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Original Article

Serum and hepatic lipids in the rat fed a Hijiki-diet.

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Summary Male Sprague-Dawley rats, 3 weeks old, were fed a Hijiki diet for 13 days, and serum and hepatic lipids were measured to give some observations relating to lipid metabolism in comparison with those fed a control diet containing cellulose as dietary fiber. The mean concentration of serum HDL-cholesterol was significantly higher, while that of hepatic triglyceride concentration was significantly lower in the Hijiki diet group, than in the control group. The mean concentrations of serum triglycerides, serum total cholesterol, serum glucose, cecal short chain-fatty acids were higher in the Hijiki diet group than those in the control group. The mean concentration of hepatic total cholesterol was lower, while the mean wet weights of cecum and colon contents were higher, in the Hijiki diet group than those in the control. The mean weight of testes of the Hijiki diet group was significantly higher than that of the control. These results suggest that Hijiki may have some effects on lipid metabolism in the rat.
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Keywords : Hijiki, *Sargassum fusiforme* (Harvey) Setchell, food efficiency, rats, serum HDL-cholesterol, hepatic triglyceride

Introduction

The seaweed Hijiki, *Sargassum fusiforme* (Harvey) Setchell, has been widely eaten by Japanese for a long time as a useful foodstuff supplying dietary fiber as well as beneficial minerals[1, 2, 3]. Traditional Japanese style meals, mainly consisting of various vegetables and seaweeds, are rich in dietary fiber. However, the recent eating habits of Japanese people have changed to European styles, and the intake of dietary fiber is decreasing in total and the people are inclined to contract abnormal metabolic syndromes [4].

The authors intended to know whether the Hijiki diet has some useful effect on lipid metabolism in the rat in comparison with the cellulose diet.

Experimental

Animals:

Twelve male Sprague-Dawley rats, 3 weeks old, were first fed a pellet food, MF (Oriental Yeast Co., Ltd), for 4 days, and then divided into two groups of 6 rats; one group was fed a Hijiki diet and the other was fed a control diet containing cellulose as dietary fiber for 13 days (Table 1). The rats, housed separately in a stainless cage, were fed the diets *ad libitum* for 13 days at 23 ± 1℃ under the cycle of 12 hours each of light (6:00~18:00) and dark (18:00 ~6:00) periods.

Diet compositions:

The Hijiki diet contained 5% dried Hijiki powder instead of cellulose of the control diet (Table 1).

Corn starch was generously donated by SANWA Starch Co., Ltd. Corn oil was a product of Ajinomoto Co., Ltd. Casein, a mineral mixture* and a vitamin mixture** were purchased from Oriental Yeast Co., Ltd. The cellulose powder was crystalline cellulose (Avicel PH-101, Asahi Kasei Co., Ltd.).

The Hijiki diet was prepared as follows: Dried Hijiki samples, harvested and prepared as commercial products in the Tsushima Archipelago, were soaked in 10 volumes of distilled water for 30 min and filtered off. After this process was repeated three more times, the samples were washed with enough water and dried in the shade. The Hijiki samples were kept in a drying cabinet at 35℃ for a week, and then pulverized.

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Table 1. Diet composition (g/100g)

Ingredient	Dietary group	
	Control diet	Hijiki diet
Corn starch	63	63
Casein	20	20
Corn oil	5	5
Mineral mixture*	5	5
Vitamin mixture**	2	2
Cellulose	5	-
Hijiki	-	5

* The mineral mixture, expressed in %, was composed of $CaHPO_4 \cdot 2H_2O$ 14.56, KH_2PO_4 25.72, NaH_2PO_4 9.35, NaCl 4.66, Ca-lactate 35.09, Fe-citrate 3.18, $MgSO_4$ 7.17, $ZnCO_3$ 0.11, $MnSO_4 \cdot 4{\sim}5H_2O$ 0.12, $CuSO_4 \cdot 5H_2O$ 0.03, and KI 0.01.

** The vitamin mixture was composed of vitamin A acetate 50,000IU, D_3 10,000IU, E acetate 500mg, K_3 520mg, B_1 hydrochloride 120mg, B_2 400mg, B_6 hydrochloride 80mg, B_{12} 0.05mg, C 3,000mg, D-biotin 2mg, folic acid 20mg, calcium pantothenate 500mg, p-aminobenzoic acid 500mg, nicotinic acid 600mg, inositol 600mg, choline chloride 20g, and cellulose powder 73.05g.

The body weight, food intake and feces:

The body weights, and the amounts of food intake and feces were measured every 2 or 3 days.

