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Seaweed fucoxanthin supplementation improves obesity parameters in mildly obese Japanese subjects

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ABSTRACT

Background: Fucoxanthin is a seaweed xanthophyll that has demonstrated an anti-obesity effect in rodents. However, clinical investigations of its influence on mildly obese subjects has not been performed. We conducted a clinical trial of fucoxanthin supplementation in Japanese obese subjects.

Methods: We examined the effect of fucoxanthin (1 or 3 mg daily) in a double-blind placebo-controlled study. Capsules containing fucoxanthin or placebo capsules were administered for 4 weeks to male and female Japanese adults with a body mass index (BMI) of more than 25 kg/m^2. Before and after treatment, the body weight, body composition, abdominal fat area, and the circumferences of the neck, arm, and thigh were evaluated.

Results: There was significant reduction of the relative (ratio versus before treatment) body weight,

BMI, and visceral fat area in the 3 mg/day fucoxanthin group compared to the placebo group. Relative values of total fat mass, subcutaneous fat area, waist circumference, and right thigh circumference were also significantly lower in the 1 mg/day fucoxanthin group than the placebo group. A significant decrease of the absolute right thigh circumference was noted in the 1 mg/day fucoxanthin group compared to the placebo group. In the subjects ingesting fucoxanthin, there were no abnormalities of the blood pressure, pulse rate, blood parameters, and urinalysis parameters, which thereby suggests adverse effects.

Conclusions: Fucoxanthin reduced body weight, BMI, and abdominal fat by acting on both visceral and subcutaneous fat. Consequently, Fucoxanthin may be able to improve a moderate overweight state in both men and women.

Keywords: Randomized, double-blind, placebo-controlled crossover trial; fucoxanthin; body mass index; body weight; subcutaneous fat; adipose tissue

INTRODUCTION

Under the Japanese food health claims system, several natural ingredients with health benefits have been approved for health claim in relation to weight loss. It is permitted to claim a weight loss effect for green tea catechins[1] and chlorogenic acids[2] from green coffee beans specifically produced by processed food companies as Japanese Food for Specified Health Uses (FOSHU). These polyphenols enhance hepatic fat metabolism by increasing mitochondrial β-oxidation[3] or by promoting the uptake of free acids into the mitochondria of hepatocytes[4]. Since 2015, similar weight loss claims have also been permitted for pueraria flower isoflavonoids[5] and licorice flavonoids[6] under the Japanese Foods with Function Claims system. Despite weight loss claims being allowed for several polyphenolic compounds, no carotenoids have yet been confirmed to achieve clinical weight loss effects. Although astaxanthine[7], β-cryptoxanthine[8] from citrus fruits, and lutein[9] from marigold flowers have confirmed health benefits other than weight loss and health claims can be made on the packaging, weight loss claims are not allowed for these products so far.

Fucoxanthin is a marine carotenoid that is found in seaweeds including kelp, kombu (*Laminaria japonica*), and wakame (*Undaria pinnatifida*). An anti-obesity effect of fucoxanthin was first reported by Japanese researchers who administered a diet containing 0.4% fucoxanthin fractions (fucoxanthin content: 67.4%) to KKAy obese mice and found significantly enhanced expression of mitochondrial uncoupling protein (UCP) 1 in visceral fat[10]. UCP1 releases the

electrochemical potential of the mitochondria for ATP production that leads to energy consumption. As another anti-obesity mechanism of fucoxanthin, the same research group confirmed that it suppresses lipid absorption in oil emulsion-loaded rats.[11] On the other hand, fucoxanthinol, a metabolite of fucoxanthin was reported to improve glucose tolerance in diabetic mice[12] which also suppresses differentiation of 3T3-L1 adipocytes[13]. Furthermore, it was confirmed that fucoxanthin enhanced energy expenditure, β-oxidation, and adipogenesis by up-regulating peroxisome proliferator-activated receptor (PPAR)α, PPARγ co-activator (PGC)1α, PPARγ, and UCP1[14]. Ha *et al*.[15] reported that fucoxanthin enhanced the expression of lipid-metabolizing enzymes, including carnitine palmitoyltransferase (CPT)-1, in rats on a high fat diet. However, there is only one report about the clinical weight loss effect of fucoxanthin mixture by Abidov *et al*.[16], who performed a 16-week clinical trial in 151 female subjects using a dietary supplement containing fucoxanthin from algae extract and pomegranate seed oil. Significant weight loss and reduction of abdominal circumference were confirmed in obese subjects [body mass index (BMI) more than 30 kg/m^2]. In light of this background, we conducted clinical trial to assess the effects of fucoxanthin on weight and fat accumulation in obese Japanese subjects with moderate obesity in order to evaluate the weight loss effect of fucoxanthin.

