Potential Beneficial Effects of Crab-Flavored Seafood Intake in Young Rats

Kazunari Kadokura, Tsuyoshi Tomita and Kohei Suruga

Research & Development Division, Development Department, Kibun Foods Inc., Inagi, Tokyo, Japan.

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ABSTRACT

BACKGROUND: Crab-flavored seafood is a well-known traditional Japanese product that is sold as "imitation crab" worldwide. Although it is a low-cost, low-fat, high-protein food, there are few data on its potential health benefits. Here, we have assessed the effects of crab-flavored seafood consumption on organ weight and serum biomarkers levels in rats.

METHODS: Sprague–Dawley rats (male; aged 6 weeks) were fed a normal diet (n = 8) or a normal diet with 5% dried crab-flavored seafood (n = 8) for 84 days. Food intake and overall body weight were measured every week; organ weight and blood biochemistry were evaluated at the end of the administration period.

RESULTS: After 84 days, there were no significant differences in food intake, overall body weight, or organ weight between the 2 groups; however, the muscle weight of rats fed crab-flavored seafood tended to be higher than that of rats fed the normal diet. Several serum biomarkers did not differ between the 2 groups, but serum high-density lipoprotein, total bilirubin, and indirect bilirubin levels were significantly raised in rats fed crab-flavored seafood. Moreover, blood urea nitrogen was significantly lower, and some liver function parameters tended to be lower in rats fed crab-flavored seafood.

CONCLUSIONS: Consumption of crab-flavored seafood may be effective for promoting muscle protein synthesis and improving serum biomarkers associated with disorders such as cardiovascular disease and stroke. Thus, crab-flavored seafood may have application as a functional food for the global management of human health.

KEYWORDS: Crab-flavored seafood, imitation crab, HDL cholesterol, atherogenic index, liver function

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CORRESPONDING AUTHOR: Kohei Suruga, Research & Development Division, Development Department, Kibun Foods Inc., 86 Yanokuchi, Inagi, Tokyo 206-0812, Japan. Email: kouhei_suruga@kibun.co.jp

Background

Fish paste products are made from mainly minced fish (surimi), and have been eaten as a traditional food since ancient times in Japan.[1] These products are commonly produced from fish muscle, which is minced with salt to solubilize actin, myosin and other myofibrillar proteins.[2] Fish pastes can be made from many fish species, including pollack, pike eel, threadfin bream, and blue shark among others.[3] Typical products include kamaboko (fish cake), hanpen (marshmallow-like and soft texture products), tsumire (fish balls made from sardines and mackerels), and satsuma-age (fried kamaboko), while the most popular fish paste products worldwide are crab-flavored seafood (CFS) or "imitation crab sticks." Indeed, many fish paste products, including CFS and imitation scallops and lobster, are manufactured from low-cost fish materials to imitate high-cost fish products.[4]

As mentioned above, CFS is a popular fish paste product worldwide. It was developed in Japan as an alternative to crab leg meat in 1970s and soon thereafter was manufactured and sold in Asian countries. Consumption of CFS subsequently increased rapidly in the United States and European countries in the 1980s and 1990s.[5,6] In the latter countries, CFS is eaten as an ingredient of California roll and seafood salad. The United States now has its own fish paste processing industry and surimi has been marketed as a new American product,[7] while several European companies are manufacturing CFS products using surimi from the United States and Argentina.[4]

In general, CFS is a low-cost, low-fat, and high protein food. Although some studies have reported the health effects of surimi ingredients such as pollack meat protein and peptides,[8,9] there are few data on the health benefits of CFS itself. We are interested in the health effects and benefits of the fish paste products consumed as Japanese traditional foods. In our previous study in Sprague–Dawley rats, we evaluated the effect of administration of hanpen on organ weight and biomarker levels, demonstrating that total cholesterol (T-CHO) and high-density lipoprotein (HDL-C) were higher, while lactate dehydrogenase (LDH) levels were markedly lower, in rats fed hanpen than in control rats after 12 weeks.[10] In addition, consumption of tsumire, which is rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has been demonstrated to significantly lower the levels of some serum biomarkers related to liver function, suggesting that intake of tsumire may prevent liver function from declining in both young (6 weeks) and aged (80 weeks) Sprague-Dawley rats.[11,12] Moreover, a recent trial evaluating the consumption of satsuma-age in aged healthy

Figure 1. KIBUN crab-flavored seafood ("imitation crab" sticks).

humans found that left and right grip strength, as well as back strength were significantly higher after 28 days of intake of satsuma-age.[13]

To our knowledge, the health effects of CFS intake have not been evaluated in young rats. Here, therefore, we have measured changes in biomarker levels and organ weight in young (6 weeks) Sprague–Dawley rats administered a CFS diet for 12 weeks.

