Effect of Oral Paprika Xanthophyll Intake on Abdominal Fat in Healthy Overweight Humans: A Randomized, Double-blind, Placebo-controlled Study

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Abstract: PURPOSE: Xanthophylls that exist in various vegetables and fruits have beneficial actions, such as antioxidant activity and an anti-metabolic syndrome effect, and daily intake of xanthophylls could play an important role in preventing lifestyle-related diseases. We investigated whether intake of xanthophylls from red paprika could decrease the abdominal fat area in the healthy overweight volunteers with a body mass index (BMI) ranging from 25 to < 30 kg/m².

METHODS: In a randomized, double-blind, placebo-controlled, parallel-group study, 100 healthy volunteers were assigned to oral administration of paprika xanthophyll capsules (containing 9.0 mg of paprika xanthophylls) or placebo capsules for 12 weeks. The primary endpoint was the effect of paprika xanthophyll intake on the abdominal visceral fat area (VFA) as determined by computed tomography. The secondary endpoints were as follows: 1) changes of the abdominal subcutaneous fat area (SFA), total fat area (TFA), and BMI; 2) changes of lipid metabolism parameters, glucose metabolism parameters, and other blood parameters.

RESULTS: After 12 weeks, VFA was smaller in the paprika xanthophyll group than in the placebo group. In the paprika xanthophyll group, there was a significant decrease of SFA, TFA, and BMI after 12 weeks compared with baseline, and the reduction of SFA, TFA, and BMI was significantly greater in the paprika xanthophyll group than in the placebo group. Moreover, total cholesterol and low-density lipoprotein cholesterol decreased significantly in the paprika xanthophyll group, but not in the placebo group. No adverse effects were caused by intake of paprika xanthophyll capsules.

CONCLUSIONS: Intake of paprika xanthophylls for 12 weeks significantly reduced the abdominal fat area and BMI in healthy overweight volunteers without causing any adverse effects. These findings suggest that paprika xanthophyll is a safe food ingredient that improves lipid metabolism and reduces abdominal fat.

Trial registration: UMIN-CTR UMIN000021529

Key words: ripe paprika, paprika xanthophyll, obesity, abdominal fat, randomized controlled study

1 INTRODUCTION

Obesity is a state in which the body weight is higher than normal due to excessive fat accumulation. Development of obesity, especially accumulation of visceral fat, causes metabolic syndrome and is a risk factor for various conditions, such as impaired glucose tolerance (insulin resistance), dyslipidemia, hypertension, hyperuricemia, gout, and arteriosclerosis¹⁻⁴⁾. Clinical studies have revealed that maintaining an appropriate body mass index (BMI) is extremely important for healthy longevity⁵⁾. In fact, a large-scale

study investigating the relationship between overweight/obesity and lifespan showed that the life expectancy of severely obese people may be reduced by an average of 10 years⁶⁾. According to the WHO, people with a BMI from 25 to less than 30 kg/m² are defined as "overweight", and people with a BMI $\geq$ 30 kg/m² are defined as "obese"⁷⁾. Because overweight and obese people are increasing rapidly worldwide, development of medications and functional foods for prevention of obesity is required.

Carotenoids are natural pigments that exist in various

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vegetables and fruits. They are classified as carotenes, which are hydrocarbons, and xanthophylls that contain oxygen atoms in addition to carbon/hydrogen atoms. Both xanthophylls and carotenes are known to have strong antioxidant activity, but the activity is higher in xanthophylls⁽⁸⁾, and its various effects on human physiology have also been reported. For example, β -cryptoxanthin has been suggested to reduce the risk of obesity, diabetes, and arteriosclerosis^(9–11). It was also reported that astaxanthin improved the brain function of healthy volunteers who were aware of forgetfulness^(12, 13). Furthermore, it is known that lutein and zeaxanthin accumulate in the eyes and are effective for prevention of age-related macular degeneration^(14–16). Thus, various xanthophylls have potential as functional food ingredients that can contribute to human health. Ripe red paprika differs from other carotenoid-rich vegetables (e.g.; carrots, tomatoes, and spinach) with respect to the abundance and composition of xanthophylls^(17–20). It has been reported that characteristic paprika xanthophylls like capsanthin, capsorubin, and cryptocapsin have strong antioxidant, anti-inflammatory, and anti-tumor effects^(21–28). Since paprika also contains an abundance of other xanthophylls, including β -cryptoxanthin, zeaxanthin, and cucurbitaxanthin A, it is a healthy functional food. We established a method of producing a paprika xanthophyll preparation containing at least seven different xanthophylls (capsanthin, capsorubin, cucurbitaxanthin A, β -cryptoxanthin, zeaxanthin, cryptocapsin and capsanthin 3,6-epoxide) together with a relatively small amount of β -carotene, and we have been investigating the properties, distribution, and function of these xanthophylls^(21–24). In the present study, we investigated whether oral administration of paprika xanthophylls could improve parameters related to obesity in overweight adult volunteers (BMI ≥ 25 kg/m² to <30 kg/m²). In brief, 100 subjects were randomly divided into two groups, and changes of the abdominal fat area (visceral fat area, subcutaneous fat area, and total fat area), BMI, and laboratory parameters were compared between the group taking capsules containing paprika xanthophylls and the group taking placebo capsules. After 12 weeks, a significant decrease of the abdominal fat area and BMI was observed in the paprika xanthophyll group compared with the placebo group. To our knowledge, this is the first evidence that intake of paprika xanthophylls can reduce fat stores in humans. Our findings suggest that paprika xanthophylls may be an effective food ingredient for suppressing obesity.