METHODS

1. Participants

To recruit subjects for the study, participants registered with the monitor bank of TES Holdings Co., Ltd. completed a questionnaire. Among the subjects, 50 men and women aged 20 to 59 years were selected because their answers satisfied the "selection criteria" and did not meet the "exclusion criteria" below. Based on the body fat parameters obtained by the screening test, 33 participants with relatively higher levels of BMI, total fat mass, visceral fat mass, and subcutaneous fat mass were selected as the subjects. The target male:female ratio of the subjects was 1:1. Subjects had a BMI greater than 25 and less than 30 kg/m^2, with abdominal circumference being at least 85 cm for men and 90 cm for women. Exclusion criteria were the following:

1. Current medication for chronic symptoms.
2. Previous severe allergic reaction to food or medicine.
3. Taking medication that may influence test results, including treatment for metabolic syndrome or dietary supplements for weight loss.
4. Current or previous cardiovascular disease, nephritis, hepatitis, and other disorders.
5. Participants with AST, ALT, or γ-GTP more than 2.5 times the upper limit of normal.

6. Participants with serum uric acid more than 9.0 mg/dL.
7. Participants with severe anemia.
8. Pregnant or breastfeeding women.
9. Regular alcohol intake of 60 g or more almost every day.
10. Subjects participating in another clinical trial.
11. Subjects determined to be unsuitable for this study by the attending physician.

Finally, 11 subjects each were allocated to 3 groups (33 in total). They were grouped so that the average values of total fat mass, visceral fat mass, subcutaneous fat mass, body weight, BMI, age, and sex were similar in each group.

Intake of alcohol was prohibited on the day before the test. The subjects were ordered to avoid an irregular lifestyle (e.g. lack of sleep, overeating, and overdrinking) during the study period. Concerning diet, a similar quantity and quality of food were maintained relative to the diet before the study. Excessive exercise, overeating, overdrinking, and lack of sleep were strictly prohibited on the day before the test. Taking medicines that could influence assessment of the effect of the test substance (e.g. weight loss and lipid-lowering agents) was prohibited, as were dietary supplements including vitamins and slimming agents. Blood donation was prohibited during the study period.

2. Preparation and allocation of test samples

The test samples (capsules containing two different doses of fucoxanthin and placebo capsules) were provided by Oryza Oil & Fat Chemical Co., Ltd. The test substance was provided in hard capsules containing Fucoxanthin-P1 (Oryza Oil & Fat Chemical Co. Ltd., a powder with 1% fucoxanthin). Fucoxanthin capsules (capsules A and B) contained 0.5 and 1.5 mg of fucoxanthin. Placebo capsules (capsule C) had an identical appearance to the fucoxanthin capsules and the same components except for fucoxanthin. The composition and nutritional data for the capsules are indicated in Tables 1 and 2. Tocopherol had been reported to decrease BMI in an intervention study[17]. However, 100 mg/day or more tocopherol was required for the effect. Some manufactured kelp extract contains fucoidan, which exhibits an anti-obese effect[18]. However, the effect requires 500 mg/day. As the contents of mixed tocopherol and kelp extract in the capsules are less than 10 mg/day, both ingredients were considered to give no influence on obesity parameters in the study. The appearance of each type was indistinguishable so colored identification tags were attached to the packages of capsules (green, red, and white). Information about test sample allocation was

strictly protected by study treatment allocation controllers from an outsourcer who were not directly involved in the study. The information was not disclosed to any other party until the cases to be analyzed were determined at a clinical conference after the study was completed.

Table 1. Composition of the test capsules

Component (mg)	Capsule A (Fucoxanthin 0.5 mg)	Capsule B (Fucoxanthin 1.5 mg)	Capsule C (Placebo)
Vegetable oil and fat	5 .0	15 .0	5.0
Kelp extract	0.8	2.3	0.8
Cyclodextrin	142.5	127.5	143.0
Mixed tocopherol	1.3	3.8	1.3
Fucoxanthin	0.5	1.5	0.0

Table 2. Nutritional content of the test capsules

Nutrient	Capsule A Fucoxanthin 0.5 mg)	Capsule B (Fucoxanthn 1.5 mg)	Capsule C (Placebo)
Water (g/100 g)	4.4	5.1	5.1
Protein (g/100 g)	25.3	25.4	25.3
Fat (g/100 g)	2.5	7.3	7.3
Carbohydrate (g/100 g)	67.6	61.9	62.2
Sodium (mg/100 g)	56.0	69.1	39.0
Calories (kcal/100 g)	394.3	414.6	415.3

3. Study protocol

The study was carried out at the Oriental Occupational Health Association Tokyo Branch Oriental Ueno Detection Center and statistical analysis was done by TES Holdings Co., Ltd. The study (protocol No. HR-2010-O03) was a placebo-controlled double-blind comparison among three groups treated concurrently. Main evaluation parameters were body weight, body composition, abdominal fat area on computerized tomography (CT, total fat, visceral fat, and subcutaneous fat), and various circumferences (waist, upper arms, neck, and thighs). Secondary outcomes were

the blood pressure and pulse rate, hematological parameters, blood biochemical parameters, and urinalysis parameters. Analysis of blood and urine was performed by Health Science Research Institute Co., with Ltd. Daily reports written by the subjects were collected, including a simplified dietary survey and questionnaire. In the study, two capsules of the assigned treatment were ingested with water after meal once a day by the subjects for 4 weeks. Then the efficacy and safety of fucoxanthin were evaluated based on the data and survey items described above.