Methods

Materials

The CFS used in this study was commercial KIBUN CFS (Figure 1), which is prepared from minced fish and surimi pollack ground with salt, starch, egg white, and other ingredients. For the production of KIBUN CFS, mixed fish paste is formed into a sheet, and steam heated at 89°C for 2 minutes 30 seconds. The dried CFS (100 g) provided 378.0 kcal of energy, 40.3 g of protein, 5.2 g of fat, and 42.6 g of carbohydrate.

Animal studies

Animal experiments were conducted at Kitayama Labes Co. Ltd. (a subsidiary of Oriental Yeast Co., Ltd., Tokyo, Japan). The study was authorized by the Japanese Government and sanctioned by the laboratory ethics committee (approval no. IBC59-047). All experiments were performed in accordance with both ethical guidelines for laboratory animals and standard operating procedures.

Experiments was done as described previously with slight modifications.[10-12] In brief, Sprague–Dawley rats (male, aged 6 weeks; SLC Japan, Inc., Shizuoka, Japan) were placed in separate cages under a 12/12 hour light (08:00)/dark (20:00) cycle at 23°C ± 5°C and 55% ± 25% relative humidity. Tap water and food were freely available. Animals in group I (n = 8) were fed AIN-93G; those in group II (n = 8) were fed AIN-93G with 5% dried CFS (see below for diets). The amount of food eaten and body weight were measured each week for 84 days and then the animals were killed using 4% isoflurane anesthesia. Blood samples were immediately collected, centrifuged (3500 rpm, 4°C, 10 minutes) and stored at −80°C. The kidney, spleen, liver, skeletal muscles, and white and interscapular brown adipose tissues were dissected and weighed. Organ weight relative to body weight (%) was calculated where appropriate.

Table 1. Ingredients in the control and CFS diets.

	GROUP I DIET (CONTROL)	GROUP II DIET (5% CFS)
Casein*1	20.0	17.8
L-cystine	0.30	0.27
β-cornstarch	39.7486	34.5986
α-cornstarch	13.2	13.2
Sucrose	10.0	10.0
Soybean oil	7.0	6.8
Cellulose	5.0	5.0
Mineral mix, AIN-93G-MX*2	3.5	-
Modified mineral mix	-	3.5
Calcium carbonate	-	1.0
Monopotassium phosphate	-	0.7
Magnesium oxide	-	0.08
Tripotassium citrate	-	0.8
Vitamin mix, AIN-93-VX*3	1.0	1.0
Choline bitartrate	0.25	0.25
Butylhydroquinone	0.0014	0.0014
CFS dry powder	-	5.0
Total (%)	100	100

Experimental diets were prepared as described previously[10-12] with slight modifications.
*1Casein came from milk.
*2AIN-93G-MX mineral mix contains $CaCO_3$, KH_2PO_4, $K_3C_6H_5O_7$ H_2O, NaCl, K_2SO_4, MgO, $FeC_6H_5O_7$ XH_2O, $ZnCO_3$, $MnCO_3$, $CuCO_3$ $Cu(OH)_2$ H_2O, KIO_3, Na_2SeO_4, $(NH_4)Mo_7O_{24}$ $4H_2O$, Na_2SiO_3 $9H_2O$, $CrK(SO_4)$ $12H_2O$, LiCl, H_3BO_3, NaF, $NiCO_3$ $2Ni(OH)_2$ $4H_2O$, NH_4VO_3, and sucrose.
*3AIN-93-VX vitamin mix contains nicotinic acid, thiamine hydrochloride, riboflavin, folic acid, D-biotin, potassium pantothenate, pyridoxine hydrochloride, sucrose, and vitamins B_{12}, E, A, D_3, and K_1.

Experimental diets

The control diet for group I comprised AIN-93G (Oriental Yeast Co., Ltd.). The diet for group II comprised AIN-93G with 5% dried CFS; the CFS replaced the casein, L-cystine, and β-cornstarch ingredients in AIN-93G. The ingredients and nutrition provided by the diets are listed in Tables 1 and 2, respectively.

Measurement of serum biomarkers

The following serum biomarkers were determined by using commercial kits and a 7180 Clinical Analyzer (Hitachi, Ltd.,

Table 2. Nutritional composition of the control and CFS diets.