Separation and sampling of organs:

The rats of 13th day were starved for 20 hours, and anesthetized by intraperitoneal injection of a Nembutal solution (2 mg Nembutal /100g body weight). Blood samples, taken from the aorta, were immediately centrifuged at 4,000 rpm for 15 min at 4℃ and the serum samples were stored below -30℃ before glucose and lipid determination. The cecal contents were removed and stored at -30℃ for determination of short chain-fatty acids. The heart, lung, liver, spleen, kidney, adrenal gland, testes and adipose tissues (intestinal, abdominal and epididymal) were excised and weighed.

The weights of cecum and colon, and their contents:

The colon and cecum, with their contents, were weighed (expressed as ***a***). After removal of the contents, the washed colon and cecum were weighed (expressed as ***b***), and the weight of the contents was calculated (***a*** minus ***b***).

Determination of short chain-fatty acids in the cecal contents:

To 0.50g of the cecal contents was added 2 ml of 1N sodium hydroxide containing crotonic acid as an internal standard, and the mixture was stirred and centrifuged at 15,000rpm for 15 min. To 0.6ml of the supernatant was added 0.6ml of chloroform, and the mixture was stirred and centrifuged at 4,000rpm for 15 min. The supernatant, filtered by a membrane filter, was injected to HPLC, and the amounts of short chain-fatty acids were determined chromatographically.

Determination of serum glucose:

Glucose in the serum was determined by measuring the red colored product of quinone formed after the successive reactions of mutarotase and glucose oxidase onto glucose in the assay using Glucose C II-Test Wako[5].

Determination of total cholesterol in serum:

Serum was treated with cholesterol esterase and cholesterol oxidase to produce hydrogen peroxide. Successive treatment with peroxidase formed a blue colored product with 3,5-dimethoxy-N-ethyl-(2-hydroxy-3-sulfopropyl)-aniline natrium (DAOS), and 4-aminoantipyrine. Serum cholesterol was determined by measuring the blue color in the assay using Cholesterol E-Test Wako [6].

Determination of serum HDL-cholesterol:

Lipoproteins other than high density lipoprotein in the serum were precipitated by heparin and manganese. HDL-cholesterol in the supernatant was treated with cholesterol esterase and cholesterol oxidase, successively, and the red color product formed from the reaction with 4-aminoantipirine and p-chlorophenol was determined by using HDL-Cholesterol-Test Wako [7].

Determination of serum triglycerides:

The glycerol, produced from triglycerides by treatment with lipoprotein lipase, was determined by the blue color of DAOS formed after the reaction with glyxcerol kinase, glycerol-3-phosphate oxidase and peroxidase, in the assay using Triglyceride E-Test Wako[8].

Hepatic lipid determination:

Liver samples of 0.50g were homogenized in methanol (5ml) - chloroform (10ml), and stirred for 30 min. After adding 3.2 ml of 0.9% sodium chloride solution, the mixture was kept in a refrigerator overnight. The lower layer was concentrated in a rotary evaporator successively in a nitrogen stream[9]. The concentrated lipids were weighed.

Determination of hepatic total cholesterol:

The liver samples were homogenized with 3 volumes of a chloroform-methanol (2:1 v/v) mixture and the extracts were separated from the residues by centrifugation at 10,000rpm for 10 min. The cholesterol in the extract was determined as described for serum cholesterol.

Determination of hepatic triglycerides:

The liver samples were homogenized with 10 volumes of a chloroform-methanol (2:1 v/v) mixture and the extracts were separated from the residues by centrifugation at 10,000rpm for 10 min. The amount of triglycerides in the extract was determined as described for serum triglycerides.

Statistical analysis:

Results were expressed in mean values ± SE and were analyzed by Student's t-test. The significant differences from the control group were assessed at $p < 0.05$ or $p < 0.01$.

Results

Amounts of food intake, body weight gains and food efficiencies:

When compared in terms of the total period of 13 days, the amount of daily food intake by the Hijiki diet group was not different from that by the control group, but it was significantly less in the Hijiki group for the period of the last four days, 10th to 13th (Table 2). The mean daily body weight gain of the Hijiki diet group for the whole period of 13 days was less than the control, and this decrease was conspicuous for the last 4 days, showing a statistically significant difference (Table 2). The food efficiency in the control diet group was 0.39 in contrast to 0.34 in the Hijiki diet group for the whole period of 13 days, representing their values being significantly different. Both groups showed lower values during the last four days than those during the earlier nine days (Table 2).

The feces:

In spite of the less daily food intake in the Hijiki group for the last four days (Table 2), the mean daily weights of feces in both groups were not different from each other throughout the whole period. (Table 3).

