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Effects of the consumption of Acacia bark-derived proanthocyanidins on visceral fat: A randomized, double-blind, placebo-controlled, parallel-group comparative study

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ABSTRACT

Background: Obesity is an important risk factor for diabetes, kidney, liver, and cardiovascular diseases, and chronic obstructive pulmonary disease. Factors such as dietary choices, decreased physical activity due to urbanization and economic expansion, and increased nutritional consumption have made obesity a growing global issue. Acacia bark extract contains proanthocyanidins, which are believed to inhibit accumulation of fat and promote the metabolism of body fat.

Objective: This study was designed to investigate the effects of Acacia bark-derived proanthocyanidins on fat accumulation among healthy Japanese adults with a body mass index (BMI) of 25–30 kg/m^2.

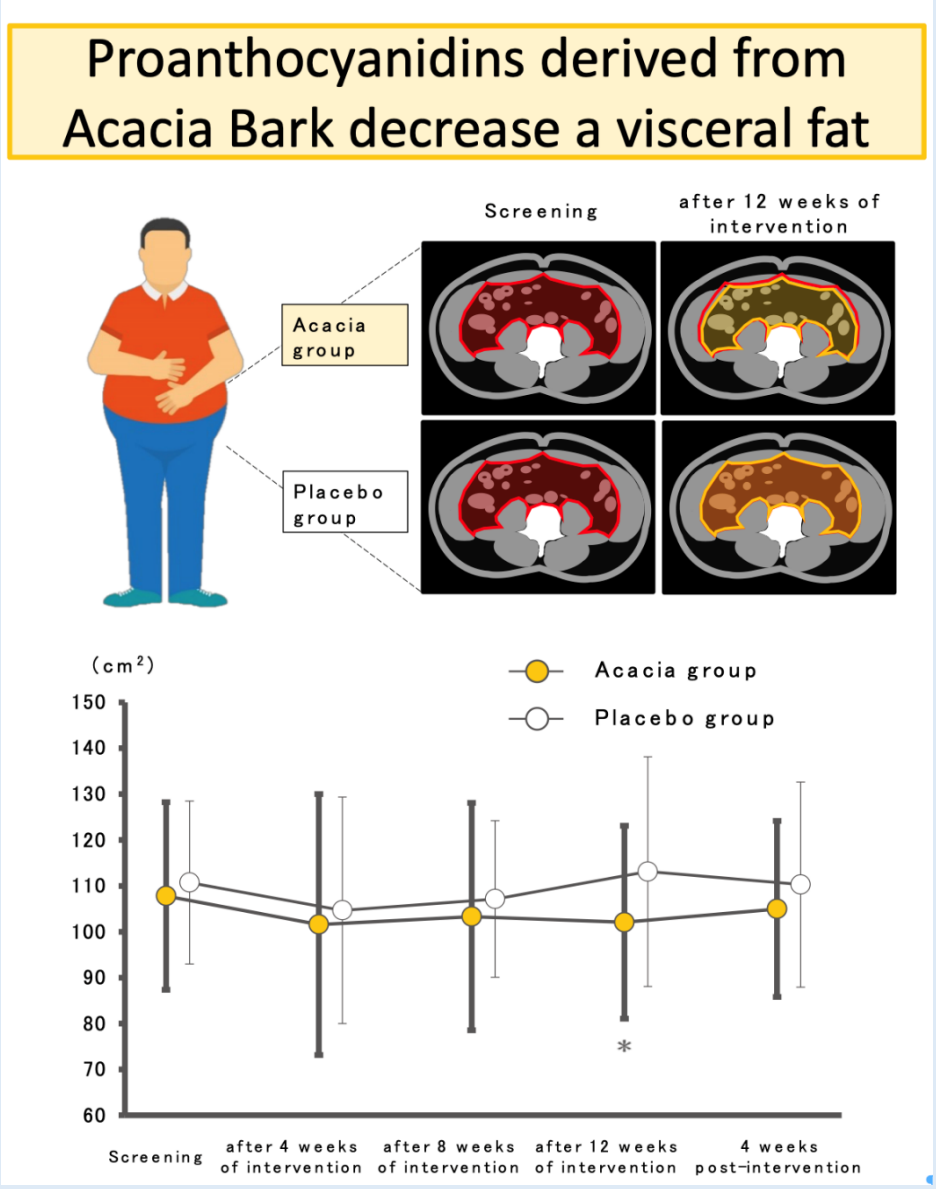
Methods: This study was performed as a randomized, placebo-controlled, double-blind, parallel-group study. Out of 199 participants who signed informed consent, 68 were randomized to two groups (n = 34/group) with a computer-generated allocation table. The participants took six tablets containing either Acacia bark-derived proanthocyanidins (Acacia group) or placebo (Placebo group) daily for 12 weeks. Their abdominal total, subcutaneous, and visceral fat areas via X-ray computed tomography, body weight, BMI, fat and muscle mass, and waist, abdominal, and hip circumferences were measured.

Results: After accounting for dropouts, the Acacia and Placebo groups had 32 and 33 participants, respectively. At the end of the 12-week intervention, the Acacia group had significantly lower abdominal visceral fat area compared with the Placebo group.

Conclusions: Acacia proanthocyanidins were found to reduce visceral fat.

Keywords: Acacia bark extract, proanthocyanidins, polyphenol, visceral fat, obesity

Trial registration number: UMIN000050201



Graphical Abstract

INTRODUCTION

Obesity refers to excessive fat buildup in adipose tissue. In Japan, obesity is defined as a body mass index (BMI) ≥25 kg/m^2[1]. Being obese is a key hazard factor for diabetes; kidney, liver, and cardiovascular diseases; and chronic obstructive pulmonary disease [2–4]. Obesity is now becoming a global issue because of poor dietary choices, decreased physical activity, and increased nutritional consumption [5,6]. According to the World Health Organization, 43% of male and female older than

18 years with a BMI of ≥25 kg/m^2 are obese in 2022 [7]. In the 2019 National Health and Nutrition Survey, 27.2% of Japanese both sexes aged older than 20 with a BMI of ≥25 kg/m^2 are obese [8]. Large quantities of inflammatory cytokines (e.g., tumor necrosis factor-α (TNF-α) and interleukin-6) are produced by visceral adipose tissues [9], and the expression of these cytokines promotes skeletal muscle degradation [10]. Recently, several studies have found that increased visceral fat area is linked to lower skeletal muscle mass [11–13]. Notably, the loss of skeletal muscles is associated with decreased quality of life (QOL), basal metabolic rate, and bone mineral density and increased fracture risk [14]. In Japan, the leading causes of death include cardiac and cerebrovascular diseases, pneumonia, and renal failure [15], wherein obesity is a risk factor. Thus, it is important to reduce excess body fat accumulation to maintain the QOL and extend the healthy lifespan of the Japanese population.