COMPONENT	GROUP I DIET (CONTROL)	GROUP II DIET (5% CFS)
Energy (kcal)	368.00	369.20
Water (g)	9.00	9.00
Crude protein (g)	18.10	18.00
Crude fat (g)	7.30	7.20
Crude fiber (g)	5.00	5.00
Crude ash (g)	3.10	2.80
Nitrogen free-extract (g)	57.60	58.10
Calcium (g)	0.52	0.53
Phosphorus (g)	0.32	0.33
Magnesium (g)	0.05	0.05
Sodium (g)	0.10	0.16
Potassium (g)	0.36	0.37

Experimental diets were prepared as described previously[10-12] with slight modification.

Tokyo, Japan). Total protein (TP) and albumin (ALB) were determined by TP-HR II and ALB II HA-Test Wako kits, respectively (both FUJIFILM Wako Pure Chemical Corporation [FUJIFILM Wako]). We determined blood urea nitrogen (BUN) levels by UN-S reagent (Denka Company, Ltd., Tokyo, Japan), And creatinine (CRE) levels by a L-type Wako CRE M kit (FUJIFILM Wako). Sodium, potassium, and chloride were analyzed by iron electrode reagents (FUJIFILM Wako). Calcium and iron were analyzed by Accuras Auto Ca II and Quick Auto Neo Fe, respectively (both Shino-Test Corporation, Tokyo, Japan). Inorganic phosphorus (IP) was determined by Determiner L IP II (Minaris Medical Corporation, Ltd, Tokyo, Japan). We measured aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and LDH by L-type Wako AST J2, ALT J2, ALP J2, and LD J kits, respectively (all FUJIFILM Wako). Leucine aminopeptidase (LAP) was determined by Iatro-LQ_LAP rate II (LSI Medience Corporation, Tokyo, Japan). Amylase (AMY) and γ-glutamyl transpeptidase (γ-GT) were determined by L-type Wako amylase and γ-GT J, respectively (both FUJIFILM Wako). Cholinesterase was analyzed by Quick Auto Neo Ch-E (Shino-Test Corporation). T-CHO, free cholesterol, and triglycerides (TG were determined by L-type Wako CHO M, free cholesterol, and TG M, respectively (all FUJIFILM Wako). Low-density lipoprotein cholesterol (LDL-C) and HDL-C were measured by Cholestest LDL and Cholestest N HDL, respectively (both Sekisui Medical Co. Ltd., Tokyo, Japan). Glucose (GLU) was determined by Quick Auto Neo GLU-HK (Shino-Test Corporation). Total bilirubin (T-BIL) and direct bilirubin (D-BIL) were determined by Nescote VL T-Bill and VL D-Bill, respectively (Alfresa Pharma Corporation, Osaka, Japan). Total bile acids (TBA) were analyzed by Aqua-auto Kainos TBA (Kainos Laboratories Inc., Tokyo, Japan). The atherogenic index of plasma (AIP) was calculated by using the equation Log (TG/HDL-C) with TG and HDL-C values in mmol/L (converted from mg/mL by division by 88.57 and 38.67, respectively).[14]

Statistical analysis

The results are presented as mean ± standard error. We used Student t-test to assess differences between groups I and II, which were considered statistically significant at $P < .05$.

The sample size was estimated by using the following relationship of Charan and Kantharia[15]:

$$\text{Sample size} = 2\text{SD}^2 \left(Z^{\alpha/2} + Z^{\beta}\right)^2 / \text{d}^2$$

where SD is standard deviation, $Z^{\alpha/2}$ is 1.96 at a type 1 error of 5%, Z^{β} is 0.842 at 80% power, and d is the difference between mean values. Coupled with the effect of KIBUN hanpen administration on rat liver weight reported previously,[10] this yielded a sample size of 7.85 animals per group. As a result, 8 rats were included in each groups.

Analysis of amino acids in dried CFS

To determine the majority of amino acids, CFS was hydrolyzed by incubation for 24 hour with 6 N hydrochloric acid at 110°C.[16] The resulting solution was adjusted to pH 2.2 by the addition of 3 mol/L sodium hydroxide solution (FUJIFILM Wako), passed through a filter (0.45 μm), and applied to an amino acid analyzer. For determination of cystine and methionine, hydrolysis was performed after oxidation with performic acid solution. For determination of tryptophan, alkaline hydrolysis was carried out with barium hydroxide and thiodiethylene glycol (FUJIFILM Wako).

Analysis of fatty acids in dried CFS

Lipids were extracted from CFS by the Folch et al[17] method. The fatty acid content was determined by adding heptadecanoic acid, an internal standard, to the extracted lipids and saponifying with 2 mol/L sodium hydroxide-methanol (FUJIFILM Wako). The fatty acids were then methyl esterified with boron trifluoride methanol reagent (FUJIFILM Wako) and measured by gas chromatography.