4. Measurement of body fat parameters

A body composition meter (MC-180, Tanita Co. Japan) was used for the measurement of body weight and body composition. The subjects wore the specified test clothing and measurements were carried out with the meter set at 500 g. Body weight, percent body fat, fat mass, fat-free mass, muscle mass, BMI, and basal metabolic rate were measured. The abdominal area was determined from a CT scan obtained at the level of the umbilicus while holding the breath. For measurement of the fat area, CT scans were converted into the BMP format and images were analyzed by using software for visceral fat measurement (Fat Scan N2 System, East Japan Institute of Technology Co. Ltd., Japan) to calculate the visceral fat area, subcutaneous fat area, and total fat area. Measurements of the waist, neck, upper arm, and thigh circumferences were obtained as the following.

Waist circumference: This was measured at the umbilicus in the standing position after the subject exhaled lightly. When a subject had a significant amount of fat and the umbilicus was dependent, the waist circumference was measured at the midpoint between the lowest rib and the anterior superior iliac spine.

Neck circumference: This was measured at the base of the neck.

Upper arm circumference: This was measured at the midpoint of the left and right upper arms.

Thigh circumference: This was measured at the most proximal part of the left and right thighs.

All measurements were done in the standing position.

5. Laboratory tests

A fasting venous blood sample was collected after measurement of fat parameters. The red blood cell, leukocyte and platelet counts, hemoglobin, hematocrit, MCV, MCH, and MCHC were measured as hematology parameters. Protein, albumin, urea N, creatinine, uric acid, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, free fatty acid, AST, ALT, γ-GTP, ALP, CK, and blood glucose were determined as biochemical parameters. A urine sample was collected after the blood sample and the specific gravity and pH were determined.

6. Ethics, adherence, and compliance

This study was performed according to the Helsinki Declaration (revised at the Edinburgh General Assembly in 2000) and was carried out in line with ethical considerations. Each subject's human rights and safety were taken into consideration, and it was ensured that the data were reliable in compliance with Ministry of Health and Welfare Ordinance No. 28 "Standards for the Implementation of Clinical Trials of Pharmaceutical Products (GCP)" dated March 27, 1997. The Ethics Committee of TES Holdings was made up of professors of a pharmaceutical university, a medical doctor, a hospital consultant, and a lawyer. The Ethics Committee was convened to deliberate on the ethicality and appropriateness of the protocol. This study was implemented according to the protocol approved by the Ethics Committee and any substantial deviations from the protocol required authorization by the committee.

7. Investigation of adverse events

Evaluation of each adverse event was carried out, with the causal relationship with the test substance being investigated and determined. A decision about whether or not to continue the study was made by the doctor in charge if necessary. Adverse events were defined as symptoms/signs resulting from ingesting the test substance that made the subject feel unpleasant. If a subject made a request to discontinue the study, the study was promptly stopped with considerations taken to prevent any disadvantage to the subject.

8. Exclusion criteria for data analysis

If any of the following occurred, the case was discussed at the clinical conference and the subject in question was to be excluded from analysis after review.

1. Cases where a test subject delayed attending for each visit by at least 7 days.
2. Cases where the number of days without ingestion (where the stated daily dose was not reached) was greater than 15% of the total number of planned days.
3. Cases where it was proven that the restrictions outlined in this document were disregarded considerably during the study period.
4. Cases where there were major issues regarding reliability of the data due to problems with testing or other reasons.
5. Other reasons that made it appropriate to exclude the subject.

9. Statistical analysis

Results were reported as the mean and SE. A two-tailed paired *t*-test was used for analysis of

comparisons between before and after ingestion. Dunnett's two-tailed test was used for comparison of the placebo group with the fucoxanthin groups. A probability of less than 5% was considered to be significant. Moreover, a Dunnett's multiple range test would negatively affect the explorative nature of the study and would increase the chance for false-negative results. For these reasons, we decided to set α value at 0.01.

RESULTS

1. Study performance

The study was performed from March 21 to April 25, 2010. During the treatment period, 2 subjects withdrew from each group for personal reasons. In total, 9 subjects remained in each group at the final evaluation. The age of the subjects was 42.6±6.4 years in the placebo group, 45.4±6.7 in the 1 mg/day fucoxanthin group, and 40.2±9.6 in the 3 mg/day fucoxanthin group.

2. Body fat parameters

Table 3 shows the body fat parameters measured in the study. After ingestion of fucoxanthin at 3 mg/day, relative body weight and BMI were significantly decreased compared to placebo and the values before ingestion. Body fat ratio, fat mass, lean mass, muscle mass, and basal metabolic rate showed no significant changes. Calorie intake and fat intake were not different among the groups. In regards to CT parameters, fucoxanthin (1 mg/day) significantly decreased both the absolute and relative total fat areas and the relative subcutaneous fat area compared to before ingestion (Table 4). A significant difference of the relative total fat area from placebo was also detected in the 1 mg/day fucoxanthin group. Additionally, fucoxanthin (3 mg/day) significantly decreased the relative visceral fat area.

