General Test Report

Test Title

Clinical Study on the Slimming Effect of Dietary Supplements Containing Fucoxanthin

Protocol No.: HR-2010-OY03
Test Period: March 21, 2010 to April 25, 2010
Date of Reporting: June 18, 2010

Table of Contents

1. Outline of the Test

Title of the Test	Clinical study on the slimming effect of dietary supplements containing fucoxanthin
Test Materials	Group A: Ingestion of 1 mg/day of fucoxanthin Group B: Ingestion of 3 mg/day of fucoxanthin Group C: Ingestion of a placebo
Test Design	Placebo-controlled double-blind test, comparison between concurrently-tested groups (comparison among three groups)
Test Period	SCR & pre-ingestion session (visit-1), after four weeks of ingestion (visit-2), four weeks in total
Objective	The test was carried out to examine the slimming effect of dietary supplements containing fucoxanthin. Specifically, the slimming effect of the ingestion of dietary supplements containing fucoxanthin for four weeks was examined on male and female adults with a BMI of 25 or higher by a double-blind test using two dietary supplement products containing fucoxanthin and also one placebo supplement product as the control. Main evaluation items were: (1) CT scan of abdominal fat area (total fat, visceral fat, and subcutaneous fat) (2) Measurement of body composition (3) Circumference measurements (waist, upper arms, lower neck and thighs) Secondary evaluation items were: (1) Measurement of blood pressure level and pulses (2) Blood and urine tests Other survey items were: (1) Daily report written by the test subjects (2) Simplified survey and instruction on diet (3) Questionnaire Effectiveness and safety of the ingestion of fucoxanthin were examined and evaluated based on the results obtained from the evaluation items and survey items described above.
No. of Test Subjects	11 persons/group × 3 groups, 33 persons in total (Guaranteed data: 30 persons in total)
Target No. for Screening	50 persons (Target male/female ratio was 1:1.)
No. of Participated Test Subjects	・ SCR & pre-ingestion session (visit-1): 50 persons ・ After four weeks of ingestion (visit-2): 33 persons
Test Subjects (Self-report from test subjects)	・ Male and female aged between 20 and 59 at the time where they received the explanation about the agreement on the participation in the test. (Target male/female ratio was 1:1.) ・ Persons with BMI between 25 and 30 ・ Persons with an abdominal circumference of at least 85 cm (male) or 90 cm (female) ・ Persons that the doctor responsible for the test approved for participation in the test

Exclusion Criteria	・ People currently taking medication for some type of chronic symptom ・ People with a potential of severe allergic reaction to foods or pharmaceuticals ・ People continuously ingesting pharmaceuticals or Oriental crude drugs that may influence test results (such as anti-metabolic syndrome drugs) or health foods for slimming purpose (e.g. low fat oils, healthy beverages, fat level resetting products, fat-reducing beverages, black oolong tea, and hydrolysate of globin protein) ・ People with a current symptom or a history of cardiovascular disease, nephritis, hepatitis, or any other disorder ・ People with an AST (GOT), ALT (GPT), or γ-GT (γ-GTP) level 2.5 times higher than the standard value ・ People with a uric acid (UA) level of 9.0 mg/dl or higher ・ People with severe anemia ・ Women who are pregnant, ready to be pregnant, or under breast-feeding ・ People drinking alcohol excessively (60 g or more of alcohol almost every day) ・ People participating in another human clinical test ・ People determined as not suitable as a subject for the test by the doctor implementing the test
Test Subjects Controlling Matters	・ Alcohol intake on the day before the test was prohibited. ・ Irregular lifestyle (e.g. lack of sleep, overeating, or overdrinking) was avoided during the test period. ・ Concerning diet, quantity and quality of food similar to that eaten before the test were maintained. Excessive exercise, overeating, overdrinking, and lack of sleep on the day before the test were strictly prohibited. ・ Ingestion of pharmaceuticals or Oriental crude drugs with a function related to the test material (e.g. anti-metabolic syndrome drugs) or health foods released for slimming or body fat-reducing effect (e.g. low fat oils, healthy beverages, fat level resetting products, fat-reducing beverages, black oolong tea, and digest of globin protein) was prohibited. ・ Test subjects who constantly take dietary supplements (e.g. vitamins) kept taking the regular amount. ・ Blood donation was prohibited during the test period.
Items Implemented at SCR and Each Visit	・ Test explanation was given and the agreement was submitted (SCR only). [Main evaluation items] ・ Measurement of body composition ・ Circumference measurement (waist, upper arms, neck, and thighs) ・ CT scan of abdominal fat area (total fat, visceral fat, subcutaneous fat) [Secondary endpoints] ・ Measurement of blood pressure level and pulses ・ Blood and urine tests [Other] ・ Daily report written by test subjects every day ・ Simplified survey and instruction on diet
Test Subjects Selection Criteria	Based on the test results of SCR and visit-1, 33 persons with higher BMI, total fat mass, visceral fat mass, and subcutaneous fat mass were selected among people applicable as the test subjects.

Test Subjects Grouping Method	Test subjects were grouped so that the average values for total fat mass, visceral fat mass, subcutaneous fat mass, body weight, BMI, age, and sex are close to equal in each group based on the results of SCR and the measurements at visit-1.
Ingestion Method/Amount	Described in the main text of this protocol.
Statistical Processing	Appropriate statistical processing was performed. A two-tailed test with a significance level of 5 % max was used.
Report	Test report and image report
Date to Submit a Draft of Report	40 days after the test finished
Ethical Considerations	To carry out the test, an ethics committee was separately established. Under approval by the committee, the test was carried out conforming to the protocol and ethical principles of the Helsinki Declaration.
How the Test is Carried Out	Described in this protocol.