HPLC analysis of carotenoids in dried CFS

Carotenoids levels in CFS were measured as reported by Xu et al.[18] In brief, lyophilized CFS powder (1 g) was added to ethanol containing 3% pyrogallol and 60% potassium hydroxide, and heated at 70°C for 30 minutes. Next, 1% sodium chloride solution was added, and the carotenoids were extracted 3 times with hexane: ethyl acetate (9:1). The extracted carotenoids

Table 3. Effect of CFS intake for 84 days on overall body weight, and tissue weight in rodents.

PARAMETER	GROUP I (WITHOUT CFS) N = 8	GROUP II (WITH CFS) N = 8
Total food intake (g)	1654.50 ± 64.59	1840.40 ± 51.40
Initial body weight (g)	190.5 ± 3.15	191.30 ± 2.42
Final body weight (g)	519.30 ± 17.59	545.90 ± 15.85
Liver (g)	17.35 ± 0.99	18.46 ± 0.94
Kidney (g)	2.79 ± 0.09	2.93 ± 0.10
Spleen (g)	0.80 ± 0.04	0.97 ± 0.07
White adipose tissue (g)	35.11 ± 2.74	38.12 ± 1.67
Perirenal (g)	15.93 ± 1.25	17.07 ± 0.92
Epididymal (g)	12.63 ± 0.99	14.00 ± 0.65
Mesenteric (g)	6.56 ± 0.68	7.05 ± 0.44
Brown adipose tissue (g)	0.65 ± 0.05	0.67 ± 0.06
Muscle (g)	6.36 ± 0.18	6.75 ± 0.28
Soleus (g)	0.39 ± 0.01	0.42 ± 0.03
Gastrocnemius (g)	5.97 ± 0.18	6.33 ± 0.25

Data are mean ± standard error. Differences between groups were evaluated by Student's *t*-test. *$P < .05$ Versus group I.

were dried under reduced pressure, dissolved in ethanol, and subjected to HPLC analysis using a Chromaster DAD system (Hitachi, Tokyo, Japan) with an InertSustain C18 (4.6 i.d. × 150 mm) column (GL Sciences, Tokyo, Japan) and a column oven temperature of 30°C. The carotenoids were separated by a linear gradient of solvent A (acetone) and solvent B (water) as follows: 0 to 5 minutes, 75% solvent A; 5 to 10 minutes, 75%–95% solvent A; 10 to 17 minutes, 95% solvent A; 17 to 22 minutes, 95%–100% solvent A; and 22 to 27 minutes, 100%–75% solvent A. The injection volume was 1.0 µL, and the flow rate was 1.0 mL/min. Detection was at 450 nm. Standard calibration curves were generated to obtain quantitative data on the carotenoid content of CFS.

Results

Effect of CFS administration on overall body weight and tissue weight

The effects of feeding a 5% CFS diet to Sprague–Dawley rats for 84 days on the overall weight, and weights of various organs and tissues are given in Table 3. In summary, there was no significant difference in food intake, overall body weight, or the individual weight of the liver, spleen, adipose tissue, or muscle between group I given the control diet and group II given the CFS diet for 84 days. However, the muscle weight of group II (soleus, 0.42 ± 0.03 g; gastrocnemius, 6.33 ± 0.25 g) tended to be elevated as compared with that of group I (soleus, 0.39 ± 0.01 g; gastrocnemius, 5.97 ± 0.18 g).

Effect of CFS intake on serum biomarkers

The effects of feeding a diet of 5% CFS diet to Sprague–Dawley rats for 84 days on serum biochemistry are summarized in Table 4. There were few significant differences between group I given the control diet and group II given the CFS diet; however, levels of BUN were significantly lower in group II, while K, Ca, IP, HDL-C, T-BIL, and I-BIL were significantly raised in group II. Moreover, AST, ALP, LDH, AMY, TG and AIP tended to have lower levels in group II than in group I.

Amino and fatty acid content in dried CFS

The content of amino acids and fatty acids in dried CFS is summarized in Tables 5 and 6, respectively. The amino acid analysis showed that high levels of alanine, arginine, aspartic acid, isoleucine, leucine, lysine, glutamic acid, glycine, and valine are present in dried CFS (Table 5), while the fatty acid analysis indicated that dried CFS contains high levels of linoleic acid and oleic acid (Table 6).