Acacia bark extract is a hydrothermal extract that is derived from the bark of *Acacia mearnsii* De Wild.. Acacia bark is composed of various compounds, including monomers, dimers, trimers, and condensed polymers of the basic backbone of flavan-3-ols (i.e., gallocatechin and robinetinidol) [16,17]. Among them, polyphenols comprise approximately 80% of the extract [18,19]. The proanthocyanidins derived from grape seeds [20] and pine bark [21] contain a polymerized structure with two hydroxy groups each in the A and B rings; by contrast, the proanthocyanidins from Acacia bark also have a polymerized structure but with one and three hydroxy groups in the A and B rings, respectively [22,23]. Acacia bark extract prevented fat absorption in the body of mice by restricting lipase activity [24,25]. According to studies using mouse models of obesity and diabetes (KK-Ay), Acacia bark extract can (1) activate AMP kinase (a central regulator in lipid metabolism), (2) improve lipid metabolism by reducing fat synthesis and accumulation and promoting hepatic lipolysis, and (3) increase energy expenditure in skeletal muscle [26]. Therefore, Acacia bark-derived proanthocyanidins may suppress body fat accumulation and promote body fat metabolism and may be considered as functional food [27,28].

This study was designed to evaluate the anti-obesity effects of Acacia bark-derived proanthocyanidins on Japanese adults with a BMI of 25–30 kg/m^2 (class I obesity) without health problems.

METHODS

Study design: A 1:1 allocation ratio was used in this randomized, placebo-controlled, double-blind, parallel-group study. The study protocol had the approval of the Institutional Ethical Review Board at Medical Corporation Seishinkai, Takara Clinic (approval date: January 18, 2023, approval number: 2301-04148-0041-0C-TC). The protocol of this trial was registered in the University Hospital Medical Information Network Clinical Trial Registry (UMIN-CTR; trial registration number: UMIN000050201) in advance. Moreover, the study faithfully adhered to the tenets of the Declaration of Helsinki (revised version of 2013) and the Ethical Guidelines for Medical and Health Research Involving Human Subjects.

Eligibility criteria: The following selection criteria were established: (1) Japanese adults of both sexes, (2) BMI between 25 kg/m^2 and 30 kg/m^2, and (3) the top 68 individuals with the largest visceral fat area at screening examination (Scr) among those meeting criteria (1) and (2). The details of the exclusion criteria are registered in the UMIN-CTR (https://center6.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000057170).

The participants were gathered through GOTOROKU (https://www.go106.jp/), a volunteer recruitment site administered by ORTHOMEDICO Inc. (Tokyo, Japan). Those who wished to participate in this study were fully informed of the study's content via the network, and consent was obtained electronically. Meanwhile, those who were concerned with the lead or sponsor companies of the study were excluded. Medical Corporation Seishinkai, Takara Clinic (Tokyo, Japan) was the institution responsible for conducting this study, evaluating the obtained data and managing the participants' physical condition. Examinations were performed together with Nerima Medical Association, Minami-machi Clinic (Tokyo, Japan).

Intervention: The nutritional facts per daily intake for each test food are described in Table 1. The Acacia bark extract, one of the test food ingredients, was provided as described below: Acacia bark was crushed and extracted with boiling water, and the product was spray-dried. The product is rich in proanthocyanidins, commonly known as wattle tannins[25]. The Folin–Ciocalteu method (gallic acid equivalence method) was used for measuring the total amount of proanthocyanidins in the test food [27]. Moreover, the concentration of robinetinidol-(4α,8)-catechin concentration of the tablet was analyzed by high-performance liquid chromatography in accordance with a previously described qualitative analysis method [29]. This study participants took six pieces per day of tablet containing Acacia bark-derived proanthocyanidins (Acacia group; dose: 245 mg/day) or placebo (Placebo group). The tables were consumed before meals with water and without chewing. The intervention period was 12 weeks. The indistinguishable appearance of the two tablets was confirmed by the ethics committee. The Acacia bark-derived proanthocyanidins were produced by Acacia-No-Ki Co., Ltd. (Hiroshima, Japan), and the intervention foods were provided by Acacia-No-Ki Co., Ltd.

Outcomes: The study schedule is shown in Table 2. Assessments for each item were performed at Scr and after 4, 8, and 12 weeks of intervention. Moreover, an additional post-intervention examination was conducted at 4 weeks after completing the 12-week intervention.

This study's primary outcome was the measured value of abdominal visceral fat area at 12 weeks. The secondary outcomes included the abdominal visceral fat area; total abdominal fat area; abdominal subcutaneous fat area; body weight; BMI; body fat percentage; fat and muscle mass; and waist, abdominal, and hip circumferences at 4, 8, and 12 weeks and at 4 weeks post-intervention.

(1) Measurement of fat areas: The subcutaneous, visceral, and total fat areas of the cross-sectional area of the umbilical region were determined via X-ray computed tomography (NAEOTOM Alpha, Siemens, Munich, Germany).

(2) Physical examination: Body weight, BMI, body fat percentage, and fat and muscle mass were evaluated using a multi-frequency body composition monitor (MC-780A-N, TANITA Corporation, Tokyo, Japan). The waist, abdominal, and hip circumferences were measured using a cloth measuring tape.

Table 1. Nutritional facts per daily intake (6 tablets)

	Acacia bark-derived proanthocyanidins tablets		Placebo tablets	
Water	0.0630	mg	0.0504	mg
Proteins	0.0522	mg	0.0468	mg
Fat	0.0558	mg	0.0540	mg
Ash	0.0198	mg	0.0126	mg
Carbohydrates	1.6092	mg	1.6362	mg
Calories	7.1460	mg	7.2180	mg
Sodium	0.3492	mg	0.1548	mg
Salt equivalents	0.0009	mg	0.0004	mg
Proanthocyanidins	0.1980	mg	ND	
Proanthocyanidins derived from Acacia bark	0.2694	mg	ND	

ND, not detectable

(3) Confirmation of adverse events and safety items: The safety assessment included physical measurements, urinalysis, and blood tests. The percentage of participants with previously normal urinalysis and blood test data at Scr who developed abnormalities after the intervention was observed. The group and individual data of these safety assessments were also reviewed for health management.

The physical measurements included body weight, BMI, and systolic and diastolic blood pressure. BMI was

calculated using the height measured at Scr. Body weight was evaluated using the multi-frequency body composition monitor (MC-780A-N, TANITA Corporation). Systolic and diastolic blood pressures were measured using an automatic blood pressure measuring device (Omron HEM-6022, OMRON Corporation, Kyoto, Japan).

In urinalysis, protein, glucose, pH, and occult blood were measured. LSI Medience Corporation (Tokyo, Japan), the clinical inspection company, was entrusted with the measurement of each item.

The following items were evaluated in the blood tests: white and red blood cell counts, hemoglobin, hematocrit, platelet count, aspartate and alanine aminotransferases, γ-glutamyl transpeptidase, total bilirubin, total protein, urea nitrogen, creatinine, uric acid, sodium, potassium, chloride, serum amylase, total cholesterol, high-density and low-density lipoprotein-cholesterols, triglyceride, glucose, and hemoglobin A1c (NGSP). The standard method of LSI Medience Corporation was used to measure each item.

A count of the number of adverse events was performed. In such cases, the physician was instructed to immediately take the necessary and appropriate measures. They also decided whether the participant could continue in the study or not, and whether the emergency key should be opened. Furthermore, the physician was instructed to assess and report the association between the adverse event and the intervention.