2 Materials and Methods

2.1 Study procedures

The study protocol and informed consent document conformed to the Declaration of Helsinki, and were approved by the ethics committee of AMC Nishi-umeda Clinic

(Osaka, Japan). All of the subjects provided written informed consent prior to participation. This study was registered with the UMIN Clinical Trials Registry (UMIN000021529; Title: Verification of the antiobesity effect of a plant extract ingredient G-PX). The study was conducted in compliance with the protocol and was performed by a contract research organization (A2 Healthcare Corporation, Tokyo, Japan) from February to September 2016 at the AMC Nishi-umeda Clinic (Osaka, Japan) and the Nakanoshima Clinic (Osaka, Japan).

2.2 Subjects

Healthy Japanese volunteers were recruited if they were aged >20 years and had a BMI from 25 to <30 kg/m², which is classified as overweight by the WHO. The candidate subjects were not using medications that could potentially influence the results. Exclusion criteria were as follows: 1) serious diseases (e.g., hepatic, renal, or cardiovascular disease, asthma, cancer, or diabetes); 2) a past history of gastrectomy or intestinal resection that could affect digestion or absorption; 3) current use of prescription medication; 4) pregnancy or breastfeeding; 5) severe food allergy; 6) use of supplements (astaxanthin, β -carotene, and lutein) that could influence the results; 7) current participation in another clinical study; 8) donation of >200 mL of blood or blood components within 4 weeks prior to enrollment in this study; 9) donation of >400 mL of blood within 16 weeks prior to enrollment in this study; 10) donation of >1000 mL of blood within 64 weeks prior to enrollment in this study; and 11) any other reason for which a subject was judged to be ineligible by the principal investigator or the study site investigators.

2.3 Paprika xanthophyll preparation and quantitative analysis of plasma carotenoids

A commercial paprika xanthophyll preparation ("PapriX"; Glico Nutrition Co., Ltd, Osaka, Japan) was employed for this study. Its xanthophyll composition is shown in Table 1. This preparation contained mainly seven xanthophylls (capsanthin, cucurbitaxanthin A, β -cryptoxanthin, zeaxanthin, capsorubin, cryptocapsin, and capsanthin 3,6-epoxide) and one carotene (β -carotene)⁽²¹⁾. Gelatin capsules containing PapriX-oil (total xanthophyll content of 9.0 mg per capsule) were used as the test substance. Gelatin capsules containing vegetable oil and caramel coloring that were indistinguishable from the test substance were used as the placebo. The subjects took one capsule daily after the evening meal for 12 weeks.

Plasma levels of several carotenoids were determined as described previously⁽²¹⁾. After the blood sample was let stand at room temperature for 30 min, plasma was obtained by centrifugation (5,000 rpm for 10 min at room temperature) and was stored at -80°C until analysis of carotenoids. After thawing, 2 mL of ethanol was added to 1

Table 1 Xanthophyll contents of the active and placebo capsules.

	Paprika xanthophyll (Active) (per capsule)	Placebo (per capsule)
Capsanthin (mg)	5.0	0
β-Cryptoxanthin (mg)	0.50	0
Others (mg)	3.5	0
Total xanthophylls (mg)	9.0	0

*Active or placebo capsules were ingested once daily (1 capsule/day).

mL of plasma and stirred, followed by extraction of carotenoids by using 5 mL of ether : hexane (2 : 8, v/v) solution with stirring. After being let stand for 10 min, the upper layer was filtered through a Millipore membrane filter (Millipore Corporation, Massachusetts, USA) and was evaporated to dryness. Then the residue was dissolved in acetone : hexane (2 : 8, v/v) and was subjected to high performance liquid chromatography (HPLC) for analysis of plasma carotenoids by our routine method²⁹⁾. In brief, HPLC was performed with a Hitachi L-6000 intelligent pump, an L-4250 UV-VIS detector set at 450 nm, and a 5 mm Cosmosil 5SL-II (250 × 4.6 mm i.d.) column (Nacalai Tesque, Kyoto, Japan). The mobile phase was acetone : hexane (2 : 8, v/v) at a flow rate of 1.0 mL/min. Identification of each carotenoid was performed by comparing its retention time with those of authentic paprika carotenoids and the content of each carotenoid was calculated from the peak areas.

2.4 Study design

This was a randomized, double-blind, placebo-controlled intervention study that was conducted according to the Consolidated Standards of Reporting Trials (CONSORT) statement. This study consisted of an 8-week screening period, followed by a 12-week treatment period. During the screening period, subjects were screened for eligibility by performing an interview with an investigator and undergoing measurement of anthropometric parameters (height, weight, BMI, and body fat ratio), measurement of physical parameters (blood pressure and pulse rate), and blood and urine tests. The 100 eligible subjects were randomly divided into two groups (the paprika xanthophyll group and the placebo group) by the grouping controller. The controller randomized the subjects to each group at a 1:1 ratio using a table of random numbers, and stored the assignment list in a sealed container until completion of all analyses. Apart from the controller, all of the investigators and data processors, as well as all of the subjects, were blinded to the treatment assigned until the end of this trial.

