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Effects of daily intake of Harudori-kombu: A randomized, double-blind, placebo-controlled, parallel-group study

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ABSTRACT

Background: Kombu (*Laminariaceae*) is traditionally consumed in Japan. "Harudori-kombu" is young kombu harvested in spring. Harudori-kombu contains functional components, such as fucoxanthin—a carotenoid—which confer various biological effects. This study aimed to test the intake of Harudori-kombu and the effects on adiponectin levels and body fat.

Methods: In this study, we investigated effects of the continuous intake of dried Harudori-kombu (2.0 g/day) for 6 weeks. We conducted a randomized, double-blind, placebo-controlled, parallel-group study including 70 healthy Japanese subjects with body mass index between 22 and 30 kg/m^2 and low-density lipoprotein cholesterol levels between 120 and 160 mg/dL. Subjects were randomly assigned to either Harudori-kombu group or placebo group. Subjects ingested 9 capsules per day for 6 weeks. We conducted medical interviews, vital sign examinations, and blood sampling at weeks 0 (baseline), 2, and 6. Moreover, we assessed body composition at weeks 0 and 6.

Results: Harudori-kombu intake did not improve the lipid profile but did reduce body fat. In addition, adiponectin levels were significantly improved.

Conclusions: Harudori-kombu intake showed beneficial health effects, such as reduction in body fat and improvement of adiponectin levels and was deemed safe at the dose administered in this study.

Clinical trial registration: UMIN000030418

Keywords: Harudori-kombu, *Laminariaceae,* fucoxanthin

BACKGROUND

Several types of brown algae are traditionally consumed in Japan, and kombu (*Laminariaceae*) is one of the most important edible algae. Kombu is a rich source of amino acids, minerals, vitamins, and dietary fibers and contains bioactive components such as fucoxanthin. Fucoxanthin is a red-to-red–orange carotenoid contained in edible brown algae, such as kombu, wakame (*Undaria pinnatifida*), and hijiki (*Sargassum fusiforme*) [1]. Beneficial effects of fucoxanthin, such as its antioxidant [2], antiangiogenesis [3], and anti-inflammatory [4] properties, have been reported.

A previous clinical trial has demonstrated that fucoxanthin intake (1 or 3 mg/day) for 4 weeks reduced body weight (BW), body mass index (BMI), and visceral fat in subjects with class 1 obesity (BMI = 25.0–29.9 kg/m^2) compared with placebo [5]. Another clinical trial has demonstrated that the intake of fucoxanthin-enriched akamoku oil (fucoxanthin amount = 1 or 2 mg/day) for 8 weeks reduced glycated hemoglobin (HbA1c) levels in subjects with BMI ≥ 22 kg/m^2 compared with placebo [6].

Biological mechanisms underlying benefits of fucoxanthin include increased mRNA and protein expressions of uncoupling protein 1 (UCP1) in white adipose tissue [7]. UCP1 is a protein present on the inner mitochondrial membrane; it is highly expressed in brown adipose cells, where it plays a role in fatty acid oxidation and heat production [8, 9]. UCP1 expression is downregulated in white adipose tissue; however, specific functional foods induce UCP1 expression in white adipose tissue [10]. In addition, a previous *in vivo* study has suggested that induction of UCP1-positive cells augmented energy metabolism [11]. Therefore, UCP1 expression in white adipose tissue represents an important mechanism underlying anti-obesity properties of functional foods, such as fucoxanthin [12, 13].

The effectiveness of fucoxanthin or kombu extract has been investigated in *in vitro* and *in vivo* studies as well as in clinical trials; however, the effectiveness of dried kombu has not been tested well, especially in clinical trials. "Harudori-kombu" is young kombu harvested in spring, whereas mature kombu is typically harvested in summer. Harudori-kombu is easy to consume owing to its soft texture; thus, the intake of effective amounts of bioactive components is enabled, which can be tested in clinical trials.

Therefore, we conducted a randomized, double-blind, placebo-controlled, parallel-group study to evaluate effects of the intake of dried Harudori-kombu in healthy subjects.

METHODS

Study design

The clinical study was conducted as a placebo-controlled, randomized, double-blinded, and parallel-group study in Hokkaido Information University, Health Information Science Research Center (Ebetsu city, Hokkaido, Japan). Schedule for the study is summarized in Table 1. Written informed consent was obtained from subjects before their enrollment at visit 1. Subsequently, medical interviews, vital sign examinations, and hematological and biological assessments were performed at visits 2 (week 0; baseline), 3 (week 2; excluding adiponectin), and 4 (week 6); body composition measurements were performed at visits 2 and 4 alone. In addition, all subjects completed the Food Frequency Questionnaire Based on Food groups (FFQg) (Kenpakusha, Tokyo, Japan) at visits 2 and 4. During the entire course of this trial, subjects were asked not to change their daily activities, including food consumption and exercise habits. Subjects recorded their daily activities in a diary, and the records were reviewed by a medical doctor or nurse at each visit.