HPLC analysis of dried CFS for carotenoids

The HPLC chromatogram of the carotenoids present in dried CFS is shown in Figure 2. Overall, 5 main peaks were observed in the HPLC chromatogram. The carotenoids were identified as capsanthin (peak 1), β-cryptoxanthin (peak 2), lycopene (peak 4), and β-carotene (peak 5). Peak 3 was unidentified, but might be cryptocapsin. The quantities of capsanthin, β-cryptoxanthin, lycopene, and β-carotene was, respectively, 4.55 ± 0.07, 2.34 ± 0.07, 4501.71 ± 103.04, and 4.68 ± 0.01 µg per g of dried CFS.

Discussion

Crab-flavored seafood is familiar to people in regions across the world, including Asia, the United states and Europe, as "imitation crab." Even though it is approximately 45 years since CFS was first produced, developers are continuing to make products with high similarity to real crab leg meat.[19] In general, CFS is produced from fish muscle proteins such as Alaska pollack, starch, flavorings and colorants, and is available in different sizes and types (eg, flake type, stick type and other types).[20] Although it is well known as a low-cost, low-fat, high-protein food, to the authors' knowledge, there are no data on the health benefits of CFS. In this preliminary study, therefore, we evaluated the effect of the intake of KIBUN CFS for 84 days on organ weight and serum biomarkers in rat. In addition, we qualitatively analyzed the compounds present in CFS by HPLC.

Table 4. Effect of CFS intake for 84 days on serum biomarkers in rodents.

BIOCHEMICAL PARAMETER	DAY 0 OF ADMINISTRATION N = 16	DAY 84 OF ADMINISTRATION	
		GROUP I (WITHOUT CFS) N = 8	GROUP II (WITH CFS) N = 8
Total protein (g/dL)	6.34 ± 0.05	6.70 ± 0.10	6.68 ± 0.15
Albumin (g/dL)	4.54 ± 0.03	4.48 ± 0.06	4.49 ± 0.09
A/G	2.55 ± 0.08	2.03 ± 0.06	2.08 ± 0.08
BUN (mg/dL)	18.18 ± 1.01	21.50 ± 0.70	15.66 ± 0.43**
Creatine (mg/dL)	0.19 ± 0.00	0.34 ± 0.00	0.32 ± 0.01
Sodium (mEq/L)	140.75 ± 0.34	142.13 ± 0.33	141.38 ± 0.39
Potassium (mEq/L)	4.74 ± 0.06	4.49 ± 0.03	4.80 ± 0.08**
Chloride (mEq/L)	98.50 ± 0.53	100.63 ± 0.39	99.00 ± 0.64
Calcium (mg/dL)	11.54 ± 0.10	10.61 ± 0.07	10.90 ± 0.07*
Inorganic phosphorus (mg/dL)	8.94 ± 0.34	5.39 ± 0.24	6.90 ± 0.11**
Iron (μg/dL)	298.88 ± 8.73	182.75 ± 17.67	150.25 ± 4.41
AST (IU/L)	102.63 ± 2.89	93.13 ± 12.76	79.50 ± 5.96
ALT (IU/L)	32.88 ± 0.98	29.38 ± 2.93	30.13 ± 4.85
ALP (IU/L)	1718.75 ± 99.73	484.63 ± 25.86	469.63 ± 54.49
LDH (IU/L)	1378.88 ± 109.87	1044.75 ± 44.09	904.38 ± 91.82
LAP (IU/L)	55.38 ± 0.73	52.13 ± 1.60	52.50 ± 1.00
Amylase (IU/L)	2355.00 ± 110.20	2137.25 ± 105.95	1977.38 ± 55.25
γ-GT (IU/L)	<3.00	<3.00	<3.00
Cholinesterase (IU/L)	11.38 ± 0.64	8.38 ± 0.71	7.50 ± 0.56
Total cholesterol (mg/dL)	98.75 ± 4.11	87.13 ± 4.84	102.38 ± 5.01
Free cholesterol (mg/dL)	21.00 ± 1.43	16.38 ± 1.03	18.75 ± 1.07
Esterified cholesterol (mg/dL)	77.75 ± 2.84	70.75 ± 3.88	83.63 ± 4.09
Esterified/Total (%)	78.88 ± 0.62	81.25 ± 0.34	82.00 ± 0.47
Triglyceride (mg/dL)	146.88 ± 17.36	177.38 ± 16.02	152.63 ± 29.16
LDL-cholesterol (mg/dL)	11.63 ± 0.47	7.00 ± 0.66	7.88 ± 0.45
HDL-cholesterol (mg/dL)	40.88 ± 1.45	29.00 ± 0.81	33.13 ± 1.10*
AIP[a]	0.17 ± 0.06	0.41 ± 0.05	0.25 ± 0.08
Glucose (mg/dL)	121.88 ± 1.85	154.50 ± 8.10	173.63 ± 7.63
Total bilirubin (mg/dL)	0.06 ± 0.00	0.06 ± 0.00	0.09 ± 0.01*
Direct bilirubin (mg/dL)	0.04 ± 0.00	0.06 ± 0.00	0.05 ± 0.01
Indirect bilirubin (mg/dL)	0.02 ± 0.01	0.01 ± 0.00	0.04 ± 0.01**
Total bile acids (μmol/L)	31.25 ± 2.95	15.38 ± 3.75	11.13 ± 2.39