At each examination, surveys and a dietary assessment using the Calorie and Nutrition Diary [30] were administered to determine the health conditions of study participants. The survey was conducted three days prior to each visit. Study participants were also required to keep a logbook of their daily living status, including test food consumption, physical condition changes, and medication consumption.

Sample size: Considering that no study has evaluated abdominal visceral fat area after the consumption of Acacia bark-derived proanthocyanidins, we anticipated a large difference between the two groups and used d = 0.80 according to the suggestion of Cohen [31]. The level of statistical significance (α) and statistical power (1-β) were determined to be 0.05 and 0.80, respectively. At least 52 participants (26 in each group) were needed, and the sample size was set at 68 (34 in each group) in expectation of withdrawal and out-of-compliance during the period of the study.

Selection, randomization, and blinding: Out of the 199 participants who agreed to be part of this study, 68 were selected by the principal physician and included. The test foods were supplied to the contract clinical trial organization (CRO) by this study's sponsor (Acacia-No-Ki Co., Ltd.). The shipping staff of the CRO, who was responsible for sending the appropriate study food to the participants, confirmed that the study foods were indistinguishable, entered and confirmed the Scr data, and provided the identifier to the allocator, who did not have direct involvement in the study.

The study participants who conformed to the eligibility criteria were randomized to either the Acacia or Placebo groups on a 1:1 basis using a computer-generated allocation table (34 participants per group) supplied by the allocator. The allocation table was generated by stratified block random assignment using SPSS version 23 (IBM Japan, Ltd.; Tokyo, Japan), with sex (male and female) as the stratification factor. This table was only used by the shipping staff of the CRO. All of the physicians, study site administrators, monitoring personnel, statistical analysts, ethics committee constituents, and clinical laboratories were blinded to the allocation. Until the statistical analysis methods were fixed, the allocation table was placed under seal and kept by the allocator.

Table 2. Schedule of enrolment, interventions, and assessments

		Briefing session	Scr	Registration	Allocation	Study periods				
						Start intervention	4 weeks	8 weeks	12 weeks	4 weeks post-intervention
Enrollment	Eligibility screen	X		X						
	Informed consent (include other procedures)	X								
	Allocation				X					
Interventions	Acacia bark-derived proanthocyanidins tablets					◆	—	—	◆	
	Placebo tablets					◆	—	—	◆	
Assessments	X-ray CT scan (visceral fat area, subcutaneous fat area, and total fat area)		X				X	X	X	X
	Circumference of abdominal, waist, and hip		X				X	X	X	X
	Physical examination		X				X	X	X	X
	Urinalysis		X				X	X	X	X
	Blood test		X				X	X	X	X
	Interview (usually medical)		X				X	X	X	X
	Dietary Survey*		X				X	X	X	X
	Logbook					◆	—	—	—	◆

Scr, screening examination; 4, 8, and 12 weeks, after 4, 8, and 12 weeks of intervention; 4 weeks post-intervention, additional post-intervention examination done 4 weeks after the end of the 12-week intervention.

*For the dietary survey, data was obtained for the three days prior to each examination date.

Statistical analysis: All statistical analyses were performed using the two-tailed method with a 5% level of significance by SPSS version 23 (IBM Japan, Ltd.). The primary outcome was a between-group comparison of the visceral fat area at 12 weeks, and no adjustments were made for multiple items or time points in the secondary outcomes.

The characteristics of participants were demographically aggregated for inclusion and analysis of participants. Sex was represented as the number of participants and percentage within the group. Age, height, body weight, BMI, and systolic and diastolic blood pressure were indicated as the mean and standard deviation (SD), minimum (Min), and maximum (Max).

The primary outcome, secondary outcomes, and safety assessment items were reported as the mean and SD for measured values. Values at Scr were considered as the baseline and compared across groups with Welch's *t*-test. For between-group comparisons of measures of the primary and secondary outcomes at other timepoints (4, 8, and 12 weeks, and 4 weeks post-intervention), a linear mixed model with baseline values as covariates was used. The between-group differences (Acacia group minus Placebo group) and their 95% confidence intervals (CIs) were also shown. Between-group differences were represented at Scr and post-intervention as the mean and estimated marginal mean (EMM), respectively.

For safety evaluation, the adverse event frequency in each group was calculated, and the 95% CIs for the occurrence of adverse events in each group and the deviation of the occurrence between groups were calculated. The chi-square test was used to provide a measure of the adverse event frequency in each group and the percentage of participants who developed abnormal urinalysis and peripheral blood test results after the intervention.

Welch's *t*-test was used to compare dietary survey data, which were indicated as the mean, SD, median (Med), Min, and Max, with differences between groups shown as the mean difference (Acacia group minus Placebo group) and 95% CI.

The pharmaceutical guideline (ICH-E9 [32]) was used to define the datasets in this study.

RESULTS

Participant flow and characteristics: Figure 1 shows a flowchart of the study participants. Recruitment of the participants was conducted between January 31 and February 24, 2023, and the study period was from February 4 to October 1, 2023.

All participants received their assigned intervention. Notably, three participants did not undergo any evaluations after Scr (Acacia group, n = 2; Placebo group, n = 1) and were excluded from the analysis because they did not receive any intervention after assignment. One participant was unable to undergo evaluation at 4 weeks. The efficacy and safety analysis datasets for this study included the full analysis set and safety analysis population, both of which included 65 participants (Acacia group, n = 32; Placebo group, n = 33). Table 3 shows the patient characteristics in each analysis dataset, including intention to treat.

Fat areas in the transverse section of the umbilical region: No significant intergroup differences in terms of visceral, subcutaneous, and total fat areas were observed at Scr. The primary outcome (visceral fat area at 12 weeks) was significantly lower in the Acacia group than in the Placebo group (EMM ± standard error [SE]: 103.5 ± 2.8 vs. 111.6 ± 2.8 cm^2; between-group difference [95%CI], −8.1 cm^2 [−15.9, −0.3]; P = 0.041; Figure 2 and Table 4). Conversely, the Acacia group had a significantly higher subcutaneous fat area at 12 weeks and at 4 weeks post-intervention compared with the Placebo group (EMM ± SE: 12 weeks, 264.1 ± 4.1 vs. 246.8 ± 4.1 cm^2; between-group difference [95%CI], 17.3 cm^2 [5.8, 28.9]; P = 0.004; 4 weeks post-intervention, 262.7 ± 4.1 vs. 248.5 ± 4.1 cm^2; between-group difference [95%CI], 14.3 cm^2 [2.7, 25.8]; P = 0.016; Figure 2 and Table 4). Total fat area was not significantly different between

groups.

Body composition and abdominal, waist, and hip circumferences: No significant intergroup difference in body composition and abdominal, waist, and hip circumferences was observed at all time points (Table 5).