Primary outcome was lipid profiles, including total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), LDL-C/HDL-C ratio, and non-HDL. Secondary outcomes were body composition, including BW; body fat rate; BMI and waist circumference; adiponectin; blood pressure; and blood glucose profiles, including fasting plasma glucose (FPG), HbA1c, insulin, and homeostasis model assessment of insulin resistance (HOMA-IR). The efficacy of active test food was evaluated at week 6 and its safety at weeks 2 and 6.

Table 1. Schedule of the clinical trial

	Guidance & agreement	Screening	Randomization	Test food intake period: Week 0	Week 2	Week 6
Visit	Visit 1		—	Visit 2	Visit 3	Visit 4
Date	Nov 24 — 28, 2017	Nov 24— 28, 2017	Dec 13, 2017	Jan 12— 13, 2018	Jan 26— 27, 2018	Feb 23— 24, 2018
Medical interview	—	○	—	○	○	○
Vital sign examination	—	○	—	○	○	○
Body composition measurement	—	○	—	○	—	○
Blood sampling	—	○	—	○	○	○
Food Frequency Questionnaire	—	—	—	○	—	○
Diary record	—	—	—	←――――――――――――――――→		

Study subjects

A total of 140 subjects volunteered to participate in the screening examination at visit 1. All volunteers provided written informed consent to participate in this clinical study. Through screening tests, 70 healthy Japanese subjects we enrolled (age ≥ 30 to < 70 years; LDL-C ≥ 120 to < 160 mg/dL; and BMI ≥ 22 to <30 kg/m^2). Inclusion and exclusion criteria are summarized in Table 2. The eligible subjects were randomly assigned to one of two groups: the active test food (Harudori-kombu) group or the placebo group, stratified by sex, age, LDL-C, and BMI at visit 1. Assignments were computer-generated using stratified block randomization at a third-party data center (Media Educational Center, Hokkaido Institute of Information Technology, Ebetsu city, Hokkaido). Medical doctors, nurses, clinical research coordinators, and statistical analysts were blinded to assignment information during the trial period. The information was only disclosed after the laboratory and analytical data were collected and the method of statistical analysis was finalized.

Table 2. The inclusion and exclusion criteria

Inclusion criteria	1. LDL-C ≥ 120 to < 160 mg/dL 2. BMI ≥ 22 to < 30 kg/m^2 3. Age ≥ 30 to < 70 years
Exclusion criteria	1. Receiving physician's advice, treatment, and/or medication for dyslipidemia, diabetes, obesity, and/or thyroid disease 2. Presenting with familial hypercholesterolemia 3. Presenting with serious cerebrovascular, cardiac, hepatic, renal, or gastrointestinal disease 4. Presenting a history of a major surgical intervention related to the digestive system 5. Presenting unusually abnormal hematological data or serious anemia 6. Regularly receiving medicine, functional foods, and/or supplements that can affect lipid and/or blood glucose metabolism 7. Heavy smokers, alcohol addicts, or subjects with an irregular lifestyle 8. Presenting with severe allergies to foods, seaweed, and/or gelatin 9. Participating in or having participated within the 4 weeks prior to the current study in other clinical trials 10. Donors of 400 ml of whole blood within 16 weeks (women) or 12 weeks (men), 200 ml of whole blood within 4 weeks (men and women), or blood components within 2 weeks (men and women) prior to the current study 11. Pregnant or lactating women or women who expect to be pregnant during this study 12. Other medical reasons, as judged by the principal investigator

BMI: body mass index; LDL-C: low-density lipoprotein cholesterol

Preparation of the test food

The manufacture of dried Harudori-kombu powder is detailed here. First, Ma-kombu (*Saccharina japonica* ver. *japonica*) was harvested in Hakodate (Hokkaido, Japan) and was used for preparing kombu samples. Second, raw kombu was boiled for a few minutes in seawater after harvesting and salted for a few days. Third, the salted kombu was washed, dried, and crushed. Finally, the coarse powder was ground into a fine powder, and 2.0 g of the powder was encapsulated in a gelatin capsule. The intake level of active test food was determined with reference to the intake level of fucoxanthin at previous trials (1-3 mg/day) [5, 6]. Placebo capsules contained dextrin powder instead of kombu powder. Nutrient compositions of the active test and placebo foods used in this study are presented in Table 3. Fucoxanthin levels were analyzed using high-performance liquid chromatography. Nutrient composition analyses were performed using the methods established by the Japan Food Research Laboratories (Hokkaido, Japan). The active test and placebo foods were manufactured under strict quality control protocols and were identical in appearance.