Table 2. Food intake and body weight gain

	Food intake (g/day)	
	Control diet	Hijiki diet
13 days	19.90 ± 0.82[a]	18.71 ± 0.45[a]
1st～ 9th	18.62 ± 1.21[a]	18.89 ± 0.41[a]
10th～13th	22.78 ± 0.80[a]	18.17 ± 1.20[b]
	Body weight gain (g/day)	
	Control diet	Hijiki diet
13 days	8.43 ± 0.35[a]	6.85 ± 0.19[b]
1st～ 9th	8.40 ± 0.46[a]	7.36 ± 0.29[a]
10th～13th	8.47 ± 0.93[a]	5.83 ± 0.21[b]
	Food efficiency	
	Control diet	Hijiki diet
13 days	0.391 ± 0.007[a]	0.339 ± 0.007[b]
1st～ 9th	0.403 ± 0.021[a]	0.390 ± 0.008[c]
10th～13th	0.370 ± 0.031[a]	0.321 ± 0.013[a]

The values were expressed in means ± SE of six rats.
The different superscript letter signifies the presence of a significant difference from the control group (b: $p<0.05$; c: $p<0.01$).
The food efficiency was calculated as the body weight gain / food intake, during the indicated period.

Table 3. Feces (g/day)

	Control diet	Hijiki diet
13days	2.98 ± 0.20[a]	3.21 ± 0.14[a]
1st～ 9th	2.89 ± 0.14[a]	3.35 ± 0.14[a]
10th～13th	3.16 ± 0.35[a]	2.93 ± 0.20[a]

The values were expressed in means ± SE of six rats.
The same superscript letters signify no significant difference from the control group.

The wet weights of various organs:

Out of the various organs, only the testes of the Hijiki diet group showed a significantly greater mean wet weight than those of the control diet group (Table 4).

Table 4. The wet weight of various organs (g/100g Body weight)

	Control diet	Hijiki diet
Heart	0.42 ± 0.02[a]	0.47 ± 0.02[a]
Lung	0.58 ± 0.01[a]	0.60 ± 0.01[a]
Liver	3.69 ± 0.05[a]	3.62 ± 0.06[a]
Spleen	0.31 ± 0.02[a]	0.27 ± 0.01[a]
Kidney	0.48 ± 0.01[a]	0.48 ± 0.01[a]
Adrenal gland ($x10^{-3}$)	10.06 ± 0.62[a]	10.74 ± 0.52[a]
Testes	0.53 ± 0.02[a]	0.62 ± 0.02[b]
Adipose tissue	2.68 ± 0.24[a]	2.44 ± 0.13[a]
Around intestine	1.21 ± 0.08[a]	1.07 ± 0.04[a]
Abdominal	0.63 ± 0.13[a]	0.55 ± 0.06[a]
Epididymal	0.85 ± 0.06[a]	0.76 ± 0.06[a]

The values were expressed in means ± SE of six rats.
The different superscript letter signifies the presence of significant difference from the control group ($p<0.05$).

The wet weight of intestinal contents:

The weights of cecal and colon contents were greater in average in the Hijiki diet group than in the control diet group, although there were no significant differences (Table 5).

Table 5. The wet weight of intestinal contents (g/100g Body weight)

	Control diet	Hijiki diet
Cecum	0.44 ± 0.07[a]	0.51 ± 0.05[a]
Colon	0.09 ± 0.03[a]	0.11 ± 0.02[a]

The values were expressed in means ± SE of six rats.
The same superscript letters signify no significant difference from the control group.

Cecal short chain-fatty acids:

The short chain-fatty acids of the cecal contents, acetic acid, propionic acid, *i*-butyric acid, *n*-butyric acid, *i*-valeric acid, *n*-valeric acid, succinic acid and lactic acid were determined; the mean concentrations of *i*- and *n*-valeric acids of the Hijiki group were higher than those of the control. Those of succinic acid, acetic acid and propionic acid were also higher and those of the other acids were lower in the Hijiki diet group than those of the control, although there were no significant differences (Table 6).

Table 6. Cecal organic acids (mmol/litter)

	Control diet	Hijiki diet
Succinic acid	11.75 ± 3.57[a]	13.67 ± 5.29[a]
Lactic acid	33.31 ± 14.28[a]	29.27 ± 18.14[a]
Acetic acid	144.22 ± 35.73[a]	170.96 ± 38.13[a]
Propionic acid	48.11 ± 11.62[a]	59.60 ± 13.87[a]
i-Butyric acid	0.00 ± 0.00[a]	0.00 ± 0.00[a]
n-Butyric acid	25.65 ± 8.47[a]	25.14 ± 4.73[a]
i-Valeric acid	2.23 ± 1.05[a]	6.36 ± 2.19[a]
n-Valeric acid	2.18 ± 1.70[a]	6.03 ± 2.12[a]
Total	267.44 ± 64.17[a]	311.03 ± 77.08[a]

The values were expressed in means ± SE of six rats.
The same superscript letters signify no significant difference from the control group.