Confirmation of adverse events and safety items: Three participants in the Acacia group experienced adverse events. One participant had toothache, cough, colds, stomach pain, and back pain; another participant had common colds; and the last participant had periodontal inflammation. Meanwhile, three participants in the Placebo group also reported adverse events. One participant had common colds, another participant had dysmenorrhea, and the last participant had dizziness. All symptoms were deemed by the study physician as not causally related to the food intervention in the study.

With regard to urinalysis and blood test data, significant intergroup differences were observed for urinary protein, total protein, total cholesterol, and low-density lipoprotein-cholesterol (Supplementary Table 1). Considering that total cholesterol and low-density lipoprotein-cholesterol fluctuated outside the reference values after the intervention in the Acacia group, each blood test was reviewed by the study physician on a group basis (Supplementary Table 4), and the fluctuations were deemed as not medically significant (Supplementary Table 4). Moreover, the study physician reviewed the safety assessment items (physical examination, urinalysis, and blood tests) on a group (Supplementary Tables 2–4) and individual basis (data not shown). No medically relevant changes related to test food consumption were observed.

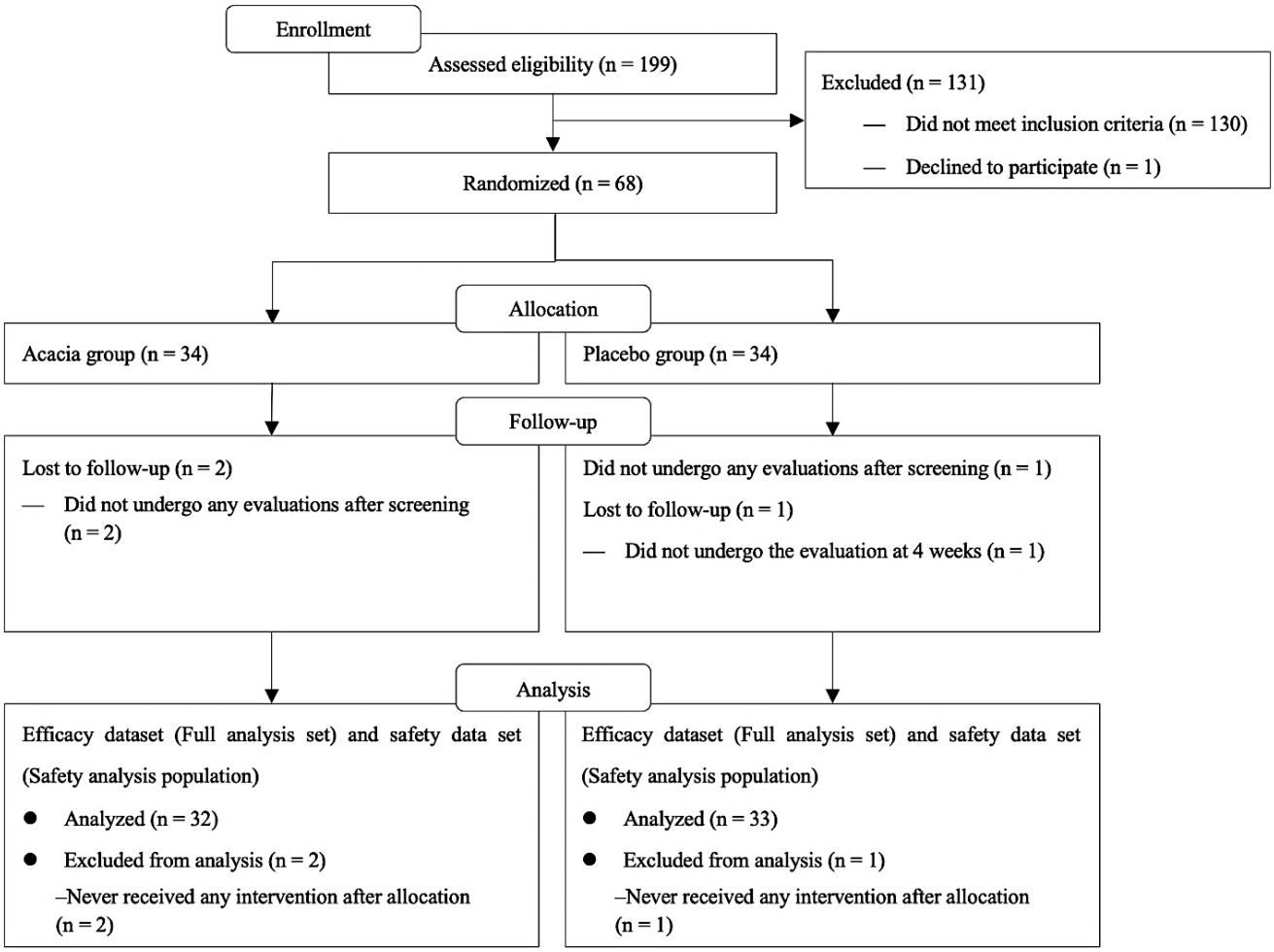


Figure 1. Flow diagram

Table 3. Participant characteristics

		ITT		FAS, SAF	
		Acacia group (n = 34)	Placebo group (n = 34)	Acacia group (n = 32)	Placebo group (n = 33)
Sex	Male	17 (50.0%)	17 (50.0%)	17 (53.1%)	17 (51.5%)
	Female	17 (50.0%)	17 (50.0%)	15 (46.9%)	16 (48.5%)
Age (years)	Mean (SD)	49.3 (8.9)	49.6 (10.4)	49.1 (8.8)	49.8 (10.5)
	Min–Max	31–67	29–74	31–67	29–74
Height (cm)	Mean (SD)	163.3 (7.2)	163.2 (6.7)	163.6 (7.3)	163.2 (6.8)
	Min–Max	149.0–179.5	151.2–174.3	149.0–179.5	151.2–174.3
Body weight (kg)	Mean (SD)	72.1 (6.7)	72.8 (7.9)	72.3 (6.8)	72.9 (8.0)
	Min–Max	58.8–88.2	57.1–87.9	58.8–88.2	57.1–87.9
Body mass index (kg/m^2)	Mean (SD)	27.0 (1.5)	27.3 (1.4)	27.0 (1.6)	27.3 (1.4)
	Min–Max	25.0–29.8	25.0–29.6	25.0–29.8	25.0–29.6
Systolic blood pressure (mmHg)	Mean (SD)	132.3 (19.7)	125.4 (16.9)	132.4 (20.3)	125.6 (17.2)
	Min–Max	101–201	96–177	101–201	96–177
Diastolic blood pressure (mmHg)	Mean (SD)	86.6 (14.4)	82.3 (13.5)	86.7 (14.8)	82.5 (13.6)
	Min–Max	62–126	59–116	62–126	59–116

Sex is indicated by the number of participants and their percentage in the group. Other items are indicated by mean, standard deviation (SD), minimum (Min), and maximum (Max).

