Journal of Food & Nutritional Disorders

RESEARCH ARTICLE

A SCITECHNOL JOURNAL

Dried Seaweed (*Porphyra yezoensis*) Extract Promotes Fat Metabolism in Fatty Liver Cell and Mouse Models

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Abstract

Seaweed has been consumed in Japan for over a thousand years. This is a staple in Japanese cuisine with nearly 10 billion pieces produced every year. In this study, we investigated the effect of seaweed extract on fat metabolism using fatty liver cell and mouse models. The results showed that seaweed extract significantly reduced the number of Nile red-positive cells. Furthermore, the administration of seaweed extract resulted in a significant decrease in the levels of cholesterol and triglycerides in fatty liver cell models. An in vivo experiment was performed using fatty liver mice models. The body weight as well as the intraperitoneal fat and liver weight of the fatty liver mice decreased when they were fed seaweed extract. From the micrographs of the liver tissue sections stained with Oil Red O, positive cells were significantly decreased in the seaweed extract group. Upon quantification, the levels of total cholesterol and triglycerides in the liver significantly decreased.

Keywords

Porphyra yezoensis; Porphyrin; Metabolic syndrome; Fatty liver; fat metabolism effects

Introduction

Nori or dried seaweed has been consumed in Japan for more than 1000 years. This is a staple in Japanese cuisine with nearly 10 billion pieces produced every year [1]. Seaweed contains many nutrients, such as vitamins, minerals, dietary fibre, and sugars, and may prevent various diseases. It has been reported that seaweed contains about 30% of the polysaccharide, porphyran, and that porphyran and porphyran-derived oligosaccharides present in seaweed can activate the immune system of mice [2,3]. It has been reported that when porphyran, a water-soluble dietary fibre, was fed in vivo to rats for 3 weeks, serum cholesterol level and renal fat accumulation decreased, demonstrating that porphyran is involved in fat metabolism [4,5]. While the beneficial effect of seaweed has been demonstrated, the amount of waste seaweed has been increasing yearly. Therefore, there is a need for the effective use of seaweed in pharmaceuticals and cosmetics.

Our laboratory is conducting a research focusing on the immunostimulatory effect of seaweed extract. In the previous year, short-term oral administration of seaweed extract to B16-F0 melanoma cell subcutaneous transplant model mice led to an increase in IFN-γ level, and increased levels of IgA and IgG were also observed due to the activation of gut immunity. These results showed that seaweed extract-induced immunostimulation has therapeutic and preventive effects against cancer [5,6].

In recent years, there has been increased attention on the dietary effect of seaweed. Obesity tends to be neglected while lifestyle-related diseases are regarded as a major health problem. Obesity is a lifestyle-related disease, and obese individuals have a high probability of developing dyslipidemia. Dyslipidemia is a factor that increases the risk of hypertension, diabetes, and cancer, and thereby increases the risk of lifestyle-related diseases [7,8]. Diet therapy, in combination with exercise therapy, is the main therapy for the treatment of obesity and other lifestyle-related diseases. Seaweeds are often incorporated into diet, and porphyran, a component of seaweed, reduces blood glucose level and lowers serum total Triglycerides (TG) and Total Cholesterol (TC).

In this study, we investigated the in vitro and in vivo effects of seaweed extract on fat metabolism using fatty liver cell model and obesity mice models (a model of metabolic syndrome and fatty liver), respectively.

Material and Methods

Samples and component extraction

For seaweed (nori), we used a seaweed extract in which the main ingredient of *Porphyra yezoensis* harvested in the Ariake Sea (Seaweed porphyrin solution, purity: 80% + Daiichi Seimo Co., Ltd., Fukuoka, Japan), which was spray-dried at Ohmoriya Co. Ltd. Osaka, Japan to obtain a powdered sample. The seaweed extract powder was dissolved in ultrapure (0.20-μm filtered) water. The mixture was sonicated (40 °C, 1 mL/mL) until completely mixed, and then autoclaved.

Cells and animals

Human hepatic carcinoma (Hep-G2) cells were purchased from the Riken BioResource Research Center, Ibaraki, Japan and cultured in DMEM medium (GIBCO, Thermo Fisher Scientific, MA, USA) containing 10% fetal bovine serum (FBS, Hyclone Laboratories, Inc., UT, USA).