2. Title of the Test and Protocol No.

Title of the Test: Clinical Study on the Slimming Effect of Dietary Supplements Containing Fucoxanthin

Protocol No.: HR-2010-OY03

3. How the Test Was Carried Out

3.1 Test Outsourcer

Oryza Oil & Fat Chemical Co., Ltd.
1 Numata Kitagata-cho, Ichinomiya-city, Aichi-pref. 493-8001 Japan
TEL: (+81) 586-86-5141/ FAX: (+81) 586-86-6191
Person in charge: Shoketsu Tan

3.2 Test Outsourcee

TES Holdings Co., Ltd.
6F Tokyo University Entrepreneurs Plaza, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033 Japan
TEL: (+81) 3-6801-8480 / FAX: (+81) 3-6801-8481
Person in charge: Toshiyasu Tamura

3.3 Doctor Responsible for the Test

Oriental Occupational Health Association Tokyo Branch
Oriental Ueno Detection Center
Yasuko Oike

3.4 Medical Institute that Carried out the Test

Oriental Occupational Health Association Tokyo Branch
Oriental Ueno Detection Center
1-20-11, Ueno, Taito-ku, Tokyo 110-0005 Japan
TEL: (+81) 03-5816-0711 / FAX: (+81) 3-5816-0712
Person in charge: Takeshi Miyanaga

3.5 Medical Institute in Charge of CT Scan

Oriental Occupational Health Association Tokyo Branch
Oriental Ueno Detection Center

3.6 Statistical Analysis Manager

TES Holdings Co., Ltd.

Yusuke Hori (Doctor of Medicine), Academic Affairs Department

3.7 Consultation Service

TES Holdings Co., Ltd.
6F Tokyo University Entrepreneurs Plaza, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033 Japan
TEL: (+81) 3-6801-8480 / FAX: (+81) 3-6801-8481

Oriental Occupational Health Association Tokyo Branch
Oriental Ueno Detection Center
1-20-11, Ueno, Taito-ku, Tokyo 110-0005 Japan
TEL: (+81) 03-5816-0711 / FAX: (+81) 3-5816-0712

3.8 Institute where Blood and Urine Tests were Outsourced

[General blood and urine tests]
Health Science Research Institute Co., Ltd.
106 Goudocho, Hodogaya-ku, Yokohama City 240-0005 Japan
TEL: (+81) 45-333-1661 / FAX: (+81) 45-333-5153

4. Objective

The objective of the test was examination of the slimming effect of dietary supplements containing fucoxanthin. Specifically, the slimming effect of the ingestion of dietary supplements containing fucoxanthin for four weeks was examined on male and female adults with a BMI of 25 or higher by a double-blind test using two dietary supplement products containing fucoxanthin and also one placebo supplement product as the control.
Main evaluation items were:
(1) CT scan of abdominal fat area (total fat, visceral fat, and subcutaneous fat)
(2) Measurement of body composition
(3) Circumference measurements (waist, upper arms, neck and thighs)
Secondary evaluation items were:
(1) Measurement of blood pressure level and pulses
(2) Blood and urine tests
Other survey items were:
(1) Daily report written by the test subjects
(2) Simplified survey and instruction on diet
(3) Questionnaire
Effectiveness and safety of the ingestion of fucoxanthin were examined and evaluated based on the results obtained from the evaluation items and survey items described above.

5. Test Subjects

5.1 No. of Test Subjects

A questionnaire was carried out when gathering people interested to participate in the test among people registered to the monitor bank of TES Holdings Co., Ltd. Among them, 50 people whose self report satisfy the “selection criteria” described below and do not fail the “exclusion criteria” were selected by an interview on the phone. Then, 33 people were selected as the test subjects by a screening test.

5.2 Test Subjects (based on self-report from the test subjects)

- Male and female aged between 20 and 59 at the time where they received the explanation about the agreement on the participation in the test. (Target male/female ratio was 1:1.)
- Persons with BMI between 25 and 30
- Persons with an abdominal circumference of at least 85 cm (male) or 90 cm (female)
- Persons that the doctor responsible for the test approved for participation in the test

5.3 Exclusion Criteria

- Persons currently taking medication for some type of chronic symptom

- Persons with a potential of severe allergic reaction to foods or pharmaceuticals
- Persons continuously ingesting pharmaceuticals or Oriental crude drugs that may influence test results (such as anti-metabolic syndrome drugs) or health foods for slimming purpose (e.g. low fat oils, healthy beverages, fat level resetting products, fat-reducing beverages, black oolong tea, and digest of globin protein)
- Persons with a current symptom or a history of cardiovascular disease, nephritis, hepatitis, or any other disorder
- Persons with an AST (GOT), ALT (GPT), or γ-GT (γ-GTP) level 2.5 times higher than the standard value
- Persons with a uric acid (UA) level of 9.0 mg/dl or higher
- Persons with severe anemia
- Women who are pregnant, may be pregnant, or breast-feeding
- Persons drinking alcohol excessively (60 g or more of alcohol almost every day)
- Persons participating in another human clinical test
- Persons determined as not suitable as a subject for the test by the doctor implementing the test

6. Agreement of Test Subjects

The test implementing organization individually obtained test subjects' informed consent concerning the following matters in a written document before starting the test. The organization thoroughly explained the test subjects that they can participate in the test on their own will and they will not suffer any disadvantages even if they do not agree to participate in the test. Then, the test subjects submitted the written agreement concerning the participation in the test. (Each test subject's signature was necessary.)