During the treatment period, each subject took 1 paprika xanthophyll capsule or 1 placebo capsule after the evening

meal every day. Subjects were instructed to continue their usual pattern of diet, exercise, sleeping, smoking, and drinking. They were also instructed to avoid blood donation, commencing a new exercise regimen, and use of dietary supplements that could potentially influence the results of the study until after the end of the study period. In week 0 (before starting administration) and week 12 of the treatment period, subjects underwent computed tomography (CT), interview by a site investigator, measurement of anthropometric, physical, blood, and urine parameters, and the confirmation of the diary record. On the day before each set of tests, subjects were prohibited from drinking alcohol and performing excessive exercise, and had to finish the evening meal by 22:00. After that, only intake of water was allowed until the tests were completed on the following day.

2.5 Anthropometric measurements and body composition

While the subject was standing, height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively. The body fat ratio and visceral fat level were measured by bioelectrical impedance analysis using a body composition monitor (InBody Dual RD-501; Tanita Co., Tokyo, Japan). The BMI was calculated as body weight (kg) divided by the square of height (m). Blood pressure and pulse rate were measured once with the subject in the sitting position after resting.

2.6 Measurement of the abdominal fat area

The abdominal visceral fat area (VFA) and the abdominal subcutaneous fat area (SFA) were measured on CT scans acquired with an Aquilion 64 scanner (TSX-101A, Toshiba Medical Systems Co., Ltd.) at Nakanoshima Clinic (Osaka, Japan). With the subject in the supine position, four CT scans (120 kV, 6 mm slice thickness) were acquired at the level of the L4-5 vertebrae. Then VFA, SFA, and total fat area (TFA = VFA + SFA) were calculated from the CT data.

2.7 Biochemical parameters

Fasting blood samples were collected for determination

of hematology parameter (white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (Ht), and platelet count (Plt)) and blood chemistry parameters (aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ -glutamyl transpeptidase (γ -GTP), alkaline phosphatase (ALP) lactate dehydrogenase (LDH), total bilirubin (T-Bil), creatinine (Cre), blood urea nitrogen (BUN), total cholesterol (T-cho), high-density lipoprotein cholesterol (HDL-cho), low-density lipoprotein cholesterol (LDL-cho), triglycerides (TG), glucose (Glc), uric acid (UA), albumin (Alb), creatine phosphokinase (CPK), total protein (TP), sodium (Na), potassium (K), chloride (Cl), calcium (Ca), hemoglobin A1c (HbA1c), adiponectin (Adn),

leptin (Lpt), and glycoalbumin (GA)). In addition, fasting urine samples were obtained to measure urinary protein, glucose, and urobilinogen. All samples were analyzed by LSI Medience Corporation.

2.8 Endpoints

The primary endpoint was the change of abdominal VFA after the 12-week treatment period. The secondary endpoints were as follows: 1) changes of abdominal SFA and TFA; 2) change of BMI; and 3) changes of lipid-related parameters (T-cho, HDL-cho, LDL-cho, TG, Adn, and Lpt), glucose metabolism-related parameters (Glc, HbA1c, GA), and other blood parameters (AST, ALT, γ -GTP, and UA).

Fig. 1 Flow diagram of the study.

2.9 Statistical analysis

Results were expressed as actual values or as the changes from week 0. The paired *t*-test was used to evaluate the significance of changes from before treatment in each group, while the unpaired *t*-test was used to compare differences between the paprika xanthophyll group and the placebo group. In addition, Bowker's test and the Wilcoxon rank sum test were used for analysis of data on the body fat ratio and on urine protein, glucose, and urobilinogen. A difference of $p < 0.05$ was considered significant. All statistical analyses were performed using SAS software (release 9.2, SAS Institute Japan Co., Ltd.) or Microsoft Excel 2010.

3 RESULTS

3.1 Disposition of the subjects

We provisionally recruited 171 subjects from March to April 2016, but 71 subjects were ineligible according to the inclusion and exclusion criteria. The remaining 100 participants were judged to be eligible by the site investigators and were randomized to the paprika xanthophyll group and the placebo group (50 subjects per group). **Figure 1** shows a flow diagram of this study. Two subjects dropped out for personal reasons unrelated to ingestion of the study capsules. Among the 98 remaining subjects who finished the study (48 in the paprika xanthophyll group and 50 in the placebo group), 18 subjects (7 and 11, respectively) were excluded from analysis before unblinding due to significant lifestyle changes, initiation of new exercise, or serious compliance violations. In addition, 5 subjects (4 and 1, re-

spectively) were excluded from analysis of the CT data (abdominal VFA, SFA, TFA, and abdominal circumference) because the site investigators judged their CT scans to be of insufficient quality. Thus, there were 41 subjects for analysis in the paprika xanthophyll group and 39 subjects in the placebo group, with analysis of CT data being done in 37 and 38 subjects, respectively. There were no significant differences of baseline data between the paprika xanthophyll and placebo groups (**Table 2**). The test capsule ingestion rate was 99.8% in the paprika xanthophyll group and 99.5% in the placebo group.

3.2 Effect of paprika xanthophyll supplementation on the abdominal fat area

Table 3 shows the VFA, SFA, TFA, and waist circumference determined by CT scanning at weeks 0 and 12, as well as the changes from week 0 (baseline). The decrease of VFA from week 0 to week 12 was $2.82 \pm 2.19 \text{ cm}^2$ in the paprika xanthophyll group and $0.43 \pm 2.47 \text{ cm}^2$ in the placebo group. Although the change of VFA after 12 weeks was larger in the paprika xanthophyll group than in the placebo group, there was no significant difference between the two groups.