Five-week-old, female C57BL/6NJcl mice were purchased from CLEA Japan, Inc. (Tokyo, Japan), respectively.

Observation of fatty liver cells using Nile Red staining

Approximately 4 mL of 2.0×10^5 cells/mL Hep-G2 cell suspension was seeded in 60-mm dishes. The cells were cultured in a culture medium (DMEM containing 10% FBS) for 24 h. Following this, the cells were cultured in DMEM containing 0.75 mM oleic acid (Nacalai Tesque, Inc., Tokyo, Japan), 1.5% BSA, and seaweed extract (0, 50, 500, and 1000) μg/mL for 24 h. Next, the cells were collected, and the cell suspension was washed with PBS (-), fixed with 10% neutral buffered formalin solution (10 min), and stained with Nile Red (FUJIFILM Wako Chemicals Co., Osaka, Japan) (10 min). After staining, analysis was performed using flow cytometry (Ex: 488 nm, Em: 585 nm, Epics

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Received: September 01, 2021 **Accepted:** September 09, 2021 **Published:** September 24, 2021

Table 1: Feed served in each group.

Group		Feed	Content of seaweed extract
Normal		CE-2	0
Control		HFD32	0
Content of seaweed extract	0.02w/w%		0.02w/w%
	0.05w/w%		0.05w/w%

XL system II flow cytometer, Beckman Coulter, Brea, CA, USA).

Preparation of feed and dosing sample

The animal experiment was conducted in compliance with the Sojo University Animal Experiment Code of Ethics and related laws and regulations in Japan. C57BL/6NJcl mice (5 weeks old, female) were obtained from CLEA Japan. The mice were allowed to acclimatize for 1 week before use in the experiment. Seaweed extract powder was provided by Omoriya Co., Ltd. (Osaka, Japan). Normal mice were fed a standard diet (CE-2, CLEA Japan, Inc., Tokyo, Japan). High-Fat Diet 32 (HFD32, CLEA Japan, Inc., Tokyo, Japan) for diabetes and obesity study was used as feed for the obesity mice models. In the seaweed extract intake group, 0.02 w/w% and 0.05 w/w% of seaweed extract were mixed accordingly with HFD32 The mice had free access to the feed in their respective groups. (Table 1).

Inhibitory effect of seaweed extract on fat accumulation in obese mice models

The mice were divided into four groups (control, normal, seaweed extract 0.02 %, and seaweed extract 0.05 %). The normal group was fed CE-2, the control group was fed only HFD32, and the 0.02 % and 0.05% seaweed extract groups were fed HFD32 with seaweed extract at concentrations of 0.02w/w% and 0.05w/w%, respectively. The mice had free access to water. The mice were observed for 7 weeks, and the mice were weighed and followed up during the observation period.

After the rearing period, the rats were dissected under anesthesia, and the liver, as well as the intra-abdominal fat, of mice from each group were collected and weighed. The liver weight and intra-abdominal fat weight were analysed.

Measurement of serum cholesterol in obese mice treated with seaweed extract

After the end of the rearing period, the mice were dissected under anesthesia, and serum samples from each group were collected. Cholesterol E-Test Wako was used for colour measurement using a microplate. Using the Total serum Cholesterol (TC) concentration of each mice group, we evaluated the decrease in serum TC concentration induced by seaweed extract in the obese mice.

Observation of fat accumulation in the liver of obese mice treated with seaweed extract using Oil Red O-staining

After the dissected liver was weighed, a small amount was embedded in OCT compound for cryopreservation. Following this, frozen liver tissue sections were prepared using a cryostat frozen section preparation device, stained with Oil Red O (FUJIFILM Wako Chemicals Co., Osaka, Japan), and evaluated by observation. The prepared liver sections were incubated in propylene glycol (FUJIFILM Wako Chemicals Co., Osaka, Japan) solution for 5 min, Oil Red O stain for 10 min, and 80 % propylene glycol (FUJIFILM Wako Chemicals Co., Osaka, Japan) solution for 1 min, followed by rinsing with deionized water. Next, it was soaked in hematoxylin solution for 2 min, washed with ion-exchanged water, dried, covered with glass from above, and fixed with a top and base coat. Stained tissue sections were observed with a light microscope.