ITT, intention to treat; FAS, full analysis set; SAF, safety analysis population

Table 4. Visceral fat area, subcutaneous fat area, and total fat area in transverse section of umbilical region

		Acacia group			Placebo group			Group difference [95%CI]	P value
		n	Mean (EMM)	SD [95%CI]	n	Mean (EMM)	SD [95%CI]		
Total fat area (cm^2)	**Scr**	32	344.8	72.2	33	365.6	62.0	–20.8 [–54.2, 12.6]	0.218
	4 weeks	32	348.6 (358.5)	70.4 [348.9, 368.0]	32	367.8 (358.8)	66.1 [349.4, 368.3]	–0.4 [–13.9, 13.2]	0.959
	8 weeks	32	350.7 (360.1)	68.8 [350.6, 369.7]	33	364.8 (355.5)	63.1 [346.1, 364.9]	4.6 [–8.9, 18.1]	0.500
	12 weeks	32	357.7 (367.0)	68.5 [357.4, 376.6]	33	368.3 (359.1)	64.0 [349.7, 368.5]	7.9 [–5.6, 21.4]	0.248
	4 weeks post-intervention	32	359.2 (369.1)	71.2 [359.5, 378.7]	33	367.1 (357.3)	67.2 [347.9, 366.7]	11.8 [–1.7, 25.3]	0.087
Visceral fat area (cm^2)	**Scr**	32	107.8	20.4	33	110.7	17.8	–2.9 [–12.4, 6.6]	0.542
	4 weeks	32	101.6 (102.8)	28.5 [97.3, 108.3]	32	104.7 (103.7)	24.7 [98.2, 109.2]	–0.9 [–8.7, 6.9]	0.821
	8 weeks	32	103.3 (104.5)	24.8 [99.0, 110.1]	33	107.1 (105.9)	17.1 [100.4, 111.3]	–1.3 [–9.1, 6.4]	0.736
	12 weeks	32	102.1 (103.5)	21.0 [98.0, 109.0]	33	113.1 (111.6)	25.0 [106.2, 117.1]	–8.1 [–15.9, –0.3]	0.041*
	4 weeks post-intervention	32	105.0 (106.1)	19.2 [100.6, 111.6]	33	110.3 (109.1)	22.4 [103.7, 114.5]	–3.0 [–10.8, 4.8]	0.449

Table 4. Visceral fat area, subcutaneous fat area, and total fat area in transverse section of umbilical region (Continued)

		Acacia group			Placebo group			Group difference [95%CI]	P value
		n	Mean (EMM)	SD [95%CI]	n	Mean (EMM)	SD [95%CI]		
Subcutaneous fat area (cm^2)	**Scr**	32	237.0	70.0	33	254.9	67.2	–17.9	0.297
								[–51.9, 16.1]	
	4 weeks	32	247.0	69.5	32	263.1	77.0	1.6	0.791
			(256.2)	[248.0, 264.3]		(254.6)	[246.5, 262.7]	[–10.0, 13.2]	
	8 weeks	32	247.4	68.4	33	257.7	64.2	5.9	0.313
			(255.6)	[247.4, 263.8]		(249.7)	[241.6, 257.7]	[–5.6, 17.5]	
	12 weeks	32	255.6	69.0	33	255.1	68.7	17.3	0.004*
			(264.1)	[255.9, 272.3]		(246.8)	[238.7, 254.9]	[5.8, 28.9]	
	4 weeks post-intervention	32	254.2	71.1	33	256.8	65.9	14.3	0.016*
			(262.7)	[254.5, 270.9]		(248.5)	[240.4, 256.5]	[2.7, 25.8]	

Data are indicated as mean, standard deviation (SD), estimated marginal mean (EMM), 95% confidence interval (CI), and between-group difference and its 95% CI. Scr, screening examination; 4, 8, and 12 weeks, after 4, 8, and 12 weeks of intervention; 4 weeks post-intervention, additional post-intervention examination done 4 weeks after the end of the 12-week intervention. *$P < 0.05$

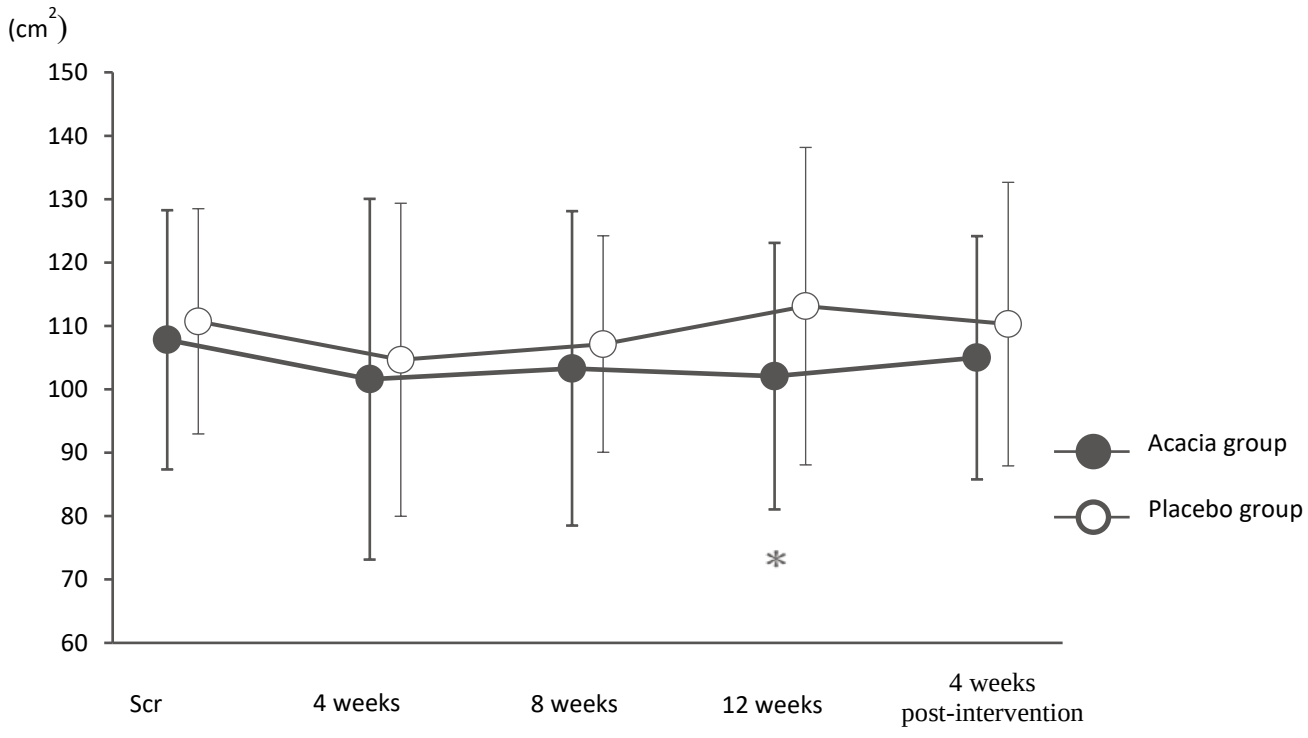


Figure 2. Changes in visceral fat area in transverse section of umbilical region

Data are presented as mean, standard deviation (SD). Scr, screening examination; 4, 8, and 12 weeks, after 4, 8, and 12 weeks of intervention; 4 weeks post-intervention, additional post-intervention examination done 4 weeks after the end of the 12-week intervention. *$P < 0.05$

Table 5. Body composition and abdominal, waist, and hip circumferences.