With the body circumference parameters, waist circumference decreased significantly after ingestion in both the placebo and 3 mg/day fucoxanthin groups compared to the values before ingestion (Table 5). In the 1 mg/day fucoxanthin group, the relative waist circumference increased significantly compared with before ingestion. However, fucoxanthin (1 mg/day) significantly suppressed the circumference of the left upper arm and thigh compared to before ingestion. Significant decreases were also observed in the relative right thigh circumference and absolute left thigh circumference compared with the placebo group. Relative values show the ratio after ingestion to before ingestion. Data are represented as the mean ±S.E. (n=9). Asterisk denotes a significant difference from before ingestion at *: $p<0.05$. Hash denotes a significant difference from placebo at #: $p<0.05$.

Table 3. Changes of body fat parameters

		Before	After 4 weeks of ingestion (Relative value)	Δ
Body weight (kg)	Placebo	76.7±2.3	76.7±2.3(100.1±0.2)	0.0(0.1)
	Fucoxanthin (1 mg/day)	76.1±2.7	75.4±2.5(99.2±0.4)	-0.7(-0.8)
	Fucoxanthin (3 mg/day)	76.2±3.2	74.9±3.1(98.3±0.7*,#)	-1.3(-1.7)
BMI (kg/m^2)	Placebo	27.5±0.5	27.6±0.6(100.1±0.3)	0.1(0.1)
	Fucoxanthin (1 mg/day)	27.4±0.4	27.2±0.4(99.2±0.4)	-0.2(-0.8)
	Fucoxanthin (3 mg/day)	26.9±0.7	26.4±0.5(98.3±0.7*,#)	-0.5(-1.7)
Body fat ratio (%)	Placebo	30.3±2.7	29.9±2.5(99.3±0.8)	-0.4(-0.7)
	Fucoxanthin (1 mg/day)	30.5±2.1	30.4±2.1(99.6±1.4)	-0.1(-0.4)
	Fucoxanthin (3 mg/day)	29.9±2.5	29.2±2.4(98.1±1.4)	-0.7(-1.9)
Fat mass (kg)	Placebo	22.9±1.7	22.7±1.6(99.4±0.8)	-0.2(-0.6)
	Fucoxanthin (1 mg/day)	22.8±1.1	22.5±1.0(98.8±1.7)	-0.3(-1.2)
	Fucoxanthin (3 mg/day)	22.3±1.4	21.5±1.3(96.5±1.9)	-0.8(-3.5)
Lean mass (kg)	Placebo	53.7±3.2	54.0±3.0(100.6±0.6)	0.3(0.6)
	Fucoxanthin (1 mg/day)	53.2±3.3	52.9±3.2(99.4±0.4)	-0.3(-0.6)
	Fucoxanthin (3 mg/day)	53.9±3.7	53.4±3.7(99.3±0.5)	-0.5(-0.7)
Muscle mass (kg)	Placebo	50.8±3.1	51.0±3.0(100.6±0.6)	0.2(0.6)
	Fucoxanthin (1 mg/day)	50.3±3.2	50.0±3.1(99.5±0.4)	-0.3(-0.5)
	Fucoxanthin (3 mg/day)	50.9±3.6	50.5±3.5(99.3±0.5)	-0.4(-0.7)
Basal metabolic rate (kcal)	Placebo	1558±76	1563±71(100.5±0.6)	5(0.6)
	Fucoxanthin (1 mg/day)	1535±82	1524±81(99.3±0.4)	-11(-0.7)
	Fucoxanthin (3 mg/day)	1566±91	1549±88(99.0±0.5)	-17(-1.0)
Calorie intake (kcal)	Placebo	6111±709	5239±201	-872
	Fucoxanthin (1 mg/day)	6183±256	5715±275	-468
	Fucoxanthin (3 mg/day)	6658±550	6091±641	-567
Fat intake (g)	Placebo	228±35	166±13	-62
	Fucoxanthin (1 mg/day)	210±14	183±17	-27
	Fucoxanthin (3 mg/day)	238±21	216±36	-22

Relative values show the ratio after ingestion to before ingestion. Data are represented as the mean ±S.E. (n=9). Asterisk denotes a significant difference from before ingestion at *: $p<0.05$. Hash denotes a significant difference from placebo at #: $p<0.05$.