Measurement of intrahepatic lipid in obesity mice model using seaweed extract

After weighing the collected liver, it was immersed in formalin and stored. Following this, 100 mg of liver and 250 µl of saline was added to BioMasher, homogenated, and centrifugated for 5 min. The supernatant was collected, and the levels of TC and TG were measured using cholesterol E-Test Wako (FUJIFILM Wako Chemicals Co., Osaka, Japan) and triglyceride E-Test Wako, respectively

Statistical analysis

Data were statistically analyzed by a Student's t-test. All data are presented as the mean S.D. p values less than 0.05 were considered to be statistically significant.

Results and Discussion

Effect of seaweed extract on fat metabolism in fatty liver cells treated with seaweed extract

The effect of seaweed extract on fat metabolism in fatty liver cells was evaluated using Nile Red staining. The results of flow cytometry analysis are shown in (Figure 1). The number of Nile Red-positive cells, which indicated the number of cells with high levels of TG, did not decrease with 50 ng/mL of seaweed extract. However, the proportion of Nile Red-positive cells significantly decreased with 500 and 1000 ng/ml seaweed extract. The results showed that seaweed extract reduced the level of fat in fatty liver cells.

Weight change in obese mice treated with seaweed extract

We investigated the suppression of fat accumulation in obese mice treated with seaweed extract. Figure 2 shows the body weight of obese mice up to Day 47. On Day 47, the control mice weighed 27 g and normal mice weighed 21 g, indicating a difference of 6 g and that we successfully created obese mice models with HFD32. The body weight

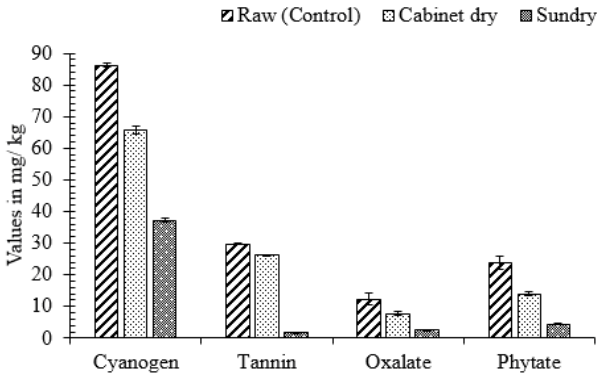

Figure 1: Reduction of triglyceride concentration caused by seaweed extract in fatty liver cells.

of the mice in the 0.02 % seaweed extract group on Day 47 was 29 g, with no significant difference observed in comparison with the weight of the control mice. The body weight of the 0.05% seaweed extract group on Day 47 was 24 g, and a significant difference was observed when compared with the weight of the control mice, indicating that the administration of seaweed extract inhibited lipid absorption. Therefore, we confirmed that the seaweed extract suppressed the increase in body weight and inhibited lipid absorption.

Seaweed extract reduced liver weight and intra-abdominal fat weight in obese mice

The effects of seaweed extract on liver weight and intra-abdominal fat weight in obese mice were evaluated. Figure 3 (a) and (b) show the liver weight and intra-abdominal fat weight of obese mice. We compared the 0.02 % seaweed extract group with the 0.05% seaweed extract group. Both the liver weight and the intra-abdominal fat weight decreased in the 0.05% seaweed extract group. This showed that seaweed extract suppressed liver weight and intra-abdominal fat weight in obese mice.

Seaweed extract decreased serum cholesterol in obese mice

We investigated the effect of seaweed extract on serum TC concentration in obese mice. Figure 4 shows the results of the serum TC concentration of the mice. Comparing the 0.02% seaweed extract group and 0.05 % seaweed group, a lower concentration of serum TC was observed with 0.05% seaweed extract. Comparing the control group and the 0.05% seaweed extract group, a decrease in serum TC concentration was observed in the 0.05% seaweed extract group. This showed that seaweed extract had an inhibitory effect on the serum TC concentration in obese mice.