		Acacia group			Placebo group			Group difference [95%CI]	P value
		n	Mean (EMM)	SD [95%CI]	n	Mean (EMM)	SD [95%CI]		
Body weight (kg)	Scr	32	72.3	6.8	33	72.9	8.0	–0.6 [–4.3, 3.1]	0.739
	4 weeks	32	72.2 (72.6)	7.2 [72.0, 73.2]	32	72.7 (72.1)	8.8 [71.5, 72.8]	0.4 [–0.4, 1.3]	0.330
	8 weeks	32	72.0 (72.3)	7.2 [71.7, 72.9]	33	72.0 (71.7)	8.5 [71.1, 72.3]	0.6 [–0.3, 1.4]	0.195
	12 weeks	32	72.3 (72.6)	7.3 [72.0, 73.3]	33	72.1 (71.8)	8.7 [71.2, 72.4]	0.8 [–0.1, 1.7]	0.072
	4 weeks post–intervention	32	72.3 (72.6)	6.9 [72.0, 73.3]	33	72.1 (71.9)	8.8 [71.2, 72.5]	0.8 [–0.1, 1.6]	0.083
Body mass index (kg/m^2)	Scr	32	27.0	1.6	33	27.3	1.4	–0.3 [–1.1, 0.4]	0.381
	4 weeks	32	27.0 (27.1)	1.6 [26.9, 27.4]	32	27.2 (27.0)	1.8 [26.7, 27.2]	0.2 [–0.2, 0.5]	0.305
	8 weeks	32	26.8 (27.0)	1.5 [26.8, 27.2]	33	27.0 (26.8)	1.7 [26.6, 27.0]	0.2 [–0.1, 0.5]	0.250
	12 weeks	32	27.0 (27.1)	1.5 [26.9, 27.4]	33	27.0 (26.8)	1.7 [26.6, 27.1]	0.3 [–0.1, 0.6]	0.102
	4 weeks post–intervention	32	27.0 (27.1)	1.4 [26.9, 27.4]	33	27.0 (26.8)	1.8 [26.6, 27.1]	0.3 [0.0, 0.6]	0.092

Table 5. Body composition and abdominal, waist, and hip circumferences (Continued)

		Acacia group			Placebo group			Group difference [95%CI]	P value
		n	Mean (EMM)	SD [95%CI]	n	Mean (EMM)	SD [95%CI]		
Body fat percentage (%)	Scr	32	31.6	7.7	33	33.0	7.6	–1.4 [–5.2, 2.4]	0.453
	4 weeks	32	31.6 (32.3)	8.0 [31.4, 33.2]	32	32.3 (31.7)	7.2 [30.8, 32.7]	0.5 [–0.8, 1.9]	0.407
	8 weeks	32	31.3 (31.9)	7.5 [31.0, 32.8]	33	32.2 (31.6)	7.2 [30.7, 32.5]	0.3 [–1.0, 1.6]	0.617
	12 weeks	32	31.6 (32.3)	7.7 [31.3, 33.2]	33	32.3 (31.6)	7.1 [30.7, 32.5]	0.7 [–0.7, 2.0]	0.323
	4 weeks post-intervention	32	31.8 (32.4)	7.8 [31.5, 33.4]	33	32.4 (31.7)	7.0 [30.8, 32.6]	0.7 [–0.6, 2.0]	0.271
Fat mass (kg)	Scr	32	22.6	4.9	33	23.9	5.3	–1.3 [–3.8, 1.2]	0.303
	4 weeks	32	22.6 (23.2)	5.2 [22.4, 24.0]	32	23.2 (22.7)	5.2 [21.9, 23.5]	0.5 [–0.6, 1.7]	0.372
	8 weeks	32	22.3 (22.9)	4.8 [22.0, 23.7]	33	23.1 (22.5)	5.2 [21.7, 23.3]	0.4 [–0.8, 1.5]	0.536
	12 weeks	32	22.6 (23.2)	5.0 [22.4, 24.0]	33	23.1 (22.5)	5.1 [21.7, 23.3]	0.7 [–0.4, 1.8]	0.225
	4 weeks post-intervention	32	22.8 (23.4)	5.2 [22.6, 24.2]	33	23.2 (22.6)	5.2 [21.8, 23.4]	0.8 [–0.4, 1.9]	0.191

Table 5. Body composition and abdominal, waist, and hip circumferences (Continued)

		Acacia group			Placebo group			Group difference [95%CI]	P value
		n	Mean (EMM)	SD [95%CI]	n	Mean (EMM)	SD [95%CI]		
Muscle mass (kg)	Scr	32	46.9	8.5	33	46.3	8.6	0.6 [–3.6, 4.8]	0.770
	4 weeks	32	46.9 (46.6)	8.7 [46.0, 47.2]	32	46.7 (46.8)	8.7 [46.3, 47.4]	–0.2 [–1.0, 0.6]	0.596
	8 weeks	32	47.0 (46.7)	8.5 [46.1, 47.2]	33	46.3 (46.6)	8.4 [46.1, 47.1]	0.1 [–0.7, 0.9]	0.833
	12 weeks	32	46.9 (46.6)	8.5 [46.1, 47.2]	33	46.3 (46.7)	8.6 [46.1, 47.2]	–0.1 [–0.8, 0.7]	0.887
	4 weeks post-intervention	32	46.8 (46.5)	8.3 [46.0, 47.1]	33	46.3 (46.6)	8.4 [46.0, 47.1]	–0.1 [–0.8, 0.7]	0.867
Waist circumference (cm)	Scr	32	88.0	5.2	33	89.2	4.3	–1.2 [–3.6, 1.1]	0.297
	4 weeks	32	88.2 (88.7)	5.1 [87.6, 89.8]	32	90.1 (89.5)	4.9 [88.4, 90.6]	–0.8 [–2.3, 0.8]	0.315
	8 weeks	32	88.1 (88.6)	5.4 [87.5, 89.7]	33	88.7 (88.2)	4.9 [87.1, 89.3]	0.4 [–1.1, 1.9]	0.611
	12 weeks	32	87.9 (88.4)	5.1 [87.4, 89.5]	33	88.2 (87.7)	5.1 [86.6, 88.7]	0.8 [–0.7, 2.3]	0.312
	4 weeks post-intervention	32	88.2 (88.7)	4.6 [87.6, 89.8]	33	89.1 (88.6)	5.4 [87.5, 89.7]	0.1 [–1.4, 1.6]	0.901

Table 5. Body composition and abdominal, waist, and hip circumferences (Continued)