Table 4. Changes of CT parameters

	Before ingestion	After 4 weeks of ingestion(Relative value)	Δ
Total fat area (cm^2)			
Placebo	357.8±14.9	374.0±17.0(104.6±2.8)	16.2(4.6)
Fucoxanthin (1 mg/day)	376.4±14.4	355.3±19.5*(94.1±2.5*,#)	-21.1(-5.9)
Fucoxanthin (3 mg/day)	377.7±19.7	363.2±16.8(96.7±2.7)	-14.5(-3.3)
Visceral fat area (cm^2)			
Placebo	111.6±9.7	117.7±13.0(104.3±3.9)	6.1(4.3)
Fucoxanthin (1 mg/day)	118.0±11.8	110.7±10.7(94.4±4.4)	-7.3(-5.6)
Fucoxanthin (3 mg/day)	114.0±18.0	95.4±11.6(85.7±3.3**,##)	-18.6(-14.3)
Subcutaneous fat area (cm^2)			
Placebo	246.2±20.9	256.2±20.7(104.6±3.7)	10.0(4.6)
Fucoxanthin (1 mg/day)	258.4±22.7	244.6±26.1(93.7±2.8#)	-13.8(-6.3)
Fucoxanthin (3 mg/day)	263.7±21.3	267.7±23.3(101.4±2.1)	4.0(1.4)

Relative values show the ratio after ingestion to before ingestion. Data are represented as the mean ±S.E. (n=9). Asterisks denote significant differences from before ingestion at *: $p<0.05$ and **: $p<0.01$. Hashes denote significant differences from placebo at #: $p<0.05$ and ##: $p<0.01$.

Table 5. Changes of circumference parameters

	Before ingestion	After 4 weeks of ingestion(Relative value)	Δ
Waist circumference (cm)			
Placebo	99.7±1.4	97.4±1.0*(97.7±0.8*)	-2.3(-2.3)
Fucoxanthin (1 mg/day)	99.8±1.2	100.1±1.2(100.3±0.3#)	0.3(0.3)
Fucoxanthin (3 mg/day)	100.6±1.5	98.4±1.1*(97.9±0.8*)	-2.2(-2.1)
Neck (cm)			
Placebo	39.3±1.1	38.7±0.9(98.6±1.4)	-0.6(-1.4)
Fucoxanthin (1 mg/day)	39.5±0.9	38.8±1.0(98.2±1.3)	-0.7(-1.8)
Fucoxanthin (3 mg/day)	40.1±1.5	39.1±1.5(97.6±1.9)	-1.0(-2.4)
Right upper arm (cm)			
Placebo	35.0±0.9	34.1±0.5(97.7±1.5)	-0.9(-2.3)
Fucoxanthin (1 mg/day)	34.7±0.8	33.6±0.6(97.0±1.9)	-1.1(-3.0)
Fucoxanthin (3 mg/day)	34.3±0.8	33.9±0.4(98.9±1.3)	-0.4(-1.1)
Left upper arm (cm)			
Placebo	35.0±1.0	34.3±0.5(98.5±1.9)	-0.7(-1.5)
Fucoxanthin (1 mg/day)	35.3±0.8	33.7±0.5*(95.6±1.8*)	-1.6(-4.4)
Fucoxanthin (3 mg/day)	34.7±0.8	34.2±0.5(98.8±2.1)	-0.5(-1.2)
Right thigh (cm)			
Placebo	62.5±1.3	61.7±1.5(98.6±0.7)	-0.8(-1.4)
Fucoxanthin (1 mg/day)	60.9±0.6	58.1±1.0**(95.3±1.1**,#)	-2.8(-4.7)
Fucoxanthin (3 mg/day)	61.1±1.1	60.0±1.1(98.1±1.0)	-1.1(-1.9)
Left thigh (cm)			
Placebo	62.7±1.0	61.4±1.6(97.9±1.4)	-1.3(-2.1)
Fucoxanthin (1 mg/day)	60.3±0.8	57.4±1.0*#(95.4±1.8*)	-2.9(-4.6)
Fucoxanthin (3 mg/day)	61.2±0.9	60.0±0.9(97.8±1.1)	-1.2(-2.2)

Relative values show the ratio after ingestion to before ingestion. Data are represented as the mean ±S.E. (n=9). Asterisks denote significant differences from before ingestion at *: $p<0.05$ and **: $p<0.01$. Hash denotes significant difference from placebo at #: $p<0.0$

3. **Safety parameters**

With regards to blood pressure, ingestion of fucoxanthin at 3 mg/day significantly reduced both the diastolic and systolic blood pressures compared to the placebo group (Table 6), although both pressures were within the normal range. In the 1 mg/day fucoxanthin group, the pulse rate was significantly lower compared to placebo group. However, a significant increase of the pulse rate was observed in the placebo group. Hematology parameters did not change during ingestion of placebo or fucoxanthin, except that MCHC increased significantly in both groups ingesting fucoxanthin (1 and 3 mg/day) compared with ingestion. However, the increase was slight and within the normal range in both groups.

