS) are involved in the development and maintenance of hypertension in SHR rats, it is believed that the decrease in the production and removal of ROS suppress the potential blood pressure increase [7]. According to other studies, consumption of red berry-derived polyphenols, known to have vasorelaxant effects, could significantly decrease blood pressure and increase SOD activity and NO in SHR rats [19]. Therefore, it is suggested that proanthocyanidins derived from acacia bark can inhibit the increase in blood pressure by increasing SOD activity and exerting antioxidant effects

[7].

In this study, subjects were categorized in accordance with their high-normal blood pressure, DBP  $\leq 89$  mmHg and/or SBP between 130 and 139 mmHg, and were provided with proanthocyanidins derived from acacia bark. As a result, the measured values and changes in SSBP at 4w, 8w, and 12w were significantly lower in the Acacia group compared to the placebo group. In addition, the ratio of the number of subjects whose SSBP  $< 130$  mmHg and SDBP  $\leq 89$  mmHg was significantly higher in the Acacia group than in the placebo group. Our findings support the preceding clinical trial report in that grape seed proanthocyanidin extract could significantly lower SBP in prehypertension Japanese men and women (SBP of 130–139 mmHg and/or DBP of 85–89 mmHg) [20]. According to the “Guidelines for the management of hypertension, 2019” issued by The Japanese Society of Hypertension (2019), the target blood pressure goal for adults under 75 years of age is set at SBP  $< 130$  mmHg and DBP  $< 80$  mmHg. These values were based on the results of clinical trial meta-analyses in which the risk of coronary artery disease and death was significantly lower in groups with systolic blood pressures lower than 130 mmHg compared to groups with SBP between 130 and 139 mmHg. In this study, the mean value of SSBP in the Acacia group, which was above 130 mmHg before test-food consumption, was reduced to below 130 mmHg postintervention, and the ratio of the number of subjects whose SSBP was less than 130 mmHg and SDBP value was  $\leq 89$  mmHg was significantly higher in the Acacia group. Therefore, it can be suggested that the test-food intervention is useful in terms of reducing the

risk of cardiovascular diseases.

In light of the results of the precedence studies, it was expected that the decrease in SSBP that was observed in this study was due to an increase in SOD activity and a decrease in ROS caused by the action of proanthocyanidins in the test food, which have a high antioxidant capacity [21]. However, there was no increase observed in SOD activity following the intake of the test food in this study. Although vascular endothelium dysfunction in SHR rats is related to excessive production of ROS [18] and the blood ROS levels in hypertensive people are higher than those in normotensives [22], few clinical trials have found an association between increased SOD activity with food intake and decreased blood pressure. In adult men and women with elevated blood pressure and obesity, there was no change in SOD observed when blood pressure was lowered by a dietary intervention [23]. Some reports have shown that the digestion of proanthocyanidins is affected by ingested nutrients such as carbohydrates and proteins [24], and it may have been possible that the increase in SOD activity by the intake of the test food could not be confirmed because of individual differences in digestion caused by the respective dietary content. Alternatively, the time period between test-food intake and SOD assessment may have affected the measured values. This means that even though SOD levels may have increased after test-food intake, we might have been unable to observe this increase at the timing of SOD assessment in this study. The goal of a future study will be to confirm the increase in SOD activity by observing changes in SOD overtime after ingestion. A non-SOD mediated pathway

to reduce ROS has also been suggested for blood pressure reduction [18,25,26]. Polyphenols are known to exert their antioxidant effects by providing electrons and increasing the stability of ROS [25]. In addition, administration of a polyphenol-rich extract of camu camu (*Myrciaria dubia*) pericarp to SHR rats has been shown to decrease the expression of AT<sub>1</sub> receptors, a type of receptor for angiotensin (AT) II, a vasoconstrictor that stimulates the activity of ROS-producing cofactors [26]. Indeed, antihypertensive drugs, which block AT II receptors, are reported to suppress oxidative stress in clinical trials. Consequently, it is suggested that oxidative stress reduction improves endothelial function and lowers blood pressure by allowing NO to act as a vasorelaxant factor [18]. As a result, the mechanism of proanthocyanidins' antioxidant effect on improving blood pressure and blood flow in people with high-normal blood pressure could be examined in more detail by standardizing the subjects' diet and measuring other oxidative stress markers in addition to SOD in future studies.

Furthermore, one of the enzymes involved in the development of hypertension is the AT-converting enzyme (ACE). ACE is known to produce AT II, a potent vasoconstrictor, from AT I. We attempted to determine the half-maximal inhibitory concentration (IC<sub>50</sub>) of ACE of acacia bark extract, proanthocyanidins fraction (71.7%) from acacia bark extract, and other fractions mainly containing sugar (28.3%) from acacia bark extract, and the results were 0.021 mg/mL, 0.014 mg/mL, and N/A, respectively (unpublished data). On the basis of these results and considering that only proanthocyanidins derived from acacia bark can affect

ACE, the theoretical half-maximal inhibitory concentration ( $IC_{50}$ ) becomes

$$0.021 \text{ [mg/mL]} \times 71.7 \text{ [%]} = 0.015 \text{ [mg/mL]}.$$

As mentioned above, the experimental  $IC_{50}$  value of the proanthocyanidins fraction was 0.014 mg/mL. Therefore, the effects of acacia bark extract can be explained by the proanthocyanidins fraction alone. Compared to other plants that are known to have strong ACE inhibitory effects, such as kuromoji (*Lindera umbellata*) extract ( $IC_{50}$ : 0.29 mg/mL)[27], green tea leaves ( $IC_{50}$ : 0.125 mg/mL), and blueberry leaves ( $IC_{50}$ : 0.046 mg/mL)[28], the ACE inhibitory effect of proanthocyanidins derived from acacia bark was confirmed to be strong. Therefore, it can be inferred that proanthocyanidins derived from acacia bark act as a functionally involved ingredient and suppress the increase in blood pressure by acting as ACE inhibitors.