		Acacia group			Placebo group			Group difference [95%CI]	P value
		n	Mean (EMM)	SD [95%CI]	n	Mean (EMM)	SD [95%CI]		
Abdominal circumference (cm)	Scr	32	94.4	5.3	33	95.9	4.3	–1.5 [–3.9, 0.9]	0.220
	4 weeks	32	95.3 (95.9)	4.4 [94.7, 97.2]	32	95.3 (94.7)	6.0 [93.5, 95.9]	1.2 [–0.5, 2.9]	0.170
	8 weeks	32	95.2 (95.7)	5.2 [94.5, 96.9]	33	96.3 (95.8)	4.0 [94.6, 97.0]	–0.1 [–1.8, 1.6]	0.933
	12 weeks	32	95.4 (95.9)	4.8 [94.7, 97.1]	33	95.2 (94.7)	4.8 [93.5, 95.8]	1.3 [–0.4, 3.0]	0.143
	4 weeks post-intervention	32	95.4 (95.9)	5.9 [94.7, 97.1]	33	95.4 (94.9)	4.0 [93.7, 96.1]	1.0 [–0.7, 2.8]	0.229
Hip circumference (cm)	Scr	32	99.6	4.5	33	99.8	4.0	–0.2 [–2.3, 2.0]	0.874
	4 weeks	32	99.7 (99.8)	4.6 [99.1, 100.5]	32	99.5 (99.4)	4.0 [98.7, 100.1]	0.4 [–0.6, 1.4]	0.474
	8 weeks	32	99.8 (99.8)	4.7 [99.1, 100.6]	33	99.4 (99.3)	4.4 [98.6, 100.0]	0.6 [–0.5, 1.6]	0.280
	12 weeks	32	99.5 (99.6)	4.6 [98.9, 100.3]	33	99.4 (99.3)	4.1 [98.6, 100.0]	0.3 [–0.7, 1.3]	0.586
	4 weeks post-intervention	32	99.2 (99.3)	4.5 [98.6, 100.0]	33	99.1 (99.0)	4.1 [98.3, 99.7]	0.2 [–0.8, 1.3]	0.629

Data are presented as mean, standard deviation (SD), estimated marginal mean (EMM), 95% confidence interval (CI), and between–group difference and its 95% CI. Scr, screening examination; 4, 8, and 12 weeks, after 4, 8, and 12 weeks of intervention; 4 weeks post–intervention, additional post–intervention examination done 4 weeks after the end of the 12–week intervention.

DISCUSSION

This study aimed to determine whether Acacia bark-derived proanthocyanidins can reduce the visceral fat area in Japanese adults with a BMI of 25−30 kg/m^2 and a relatively large baseline visceral fat area.

The Japan Society for the Study of Obesity defines visceral adiposity as having a visceral fat area of ≥100 cm^2 on an abdominal computed tomography scan [1]. In the present study, the selection criteria included participants with a BMI of 25−30 kg/m^2 and a large visceral fat area. The mean visceral fat area decreased from 107.8 cm^2 at Scr to 102.1 cm^2 at 12 weeks in the Acacia group, whereas it increased from 110.7 to 113.1 cm^2 in the Placebo group (EMM group difference: −8.1 cm^2). Diet and lifestyle have been thought to influence fat accumulation [33-34]. Previous studies have reported that (1) dietary components (e.g., soluble dietary fiber, minerals, and vitamins) decrease the visceral fat area [33], (2) monounsaturated fatty acids increase the visceral fat area [33], and (3) physical activity decreases the visceral fat area [34]. In the present study, dietary assessments conducted three days before each examination period revealed no significant intergroup differences regarding the aforementioned nutrients (Supplementary Table 5). With regard to physical activity, the participants were asked by the physician to avoid binge eating and drinking, as well as retain their usual lifestyle. Taking these factors into account, the effect of diet and lifestyle changes on visceral fat area in the participants was considerably small. Therefore, the significantly lower abdominal visceral fat area in the Acacia group than in the Placebo group was considered as a visceral fat reduction effect caused by proanthocyanidins. Furthermore, the abdominal visceral fat area approached 100 cm^2, which is the cutoff value proposed by the Japan Society for the Study of Obesity [1]. Asian people, including the Japanese, are more prone to visceral fat accumulation than other races [35]. A cohort study of Japanese Americans found that visceral fat, along with BMI and waist circumference, was positively associated with type 2 diabetes risk, but not subcutaneous fat in the abdomen and thighs [36]. This finding suggests the importance of reducing visceral fat rather than subcutaneous fat to lower the risk of obesity complications. Thus, the consumption of Acacia bark-derived proanthocyanidins may contribute to risk reduction of obesity-related diseases in the future.

Previous *in vitro* and *in vivo* studies suggest that the following mechanisms may be responsible for the visceral fat area reduction effect of Acacia bark-derived proanthocyanidins: (1) inhibitory activities of lipase, glucosidase, and α-amylase, (2) increased expression of heat production–related genes in the skeletal muscle and liver, (3) suppressed synthesis and uptake of fat in the liver, and (4) suppression of TNF-α secretion and enhancement of adiponectin secretion [24–26,37]. Stomach acid degrades procyanidins to epicatechins and catechins, which are then absorbed through the small intestine [38]. Catechins exert an anti-obesity effect by inhibiting digestive activity and macronutrient absorption in the digestive tract; suppressing anabolism and promoting catabolism in the liver, muscle, and adipose tissue; and inhibiting glycogenesis, synthesis of fatty acid, insulation, and heterotopic fat deposition in the muscle and liver [39]. In fact, previous studies in healthy humans have reported that the ingestion of catechins resulted in significant reductions in body weight and waist circumference [40], besides significant increases in energy expenditure [41]. Therefore, catechins, which are metabolites of procyanidins in Acacia bark, may contribute to the molecular mechanism behind the anti-obesity effect.

Conversely, visceral fat reduction could also be caused by changes in intestinal microbiota after consuming proanthocyanidins. The relationship between obesity and gut microbiota has been demonstrated in

many studies [42–44]. A previous study investigated the intestinal microbiota across healthy Japanese subjects stratified by BMI (lean, BMI < 18.5; normal, 18.5 ≤ BMI < 25; obese, BMI ≥ 25) and found that obese subjects had different microbiota compared to those with lean and normal BMI [45]. In addition, Acacia bark-derived proanthocyanidins were also reported to modify the intestinal microbiota in the feces of dermatitis mouse models [46]. Notably, intestinal microflora is reportedly involved in the host's lipid metabolism [47,48]. Therefore, the consumption of Acacia bark-derived proanthocyanidins could have changed the intestinal microflora and enhanced lipid metabolism, thereby reducing visceral fat.

Visceral and subcutaneous fat are aerobically metabolized by being broken down into free fatty acids [49]. Although visceral fat reportedly has a higher capacity to release free fatty acids than subcutaneous fat [50], they have identical breakdown processes. Visceral and subcutaneous fat are collectively referred to as body fat, which increases because of the combined effects of excess energy intake and timing of food intake, as well as increased fat synthesis [51]. Moreover, there is no difference in the accumulation process of visceral and subcutaneous fat. As previously reported, Acacia bark-derived proanthocyanidins can reduce fat synthesis and uptake in the liver [26]. Therefore, we hypothesized that Acacia bark-derived proanthocyanidins could also have an anti-obesity effect by suppressing visceral and subcutaneous fat accumulation. However, the subcutaneous fat area at 12 weeks and 4 weeks post-intervention was significantly lower in the Placebo group than in the Acacia group. Furthermore, we could not confirm the effect of Acacia bark-derived proanthocyanidins on subcutaneous fat area because of variations in subcutaneous fat area at Scr. Notably, the selection criteria and allocation of this study did not consider the abdominal subcutaneous fat area. A previous study reported a weak correlation between visceral and subcutaneous fat areas [52]. Thus, it may be possible that some participants in this study had larger abdominal visceral fat area but smaller abdominal subcutaneous fat area. In fact, the maximum and minimum abdominal subcutaneous fat areas at Scr were 399.6 and 115.0 cm^2, respectively, whereas the SD of the abdominal visceral fat area was 20 cm^2. Thus, the anti-obesity effect of Acacia bark-derived proanthocyanidins was not observed in the abdominal subcutaneous fat area, possibly because of variations in the abdominal subcutaneous fat area at Scr.