- Objective and method of the test
- Explanation about the test materials, their effects, and expected development of side-effects
- Test subjects will be under sufficient control of the doctor responsible for the test during the test period.
- Test subjects will not be discriminated against even if they do not agree to participate in the test.
- Test subjects can cancel the agreement to participate in the test at any time.
- Appropriate treatment and medical services available to the test subjects in case of health damage related to the test
- If information that may influence the test subjects' desire to continue participating in the test is obtained, the information will be promptly communicated to the test subjects.
- Other matters necessary for the protection of the test subjects' human rights and disclosure of information about the test subjects
- Matters that the test subjects should follow (entire test schedule, dates to visit the doctor, dose, etc.)
- Establishment of a contact to a medical institute when the test subjects want more information about the test or their rights or when health damage occurs related to the test
- Transportation fees and cooperation fees to be given to the test subjects

7. Outline of Test Materials

7.1 Outline of Test Materials

In the test, test materials (two dietary supplements containing fucoxanthin and a placebo supplement) provided by Oryza Oil & Fat Chemical Co., Ltd. were used.

The dietary supplements containing fucoxanthin were hard capsules prepared using Oryza Oil & Fat Chemical's product Fucoxanthin-P1 (powder containing fucoxanthin 1 %).

Placebo supplement was a similar-looking hard capsule prepared using only the diluting agent that is used in the production of Fucoxanthin-P1.

7.2 Origin and Development History

Origin:

The test material is a kelp extract extracted and purified from kelp (Laminaria japonica) grown in Hokkaido in Japan using hydrous ethanol where fucoxanthin is highly-enriched.

Development history:
Fucoxanthin is a type of carotenoid contained in brown algae such as kelp, hijiki algae, and brown seaweed and also some microalgae. In Japan, functionality of fucoxanthin, which is a carotenoid specifically contained in kelp and brown seaweed, is actively studied because Japanese people eat seaweed more than people in other countries. Especially the function to reduce symptoms of metabolic syndrome is attracting many people's attention.
Oryza Oil & Fat Chemical Co., Ltd. successfully developed high-concentration fucoxanthin using kelp purely grown in Hokkaido in Japan, released it in autumn 2008, and has been selling it to food and cosmetic manufacturers. In this test, the anti-obesity effect of the ingestion of fucoxanthin was examined among test subjects in a human clinical trial.

7.3 Safety/Effectiveness Data

Safety:
Content of 507 items of agricultural chemicals in kelp extract containing no diluting agent (fucoxanthin content: 3.0 %) was examined based on the Japanese Food Sanitation Act and Agricultural Chemicals Regulation Act. As a result, all items were the standard level (measurable limit) or less.
Kelp extract (fucoxanthin content: 3.0 %) was orally administrated to rats by a dose of 2000 mg/kg and the rats were kept and monitored for 14 days. No abnormal weight change was shown as compared to the control group. No abnormality in rats' organs was shown in an autopsy after the test was finished either. Therefore, LD_{50} of kelp extract (fucoxanthin content: 3.0 %) on rats is considered to be 2000 mg/kg min.
To examine mutagenicity of kelp extract, kelp extract (fucoxanthin content: 3.0 %) was orally administrated to 8-week old ICR male mice by the dose of 500, 1000, and 2000 mg/kg once and the occurrence of micronuclei was evaluated. As a result, no abnormality in the micronuclei inducing frequency was shown in any dose. In the ratio of polychromatophilic erythrocyte to the total erythrocyte, no significant difference from the negative control group was shown in any dose and prohibition of myeloid proliferation was not observed.
In the subacute toxicity test (90 days), kelp extract (fucoxanthin content: 3.0 %) was orally administrated to F344/DuCrj rats by mixing it in feed by 1.0, 2.0, and 4.0 %. General conditions of both male and female rats did not change throughout the test period and no rat died. No significant change in their weight was shown as compared to the control group. In a urine test, a hematology test, and a biochemical examination of blood, no toxic influence related to the ingestion of kelp extract was shown. No toxic influence related to the ingestion of kelp extract was shown in either male and female rats in all groups in a histopathological test.
In literature, pure fucoxanthin product (derived from brown seaweed, 95 % min) has been confirmed to not cause any abnormalities by a single administration test (1000, 2000 mg/kg) and 30-day multiple administration test (500, 1000 mg/kg). No toxicity was shown in a 28-day multiple administration test (10 mg/kg, 50 mg/kg) on rats conducted by a different researcher.

Effectiveness:
A study group lead by Dr. Miyashita, professor at the Graduate School of Fisheries Science, Hokkaido University, administrated fucoxanthin fractions (fucoxanthin content: 67.4 %) to KKAy obese mice for four weeks by mixing them in feed (0.4 %). As a result, fat mass of the mice significantly reduced. The expression of mitochondrial uncoupling protein 1 (UCP1) in visceral fat was examined by the Western blot method and a significant increase in the expression was confirmed. UCP1 is originally responsible to release electrochemical potential of mitochondria which is used to ATP production as body heat. Therefore, fucoxanthin is considered to have an activity to burn fat as body heat by accelerating the UCP1 expression in visceral fat (white fat).
Fucoxanthin has been also reported to have an activity to inhibit fat accumulation against cultured 3T3-L1 fat cell.
Moreover, the Institute of Immunopathology, Russian Academy of Natural Science conducted a 16-week clinical trial on 151 female subjects using dietary supplements containing fucoxanthin

from algae extract and pomegranate seed oil. According to the report about the results of the test, significant weight loss and reduced abdominal circumference were confirmed.