As shown in **Table 3**, SFA and TFA both decreased significantly by week 12 compared with week 0 in the paprika xanthophyll group, but SFA and TFA increased in the placebo group. When compared with the placebo group, SFA and TFA showed a significant decrease in the paprika xanthophyll group by week 12. In addition, we analyzed the waist circumference on abdominal CT scans. Waist circumference decreased significantly from week 0 to week

Table 2 Baseline characteristics of the subjects.

	Paprika xanthophyll (Active) group	Placebo group	Total
Subjects (n)	41	39	80
Male (n)	35	32	67
Female (n)	6	7	13
Age (years)	48.9 ± 1.38	50.8 ± 1.39	49.8 ± 0.98
Height (cm)	169.2 ± 1.10	166.9 ± 1.24	168.1 ± 0.83
Body weight (kg)	77.81 ± 1.00	75.48 ± 1.14	76.68 ± 0.76
BMI (kg/m ²)	27.19 ± 0.24	27.09 ± 0.22	27.14 ± 0.16
Body fat ratio (%)	27.54 ± 0.99	28.42 ± 1.04	27.97 ± 0.71
SBP (mmHg)	130.7 ± 1.49	126.2 ± 2.33	128.5 ± 1.38
DBP (mmHg)	79.3 ± 1.01	72.4 ± 1.49	75.9 ± 0.97
Pulse rate (beats/min)	75.8 ± 1.59	73.3 ± 1.65	74.6 ± 1.15

Data are expressed as the mean $\pm$ SEM. BMI: body mass index, SBP: systolic blood pressure. DBP: diastolic blood pressure. Active or placebo capsules were ingested once daily (1 capsule/day).

Table 3 Effect of Paprika xanthophyll intake on abdominal fat area and waist circumference.

Parameter	Group	Treatment period		Change from week 0
		Week 0	Week 12	
VFA (cm ²)	Active	113.5±5.13	110.7±5.01	- 2.82±2.19
	Placebo	113.0±5.74	112.6±5.50	- 0.43±2.47
SFA (cm ²)	Active	191.2±9.23	182.9±10.1 *	- 8.29±3.13
	Placebo	186.7±8.99	188.7±9.61	2.00±3.18
TFA (cm ²)	Active	304.7±10.6	293.6±11.9 *	- 11.1±4.37
	Placebo	299.7±9.50	301.3±10.5	1.57±3.80
Waist circumference (cm)	Active	89.02±0.91	87.89±0.98 **	- 1.12±0.40
	Placebo	87.89±0.83	88.02±0.92	0.14±0.44

Data are expressed as the mean ± SEM. Paprika xanthophyll (active) group (n = 37), placebo group (n = 38), VFA: visceral fat area, SFA: subcutaneous fat area, TFA: total fat area. Differences between the two groups were determined with the unpaired *t*-test. Comparisons between week 12 and week 0 (baseline) were performed by using the paired *t*-test. #: *p* < 0.05 vs. placebo group; *: *p* < 0.05, **: *p* < 0.01 vs. week 0.

Table 4 Effect of paprika xanthophyll intake on body indices.

Parameter	Group	Treatment period		Change from week 0
		Week 0	Week 12	
Body weight (kg)	Active	77.81±1.00	76.87±1.04 **	- 0.95±0.30
	Placebo	75.48±1.14	75.36±1.10	- 0.12±0.19
BMI (kg/m ²)	Active	27.19±0.24	26.85±0.28 **	- 0.33±0.10
	Placebo	27.09±0.22	27.06±0.22	- 0.03±0.07
Body fat ratio (%)	Active	27.54±0.99	26.88±1.03 **	- 0.66±0.21
	Placebo	28.42±1.04	28.01±1.08 *	- 0.41±0.18
Visceral fat level	Active	13.35±0.34	13.06±0.33	- 0.29±0.09
	Placebo	13.37±0.40	13.32±0.39	- 0.05±0.07

Data are expressed as the mean ± SEM. Paprika xanthophyll (active) group (n = 41), placebo group (n = 39), BMI: body mass index. Analysis of visceral fat level data was performed by using the Wilcoxon rank sum test. Differences of body weight, BMI, and body fat ratio between the active and placebo groups were determined with the unpaired *t*-test. Comparisons between week 12 and week 0 (baseline) were done with the paired *t*-test. #: *p* < 0.05 vs. placebo group; *: *p* < 0.05, **: *p* < 0.01 vs. week 0.

12 in the paprika xanthophyll group and the change of waist circumference from week 0 was significantly larger than in the placebo group. Additionally, the waist circum-

ference was significantly smaller in the paprika xanthophyll group than in the placebo group by week 12. These results suggested that daily intake of paprika xanthophylls de-

Table 5 Effect of paprika xanthophyll intake on parameters of lipid and glucose metabolism.