Table 6. Changes of blood pressure, pulse, and laboratory parameters

		Before ingestion	After 4 weeks of ingestion	Δ	Standard value
Systolic pressure (mmHg)	Placebo	133.4±3.2	127.4±4.7	-0.7	<125
	Fucoxanthin (1 mg/day)	124.7±4.2	121.1±5.5	-3.6	
	Fucoxanthin (3 mg/day)	118.8±3.4	114.2±4.0#	-4.6	
Diastolic pressure (mmHg)	Placebo	82.6±3.7	80.4±3.8	-1.8	<85
	Fucoxanthin (1 mg/day)	72.6±3.8	69.8±3.3	-2.8	
	Fucoxanthin (3 mg/day)	65.3±3.2	65.7±2.8#	0.4	
Pulse (beats/min)	Placebo	74.4±2.8	80.2±3.0*	5.8	
	Fucoxanthin (1 mg/day)	68.0±2.7	68.7±2.9#	0.7	
	Fucoxanthin (3 mg/day)	73.3±2.3	75.8±3.6	2.5	
Red cells ($\times 10^4$cells/μL)	Placebo	477±9	478±10	1	Male 427-570
	Fucoxanthin (1 mg/day)	492±13	495±9	3	Female 327-500
	Fucoxanthin (3 mg/day)	497±16	490±20	-7	
Leukocytes (cells/μL)	Placebo	6189±336	6211±359	22	Male 3900-9800
	Fucoxanthin (1 mg/day)	6733±652	7444±857	711	Female 3500-9100
	Fucoxanthin (3 mg/day)	5978±347	5956±556	-22	
Hemoglobin (g/dL)	Placebo	14.2±9.5	14.2±0.5	0	Male 13.5-17.6
	Fucoxanthin (1 mg/day)	14.6±0.5	14.7±0.5	0.1	Female 11.3-15.2
	Fucoxanthin (3 mg/day)	14.9±0.5	14.9±0.5	0	
Hematocrit (%)	Placebo	43.2±1.2	42.8±1.2	-0.4	Male 39.8-51.8
	Fucoxanthin (1 mg/day)	44.2±1.1	43.9±1.2	-0.3	Female 33.4-44.9
	Fucoxanthin (3 mg/day)	45.0±1.2	44.0±1.3	-1.0	
Platelet ($\times 10^4$cells/μL)	Placebo	30.0±2.3	30.1±2.2	0.1	13.0-36.9
	Fucoxanthin (1 mg/day)	30.1±3.0	30.7±2.5	0.6	
	Fucoxanthin (3 mg/day)	25.0±1.4	25.8±1.4	0.8	
MCV (fL)	Placebo	90.6±1.6	89.4±1.7	-1.2	Male 83-102
	Fucoxanthin (1 mg/day)	89.8±1.9	88.7±2.0	-1.1	Female 79-100
	Fucoxanthin (3 mg/day)	91.0±1.4	90.1±1.8	-0.9	
MCH (pg)	Placebo	29.8±0.8	29.6±0.8	-0.2	Male 28.0-34.6
	Fucoxanthin (1 mg/day)	29.6±0.9	29.6±0.9	-0.0	Female 26.3-34.3
	Fucoxanthin (3 mg/day)	30.0±0.7	30.5±0.6	0.5	
MCHC (%)	Placebo	32.8±0.5	33.1±0.4	0.3	Male 31.6-36.6
	Fucoxanthin (1 mg/day)	32.9±0.4	33.3±0.4*	0.4	Female 30.7-36.6
	Fucoxanthin (3 mg/day)	33.0±0.4	33.9±0.3*	0.9	

Data are represented as the mean ±S.E. (n=9). Asterisk denotes significant difference from before ingestion at *: $p<0.05$. Hash denotes significant difference from placebo at #: $p<0.05$