Results are expressed as mean ± standard error. Differences between groups were evaluated by Student's *t*-test.
Abbreviations: AIP, atherogenic index of plasma; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; HDL, high-density lipoprotein; LAP, leucine aminopeptidase; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; γ-GT, γ-glutamyl transpeptidase.
[a]AIP was calculated as log (Triglyceride/HDL-cholesterol) with Triglyceride (mg/dL/88.57) and HDL-cholesterol (mg/dL/38.67) expressed in mmol/L.[14] **$P < .01$ versus group I.
*$P < .05$ versus group I.

Table 5. Analysis of amino acids in dried CFS.

AMINO ACID	CONTENT (G/100 G)
Glutamic acid	8.54
Aspartic acid	4.08
Lysine	3.46
Leucine	3.24
Arginine	2.51
Glycine	2.50
Alanine	2.42
Valine	2.07
Serine	1.98
Threonine	1.80
Isoleucine	1.86
Phenylalanine	1.65
Proline	1.55
Tyrosine	1.36
Methionine	1.15
Histidine	1.00
Cystine	0.52
Tryptophan	0.47

Table 6. Analysis of fatty acids in dried CFS.

FATTY ACID	CONTENT (G/100 G)
Total fatty acid	7.12
Polyunsaturated fatty acid	3.82
Monounsaturated fatty acid	2.16
Saturated fatty acid	1.14
n-6 Unsaturated fatty acid	2.89
n-3 Unsaturated fatty acid	0.93
Myristic acid	0.05
Pentadecylic acid	0.01
Palmitic acid	0.79
Palmitoleic acid	0.06
Heptadecanoic acid	0.01
Stearic acid	0.25
Oleic acid	1.96
Linoleic acid	2.84
Linolenic acid	0.45
Octadecatrienoic acid	0.02
Arachidic acid	0.02
Eicosenoic acid	0.09
Arachidonic acid	0.03
Eicosapentaenoic acid	0.15
Behenic acid	0.01
Docosenoic acid	0.05
4,7,10,13,16-Docosapentaenoic acid	0.02
Docosapentaenoic acid	0.02
Docosahexaenoic acid	0.29

There were no fatalities or changes in food consumption or coat condition in the CFS-fed rats (group II), suggesting that 12 weeks of CFS administration does not induce adverse effects in rats. We found that overall food intake, body weight, organ weight, and many serum biomarkers did not differ between group I and group II, probably because the nutritional content of the diets fed the 2 groups were similar (Tables 3 and 4).

After the 12-week administration period, the weight of the soleus and gastrocnemius muscles tended to be elevated in group II as compared with group I (Table 3). The principal source of protein in the control diet was casein (20%), which is reported, along with whey, to enhance amino acid supply to muscles and thereby promote muscle protein synthesis.[21] In the group II diet, the added CFS (5%) mainly replaced casein, suggesting that CFS may also promote muscle protein synthesis. Protein from cod possesses anti-inflammatory properties and can also improve skeletal muscle repair in rodents after injury.[22] Furthermore, we previously found that the fish paste products hanpen[10] and tsumire[11,12] are potential protein sources, in addition to casein, for the synthesis of skeletal muscle. Based on those reports and the present study, we propose that CFS intake may be more potent than that of hanpen or tsumire for muscle protein synthesis.

Dried CFS consists of 40.30% total protein, and 3.24%, 2.07%, and 1.86% leucine, valine, and isoleucine, respectively (Table 5). These residues are branched-chain amino acids (BCAAs), which comprise a popular type of food supplement that is consumed after resistance physical exercise to stimulate muscle protein synthesis.[23] Dried CFS also contains methionine at 1.15%, which is higher than the levels present in hanpen and tsumire. Methionine is an essential nutrient in human and a limiting amino acid[24] that is required, along with lysine, for biosynthesis of carnitine, which has an important role in cellular energy metabolism.[25] In particular, L-carnitine has been reported to increase total muscle mass, to decrease total fat mass with weight gain, and to increase walking capacity.[26] Based on these reports, we propose that the high levels of BCAAs and methionine in CFS might be related to skeletal muscle synthesis.