The secondary outcomes in this study (i.e., weight, BMI, and waist circumference) have been reported to be strongly associated with visceral and subcutaneous fat areas [53–55]. Although these parameters displayed no changes after the intervention, the data from each study showed that the values of the correlated items differed across individuals, even among those with similar levels of visceral and subcutaneous fat areas. This finding suggests that these parameters were not necessarily reduced in conjunction with significant reductions in visceral or subcutaneous fat area. With regard to the body fat percentage, the results were hypothesized to be linked to a significant reduction in visceral or subcutaneous fat area, since the bioelectrical impedance analysis method reflects total body fat [56]. However, no differences were found between groups in the present study, because the bioelectrical impedance analysis method is affected by the amount of water in the body due to its principle [56]. Therefore, the effect of the Acacia bark-derived proanthocyanidins on body fat percentage could not be confirmed due to the effect of the amount of water. Nevertheless, the primary outcome in this study (i.e., abdominal visceral fat area) was significantly smaller in the Acacia group than in the Placebo group at 12 weeks, and the abdominal visceral fat area increased at 4 weeks post-intervention. Our

results suggest that Acacia bark-derived proanthocyanidins have an anti-obesity effect on healthy Japanese with a BMI between 25 kg/m^2 and 30 kg/m^2.

This study has two limitations. One is the lack of consideration of hypertension risk and gender effects. Although abdominal subcutaneous fat area has no formal reference value, increased subcutaneous fat increases the risk of the occurrence of diabetes and metabolic syndrome [57–59], and subcutaneous and visceral fat are important risk factors of obesity-associated diseases, including hypertension [60]. In fact, the risk of developing hypertension among obese individuals has increased by 1.5-fold from 1980 to 2010 [61]. Thus, the risk of hypertension should be considered in obesity management. In a study of Japanese patients (1,046 males and 853 females) attempting to determine cutoff values for abdominal subcutaneous fat area based on hypertension prevalence, cutoff values of 114.7 and 169.3 cm^2 for subcutaneous fat area for males and females, respectively, were suggested [62]. Sex differences have been described in body fat accumulation and reduction, with men and women more likely to accumulate visceral and subcutaneous fat, respectively [63]. In particular, visceral fat is more easily reduced than subcutaneous fat in both sexes, with men having a higher fat resolution than women [64]. Accordingly, one of our future tasks is to verify the anti-obesity effect of Acacia bark-derived proanthocyanidins after considering the effects of abdominal subcutaneous fat area and sex, particularly in light of the cutoff value calculated based on hypertension risk.

Another limitation is that we did not take into account the genetic background. Differences in visceral fat mass have been observed depending on the genetic polymorphism of *Uncoupling protein 1* (*UCP1*; [*−3826A/G*]), which involves the burning of visceral fat [65]. No studies reported associations between *UCP1* genetic polymorphisms and polyphenols and visceral fat, as far as we know. However, diets rich in polyphenols and anthocyanins have been shown to modulate the activity and/or expression of the high-density lipoprotein-related enzyme paraoxonase 1 (*PON1*) [66–69]. Another study determined the role of *PON1* as a susceptibility gene to high antioxidant intake on cardiovascular health and identified four independent polymorphic protective genotypes that were significantly associated with elevated high-density lipoprotein levels under high polyphenol and anthocyanin intake [70]. In a study that investigated the effects of fucoxanthin (a type of carotenoid that is not a polyphenol) on blood glucose levels in Japanese people, those with the *G/G* allele of *UCP1* had significantly lower blood glucose levels than those with *A/A* and *A/G* alleles [71]. In these studies, genetic polymorphisms can cause different high-density lipoprotein levels and different responses to blood glucose. Thus, the degree of visceral fat burning by consuming Acacia bark-derived proanthocyanidins can also vary in this study. However, genetic polymorphisms, including *UCP1* and β3 Adrenoceptor (*ADRB3*), which are involved in fat burning, were not included in this study. Thus, it remains unclear which gene polymorphisms were actually incorporated and strongly affected by Acacia bark-derived proanthocyanidins. Future research should investigate genetic polymorphisms to determine the attributes of individuals who strongly display the visceral fat–reducing effects of Acacia bark-derived proanthocyanidins.

CONCLUSIONS

This study evaluated the effect of Acacia bark-derived proanthocyanidins on fat accumulation among healthy Japanese adults with a BMI of 25–30 kg/m^2 and a large visceral fat area. The abdominal visceral fat area at 12

weeks was significantly reduced in the Acacia group than in the Placebo group. Therefore, the consumption of Acacia bark-derived proanthocyanidins at 375 mg/day decreased the abdominal visceral fat area, exerting an anti-obesity effect, and was considered safe for use in the conditions of the present study.

List of abbreviations: 4 weeks, after 4 weeks of intervention; 8 weeks, after 8 weeks of intervention; 12 weeks, after 12 weeks of intervention; 4 weeks post–intervention, additional post–intervention examination done 4 weeks after the end of the 12–week intervention; 95%CI, 95% confidence intervals; BMI, body mass index; CAND, Calorie and Nutrition Diary; CRO, contract clinical trial organization; EMM, estimated marginal mean; FAS, full analysis set; ITT, intention to treat; Max, maximum; Med, median; Min, minimum; ND, not detectable; QOL, quality of life; SAF, safety analysis population; Scr, screening examination; SD, standard deviation; UCP1, Uncoupling protein 1

Competing interests: The sponsor of this study, Acacia–No–Ki Co., Ltd. entrusted ORTHOMEDICO Inc., with conducting the study. S.O. and K.Y. belong to Acacia–No–Ki Co., Ltd., and A.B. and N.S. belong to ORTHOMEDICO Inc. T.T. (MD), the principal investigator of this study, belongs to Medical Corporation Seishinkai, Takara Clinic.

Authors' contributions: Conceptualization, S.O. and K.Y.; methodology, A.B., S.O. and K.Y.; formal analysis, A.B. and N.S.; investigation, A.B., N.S. and T.T.; resources, S.O. and K.Y.; data curation, A.B. and N.S.; writing—original draft preparation, A.B.; writing—review and editing, A.B., S.O., K.Y., N.S. and T.T; visualization, A.B. and N.S.; supervision, T.T.; project administration, N.S. and T.T.; funding acquisition, S.O. and K.Y. All authors have read and agreed to the published version of the manuscript.

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