Table 7. Changes of biochemical parameters

		Before ingestion	After 4 weeks of ingestion	Δ	Standard value
Protein (g/dL)	Placebo	7.5±0.1	7.3±0.1*	-0.2	6.7-8.3
	Fucoxanthin (1 mg/day)	7.3±0.1	7.3±0.1	0.0	
	Fucoxanthin (3 mg/day)	7.4±0.1	7.4±0.1	0.0	
Albumin (mg/dL)	Placebo	4.26±0.04	4.26±0.07	0	3.8-5.3
	Fucoxanthin (1 mg/day)	4.20±0.06	4.31±0.06*	1.1	
	Fucoxanthin (3 mg/day)	4.33±0.05	4.42±0.06	0.9	
Urea N (mg/dL)	Placebo	10.9±0.6	10.3±0.6	-0.6	8-22
	Fucoxanthin (1 mg/day)	12.7±1.2	12.2±0.9	-0.5	
	Fucoxanthin (3 mg/day)	14.2±1.2	12.1±0.9**	-2.1	
Creatinine (mg/dL)	Placebo	0.70±0.03	0.65±0.04	-0.05	Male 0.61-1.04
	Fucoxanthin (1 mg/day)	0.66±0.04	0.63±0.04*	-0.03	Female 0.47-0.79
	Fucoxanthin (3 mg/day)	0.77±0.04	0.71±0.04*	-0.06	
Uric acid (mg/dL)	Placebo	4.6±0.3	4.8±0.4	0.2	Male 3.7-7.0
	Fucoxanthin (1 mg/day)	5.3±0.4	5.3±0.4	0.0	Female 2.5-7.0
	Fucoxanthin (3 mg/day)	5.8±0.4	5.7±0.4	-0.1	
Total cholesterol (mg/dL)	Placebo	231±10	224±10	-7	130-219
	Fucoxanthin (1 mg/day)	204±12	208±10	4	
	Fucoxanthin (3 mg/day)	191±11	195±10	4	
LDL-cholesterol (mg/dL)	Placebo	144±9	140±9	-4	70-139
	Fucoxanthin (1 mg/day)	125±10	128±8	3	
	Fucoxanthin (3 mg/day)	106±10	110±10#	4	
HDL-cholesterol (mg/dL)	Placebo	59.0±5.4	58.7±4.7	-0.3	Male 40-86
	Fucoxanthin (1 mg/day)	52.6±3.3	54.6±3.7	2.0	Female 40-96
	Fucoxanthin (3 mg/day)	63.9±5.1	63.8±7.5	-0.1	
Triglyceride (mg/dL)	Placebo	145±28	126±24	-19	35-149
	Fucoxanthin (1 mg/day)	140±22	133±12	-7	
	Fucoxanthin (3 mg/day)	109±25	98±26	-11	
Free fatty acid (mEq/dL)	Placebo	0.45±0.06	0.52±0.04	0.07	0.10-0.85
	Fucoxanthin (1 mg/day)	0.40±0.06	0.51±0.06*	0.11	
	Fucoxanthin (3 mg/day)	0.37±0.05	0.53±0.09*	0.10	
AST (U/L)	Placebo	23.4±1.4	20.8±1.6	-2.6	10-40
	Fucoxanthin (1 mg/day)	21.7±1.9	21.4±2.4	-0.3	
	Fucoxanthin (3 mg/day)	22.0±1.6	20.1±1.2	-1.9	
ALT (U/L)	Placebo	31.9±4.7	24.7±4.1*	-7.2	5-45
	Fucoxanthin (1 mg/day)	27.9±5.5	30.0±6.2	2.1	
	Fucoxanthin (3 mg/day)	21.8±3.0	21.7±2.1	-0.1	
γ-GTP (U/L)	Placebo	33.1±4.5	32.4±4.4	-0.7	Male <75
	Fucoxanthin (1 mg/day)	47.4±9.6	53.3±11.1	5.9	Female <45
	Fucoxanthin (3 mg/day)	25.9±4.4	26.1±4.7	0.2	
ALP (U/L)	Placebo	244±25	235±26	-9	110-360
	Fucoxanthin (1 mg/day)	234±16	232±13	-2	
	Fucoxanthin (3 mg/day)	194±23	199±22	5	
CK (U/L)	Placebo	100±12	114±23	14	Male 50-250
	Fucoxanthin (1 mg/day)	128±34	107±13	-11	Female 45-210
	Fucoxanthin (3 mg/day)	154±23	118±18	-36	
Blood glucose (mg/dL)	Placebo	100±2	100±3	0	70-109
	Fucoxanthin (1 mg/day)	97±3	98±4	1	
	Fucoxanthin (3 mg/day)	92±2	94±3	2	

Data are represented as the mean ±S.E. (n=9). Asterisks denote significant differences from before ingestion at*:$p<0.05$ and **: $p<0.01$. Significant differences were not detected between the placebo and fucoxanthin groups.

Regarding biochemical parameters, only LDL-cholesterol decreased significantly in subjects taking fucoxanthin at 3 mg/day compared to the value in the placebo group (Table 7). However, the baseline LDL-cholesterol level was lower in the 3 mg/day fucoxanthin group than in the placebo group, so the significant decrease was not considered to be problematic. Several other parameters changed significantly during fucoxanthin ingestion, including urea N, creatinine, and free fatty acids. However, all values were within the standard normal ranges and there were no clear changes of other associated parameters from before to after ingestion. Therefore, these changes were not considered to be caused by the test substance. Urine pH decreased in the fucoxanthin (3 mg/day) group, but stayed within the normal range (Table 8).

Table 8. Changes of urine parameters

	Before ingestion	After 4 weeks of ingestion	Standard value
Specific gravity			
Placebo	1.019±0.002	1.017±0.002	1.005-1.030
Fucoxanthin (1 mg/day)	1.020±0.002	1.018±0.001	
Fucoxanthin (3 mg/day)	1.025±0.002	1.018±0.003*	
pH			
Placebo	6.33±0.25	6.22±0.21	5.0-8.5
Fucoxanthin (1 mg/day)	6.00±0.25	6.06±0.19	
Fucoxanthin (3 mg/day)	6.11±0.20	6.28±0.30	

Data are represented as the mean ±S.E. (n=9). Asterisk denotes significant differences from before ingestion at *: $p<0.05$. Significant differences were not detected between the placebo and fucoxanthin groups.