Seaweed extract suppressed intrahepatic lipid accumulation

Oil Red O staining was used to evaluate the inhibitory effect of seaweed extract on intrahepatic lipid accumulation in obese mice. Figure 5 shows an image of a liver tissue section after Oil Red O staining. In the control group, adipose cells were dyed red throughout the liver, confirming fat accumulation. In the 0.05% seaweed extract group, we observed a part without dye, confirming a decreased in the level of fat. This indicated that the administration of seaweed extract to obese mice suppressed fat accumulation in the liver.

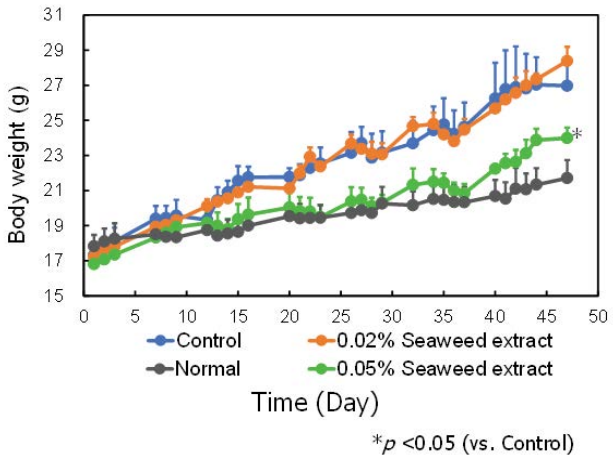

Figure 2: Changes in body weight in obese mice treated with seaweed extract.

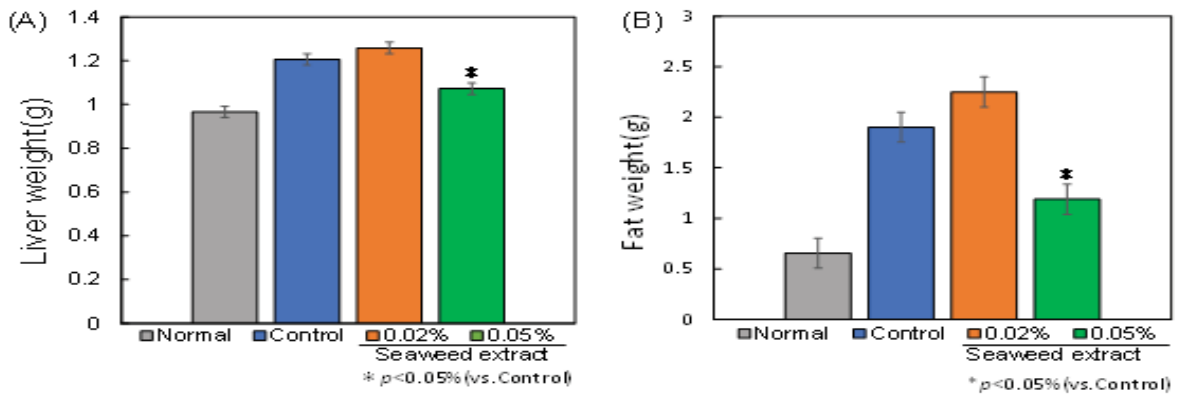

Figure 3: Decrease in liver weight; (a) and intra-abdominal fat; (b) in obese mice treated with seaweed extract.

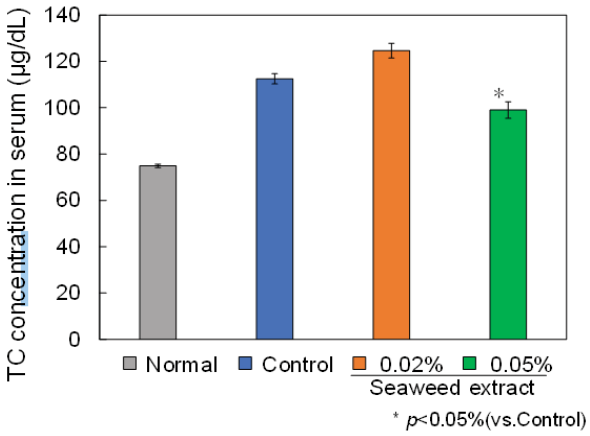

Figure 4: Decrease in serum TC concentration in obese mice treated with seaweed extract.