Table 3. Nutrient composition of the active test and placebo foods per day

Parameter	Placebo food group	Active test food group
Calories (kcal)	8.84	8.36
Water (g)	0.16	0.21
Proteins (g)	0.58	0.92
Lipids (g)	0.00	0.11
Carbohydrates (g)	1.63	0.93
Ash (g)	0.00	0.39
Sodium (mg)	0.86	0.14
Fucoxanthin (mg)	—	3.04

Physical, hematological, and biological measurements

Blood was collected after fasting for 12 h. General blood tests included the following hematological examinations: white blood cell (WBC), red blood cell (RBC), hemoglobin (Hb), hematocrit (Ht), and blood platelet (Plt). Biological examination included liver function [aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (γ-GTP), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH)]; renal function [blood urea nitrogen (BUN), creatinine (CRE), and uric acid (UA)]; lipid profiles (TC, LDL-C, HLD-C, and TG); blood glucose profiles (FPG, HbA1c, insulin, and HOMA-IR), and adiponectin levels (only at visits 2 and 4).

Blood tests were performed at Sapporo Clinical Laboratory, Inc. (Hokkaido, Japan). Body composition and blood pressure were measured using a Body Composition Analyzer DC-320 (Tanita Corp, Tokyo, Japan) and an Automatic Blood Pressure Monitor HEM-7080IC (Omron Co., Ltd., Kyoto, Japan), respectively.

Food frequency questionnaire

FFQg was used for estimating nutrient intake from the regular diet of subjects. This questionnaire comprised 29 food groups and 10 types of cooking methods. For each question, the subjects reported the amount and frequency of food intake on a weekly basis. Based on the answers, regular and nutrient intakes (calories, protein, fat, carbohydrates, dietary fiber, and salt) were estimated.

Ethics

The current clinical trial was conducted in compliance with the ethical guidelines on medical research in humans (Ministry of Education, Culture, Sports, Science and Technology, and Ministry of Health, Labor and Welfare) and the Helsinki Declaration (revised by the Fortaleza General Meeting of the World Medical Association). The trial protocol was approved by the ethics committee of Hokkaido Information University (Ebetsu, Hokkaido, Japan; approved on October 30, 2018; approval number: 2017-21). This trial was registered at www.umin.ac.jp/ctr/index.htm (registered on December 15, 2018; registration number: UMIN000030418).

Statistical analysis

Means and standard deviations of subject characteristics were calculated for each group. For the primary and secondary outcomes and food frequency questionnaire, values were analyzed using Student's t-test by comparing the means between the two groups. For subject characteristics, Fisher's exact probability test was used for sex and Mann–Whitney U-test was used for intake rate; student's t-test was used for the other subject characteristics. All statistical analyses were performed using SPSS Statistics 20 (IBM Japan, Ltd., Tokyo, Japan), and $p < 0.05$ was considered significant, while $0.05 \leq p < 0.10$ was considered tending towards significance.

Sample size

The sample size was statistically determined to obtain a power of 80% and a two-sided significance level of 5%. To demonstrate a difference in LDL-C at week 6, which was postulated to have an intergroup difference of 10 mg/dL with a standard deviation of 13 mg/dL, a sample size of 60 (30 in each group) was required. Assuming a 15% loss in the follow-up rate, 70 subjects (35 in each group) were enrolled.

RESULTS

Subject dropouts and characteristics

The flow of subjects' involvement through the trial is presented in Figure 1. Subjects who provided informed consent (n = 140) were assessed for eligibility, and a total of 70 subjects were enrolled in this study. All enrolled subjects were randomized to one of the two intervention groups (placebo group, n = 35; active test food group, n = 35). One subject dropped out for personal reasons before the trial started; one subject dropped out because of elevated liver function markers at week 0 (before the test food intake), and two subjects dropped out for personal reasons during the test food intake period. Finally, 66 subjects completed this trial: 31 in the active test food group and 35 in the placebo group. All subjects were included in the safety analysis. The efficacy analysis included 31 subjects in the active test food group and 35 in the placebo group.

Sex ratio, mean age, height, BW, body fat rate, BMI, LDL-C, and intake rate for each group are presented in Table 4. These characteristics did not significantly differ between the two groups, confirming the appropriate allocation of subjects between the active test food and placebo groups.