4. **Adverse effects**

The physical condition of the subjects tended to improve in all groups according to the results of the questionnaire, with no differences among the 3 groups. No severe events occurred in any of the subjects. According to the daily reports from the subjects, there were no abnormalities of the physical or mental condition, fatigue, back/shoulder pain, sleep impairment, digestive dysfunction, or skin abnormalities related to ingestion of the placebo or fucoxanthin. There was a slight change of safety parameters by ingestion of fucoxanthin including blood pressure, blood, and urine parameters, which were in normal ranges. As a result, intervention of fucoxanthin appears to to exhibit no adverse effects in the study condition.

DISCUSSION

There are few countries where the population regularly consumes seaweed or algae in the diet. However, the health benefits of ingredients in seaweed have been studied in many countries, including China, South Korea, and Japan. Utilization of seaweed ingredients for weight loss has shown progress, including assessment of polyphenols[19], polysaccharides[20], and alginates[21]. Fucoxanthin is a promising anti-obesity ingredient derived from brown algae that reduces fat accumulation *in vitro* and in rodents[22]. In the present clinical study, ingestion of fucoxanthin at 3 mg/day significantly decreased the body weight, BMI, and visceral fat area compared to placebo. Thus, fucoxanthin was discovered to suppress body weight and BMI by reducing visceral fat. The

Japanese Obesity Society classifies obesity into 4 grades, where Japanese individuals with a BMI ranging from 25 to 29 kg/m^2 (our subjects) belong to grade 1[23]. Grade 1 obese individuals without 11 complications, including hypertension and glucose intolerance[24], are regarded as mildly obese subjects. In our study, we excluded subjects with associated complications. Therefore, fucoxanthin was found to improve visceral fat content in Japanese men and women with grade 1 obesity. Recently published report by Mikami *et al.*[25] concluded that fucoxanthin (1 and 2 mg/day) derived from akamoku seaweed affected no influence on BMI in subjects mainly consisting of grade 1 obese Japanese. A part of the result is corresponding to our results and slight difference of dosage (2 and 3 mg/day focosanthin) might affect to the anti-obesity effect.

On abdominal CT scans, fucoxanthin (1 mg/day) significantly decreased the total and subcutaneous fat areas. However, the effect lacked dose-responsiveness and fucoxanthin (3 mg/day) and significantly suppressed the relative visceral fat area instead. In terms of the obesity criteria used in Japan, subjects with a visceral fat area of more than 100 cm^2 in addition to a BMI over 25 kg/m^2 are judged as visceral fat obesity. The mean baseline visceral fat area of our subjects was around 110 to 120 cm^2. Consequently, most of the subjects were regarded as having a high visceral fat content by the Japanese criteria and fucoxanthin (3 mg/day) which may improve visceral fat-type obesity which often leads to complications.

The neck, arm, and thigh circumferences showed similar trends to abdominal fat areas, as did the right and left thigh circumferences. In the 1 mg/day fucoxanthin group, the relative circumferences of the right and left thighs and left upper arm were significantly lower than in the placebo group. According to these results, as the fat at the body sites is subcutaneous fat, fucoxanthin appeared to reduce subcutaneous fat at 1 mg/day and it improved visceral fat at 3 mg/day. A longer-term study may be required to clarify the detailed effects of fucoxanthin on visceral and subcutaneous fat, in addition to the appropriate dosage of this agent. We should also consider the influence of UCP1 in the subjects. UCP1 expression in white adipose tissue decreases with aging in mice[26]. On the other hand, the amount of brown adipose tissue (BAT), which is the major source of UCP1 in humans, varies among individuals and is higher in young adults than in the elderly[27]. Therefore, we need to consider the actions of fucoxanthin in future clinical trials on obesity. Regarding the safety of fucoxanthin, no adverse events were caused by its ingestion during the study period.

In conclusion, oral administration of fucoxanthin demonstrated a selective anti-obesity effect on visceral fat and no adverse events occurred when 1 or 3 mg/day of fucoxanthin was ingested for four weeks. These results suggest that fucoxanthin may be an effective and safe functional food additive.

CONCLUSION

In this study, we demonstrated that a 4-week treatment with fucoxanthin (3 mg/day) reduced body weight, BMI and abdominal fats. Therefore, we discovered that fucoxanthin has weight loss effects in mildly obese adults.

LIST OF ABBREVIATIONS: Body mass index (BMI), Food for Specified Health Uses (FOSHU), Uncoupling protein (UCP), Peroxisome proliferator-activated receptor (PPAR), PPARγ co-activator (PGC), Carnitine palmitoyltransferase (CPT), Brown adipose tissue (BAT),

Computerized tomography (CT), Standards for the Implementation of Clinical Trials of Pharmaceutical Products (GCP),

Conflicts of Interest: Both authors related to this study are employees of Oryza Oil & Fat Chemical Co., Ltd. (Aichi, Japan). The authors declare no conflict of interest associated with this manuscript.

Authors contribution: Dr. Shoketsu-Hitoe conducted the study and prepared test samples. Dr. Hiroshi Shimoda supported the study and wrote the manuscript.

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