Parameter	Sex	Reference range	Group	Treatment period		Change from week 0
				Week 0	Week 12	
T-Cho (mg/dL)		120-219	Active	203.2±4.9	196.9±4.3 *	- 6.3±2.4
			Placebo	214.4±5.3	212.5±4.8	- 1.9±3.0
LDL-Cho (mg/dL)		65-139	Active	124.8±4.1	118.3±3.8 **	- 6.5±2.4
			Placebo	136.2±5.1	129.1±4.5	- 7.1±3.5
HDL-Cho (mg/dL)	Male	40-85	Active	50.9±4.18	50.6±3.90	- 0.3±2.2
			Placebo	48.1±3.44	48.3±4.99	0.2±2.7
	Female	40-95	Active	59.2±3.68	56.7±2.64	-2.5±2.1
			Placebo	61.9±5.03	61.3±6.21	-0.6±2.5
LDL/HDL ratio		≤2.0	Active	2.48±0.11	2.36±0.09 *	- 0.12±0.06
			Placebo	2.82±0.15	2.74±0.15	- 0.08±0.07
TG (mg/dL)		30-149	Active	131.8±12.6	132.9±19.4	1.1±12.0
			Placebo	141.1±13.9	164.1±17.7	23±11.8
Adiponectin (µg/mL)		≥4.0	Active	7.29±0.41	7.01±0.39 *	- 0.28±0.14
			Placebo	7.35±0.47	7.20±0.47	- 0.15±0.14
Leptin (ng/mL)	Male	0.9-13.0	Active	7.11±0.53	6.80±0.47	- 0.31±0.36
			Placebo	8.43±0.68	7.99±0.57	- 0.44±0.44
	Female	2.5-21.8	Active	24.0±3.43	22.7±3.40	- 1.28±2.0
			Placebo	18.2±1.98	20.8±2.04	2.56±2.2
Glc (mg/dL)		70-109	Active	83.4±1.3	84.9±1.3	1.5±1.2
			Placebo	85.0±1.8	87.0±1.7	2.0±2.2
HbA1c (%)		4.6-6.2	Active	5.51±0.03	5.49±0.04	- 0.03±0.03
			Placebo	5.65±0.05	5.57±0.05 **	- 0.08±0.02
GA (%)		12.3-16.5	Active	12.9±0.16	13.1±0.15 **	0.19±0.06
			Placebo	12.9±0.17	13.2±0.18 **	0.28±0.07

Data are expressed as the mean ± SEM. Paprika xanthophyll (active) group (n = 41), placebo group (n = 39), T-Cho: total cholesterol, LDL-Cho: low-density lipoprotein cholesterol, HDL-Cho: high-density lipoprotein cholesterol, TG: triglycerides, Glc: glucose, GA: glycoalbumin. Differences between the active and placebo groups were determined with the unpaired *t*-test. Comparisons between week 12 and week 0 (baseline) were performed using the paired *t*-test. *: $p < 0.05$, **: $p < 0.01$ vs. week 0.

creased the waist circumference by reducing abdominal fat stores.

3.3 Effect of paprika xanthophyll supplementation on anthropomorphic indices

Table 4 shows the values measured in weeks 0 and 12, as well as the changes from week 0, for body weight, BMI,

Table 6 Effect of paprika xanthophyll intake on liver function.

Parameter	Sex	Reference range	Group	Treatment period		Change from week 0
				Week 0	Week 12	
TP (g/dL)		6.5-8.2	Active	7.36±0.06	7.27±0.06 *	- 0.09±0.05
			Placebo	7.21±0.07	7.21±0.06	0.00±0.06
Alb (g/dL)		3.7-5.5	Active	4.53±0.05	4.45±0.04 *	- 0.08±0.03
			Placebo	4.39±0.05	4.39±0.04	0.01±0.04
T-Bil (mg/dL)		0.3-1.2	Active	0.79±0.05	0.87±0.06	0.08±0.04
			Placebo	0.73±0.04	0.74±0.05	0.01±0.04
AST (U/L)		10-40	Active	22.7±1.0	23.3±1.3	0.6±1.4
			Placebo	22.6±1.6	23.0±1.5	0.4±0.8
ALT (U/L)		5-45	Active	27.3±2.0	27.0±2.1	- 0.3±1.5
			Placebo	25.5±2.0	24.9±1.8	- 0.6±1.0
γ-GTP (U/L)	Male	≤ 80	Active	42.4±6.3	41.8±6.0	- 0.6±1.8
			Placebo	52.6±6.2	48.8±5.1	- 3.8±3.1
	Female	≤ 30	Active	20.5±1.0	20.8±2.0	0.3±1.6
			Placebo	16.7±3.1	17.9±2.7	1.1±1.4
LDH (U/L)		120-240	Active	187.9±4.3	192.5±4.0	4.7±3.2
			Placebo	180.6±5.9	190.1±6.6 **	9.5±3.0
ALP (U/L)		100-325	Active	208.0±7.3	211.1±9.3	3.1±6.7
			Placebo	221.9±14	217.2±15	- 4.7±4.4

Data are expressed as the mean ± SEM. Paprika xanthophyll (active) group (n = 41), placebo group (n = 39), TP: total protein, Alb: albumin, T-Bil: total bilirubin, AST: aspartate aminotransferase, ALT: alanine aminotransferase, γ-GTP: γ-glutamyl transpeptidase, LDH: lactate dehydrogenase, ALP: alkaline phosphatase. Differences between the active and placebo groups were determined with the unpaired *t*-test. Comparisons between week 12 and week 0 (baseline) were done with the paired *t*-test. *: $p < 0.05$, **: $p < 0.01$ vs. week 0.

body fat ratio, and visceral fat level. In the paprika xanthophyll group, BMI and body weight were significantly reduced by week 12 compared with week 0, while no significant change were observed in the placebo group. Moreover, BMI and the body weight were significantly lower in the paprika xanthophyll group than in the placebo group, and the changes of BMI and body weight were significantly larger in the active treatment group. The body fat ratio showed significant reduction from week 0 in both groups, but there was no significant difference between them. Visceral fat level did not decrease significantly in either group, although the change from week 0 was larger in the paprika xanthophyll group than in the placebo group. These find-

ings indicated that paprika xanthophyll intake can improve several body indices, such as BMI and body weight, in health overweight subjects.