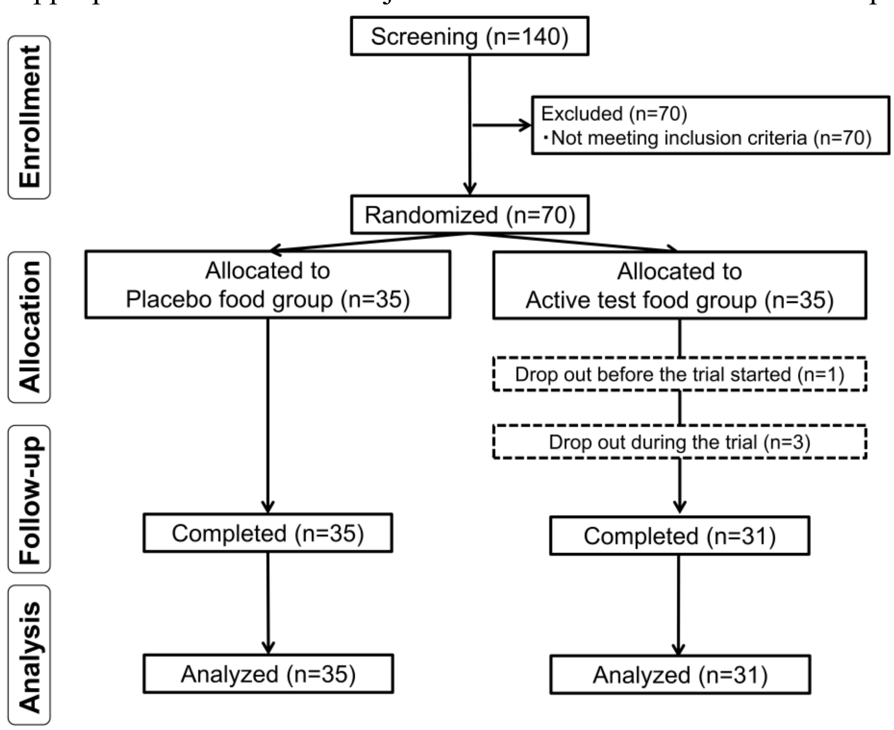


Figure 1. Flowchart of subjects' selection.

Table 4. Characteristics of subjects and intake rates of test foods in the active test food and placebo groups

Characteristic	Placebo	Active	p
Subjects (n)	35	31	-
Male (n)	17	16	> 0.99
Age (years)	56.5 ± 9.2	56.6 ± 9.8	0.96
Height (cm)	162.5 ± 8.8	160.7 ± 9.1	0.44
Body weight (kg)	63.6 ± 8.2	63.1 ± 9.4	0.81
Body fat rate (%)	28.6 ± 6.1	28.8 ± 5.6	0.85
Body mass index (kg/m^2)	24.0 ± 1.8	24.3 ± 1.8	0.55
LDL-C (mg/dL)	138.9 ± 13.1	138.7 ± 11.7	0.95
Intake rate (%)	99.4 ± 1.5	99.5 ± 1.6	0.50

Values are shown as mean and standard deviation. Fisher's exact probability test was used for sex, and Mann–Whitney U-test was used for intake rate; student's t-test was used for the other characteristics. n, number of subjects; LDL-C: low-density lipoprotein cholesterol

Efficacy of Harudori-kombu on lipid profile

Effects of Harudori-kombu on lipid profile, including TC, LDL-C, HDL-C, TG, LDL-C/HDL-C ratio, and non-HDL, are summarized in (Table 5). There were no differences between the active test food and placebo groups in terms of changes in any of the parameters.