7.4 Main Components of Test Materials and Their Contents (Per Capsule)

Component	Dietary supplement containing fucoxanthin A	Dietary supplement containing fucoxanthin B	Placebo supplement
Vegetable oil and fat	5 .0 mg	15 .0 mg	0 mg
Kelp extract	0.8 mg	2.3 mg	0 mg
Cyclodextrin	142.5 mg	127.5 mg	150.0 mg
Antioxidant (Mix tocopherol)	1.3 mg	3.8 mg	0 mg
Fucoxanthin	0.5 mg	1.5 mg	0 mg

7.5 Nutrient Components in Test Materials / 100 g

Nutrient Component	Dietary supplement containing fucoxanthin A	Dietary supplement containing fucoxanthin B	Placebo supplement
Water	4.4 g	5.1 g	5.1 g
Caloric value	394.3 kcal	414.6 kcal	415.3 kcal
Protein	25.3 g	25.4 g	25.3 g
Fat	2.5 g	7.3 g	7.3 g
Carbohydrate	67.6 g	61.9 g	62.2 g
Sodium	56.0 mg	69.1 mg	39.0 mg

7.6 Dose of Test Materials and Ingestion Method

Test Material	Dose	Ingestion method
Dietary supplement containing fucoxanthin A	2 capsules/day	Two capsules were ingested with water after meal per day.
Dietary supplement containing fucoxanthin B	2 capsules/day	
Placebo supplement	2 capsules/day	

7.7 Storage of Test Materials

Test materials were stored in a cool place protected from direct sunlight, high temperature, and high humidity.

8. Test Material Control Matters

8.1 Person in Charge of Storage of Test Materials

The test materials were appropriately stored and strictly controlled by the following persons.

- Oryza Oil & Fat Chemical Co., Ltd.
  Shoketsu Tan
- TES Holdings Co., Ltd.
  Aya Takahashi

8.2 Test Material Delivery Date and Returning Destination

Test material delivery date: Delivered by March 15, 2020.
Destination to return the test materials: Research and Development Department, Oryza Oil & Fat Chemical Co., Ltd.

8.3 Allocation of Test Materials in Blind Test

Test materials were two types of dietary supplements containing fucoxanthin and a placebo supplement. Their appearance was undistinguishable and identification color was attached on the test materials. This identification was clear on packaged test materials as well. The table of test material allocation was strictly protected by allocation controllers of the test outsourcer and

test outsourcee who were not directly involved in the test so that information was not disclosed to any other parties until the cases to be analyzed were determined in a clinical conference after the test was completed.

8.4 Delivery of Test Materials

Test materials were sent to each test subject by mail. Procedure to ingest the test materials was explained to the test subjects and a document containing the information was sent with the test materials.

9. Test Method

9.1 Test Design

Placebo-controlled double-blind test, comparison between concurrently-tested groups (comparison among three groups)

9.2 Test Period

SCR & pre-ingestion session (Visit-1), after four weeks of ingestion (Visit-2), four weeks in total

9.3 No. of Test Subjects

11 persons × 3 groups, 33 persons in total (guaranteed data: 30 persons in total)

9.4 Target No. for Screening

50 persons (Target male/female ratio was 1:1.)

9.5 Test Subjects Selection Criteria

Based on the test results of SCR and visit-1, 33 persons with higher BMI, total fat mass, visceral fat mass, and subcutaneous fat mass were selected among people applicable as the test subjects.

9.5 Test Subjects Grouping Method

Test subjects were grouped so that the average values for total fat mass, visceral fat mass, subcutaneous fat mass, body weight, BMI, age, and sex are close to equal in each group based on the results of SCR and the measurements at visit-1.

9.6 Test Subjects Controlling Matters

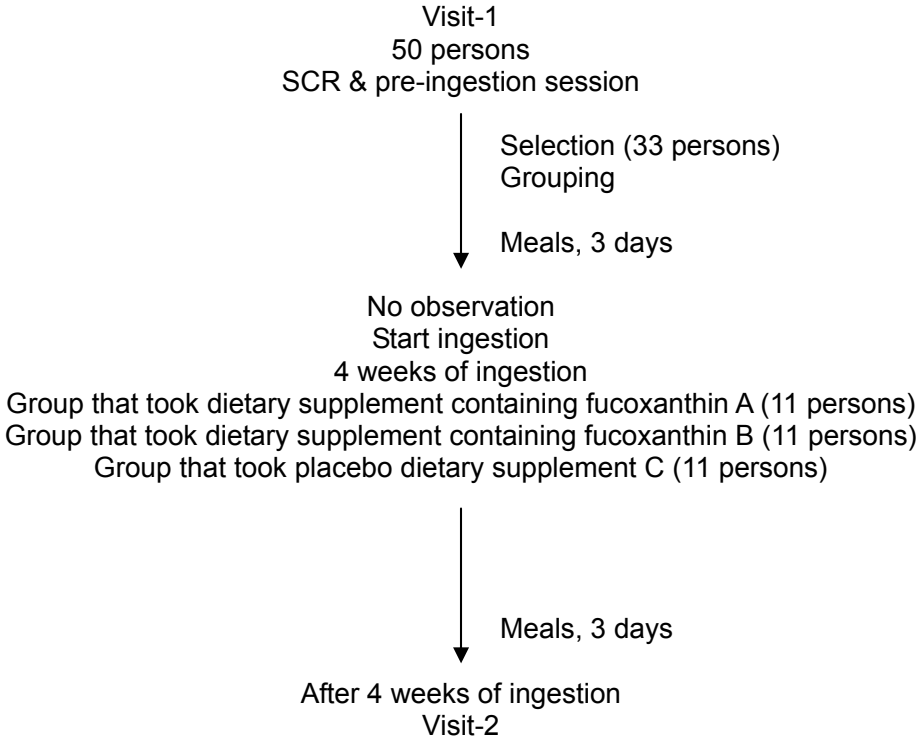
- Alcohol intake on the day before the test was prohibited.
- Irregular lifestyle (e.g. lack of sleep, overeating, or overdrinking) was avoided during the test period.
- Concerning diet, quantity and quality of food similar to that eaten before the test were maintained. Excessive exercise, overeating, overdrinking, and lack of sleep on the day before the test were strictly prohibited.
- Ingestion of pharmaceuticals or Oriental crude drugs with a function related to the test material (e.g. anti-metabolic syndrome drugs) or health foods released for slimming or body fat-reducing effect (e.g. low fat oils, healthy beverages, fat level resetting products, fat-reducing beverages, black oolong tea, and digest of globin protein) was prohibited.
- Test subjects who constantly take dietary supplements (e.g. vitamins) kept taking the regular amount.
- Blood donation was prohibited during the test period.