3.4 Effect of paprika xanthophyll supplementation on laboratory parameters

Parameters of lipid and glucose metabolism determined in weeks 0 and 12 are displayed in Table 5. In the paprika xanthophyll group, T-Chol and LDL-Chol decreased significantly after 12 weeks, but no significant changes were observed in the placebo group. The LDL/HDL ratio (an index of the risk of arteriosclerotic disease) was significantly improved by intake of paprika xanthophylls, but not by

Table 7 Effect of paprika xanthophyll intake on renal function and electrolytes.

Parameter	Sex	Reference range	Group	Treatment period		Change from week 0
				Week 0	Week 12	
UA (mg/dL)	Male	3.8-7.0	Active	6.46±0.49	6.57±0.55	-0.09±0.05
			Placebo	6.77±0.41	7.01±0.46	0.00±0.06
	Female	2.5-7.0	Active	4.97±0.22	5.50±0.15 *	-0.09±0.05
			Placebo	4.93±0.25	5.41±0.28	0.00±0.06
BUN (mg/dL)		8.0-20	Active	13.3±0.49	13.9±0.49	-0.08±0.03
			Placebo	13.4±0.52	13.8±0.53	0.01±0.04
Cre (mg/dL)	Male	0.61-1.04	Active	0.86±0.02	0.85±0.02	0.08±0.04
			Placebo	0.87±0.02	0.88±0.03	0.01±0.04
	Female	0.47-0.79	Active	0.62±0.03	0.63±0.02	0.08±0.04
			Placebo	0.64±0.04	0.67±0.05 *	0.01±0.04
CPK (U/L)	Male	60-270	Active	184.0±23.4	227.6±63.1	43.6±69.5
			Placebo	128.6±9.14	159.3±23.4	30.8±20.9
	Female	40-150	Active	62.2±7.43	64.7±4.54	2.5±5.32
			Placebo	86.6±7.03	106±17.7	19.4±16.7
Na (mEq/L)		137-147	Active	141.2±0.2	141.6±0.3	0.3±0.3
			Placebo	141.4±0.3	141.5±0.3	0.2±0.2
Cl (mEq/L)		98-108	Active	104.1±0.2	104.3±0.3	0.2±0.3
			Placebo	104.6±0.3	104.4±0.3	-0.2±0.3
K (mEq/L)		3.5-5.0	Active	4.16±0.04	4.19±0.04	0.03±0.04
			Placebo	4.22±0.04	4.22±0.04	0.00±0.05
Ca (mg/dL)		8.4-10.4	Active	9.65±0.05	9.56±0.05 *	-0.09±0.04
			Placebo	9.59±0.06	9.60±0.05	0.01±0.06

Data are expressed as the mean ± SEM. Paprika xanthophyll (active) group (n = 41), placebo group (n = 39), UA: uric acid, BUN: blood urea nitrogen, Cre: creatinine, CPK: creatine phosphorus kinase, Na: sodium, Cl: chloride, K: potassium, Ca: calcium. Differences between the active and placebo groups were determined with the unpaired *t*-test. Comparisons between week 12 and week 0 (baseline) were done with the paired *t*-test. *: *p* < 0.05 vs. week 0.

placebo intake. Although adiponectin, HbA1c, and GA showed significant differences from week 0 in one or both groups, the actual changes were very small and within the respective reference ranges. In both groups, there were no significant changes of HDL-Cho, TG, leptin, and Glc. Taken together, these results suggested that intake of paprika xanthophylls could improve parameters of lipid metabolism (T-Cho, LDL-Cho, and the LDL/HDL ratio) in healthy sub-

jects.

Tables 6 and 7 show the effects of paprika xanthophyll intake on parameters of liver function, kidney function, and electrolytes. Although TP, Alb, LDH, UA (female subjects), Cre (female subjects), and Ca showed significant changes from week 0 to week 12 in one or both groups, these changes were small and within the respective reference ranges. There were no significant variations of T-Bil, AST,

Table 8 Effect of paprika xanthophyll intake on hematological parameters.