In addition, various studies on blood pressure and vasorelaxation responses with polyphenol-containing foods have been conducted. Polyphenols in grape seeds, black tea, and red wine have been suggested to increase NO via activation of the PI3K–Akt pathway and endothelial NO synthase (NOS) [29–31]. According to a clinical trial performed on women with high-normal blood pressure, consumption of a polyphenol-rich olive oil diet was associated with significantly lower DBP, SBP, and blood asymmetric dimethylarginine (ADMA), and significantly higher blood nitrite/nitrate ratio compared to before consumption [32]. Because ADMA is a NOS inhibitor and the plasma nitrite/nitrate ratio represents a gradual reduction in NO, consumption of a high-polyphenol diet facilitated a decrease in blood pressure by means of

decreasing ADMA and increasing NO production [32]. This mechanism may also work for proanthocyanidins derived from acacia bark. Therefore, in future studies, measurement of blood ADMA and plasma nitrite/nitrate ratio can provide novel insights with respect to test-food intake contribution to blood pressure lowering.

Our study design has two limitations. First, the applicability of the results to individuals with DBP  $\geq$  90 mmHg and/or SBP  $\geq$  140 mmHg necessitates further investigations. In addition, we were unable to clarify the relationship between blood pressure and blood flow. However, it is true that the acacia bark extract showed blood-pressure-lowering effects on healthy Japanese adults with DBP < 89 mmHg and SBP between 130 and 139 mmHg. Therefore, while considering the mechanism of blood-pressure-lowering effects, the acacia bark extract may be decreasing blood pressure even in individuals whose blood pressure values are higher compared to our study. Although we could not identify any effect of the test foods on blood flow in this study, the test food may have an effect on blood flow if the decrease in blood pressure is due to vasorelaxation response improvement. In this study, the mean blood flow volume in the Acacia and placebo groups at 0w was largely different, which may have affected the mean blood flow volume after the intervention. The decline in blood flow rates is known to be involved in subjective symptoms such as coldness and stiff shoulders [33,34]. In future studies, a questionnaire pertaining to the symptoms related to blood flow will enable us to verify the effectiveness of the test food on subjective symptoms.

## CONCLUSIONS

After 12-week consumption of the acacia bark extract by healthy Japanese subjects with DBP  $\leq$  89 mmHg and SBP of 130 to 139 mmHg, the measured values and changes from baseline at 4w, 8w, and 12w of SSBP were significantly lower than those of the placebo group. Furthermore, “the ratio of the number of subjects whose SSBP < 130 mmHg and SDBP  $\leq$  89 mmHg at 12 w” after consumption of acacia bark extract was significantly higher than that of the placebo group. These findings suggest that acacia bark extract contributes to reducing the risk of coronary artery disease and death by achieving the target blood pressure for adults under 75 years as dictated in the “Guidelines for the management of hypertension, 2019” by The Japanese Society of Hypertension. Therefore, consumption of acacia bark extract is shown to attenuate blood pressure in people with high-normal blood pressure, lowering its levels to normal blood pressure (within the normal range), maintaining healthy blood pressure, and improving it to normal blood pressure. As a result, this method is extremely suitable for people who have relatively high levels of blood pressure or for people with increased anxiety levels regarding their blood pressure. In addition, the functional component that facilitated these findings is thought to be proanthocyanidins derived from acacia bark.

**List of abbreviations:** ACE: angiotensin-converting enzyme, ANCOVA: analysis of covariance, AT: angiotensin, DBP: diastolic blood pressure, NO: nitric oxide, OR: odds ratio, ROS: reactive oxygen species,

SBP: systolic blood pressure, SOD: superoxide dismutase

**Competing Interests:** The sponsor of this study, Acacia-No-Ki Co., Ltd., entrusted ORTHOMEDICO Inc. with conducting the study. Sosuke Ogawa is a member of Acacia-No-Ki Co., Ltd., and Asami Baba and Tomohiro Hoshino are employees of ORTHOMEDICO Inc. Tsuyoshi Takara (MD), the principal investigator of this study, is a staff of Medical Corporation Seishinkai, Takara Clinic, and he monitored all of the conditions of the subjects.

**Authors' Contributions:** Conceptualization, Asami Baba, Tomohiro Hoshino, Sosuke Ogawa, and Tsuyoshi Takara; Methodology, Asami Baba, Tomohiro Hoshino, and Sosuke Ogawa; Formal analysis, Asami Baba, Tomohiro Hoshino, and Tsuyoshi Takara; Investigation, Tsuyoshi Takara; Resources, Asami Baba, Tomohiro Hoshino, Sosuke Ogawa, and Tsuyoshi Takara; Writing – original draft, Asami Baba and Tomohiro Hoshino; Writing – review and editing, Asami Baba, Tomohiro Hoshino, and Tsuyoshi Takara; Supervision, Tsuyoshi Takara; Project administration, Tsuyoshi Takara

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