9.6 Test Schedule and Dates of Tests and Measurements

Tests and measurements were carried out by the following schedule.

1) Approval of the ethics committee
2) Gathering of test subjects
3) Explanation about the test to potential test subjects and obtain written agreement from them
4) SCR & pre-ingestion session (visit-1)

5) Selection/grouping of test subjects
6) Delivery of test materials
7) Start of the ingestion of test materials
8) After four weeks of ingestion (visit 2)



10. Evaluation Items (Various Tests, Measurement Items, Survey Method, and Time of Observation)

10.1 Main Evaluation Items

10.1.1 Measurement of Fat Mass by CT Scan

・Scanning equipment
Subsecond helical CT scanner (ASTEION VP3.5M) made by TOSHIBA corporation

・Scanning method
Test subjects lied down on their back on a bed, lightly breathed two or three times, and held their breath. The cross-section of their abdominal area was scanned at the position of navel while they were holding the breath.

・Fat mass measurement method
CT images were converted into the BMP format and the images were analyzed using the visceral fat measurement PC software Fat Scan (N2 System) to calculate the fat mass.

・Measurement items
Visceral fat area, subcutaneous fat area, and total fat area

・Time of observation
SCR & pre-ingestion session (visit-1), after four weeks of ingestion (visit-2)

10.1.2 Measurement of Body Composition

・Measurement equipment
MC-180 made by Tanita Corporation

・Measurement method
Test subjects wore the specified test clothing and measurements were carried out with setting the tear to 500 g.

・Measurement items

Body weight, body fat percentage, fat mass, fat-free mass, muscle mass, BMI, basal metabolic rate

・Time of observation

SCR & pre-ingestion session (visit-1), after four weeks of ingestion (visit-2)

10.1.3 Circumference Measurement of the Waist, Neck, Upper Arms, and Thighs

・Measurement equipment

Each part was measured by an examiner using a tape measure.

・Measurement positions

Waist circumference: The circumference was measured at the navel in the standing position after the test subjects lightly breathed out. When a test subject had significant amount of fat and their navel has lowered, the circumference was measured at the midpoint between the bottom rib and anterior superior iliac spine (protruding area in the front of the pelvic bone). (Japanese Society for the Study of Obesity, Research on Obesity, 2000-6 (1) 18-28)

Neck circumference: The circumference was measured at the lowest part of the neck.

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

Thigh circumference: The circumference was measured at the highest part of the left and right thighs.

* All measurements were done in the standing position.

・Time of observation

SCR & pre-ingestion session (visit-1), after four weeks of ingestion (visit-2)

10.2 Secondary Evaluation Items

10.2.1 Blood/Urine Tests

・Test items

See separate table 2.

・Institute where analysis was outsourced

Health Science Research Institute Co., Ltd.

・Time of observation

SCR & pre-ingestion session (visit-1), after four weeks of ingestion (visit-2)

10.2.2 Blood Pressure/Pulse Measurements

・Measurement equipment

listmini BP-10 made by COLIN Corporation

・Measurement method

After the test subjects rested for at least 15 minutes, their blood pressure and pulse were measured on their right upper arm.

・Measurement items

Systolic blood pressure, diastolic blood pressure, and pulse

・Time or observation

SCR & pre-ingestion session (visit-1), after four weeks of ingestion (visit-2)

10.3 Other

10.3.1 Simplified Survey and Instruction on Diet

・Survey method

The test subjects wrote what they ate for breakfast, lunch, and dinner as well as in-between meals and also the amount of alcohol they took in the specified survey form for three days before SCR & pre-ingestion session (visit-1) and the observation session after four weeks of ingestion (visit-2).

・Time of dietary instruction

SCR & pre-ingestion session (visit-1)

・Dietary instruction method

A national registered dietitian instructed the test subjects about the amount of meals and nutritious balance based on the test subjects' diet described above.

10.3.4 Test Subjects' Daily Report

・Survey method
Each test subject wrote a daily report in the survey form.
・Survey items
Adverse events, ingestion of the test materials, intake of pharmaceuticals and dietary supplements, menstrual period, etc.
・Time of observation
Every day from the day they started to ingest the test materials until the end of the test

10.3.5 Questionnaire

・Survey method
Each test subject filled out the questionnaire sheet.
・Survey items
Physical and skin conditions
・Time of observation
SCR & pre-ingestion session (visit-1), after four weeks of ingestion (visit-2)

11. Privacy Protection for Test Subjects

The privacy of test subjects was completely protected when handling case report forms, source documents used in the test, agreement forms, etc. When preparing findings reports, test subjects were identified using their subject number.

12. Statistical Analysis

A two-tailed test with a significance level of 5 % max was used.
Appropriate statistical methods were applied.

13. Final Report

A report containing aggregate data and the results of statistical analyses was submitted.
The test outsourcee created the final report based on aggregated and statistically analyzed data.

14. Discontinuation of Testing and Exclusion Criteria

If any of the events below occurred, testing would be terminated at the medical or ethical discretion of the doctor in charge. Furthermore, in the event of confirmation of 1) below, appropriate medical attention would be given to the test subjects to ensure their safety. If a test subject requested testing be discontinued, the test would be promptly stopped and consideration would be taken to prevent any disadvantages to the test subject.

1) If an adverse event such as a serious adverse reaction or subjective/objective symptoms occurred
2) If continued testing became difficult due to a concurrent medical illness or the deterioration of a coexisting disease
3) If drawing blood or carrying out tests became significantly difficult
4) If the test was discontinued in its entirety
5) If the doctor in charge of the test decided it was necessary to discontinue testing

15. Adverse Events

15.1 Definition of Adverse Events

An adverse event is any subjective symptom, which resulted from ingesting the test materials, and that makes the test subject feel unpleasant.