After 12 weeks of diet administration, serum K, Ca and IP levels were significantly elevated in the CFS-fed rats relative to

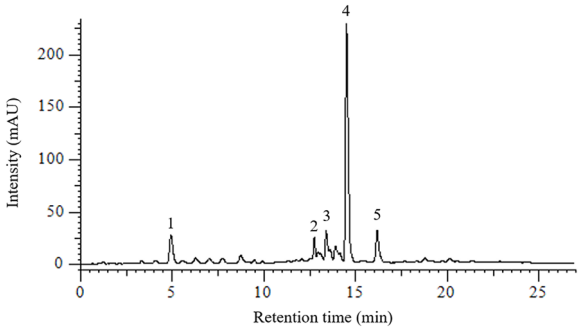

Figure 2. HPLC separation of carotenoids in dried CFS. 1, capsanthin; 2, β-cryptoxanthin; 3, unidentified compound; 4, lycopene; 5, β-carotene.

the control rats in the present study (K: $P < .01$, Ca: $P < .05$, IP: $P < .01$). However, based on standard values reported for Crj:CD(SD)IGS rats aged 10 to 32 weeks (K: 4.6-4.7 mg/dL, Ca: 9.9-10.2 mg/dL, IP: 6.0-7.7 mg/dL),[27] we consider that the K, Ca, and IP levels of the rats fed CFS were more or less the same as these standard values.

Due to the hectic pace of modern life, many individuals are affected by a shortage of sleep and tiredness.[28] In this study, the CFS-fed rats had significantly lower levels of BUN, an indicator of fatigue and a metabolic product of proteins that is produced by the liver and removed via the kidneys.[29] BUN is induced by exhaustive exercise: that is, the more exercise the body undergoes, the higher the levels of BUN. After 12 weeks, the levels of BUN were decreased by 27% in group II relative to group I. Moreover, levels of LDH, another fatigue-related biochemical marker,[30] tended to be lower in group II (904.38 ± 91.82 IU/L) than in group I (1044.75 ± 44.09 IU/L) after 12 weeks. Taken together, these results indicate that CFS might have anti-fatigue activity. Other studies have recently reported the anti-fatigue activity of natural products including polypeptides,[31] polysaccharides,[32] and proteins.[33] In particular, glycoprotein from Hairtail fish has been shown to significantly lower the levels of LDH, blood lactic acid, creatine kinase, and BUN in BALB/c mice.[34] Therefore, we propose that these anti-fatigue active compounds, including polypeptides, polysaccharides, proteins and glycoprotein, might be present in CFS. In future studies, we plan to investigate other fatigue-related biochemical markers such as liver glycogen levels, blood ammonia levels, and blood lactic acid[35] after the administration of CFS and anti-fatigue active compounds in CFS to rats.

In the present study, rats given the CFS diet also had higher levels of serum bilirubin, the cytotoxic, lipid-soluble end product of heme catabolism.[36] Bilirubin has strong anti-oxidant and anti-inflammatory activities, and has been linked to the risk of many diseases such as diabetes, heart disease, and stroke.[37] For example, serum bilirubin has been inversely correlated with risk of hypertension,[38] while low bilirubin may be an independent risk factor for stroke.[39] In addition, higher bilirubin levels have been linked to both reduced prevalence and favorable outcomes for stroke.[40] After 84 days of CFS administration, T-BIL and I-BIL levels were higher in group II, although there were no differences in D-BIL between groups I and II. It is possible, however, that CFS intake may help to prevent some diseases such as diabetes, heart diseases, hypertension, and stroke.

Regarding lipid biochemistry, HDL-C was significantly higher, while TG and AIP levels tended to be lower, in the rodents fed CFS in the present study. Hyperlipidemia is characterized, among other features, by a decrease in HDL-C,[41] while serum TG and AIP are risk factors for coronary heart disease[42,43]; therefore, CFS may be effective in protecting against hyperlipidemia and heart disease. Dried CFS contains 0.93% and 2.89% omega-3 and omega-6 fatty acids, respectively (Table 6). In particular, it contains 0.15% EPA and 0.29% DHA, 2 very long chain omega-3 fatty acids whose daily intake is endorsed by many health administrations worldwide.[44] Intake of EPA has been shown to lower plasma TG in animals,[44,45] and also TG and non-HDL-C levels in humans.[46,47] In addition, a review has concluded that EPA and DHA lower serum TG and raise HDL-C to some extent.[48] Another characteristic of CFS is the high levels of oleic acid (OA; 1.96%) and linoleic acid (LA; 2.84%). OA, a monounsaturated fatty acid abundant in olive oil,[49] has been demonstrated to reduce LDL-C[50,51] and to increase beneficial HDL-C in blood,[52] while omega-6 polyunsaturated fatty acids including LA lower the risk of coronary heart disease.[53] Given those reports, it is assumed that the effects of CFS on lipid metabolism in rats may be linked to EPA, DHA, and OA. Moreover, CFS containing LA may be effective for prevention of coronary heart disease.