Parameter	Sex	Reference range	Group	Treatment period		Change from week 0
				Week 0	Week 12	
WBC (/μL)		3300-9000	Active	6059 ± 236	5810 ± 191	- 249 ± 178
			Placebo	5862 ± 229	5931 ± 249	69.2 ± 180
RBC (x 10 ⁴ /μL)	Male	430-570	Active	500.2 ± 13.8	489.5 ± 13.3 **	-10.7 ± 3.4
			Placebo	494.4 ± 11.0	491.0 ± 10.8	- 3.44 ± 2.9
	Female	380-500	Active	446.3 ± 11.0	452.7 ± 8.87	6.33 ± 11
			Placebo	429.6 ± 7.87	428.6 ± 6.45	- 1.00 ± 7.7
Hb (g/dL)	Male	13.5-17.5	Active	15.32 ± 0.38	15.05 ± 0.41 *	- 0.26 ± 0.12
			Placebo	15.24 ± 0.37	15.23 ± 0.31	- 0.01 ± 0.11
	Female	11.5-15.0	Active	11.85 ± 0.83	12.00 ± 0.79	0.15 ± 0.30
			Placebo	11.99 ± 0.64	12.07 ± 0.57	0.09 ± 0.24
Ht (%)	Male	39.7-52.4	Active	47.04 ± 1.19	46.53 ± 1.11	- 0.51 ± 0.39
			Placebo	46.59 ± 0.94	46.70 ± 0.84	0.11 ± 0.38
	Female	34.8-45.0	Active	38.30 ± 1.78	39.28 ± 1.59	0.98 ± 0.98
			Placebo	38.73 ± 1.46	38.90 ± 1.26	0.17 ± 0.88
Plt (x10 ⁴ /μL)		14.0-34.0	Active	25.66 ± 0.77	25.10 ± 0.70	- 0.55 ± 0.44
			Placebo	26.31 ± 0.98	25.41 ± 0.81	- 0.91 ± 0.47

Data are expressed as the mean ± SEM. Paprika xanthophyll (active) group (n = 41), placebo group (n = 39), WBC: white blood cell count, RBC: red blood cell count, Hb: hemoglobin, Ht: hematocrit, Plt: platelet count. Differences between the active and placebo groups were determined with the unpaired *t*-test. Comparisons between week 12 and week 0 (baseline) were done with the paired *t*-test. *: *p* < 0.05, **: *p* < 0.01 vs. week 0.

ALT, γ-GTP, ALP, UA (male subjects), BUN, Cre (male subjects), CPK, Na, Cl, and K in both groups.

Tables 8 and **9** summarize the effects of paprika xanthophyll intake on hematology and circulatory parameters. Although RBC and Hb showed significant changes after 12 weeks in male subjects from the paprika xanthophyll group, the changes were very small and within the respective reference ranges. After 12 weeks, the DBP was not significantly lower in either group, but the change of DBP was significantly smaller in the paprika xanthophyll group than in the placebo group. On the other hand, WBC, RBC (female subjects), Hb (female subjects), Ht, Plt, SBP, and pulse rate showed no significant variations in both groups.

Figure 2 shows serum xanthophyll levels in weeks 0 and 12. After intake of the paprika xanthophyll capsule, the serum levels of capsanthin, capsanthone, zeaxanthin, β-cryptoxanthin, and cucurbitaxanthin A were significantly elevated in week 12 compared with week 0, while the serum level of lutein (not contained in the capsules) was

unchanged (**Fig. 2**). On the other hand, the serum levels of zeaxanthin, β-cryptoxanthin, and lutein showed an increase after ingestion of placebo capsules. In addition, the serum levels of capsanthin, capsanthone, zeaxanthin, β-cryptoxanthin, and cucurbitaxanthin A supplementation were significantly higher in the paprika xanthophyll group than in the placebo group after 12 weeks.

During the study period, the only adverse event reported was elevation of CRP in 1 subject from the paprika xanthophyll group. According to the investigator, there was no relation between this event and intake of paprika xanthophyll capsules.

4 DISCUSSION

This randomized, double-blind, placebo-controlled study investigated whether oral paprika xanthophyll supplementation reduced abdominal fat in healthy overweight sub-

Table 9 Effect of paprika xanthophyll intake on circulatory parameters.

Parameter	Group	Treatment period		Change from 0 week
		0 week	12 weeks	
SBP (mmHg)	Active	130.7±1.49	129.0±1.53	- 1.6±1.6
	Placebo	126.2±2.33	123.7±2.17	- 2.5±2.1
DBP (mmHg)	Active	79.3±1.01	77.6±1.06	- 1.7±1.0
	Placebo	72.4±1.49	74.5±1.55	2.1±1.4
Pulse rate (beat/min)	Active	75.8±1.59	73.8±1.51	- 2.0±1.2
	Placebo	73.3±1.65	72.2±1.78	- 1.1±1.5

Data are expressed as the mean ± SEM. Paprika xanthophyll (active) group (n = 41), placebo group (n = 39), SBP: systolic blood pressure, DBP: diastolic blood pressure. Differences between the active and placebo groups were determined with the unpaired *t*-test. Comparisons between week 12 and week 0 (baseline) were done with the paired *t*-test. #: *p* < 0.05 vs. placebo group.

jects with a BMI from 25 to <30 kg/m², revealing that the abdominal fat area (SFA and TFA) assessed by CT decreased significantly after 12 weeks in the paprika xanthophyll group compared with the placebo group. In addition, the BMI, body weight, abdominal circumference, and DBP were significantly lower in the paprika xanthophyll group than in the placebo group after 12 weeks. Furthermore, lipid metabolism parameters (including body fat ratio, T-Cho, LDL-Cho, and LDL/HDL ratio) showed significant improvement compared with week 0 in the paprika xanthophyll group. These findings suggest that oral paprika xanthophyll supplementation decreases the accumulation of abdominal fat and improves parameters of lipid metabolism in healthy overweight subjects. No adverse events related to intake of paprika xanthophyll capsules were detected during the study period, suggesting that paprika xanthophylls can be used safely as a food ingredient to improve metabolic syndrome.