Table 5. Lipid profiles

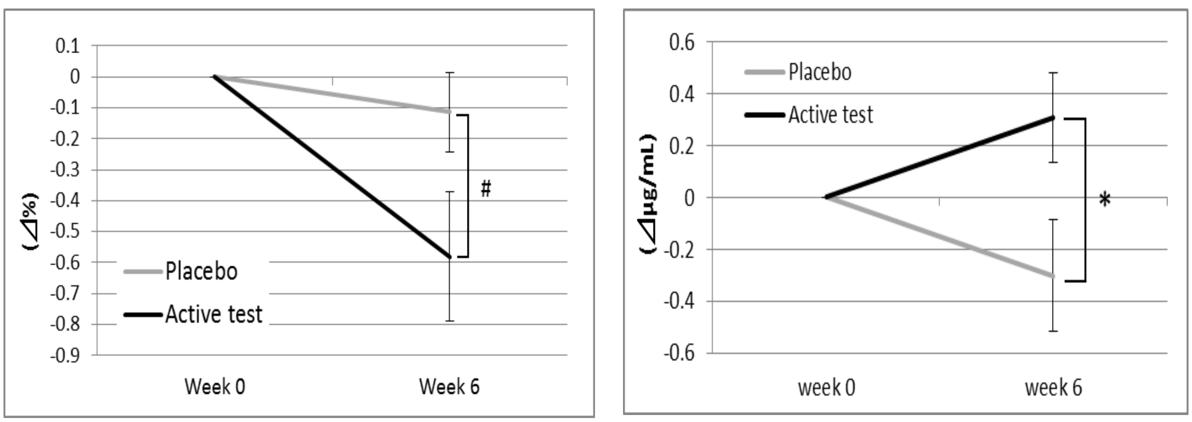
		Week 0	**Δ week 2**	**Δ week 6**
TC (mg/dL)	Placebo	242.4 ± 22.3	−12.5 ± 14.4	−2.9 ± 22.6
	Active	243.2 ± 28.3	−13.0 ± 16.8	−1.1 ± 18.0
	p	0.20	0.89	0.72
LDL-C (mg/dL)	Placebo	158.1 ± 19.3	−9.5 ± 15.7	−6.3 ± 21.4
	Active	148.3 ± 21.6	−6.7 ± 13.8	−2.0 ± 16.4
	p	0.06#	0.45	0.36
HDL-C (mg/dL)	Placebo	68.0 ± 13.9	−1.2 ± 3.6	0.7 ± 4.9
	Active	65.5 ± 16.5	−1.4 ± 4.9	1.5 ± 6.3
	p	0.50	0.81	0.54
TG (mg/dL)	Placebo	100.2 ± 43.9	−3.9 ± 32.4	1.0 ± 32.8
	Active	126.2 ± 131.0	−23.4 ± 64.0	−20.4 ± 90.0
	p	0.27	0.12	0.19
LDL-C/HDL-C ratio	Placebo	2.4 ± 0.6	−0.1 ± 0.3	−0.1 ± 0.3
	Active	2.4 ± 0.6	−0.1 ± 0.2	−0.1 ± 0.2
	p	0.72	0.39	0.63
non-HDL (mg/dL)	Placebo	174.4 ± 20.8	−11.3 ± 13.6	−3.6 ± 20.6
	Active	168.8 ± 23.1	−11.6 ± 14.0	−2.7 ± 16.2
	p	0.30	0.93	0.84

Values are shown as the mean and standard deviation. ΔWeek 2: change in value from baseline to week 2; ΔWeek 6: change in value from baseline to week 6. Student's t-test was performed.

TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglyceride; LDL-C/HDL-C ratio: LDL cholesterol/HDL cholesterol ratio; non-HDL: non-low-density lipoprotein cholesterol.

Efficacy of Harudori-kombu on body composition and adiponectin levels

To confirm effects of Harudori-kombu on body composition, changes in BW, body fat rate, BMI, and waist circumference were evaluated (Table 6). In addition, effects of Harudori-kombu on adiponectin levels were assessed (Table 6). The intake of active test food tended to improve body fat rate compared with the intake of placebo (Δweek6: Placebo, −0.1 ± 0.8%; active, −0.6 ± 1.2%; p = 0.055) [Figure 2-(a)]. Additionally, adiponectin level was improved in the active test food group compared with that in the placebo group at week 6 (Δ week 6: Placebo, −0.3 ± 1.3 µg/mL; active, 0.3±1.0 µg/mL; p = 0.034) (Figure 2-b). BW, BMI, and waist circumference did not differ between the two groups.



(a) (b)

Figure 2. Body fat rate and adiponectin levels

Values are presented as means and standard error. (a) Change in body fat rate; (b) Changes in adiponectin level. Student's t-test was performed to analyze the values. Statistical significance: *p < 0.05, #0.05 ≤ p < 0.10 vs. placebo group.

Efficacy of Harudori-kombu on blood pressure and blood glucose profile

We examined blood pressure (systolic and diastolic) and blood glucose profile (FPG, Insulin, HbA1c, and HOMA-IR). There were no statistically significant differences between the active test food and placebo groups (Table 6).