15.2 Investigation and Determination of Adverse Events

In the case where an adverse event was recognised on the basis of the definition in section 15.1,

all necessary and appropriate measures would be undertaken promptly by the doctor in charge of the test. In addition, an evaluation of the adverse event would be carried out and the causal relationship with the test materials would be investigated and determined.

The decision as to whether or not testing should continue would be determined by the doctor in charge if necessary. In addition, the doctor would fill out the following particulars on an "Adverse Event Case Report Form" and would investigate and determine the causal relationship with the test materials.

1) Type of adverse event
   The name (symptoms) of the adverse event should be written.
2) Date of occurrence
   The date of occurrence or the date on which the event was confirmed should be written. If possible, the time of occurrence should also be noted. For symptoms identified before testing commenced, the date of exacerbation of these symptoms should be stated. The date the adverse event was resolved or the date this was confirmed should be written. If possible, the time it was resolved should also be noted.
3) Severity
   The severity of symptoms should be evaluated as either (a), (b) or (c) below, and written on the "Adverse Event Case Report Form". In cases where the same symptoms developed more than once, the point at which the symptom was most severe should be applied.
   (a) Mild: The respective test subject can continue to participate in the test without any form of treatment
   (b) Moderate: The respective test subject can continue to participate in the test with some form of necessary treatment
   (c) Severe: The respective test subject cannot continue to participate in the test even after some form of necessary treatment
4) Existence of severe adverse events
   When a severe adverse event is categorised as "Yes," it has to be reported in writing to the head of the test outsourcer and the institute carrying out the test, and to the ethics committee.
5) Degree of severity
   When a severe adverse event is categorised as "Yes," the adverse event should be evaluated using (a) to (e) below, and written on the "Adverse Event Case Report Form."
   (a) Resulted in death
   (b) Was life-threatening
   (c) Resulted in permanent or serious dysfunction
   (d) Required a hospitalization or extended medical treatment
   (e) Resulted in other serious medical phenomena
6) Treatment
   Whether new treatments were employed to counter the occurrence of an adverse event should be written. In cases where new treatments were employed, the type of treatment undertaken should be noted.
   (a) Temporary suspension of test material ingestion
   (b) Termination of testing
   (c) Medical treatment or hospitalization
   (d) Other
7) Causal relationship
   The causal relationship between the test materials and the adverse event should be evaluated using (a) to (e) below.
   (a) No relationship: Inconceivable that the test materials are the direct cause of the adverse event
   (b) Probably no relationship: Due to timing, there is almost no cause-and-effect link with the test materials
   (c) Relationship may exist: Due to timing, a cause-and-effect link with the test materials is possible

(d) Relationship exists: Due to timing, there is an undeniable cause-and-effect link with the test materials and no other direct cause of the adverse event is conceivable
(e) Unknown: Impossible to determine due to lack of information to evaluate the causal relationship

8) Comments
Reasons given for the evaluation of the causal relationship should be written on the "Adverse Event Case Report Form."
9) Follow-up research
In the case that either (c) or (d) were judged as being the causal relationship by the doctor in item 7) above, follow-up research should be undertaken and the results should be reported.
10) Outcome
Outcomes should be classified based on the following standard only if follow-up research described in 9) was carried out. In addition, the date the outcome was confirmed should be noted.
(a) Recovered
(b) Symptoms were relieved
(c) Not recovered
(d) Had subsequent complications
(e) Died
(f) Other

16. Compensation for Test Subjects

In the event that this test caused damage to a test subject, or a test subject instituted a lawsuit relating to damage caused by this test, the doctor in charge of the test should promptly inform this to the test outsourcer. When health damage occurs due to deliberate intent or negligence on the institute carrying out the test, the institute should assume responsibility for compensation. However in cases where health damage is caused by the test materials, the test outsourcer should take full responsibility for compensation, provided the test subject did not present false information or intentionally cause their health damage.

17. Criteria for Exclusion from Analysis

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

- Cases where a test subject delayed to participate in each visit by at least 7 days
- Cases where the number of days with no ingestion (where the stated daily ingestion amount was not reached) was greater than 15 % of the total number of planned ingestion days
- Cases where, during the test period, it was proven that the restrictions outlined in this document were disregarded considerably
- Cases where major issues arose regarding the reliability of the data due to a problem in testing or other reasons
- Other apparent reasons believed appropriate to exclude the case

18. Notice for Filling Survey forms and Questionnaire Sheets

Test subjects filled out the survey form and questionnaire sheet using a black ballpoint pen. Corrections were made by crossing through the mistake with a double line.

19. Test Data Changes and Deficiencies

In cases where a test subject's physical condition or wishes necessitated that measurements be delayed, or gave rise to deficiencies, the test subject's physical condition or wishes were given priority in accordance with the main purport of the Helsinki Declaration. Therefore, if a portion of data was impossible to collect for a reason unrelated to this test, that data would be treated as deficient data.

20. Ethics

20.1 Adherence and Compliance Issues

- This test conforms to the Helsinki Declaration (revised at the Edinburgh General Assembly, 2000) and was carried out in line with ethical considerations.
- This test took each test subject's human rights and safety into consideration, and ensured test data was reliable in the context of Ministry of Health and Welfare Ordinance No. 28 "Standards for the Implementation of Clinical Trials on Pharmaceutical Products (GCP)" dated March 27, 1997.

20.2 Ethics Committee

The TES Holdings Ethics Committee, made up of the following members, was convened to deliberate the morality and appropriateness of the protocol. This test had to be implemented based on the protocol approved by the ethics committee and any substantial deviations from the protocol required authorization from the committee.