Serum glucose:

The mean concentration of serum glucose in the Hijiki diet group was 174.10 ± 7.39 mg/dl, higher than that (151.10 ± 11.36 mg/dl) of the control diet group, but there was no significant difference.

Serum lipids:

The mean concentration of HDL-cholesterol of the Hijiki diet group was significantly higher than that of the control group, and the mean values of total cholesterol and triglycerides also showed higher values than those of the control diet group (Table 7). However, the ratio of the HDL-cholesterol to LDL-cholesterol was almost the same between the two groups.

Table 7. Serum lipids (mg/dl Serum)

	Control diet	Hijiki diet
Total cholesterol	96.56 ± 10.31[a]	125.88 ± 9.25[a]
HDL-cholesterol	33.26 ± 4.24[a]	44.29 ± 2.21[b]
Triglyceride	109.69 ± 11.51[a]	120.69 ± 2.35[a]

The values were expressed in means ± SE of six rats.
The different superscript letter signifies the presence of significant difference from the control group ($p<0.05$).

Hepatic lipids:

The hepatic triglyceride concentration of the Hijiki diet group was significantly lower than that of the control diet group (Table 8). The mean concentration of hepatic total cholesterol was lower in the Hijiki diet group than in the control group, although there was no significant difference.

Table 8. Hepatic lipids (mg/g Tissues)

	Control diet	Hijiki diet
Total lipid	74.31 ± 10.14[a]	69.97 ± 3.61[a]
Total cholesterol	27.11 ± 4.31[a]	17.90 ± 0.11[b]
Triglyceride	32.14 ± 3.48[a]	23.27 ± 1.62[b]

The values were expressed in means ± SE of six rats.
The different superscript letter signifies the presence of significant difference from the control group ($p<0.05$).

Discussion

Dietary fiber is an essential component to prevent abnormal metabolic syndromes, and various dietary fibers will contribute to the beneficial characteristics of the respective foods which contain them. As components of Japanese daily meals, seaweeds such as Hijiki and green laver are very popular. Our study result showed that mice fed Hijiki, *Sargassum fusiforme*, tended to have a reduced amount of adipose tissues compared to those fed a cellulose diet[10]. From this result, it is interesting to investigate whether the seaweeds may suppress obesity through their effects on lipid metabolism. As shown in the Table 4 of this study, the adipose tissues surrounding the intestine as well as the abdominal and epididymal adipose tissues tended to be smaller in the Hijiki diet group than in the control diet group. The concentration of triglycerides in the liver of the Hijiki diet group was significantly lower than that of the control diet group, and the total cholesterol showed a similar tendency (Table 8).

The greater concentration of serum HDL-cholesterol in the Hijiki diet group than that in the control diet group seems to suggest an effect of the Hijiki diet to accelerate lipid metabolism (Table 7). However, the greater concentration of total cholesterol in blood (Table 7) indicates a greater concentration of LDL-cholesterol in the Hijiki diet group than in the control diet group, and thus the ratio of the HDL-cholesterol to LDL-cholesterol in the Hijiki diet group seems to remain similar to that of the control diet group.

The Hijiki diet has some characteristics as reducing the food intake and the body weight gain. That is, the Hijiki diet is to lower food efficiency than the control diet (Table 2).

Besides the above results, the Hijiki diet has other beneficial characteristics, such as high contents of calcium and iron[11, 12] and antitumor effects[10, 13], in spite of the existence of arsenic in a high concentration *in situ* [14, 15], although the arsenic in the Hijiki could easily be removed during the careful pre-cooking processes [16, 17, 18].

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ヒジキ食投与のラットにおける血清および肝臓の脂質代謝について

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要　旨

Sprague-Dawley 種の雄ラット（3 週令）に 5%ヒジキ食を与えて 13 日間飼育し，血清および肝臓の脂質について分析した。

ヒジキ食群の血清HDL-cholesterol濃度は，セルロース食（対照）群と比べて有意に高く，肝臓triglycerides濃度は有意に低かった。血清triglycerides濃度，血清総cholesterol濃度，血清glucose濃度，盲腸内容物中の short chain fatty acids濃度の各平均値は，ヒジキ食群において高値を示した。一方，肝臓total cholesterol濃度は低値を示した。

これらの結果から，脂質代謝に影響を及ぼす因子が，ヒジキ自体に固有の成分として存在することが示唆された。

キーワード：ヒジキ，*Sargassum fusiforme* (Harvey) Setchell，飼料効率，ラット，
血清HDL-コレステロール，肝臓トリグリセリド