Table 6. Body composition, adiponectin, blood pressure, and blood glucose profile

		Week 0	**Δ week 2**	**Δ week 6**
BW (kg)	Placebo	63.4 ± 8.3	—	−0.1 ± 1.0
	Active	62.4 ± 9.7	—	0.0 ± 0.9
	p	0.65	—	0.95
Body fat rate (%)	Placebo	29.0 ± 6.0	—	−0.1 ± 0.8
	Active	29.3 ± 5.9	—	−0.6 ± 1.2
	p	0.89	—	0.055#
BMI (kg/m^2)	Placebo	24.0 ± 1.8	—	0 ± 0.4
	Active	24.0 ± 1.9	—	0 ± 0.4
	p	0.91	—	0.96
Waist circumference (cm)	Placebo	83.8 ± 6.4	—	0.2 ± 3.2
	Active	83.8 ± 5.7	—	0.5 ± 2.6
	p	1.00	—	0.63
Adiponectin (μg/mL)	Placebo	10.8 ± 4.5	—	−0.3 ± 1.3
	Active	10.4 ± 5.8	—	0.3 ± 1.0
	p	0.79	—	0.034*
SBP (mmHg)	Placebo	122.1 ± 15.7	-0.2 ± 9.9	0.8 ± 10.2
	Active	128.7 ± 14.3	−4.8 ± 10.2	−2.2 ± 10.6
	p	0.08#	0.07#	0.24
DBP (mmHg)	Placebo	79.3 ± 11.8	−0.2 ± 8.5	−1.5 ± 6.9
	Active	80.5 ± 10.1	−1.7 ± 7.6	−1.1 ± 6.0
	p	0.65	0.44	0.81
FPG (mg/dL)	Placebo	93.8 ± 8.3	−0.2 ± 5.6	−0.8 ± 5.1
	Active	90.1 ± 8.0	−1.9 ± 4.4	−1.2 ± 4.3
	p	0.07#	0.18	0.72
Insulin (μU/mL)	Placebo	4.1 ± 1.8	0.6 ± 2.0	−0.2 ± 2.0
	Active	4.6 ± 2.7	0.4 ± 2.2	−0.5 ± 2.2
	p	0.47	0.67	0.56
HbA1c (%)	Placebo	5.5 ± 0.4	0.0 ± 0.1	0.1 ± 0.2
	Active	5.3 ± 0.3	0.0 ± 0.1	0.1 ± 0.2
	p	0.13	0.97	1.00
HOMA-IR	Placebo	1.0 ± 0.5	0.1 ± 0.5	−0.1 ± 0.5
	Active	1.0 ± 0.6	0.1 ± 0.5	−0.1 ± 0.5
	p	0.74	0.50	0.62

Values are shown as mean and standard deviation. Δweek 2: change from baseline to week 2; Δweek 6:

change from baseline to week 6. Student's t-test was performed to analyze the values. *p < 0.05, #0.05 ≤ p < 0.10 vs. placebo group.

BW: body weight; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FPG: fasting plasma glucose; HbA1c: glycated hemoglobin; HOMA-IR: homeostatic model assessment of insulin resistance.

Assessment of dietary nutrients of subjects during trial

The subjects completed FFQg at weeks 0 and 6 to assess dietary nutrients. Analysis revealed no statistically significant differences in intake of calories, proteins, lipids, carbohydrates, dietary fibers, and salt in dietary meal between the active test food and placebo groups (Table 7). These results suggest that dietary nutrients from the meals did not affect the results of this trial.

Table 7. Dietary nutrients of the subjects during the trial

		Week 0	**Δ week 6**
Calorie (kcal)	Placebo	1825.9 ± 349.7	−1.9 ± 267.2
	Active	1811.0 ±3 29.3	−44.7 ± 218.6
	p	0.86	0.48
Protein (g)	Placebo	64.0 ± 14.1	2.1 ± 9.0
	Active	63.5 ± 15.2	−0.9 ± 9.2
	p	0.90	0.19
Lipid (g)	Placebo	61.4 ± 16.0	4.2 ± 12.4
	Active	61.8 ± 17.2	0.3 ± 10.9
	p	0.92	0.19
Carbohydrate (g)	Placebo	241.2 ± 51.4	−12.9 ± 42.2
	Active	238.2 ± 42.0	−11.3 ± 32.0
	p	0.80	0.86
Dietary fiber (g)	Placebo	12.5 ± 4.1	−0.6 ± 2.6
	Active	12.8 ± 3.5	−0.3 ± 2.0
	p	0.82	0.67
Salt (g)	Placebo	9.6 ± 3.1	−0.4 ± 2.1
	Active	9.3 ± 2.9	0.1 ± 2.2
	p	0.61	0.42

Values are shown as mean and standard deviation. Student's t-test was performed to analyze the values.