- Chairperson
  Yasuo Watanabe (specialist) – Professor of the Nihon Pharmaceutical University, Faculty of Medicine, Medicinal Substance Treatments
- Committee Members
  Yoshifumi Oshiba (specialist) – Director of Oshiba Doctor's Surgery
  Tomio Fukuhara (specialist) – Consultant of Kosei General Hospital (Anesthesia Department Manager)
  Shizuo Yamada (specialist) – Professor of the University of Shizuoka, Faculty of Pharmaceutical Sciences
  Shigetoshi Miyamoto (non-specialist) – Lawyer, Miyamoto Legal Firm

21. Reference Materials in Separate Sheet

(Data omitted)

22. Test Results

(Data narration omitted)

22.9 Test Subjects' Daily Report

Data of test subjects' daily reports is shown in **Tables 10 to 15**.
As shown in the test material ingestion percentage (**Table 10**), no test subject fell under the analysis exclusion criteria, indicating that the test subjects took the test materials as planned.
No change caused by the test materials or subjective symptom that could influence test results was confirmed according to the doctor's opinion.

* Data of individual test subject is shown in **Tables 16 to 23**.
(The above tables were omitted)

23. Conclusion

The slimming effect of the ingestion of dietary supplements containing fucoxanthin for four weeks was examined on male and female adults with a BMI of 25 or higher by a double-blind test using two dietary supplement products containing fucoxanthin and also one placebo supplement product as the control.
Main evaluation items were:
(1) CT scan of abdominal fat area (total fat, visceral fat, and subcutaneous fat)
(2) Measurement of body composition
(3) Circumference measurements (waist, upper arms, neck and thighs)
Secondary endpoints were:
(1) Measurement of blood pressure level and pulses
(2) Blood and urine tests

Other survey items were:
(1) Daily reports written by the test subjects
(2) Simplified survey and instruction on diet
(3) Questionnaire
Effectiveness and safety of the ingestion of fucoxanthin were examined and evaluated based on the results obtained from the evaluation items and survey items described above.

Fucoxanthin is a type of carotenoid contained in brown algae such as kelp, hijiki algae, and brown seaweed and also some microalgae. Since its effect to accelerate the expression of mitochondrial uncoupling protein 1 (UCP1) (1) and effect to inhibit fat accumulation (2) have been reported, there is a growing awareness of fucoxanthin's anti-metabolic syndrome effect.

Figure 1 showed results of various parameters measured in the study. Upon CT scanning on visceral fat area and subcutaneous fat area of test subjects, both values increased in the group taking placebo. However, dose-dependent reduction in visceral fat area was demonstrated in test subjects taking fucoxanthin. A significant reduction was demonstrated in the group taking fucoxanthin 3 mg/day. Concerning the subcutaneous fat area and total fat area, a significant reduction was demonstrated in the group taking fucoxanthin 1 mg/day. These results suggest that fucoxanthin may have an effect to reduce body fat and also a high potential that the effect works on visceral fat highly selectively. Typical CT scan images of the fat area are shown in Figure 2.

Circumferences of the neck and thighs (left and right) tended to be reduced by the ingestion of fucoxanthin.

Body weight and BMI remained mostly unchanged in the group taking placebo. In groups taking fucoxanthin however, a dose-dependent reduction tendency was observed. A significant reduction was shown in the group taking fucoxanthin 3 mg/day. Although no significant difference was shown in fat mass or body fat percentage, a dose-dependent reduction was shown by the ingestion of fucoxanthin.

Concerning free-fat mass, muscle mass, or basal metabolic rate, no significant change deemed to be caused by the test was shown. In the results of blood, blood pressure level, and urine tests, statistically significant difference was shown in some items before and after the ingestion. However, all measurement values were within the standard value range and no clear change was shown in other associated measurement values before and after the ingestion. Therefore, the difference was not considered to be caused by the test. Physical conditions of test subjects demonstrated an improvement tendency in all groups according to the results of the questionnaire and test subjects' journals. However, no difference was shown among groups.

In this test, fucoxanthin's effect to reduce circumference of waist, thighs, or other parts could not be clarified. Since fat in these body parts is mainly subcutaneous fat, the effect is believed to be apparent after visceral fat is reduced. A longer-period test in the future is considered to clarify fucoxanthin's activity to reduce this type of fat.

The results above suggest that fucoxanthin has a dose-dependent effect in improving the obesity parameters in human. Namely, fucoxanthin has been proved to be effective in reducing visceral fat since it demonstrated effects to accelerate fat burning and inhibit fat accumulation. No adverse event or reaction caused by the ingestion of fucoxanthin was shown during the test period. No adverse change was observed in blood pressure and pulse measurements, blood and urine tests, or questionnaire either.

In conclusion, oral administration of fucoxanthin demonstrated selective anti-obesity effect on visceral fat and no adverse event occurred in a test where 1 to 3 mg/day of fucoxanthin was ingested four weeks. These results suggest that fucoxanthin may be an effective and safe food.

References

(1) Maeda H, Hosokawa M, Sashima T, Funayama K, Miyashita K. Fucoxanthin from edible seaweed, Undaria pinnatifida, shows antiobesity effect through UCP1 expression in white adipose tissues. *Biochem Biophys Res Commun.* **332**(2): 392-7 (2005).

(2) Maeda H, Hosokawa M, Sashima T, Takahashi N, Kawada T, Miyashita K. Fucoxanthin and its metabolite, fucoxanthinol, suppress adipocyte differentiation in 3T3-L1 cells. *Int J Mol Med.* **18**(1): 147-52 (2006).