The active capsules used in this study contained mainly seven xanthophylls (capsanthin, cucurbitaxanthin A, β -cryptoxanthin, zeaxanthin, capsorubin, cryptocapsin, and capsanthin 3,6-epoxide) and one carotene (β -carotene)²¹⁾. These carotenoids have very strong antioxidant activity and act as scavengers of reactive oxygen species (ROS). The singlet oxygen quenching activity of capsorubin, capsanthin, and cucurbitaxanthin A is more than 25 times stronger than that of β -carotene, and these xanthophylls also show greater antioxidant activity than astaxanthin, which was reported to be a strong antioxidant *in vitro* study²¹⁾. Furthermore, capsorubin, capsanthin, and zeaxanthin can quench hydroxyl radicals, which are one type of ROS²²⁾. It was previously reported that orally administered paprika xanthophylls were absorbed by the small intestine

in humans, and were subsequently found in the plasma and red blood cell membranes²¹⁾. Accordingly, paprika xanthophylls administered orally may efficiently scavenge ROS at various sites in the body, suggesting that such xanthophylls could be utilized as an antioxidant element in foods and medications. On the other hand, there is no report that antioxidant activity such as singlet oxygen scavenging ability improves lipid metabolism and decreases abdominal fat. For this reason, we speculate that the effect of paprika xanthophyll supplementation on abdominal fat degradation is involved in a mechanism different from antioxidant effect.

It has recently been reported that particular xanthophylls and carotenes possess various functions in addition to antioxidant activity. For example, it is known that supplementation of lutein and/or zeaxanthin can have a protective effect against age-related macular degeneration, which is a major cause of visual impairment in the elderly³⁰⁾. In addition, Shirasawa *et al.* reported that oral intake of astaxanthin derived from *Haematococcus pluvisialis* improved cognitive function (working memory and response time) in healthy volunteers with age-related forgetfulness^{12, 13)}. There have also been reports that β -cryptoxanthin supplementation may improve conditions such as osteoporosis, hepatic dysfunction, dyslipidemia, and arteriosclerosis^{31–37)}. Sugiura *et al.* found that the serum level of β -cryptoxanthin was closely correlated with the risk of several lifestyle-related diseases^{9–11, 31–33)}, while Suzuki *et al.* reported that a higher serum β -cryptoxanthin level was associated with a significantly lower odds ratio for metabolic syndrome³⁴⁾. Moreover, Tsuchida *et al.* reported that the abdominal fat area (VFA, SFA, and TFA) was significantly decreased by intake of β -cryptoxanthin

Fig. 2 Serum levels of xanthophylls in week 0 and week 12. Data are expressed as the mean $\pm$ SD. Paprika xanthophyll (active) group ($n = 41$), placebo group ($n = 39$). White and black bar in the graph indicate placebo group and active group, respectively. Differences between the active and placebo groups were determined with the unpaired *t*-test. Comparisons between week 0 and week 12 were performed by using the paired *t*-test. ##: $p < 0.01$, ###: $p < 0.001$ vs. placebo group. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ vs. week 0.

(0.5 mg/day) for 12 weeks in patients with mild/moderate obesity³⁵. It has been suggested that peroxisome proliferator-activated receptor (PPAR)- α and PPAR- γ expressed by adipocytes might be involved in the mechanism of fat reduction by β -cryptoxanthin^{36, 37}. These reports strongly suggest that β -cryptoxanthin has a role in reducing abdominal fat in obese subjects. In the present study, β -cryptoxanthin was one of components of the paprika xanthophyll preparation and subjects in the paprika xanthophyll group ingested 0.5 mg of β -cryptoxanthin every day. Accordingly, β -cryptoxanthin may have played an important role in reducing the abdominal fat area in the paprika xanthophyll group. In addition, Maeda *et al.* reported that other xanthophylls contained in paprika xanthophyll might contribute to improve lipid metabolism²⁸. They showed that oral intake of paprika xanthophylls improved serum levels of LDL-Chol and adiponectin in high fat fed mice, and that capsanthin and capsorubin included in paprika xanthophyll activated PPAR- γ to improve lipid metabolism by converting hypertrophy adipocyte to compact adipocyte. These reports suggest that not only β -cryptoxanthin but also other xanthophylls in paprika xanthophyll relate to enhance the differentiation to compact adipocyte. Therefore, several of the xanthophylls in the paprika xanthophyll preparation that we tested in this study may have acted together to reduce the abdominal fat area.

5 CONCLUSIONS

In summary, we performed a randomized, double-blind, placebo-controlled human trial to investigate the effect of oral paprika xanthophyll supplementation on fat reduction in healthy overweight subjects. Our findings demonstrated that paprika xanthophyll supplementation reduced the abdominal fat area. In addition, it was suggested that administration of paprika xanthophyll improves the lipid metabolic parameters of these volunteers. To the best of our knowledge, this is the first randomized controlled trial to show a fat-reducing effect of paprika xanthophyll intake in humans. Since xanthophylls have been reported to have various other actions besides fat reduction, we are currently examining such effects of paprika xanthophylls (improvement of cognitive function, antioxidant effect, and anti-inflammatory effect) in clinical randomized trials.

Conflicts of interest

The authors are employees of Ezaki Glico Co., Ltd., but this did not influence the author's adherence to the journal's policy.

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