Safety

For the safety analysis of active test food (Harudori-kombu), pulse rate; complete blood counts (WBC, RBC, Hb, Ht, and Plt); liver function (AST, ALT, γ-GTP, ALP, and LDH); and renal function (BUN, CRE, and UA) were evaluated. Minimal changes (Table 8) and minimal adverse effects (variation of clinical data and clinical observation) were observed in each group. In the active test food group, the five adverse effects were observed: diarrhea (n = 1); variation in LDH value (n = 2); variation in γ-GTP value (n =1); and variation in UA value (n =1). In the placebo group, nine adverse effects were observed: toothache (n = 1); urticarial (n = 1); variation in γ-GTP value (n = 2); variation in UA (n = 2); variation in CRE value (n = 1); variation in BUN (n = 1); and variation in WBC (n = 1). However, regarding clinical observation, these subjects presented with only mild symptoms, and all recovered within a few days. Regarding variation of clinical data, these subjects presented with no symptoms related to variation; the principal investigator judged that none of the adverse events were related to the intake of the test food. Thus, the intake of Harudori-kombu had no or minimal unfavorable effects even at a dose of 17 g/day (as raw kombu).

Table 8. Pulse rate, complete blood counts, liver function; and renal function

		Week 0	**Week 2**	**Week 6**
Pulse rate (bpm)	Placebo	69.3 ± 9.2	72.8 ± 10.9	70.9 ± 10.4
	Active	71.2 ± 8.9	71.1 ± 8.9	73.2 ± 8.2
WBC ($\times 10^3/\mu L$)	Placebo	5.7 ± 1.1	5.4 ± 1.1	5.6 ± 1.2
	Active	6.0 ± 1.5	5.7 ± 1.3	5.4 ± 1.3
RBC ($\times 10^4/\mu L$)	Placebo	481.4 ± 38.4	473.6 ± 40.5	490.7 ± 47.1
	Active	496.3 ± 44.6	481.6 ± 39.9	498.9 ± 45.9
Hb (g/dL)	Placebo	14.6 ± 1.2	14.4 ± 1.2	14.8 ± 1.4
	Active	14.7 ± 1.7	14.4 ± 1.5	14.7 ± 1.6
Ht (%)	Placebo	44.3 ± 3.1	43.4 ± 3.5	45.4 ± 4.1
	Active	44.8 ± 4.3	43.4 ± 3.7	45.5 ± 4.2
Plt ($\times 10^4/\mu L$)	Placebo	25.8 ± 5.6	24.7 ± 5.4	25.5 ± 5.9
	Active	26.4 ± 6.8	24.7 ± 5.9	25.0 ± 5.6
AST (U/L)	Placebo	25.1 ± 7.2	23.0 ± 8.0	23.1 ± 5.7

		Week 0	Week 2	Week 6
	Active	25.3 ± 15.5	21.3 ± 6.5	22.3 ± 6.8
ALT (U/L)	Placebo	25.4 ± 13.4	22.2 ± 13.9	22.2 ± 11.8
	Active	27.8 ± 31.9	20.6 ± 10.2	21.8 ± 10.7
γ-GTP (U/L)	Placebo	41.2 ± 41.3	35.2 ± 28.1	35.9 ± 26.7
	Active	31.4 ± 22.3	26.2 ± 17.6	28.0 ± 17.5
ALP (U/L)	Placebo	238.2 ± 82.8	226.9 ± 62.5	235.2 ± 64.2
	Active	211.9 ± 40.1	199.8 ± 40.7	209.2 ± 42.1
LDH (U/L)	Placebo	202.7 ± 25.2	190.5 ± 24.9	204.6 ± 21.7
	Active	201.8 ± 28.0	193.4 ± 34.8	204.7 ± 30.5
BUN (mg/dL)	Placebo	15.2 ± 3.9	15.2 ± 3.9	14.7 ± 3.6
	Active	14.4 ± 4.9	14.2 ± 3.7	13.9 ± 3.2
CRE (mg/dL)	Placebo	0.8 ± 0.2	0.9 ± 0.2	0.8 ± 0.2
	Active	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
UA (mg/dL)	Placebo	5.4 ± 1.2	5.6 ± 1.3	5.3 ± 1.2
	Active	5.4 ± 1.2	5.3 ± 1.2	5.2 ± 1.2

Values are shown as the mean and standard deviation. WBC: white blood cell; RBC: red blood cell; Hb: hemoglobin; Ht: hematocrit; Plt: platelet; AST: aspartate aminotransferase; ALT: alanine aminotransferase; γ-GTP: gamma-glutamyl transpeptidase; ALP: alkaline phosphatase; LDH: lactate dehydrogenase; BUN: blood urea nitrogen; CRE: creatinine; UA: uric acid; bpm: beats per minute.