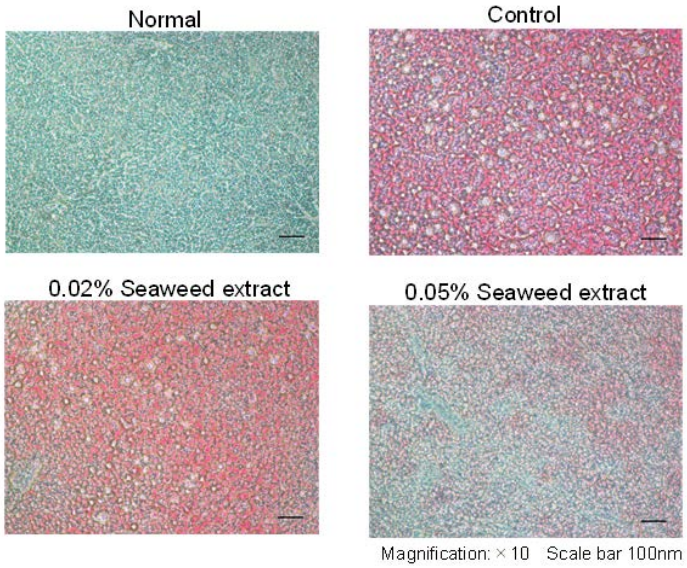

Figure 5: Micrograph of Oil Red O-stained liver tissue section of obese mice treated with seaweed extract.

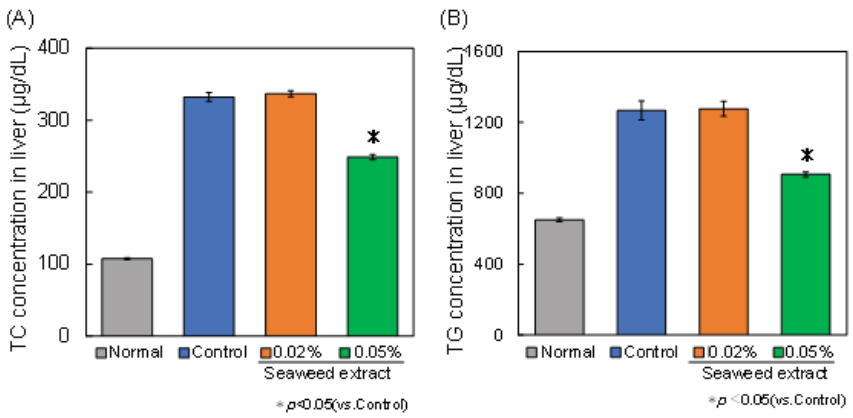

Figure 6: Decrease in liver TC; (a) and TG; (b) concentrations in obese mice treated with seaweed extract.

Seaweed extract suppressed hepatic TC and TG concentrations in obese mice

We investigated the effects of seaweed extract on hepatic TC and Triglyceride (TG) concentrations in obese mice. Figure 6 shows the results of the TC and TG concentrations in the liver. Both the hepatic TC concentration Figure 6 (a) and the hepatic TG concentration Figure 6(b) showed a decrease in the 0.05% seaweed extract group, suggesting that seaweed extract had inhibitory effect on the hepatic TC and TG concentrations in obese mice.

Conclusion

In this study, we examined the suppression of fat accumulation caused by seaweed extract in obese mice and obtained the following findings:

(1) We compared the body weights of the mice in the seaweed treatment groups with that of the control group, and there was no significant difference between the 0.02 % seaweed extract group and the control group. However, a significant difference was observed with 0.05 seaweed extract, which confirmed the suppression of body weight in the group.

(2) We compared the liver weight and intra-abdominal fat weight of the control group and 0.05 % seaweed extract group, and we confirmed that liver weight and intra-abdominal fat weight was suppressed in the 0.05 % seaweed extract group.

(3) Serum TC, hepatic TC, and hepatic TG concentrations decreased in the 0.05 % seaweed extract group.

(4) The frozen liver tissue sections of the control mice were dyed red with Oil Red O, and in contrast, the samples in the 0.05 % seaweed extract group had many undyed parts, indicating the absence or low level of fat.

Based on these findings, we believe that high-fat diet mixed with 0.05 % seaweed extract suppressed increase in body weight and fat absorption.

Disclosure statement

No potential conflict of interest was reported by the authors.

Acknowledgments

We thank for offering of seaweed extract from Ohmoriya Co. Ltd.

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