Serum levels of ALP, AST, ALT, LAP, and LDH are used as clinical markers of damage to the liver,[54] an essential organ that, among other functions, breaks down toxins, regulates metabolic functions, and maintains body homeostasis.[55] In the present study, serum AST, ALP, LDH levels tended to be lower in the CFS group than in the control group. In our previous study, some biomarkers related to liver function were significantly reduced in both young and aged rats fed diets based on hanpen and tsumire, suggesting that consumption of these fish paste products might prevent a deterioration in liver function.[10-12] In particular, levels of AST, ALT, LDH, and LAP were significantly lower in young rats fed tsumire than in those fed the control diet.[11] Relative to other fish paste products, tsumire contains high levels of EPA and DHA,[11] which, based on their antioxidant, anti-inflammatory, and hypolipidemic activities, have been recommended as a dietary regime to improve NAFLD (non-alcoholic fatty liver disease).[56,57] Furthermore, consumption of fish oil has been found to have beneficial effects in children with IFALD (intestinal failure-associated liver disease), where it improves liver function, as assessed by serum levels of ALT and AST.[58] While the lower serum AST, ALP, and LDH observed in CFS-fed rats suggest that CFS intake might prevent a decline in liver function, the content of DHA and EPA in dried tsumire is, respectively, 4.3

and 4.4 times higher than that in dried CFS; therefore, we assume that CFS consumption will not be more potent than tsumire consumption for preventing a deterioration in liver function.

Our HPLC analysis showed that carotenoids were present in dried CFS, including capsanthin, β-cryptoxanthin, lycopene, and β-carotene, as well as an unidentified compound (peak no. 3 in Figure 2), which might be cryptocapsin (analysis is ongoing). The 4 identified carotenoids are strong antioxidants found in fruits and vegetables.[59] It is thought that consumption of carotenoids can reduce the risk of type 2 diabetes, cardiovascular disease, neurodegenerative disorders and some types of cancer,[60,61] and can also affect the levels of pro-inflammatory mediators by modulating oxidative stress.[62] In particular, lycopene has been shown to significantly improve blood HDL-C levels.[63] Therefore, CFS, which contains these carotenoids, might have antioxidant and anti-inflammatory activity against oxidative stress and might improve serum HDL-C.

CFS can be manufactured to imitate the taste and texture of snow crab leg meat. Recently, Mun et al[19] evaluated similarities between commercial CFS in Korea and snow crab meat in term of sensory aspects, proximate composition, colors, rheological properties, microstructure, taste, and aroma. To our knowledge, however, no studies have compared the effects of CFS and snow crab meat consumption on animals or humans. In this paper, we have demonstrated that beneficial compounds such as OA, LA and carotenoids are present in CFS. CFS also has advantages over crab meat: it is produced from low-cost fish protein materials and is easy to eat as compared with snow crab meat because it does not require any difficult cookery skills.

Conclusions

Commercially available CFS is one of the most well-known Japanese traditional foods and is eaten across the world. Although it is known as a low-cost, low-fat, high protein food, to date there have been few reports about its actual health benefits. Here, we found that CFS intake for 84 days in Sprague–Dawley rats led to higher muscle weight and favorable changes in some biomarkers. Consumption of CFS may be more effective for promoting muscle protein synthesis than consumption of other fish paste products such as hanpen and tsumire. Moreover, CFS intake might prevent some diseases such as diabetes, heart diseases, hypertension, and stroke, as well as having anti-fatigue activity and positive effects on lipid metabolism. In summary, our findings indicate that CFS may be an effective functional food for the global management of human health.

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Author Contributions

K. Kadokura: study design, data analysis, and manuscript editing. T. Tomita: data analysis and manuscript editing; K. Suruga study design, data analysis, and manuscript writing and editing.

Animal Studies

All animal experiments were authorized by the Japanese Government. They were conducted at Kitayama Labes Co. Ltd., a subsidiary of Oriental Yeast Co., Ltd., under standard operating protocols in accordance with the ethical guidelines for laboratory animals. The study protocol was approved by the ethics committee of Kitayama Labes Co. Ltd. (approval no. IBC59-047).

Availability of Data and Material (ADM)

All data generated or analyzed during this study are included in this published article.

Consent for Publication

Not applicable.

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