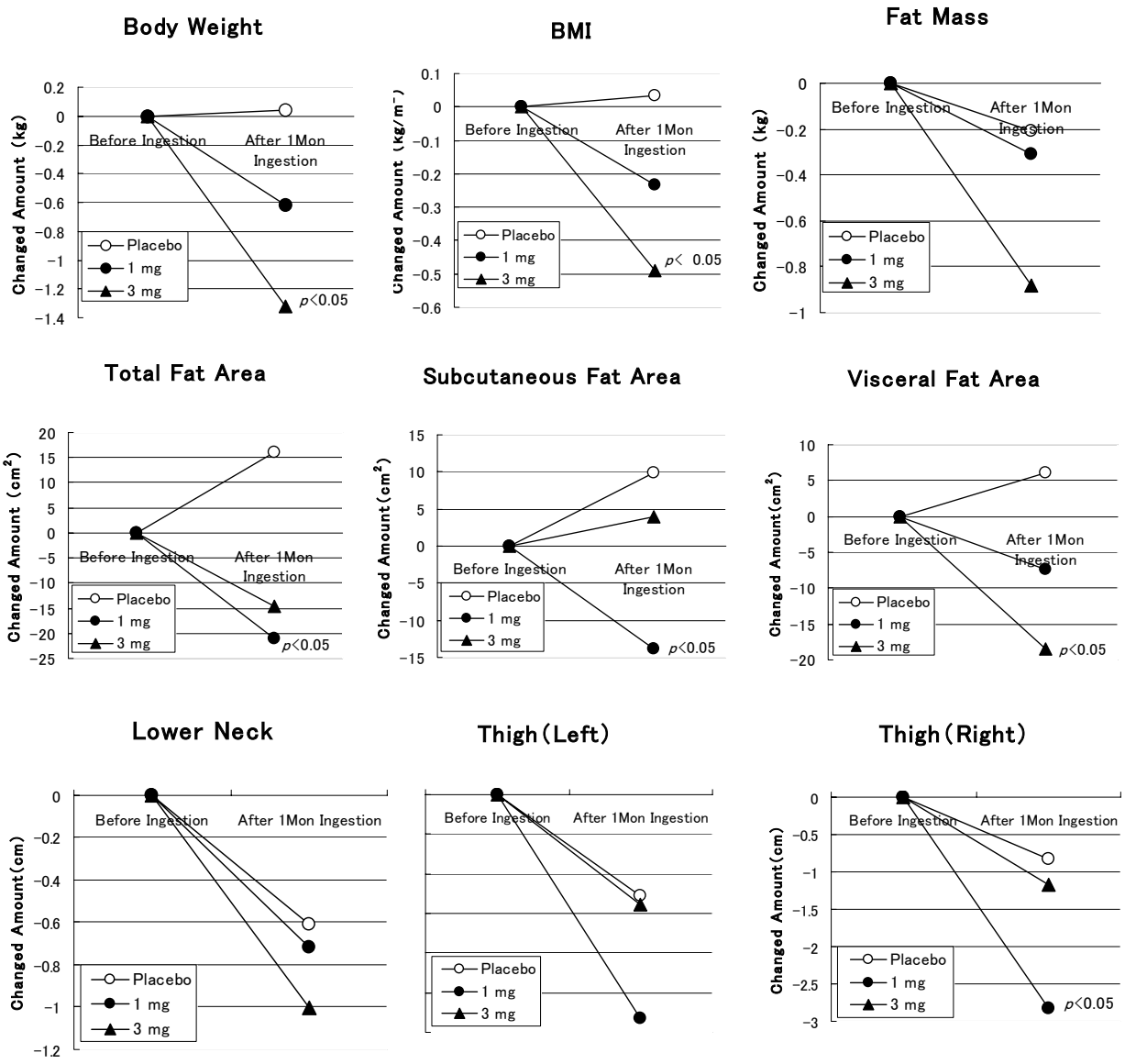


Fig.1. The effect of Fucoxanthin on obesity parameters

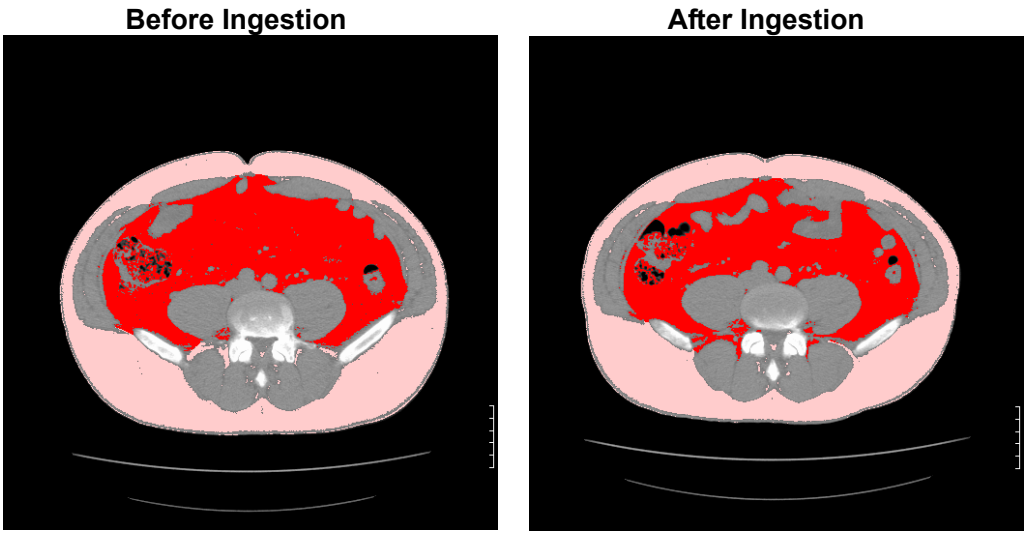


Fig.2. CT scan of abdominal fat area (an example from group consuming fucoxanthin 1mg/day. Red part: visceral fat. Pink part: subcutaneous fat)

We certify the above to be true in every particular.

Akio Hayashi
President and Chief Executive Officer
TES Holdings Co., Ltd.