DISCUSSION

In this clinical trial, we assessed the effect of daily Harudori-kombu intake for 6 weeks on lipid profile, secondary body fat rate, adipokine levels, blood glucose profile, and blood pressure. We demonstrated that Harudori-kombu intake tended to reduce body fat rate and altered adiponectin levels.

The primary outcomes of changes in the lipid profile (TC, LDL-C, HDL-C, TG, LDL-C/HDL-C ratio, and non-HDL) were not significantly different between the active test food and placebo groups. Therefore, intake duration and/or the dose of Harudori-kombu should be reconsidered.

The secondary outcome of body fat rate tended to be reduced by the intake of active test food. A previous clinical trial has demonstrated that the intake of fucoxanthin (1–3 mg/day) for 4 weeks reduced BW, BMI, and visceral fat in subjects with class 1 obesity compared with placebo [5]. In another study, Xanthigen® (brown marine algae fucoxanthin + pomegranate seed oil) intake reduced BW in obese non-diabetic women [14]. In addition, active test food in our study increased adiponectin levels. The biological mechanism of body fat reduction by fucoxanthin depends on increased UCP-1 expression at the membranes of white adipose cells, likely mediated by increase in β3 adrenaline receptor (β3AR) expression [7]. Peroxisome proliferator-activated receptor γ (PPARγ) is downstream of β3AR and targets adiponectin [15, 16]. Similar to other natural products such as *Cyclolepis genistoides* D. Don (palo azul), fucoxanthin may increase adiponectin expression via PPARγ activation [17] . Adiponectin level decreases as visceral fat increases [18]. Collectively, these results indicate that Harudori-kombu reduces body fat rate via UCP-1 or PPARγ activation, both of which elevate adiponectin levels.

In this clinical trial, we observed no effect of Harudori-kombu on blood glucose profiles. A previous clinical trial has demonstrated a significant decline in HbA1c levels in the 2 mg/day fucoxanthin group compared with that in the placebo group [6]. Moreover, adiponectin levels were associated with blood glucose profiles, such as insulin resistance and glucose uptake. Of note, obesity is an important component that exacerbates insulin resistance, and insulin resistance is often associated with increased body weight. In addition, various adipokines, such as adiponectin and resistin, are associated with insulin resistance [19]. In our clinical trial, subjects' initial FPG was normal and intake period was shorter than those in a previous trial [6]. These differences might explain unchanged blood glucose profiles in our study population.

Kombu is a rich source of dietary fibers, which confer various beneficial effects [20]. Compared with a previous trial assessing the benefits of ingesting 5–10 g of dietary fiber, active test food of our study contributed to 1 g of dietary fiber per day, suggesting that the main functional component of active test food was fucoxantin.

In this study, no side effects or severe adverse events were observed during physical and blood examinations or reported in the medical interviews. These results confirm the safety of 6-week intake of 2.0 g of Harudori-kombu daily (as dried kombu). This study included healthy subjects who had not been receiving any medications for obesity, dyslipidemia, and/or diabetes mellitus. In addition, the intake periods in this study were short. In the future, investigation of the effects of Harudori-kombu with longer intake periods is required.

CONCLUSION

In this 6-week randomized, double-blinded, placebo-controlled, parallel-group comparative study, Harudori-kombu intake improved adiponectin levels and reduced body fat. Additionally, we confirmed the safety of Harudori-kombu over 6 weeks. Kombu is an important component of the Japanese diet, and our findings support the use of Harudori-kombu as a functional food with health benefits.

List of abbreviations: total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), LDL cholesterol/HDL cholesterol ratio (LDL-C/HDL-C ratio), non-low-density lipoprotein cholesterol (non-HDL), body weight (BW), body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting plasma glucose (FPG), hemoglobin A1c (HbA1c), homeostatic model assessment of insulin resistance (HOMA-IR), white blood cell (WBC), red blood cell (RBC), hemoglobin (Hb), hematocrit (Ht), platelet (Plt), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (γ-GTP), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine (CRE), uric acid (UA).

Competing interests: This study was conducted with research funds under contract with Northern Advancement Center for Science and Technology. Under the “Hokkaido Information University Bioethics Committee Regulations,” the research director and the investigator of this study declared necessary conflicts of interest to the ethical review committee.

Authors’ contributions: M.N., M.S., M.K., and J.N. designed the clinical trial; M.S., M.K, Y.K., and H.Y. designed the test food. J.N. conducted the research; M.N. conducted statistical analysis; M.N. wrote the manuscript; All authors take responsibility for the final content of the manuscript.

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