Arsenic Metabolites Excreted into the Human Urine after Hijiki Intake

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Summary

We intended to investigate effects of Hijiki intake on biochemical modification of the arsenic ingested by the human body. The 24 hour-urine was collected either before or after starting the Hijiki diet, and urinary arsenic components were analyzed with an HPLC/ICP-MS instrument. Some differences in the urinary arsenic components were observed between before and after starting the Hijiki diet, but the differences were not the same in each individual, probably owing to different physiological or biochemical conditions.

Keywords: Arsenic metabolism, *Hizikia fusiforme* Okam., Human urine, Arsenite, HPLC/ICP-MS analysis, Neutron activation analysis, Methylarsonic acid, Dimethylarsinic acid, Arsenobetaine.

Introduction

The seaweed Hijiki (*Hizikia fusiforme* Okam.) accumulates minerals including arsenic[1, 2)] out of the environmental ocean seawater.

The experiments with rats fed a diet containing Hijiki suggested that it stimulated arsenic detoxification metabolism in rats[3)], and the arsenic components in the serum and urine of those rats indicated an accelerating effect of Hijiki on methylation of arsenic compounds[4)].

In the present study, we intended to know the composition of arsenic compounds excreted into the human urine after taking Hijiki diets.

Experimental

Experimental design

Seven female volunteers, 21 to 23 years-old students of Fukuoka Women's University participated in this project with their informed consents.

For the first three days, they took usual meals without Hijiki individually. For the following seven days they consumed constant amounts of Hijiki daily in diets prepared in our laboratory for their lunches (Fig. 1).

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14	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*
Without Hijiki-diets			**With Hijiki-diets**							
		*			*					*

Fig. 1 Experimental design.
The daily meals did not contain Hijiki before starting the Hijiki diet. After starting the Hijiki diet, Hijiki diets were served daily as lunches for 7 days. The ingredients of the Hijiki diets are given in Table 1, and the daily food contents of the nutrients and dietary fibers are shown in Table 2. On the day marked with an asterisk (3^{rd} day of the experiment before starting the Hijiki diet, 3^{rd} and 7^{th} days after starting the Hijiki diet), urine samples were collected for 24 hours and measured aliquots of them were stored until analysis.

Hijiki

Hijiki was harvested at the seashores of the Tsushima Archipelago, Japan, and prepared according to the commercial product processes. The Hijiki products were all a dried mixture of the leaves, stalks and apexes, and were stored under 4℃ until use.

Hijiki diets

Before usage, a weighed amount of Hijiki was soaked in water for 30 min and the soaking water was discarded. Five grams of the dried Hijiki, swollen in water, was used for each lunch. Hijiki diets were prepared with other foodstuffs as shown in Table 1. The intakes of nutrients from the Hijki diets are also shown in Table 1.

Dietary survey

The daily amounts of ingestion of energy, carbohydrates, proteins, lipids, vitamins and dietary fibers were surveyed for all the ten days according to the Standard Tables of Food Composition in Japan[5] by the aid of the Excel add-in software, "Eiyoukun" ver. 3.0[6]. Mineral contents as well as arsenic contents in the daily diets were estimated by calculation using the "Table of Trace Element Contents in Japanese Foodstuffs[1]".

Neutron activation analysis

Arsenic contents in Hijiki samples were determined by thermal neutron activation analysis at the Research Reactor Institute, Kyoto University[7]. The samples were placed in polyethylene Neuma capsules and irradiated by a flux of 10^{13} slow neutrons $cm^{-2}s^{-1}$ for 20 min in the center of the nuclear reactor. After the cooling time of 72 hours, the asrsenic content was determined by gamma radiation from ^{76}As.

HPLC / ICP-MS analysis

Arsenic compounds were analyzed with an ICP-MS instrument, equipped with high performance liquid chromatography (HPLC) columns. The columns of HPLC were Nucleosil 100 SA (cation-exchange, 4.6 mm i.d.x250 mm) and Nucleosil 100 SB (anion-exchange, 4.6 mm i.d.x250 mm) of Wako Pure Chemical Co., Japan. The sample solutions

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Abbreviation

Arsenic compounds and others were abbreviated as follows: ***As***, arsenic; ***As(III)***, arsenous acid, arsenite; ***As(V)***, arsenic acid, arsenate; ***MMAA***, monomethylarsonic acid; ***DMAA***, dimethylarsinic acid; ***AB***, arsenobetaine; **HPLC**, high performance liquid chromatography; **ICP / MS**, inductively coupled plasma mass spectrometry.

Table 1 Ingredients of Hijiki Diets and Intakes of Energy Nutrients, Dietary fibers and Arsenic.

1. Okonomiyaki :

Hijiki (boiled and dried), 5g; Welsh onions, 11g; Black gram sprouts, 32g; Cabbage, 92g; Scallops (boiled and dried), 5g; Soft wheat flour, 75g; Niboshi extract, 2g; Egg, 29g; Salt (common salt), 1.5g; Vegetable oil (blended), 1.5g; Worcester sauce (thick type), 25g; Mayonnaise, 10g.

Intakes of nutrients, dietary fibers and arsenic:

Energy (kcal)	Proteins (g)	Lipids (g)	Carbohydrates (g)	Dietary fibers (g)	Arsenic (μg)
289.0	15.8	13.7	74.7	6.8	287.0

2. Butanikumaki :

Hijiki (boiled and dried), 5g; Carrot, 7g; Edible burdock, 6.5g; Fried thin slices of pressed tofu (Abura-age), 3g; Sake, 1.4g; Vegetable oil (blended), 3.4g; Soy sauces (common type), 15.3g; Sweet cooking seasoning, 7g; White sugar, 5.7g; Pork, large type breeds (inside ham lean and fat), 105g; Sake, 2g; Soft wheat flour, 1.5g; Salt (common salt), 0.2g; Mixed pepper, 0.2g.

Intakes of nutrients, dietary fibers and arsenic:

Energy (kcal)	Proteins (g)	Lipids (g)	Carbohydrates (g)	Dietary fibers (g)	Arsenic (μg)
307.2	24	15.2	17.1	2.8	87.0

3. Omelette :

Hijiki (boiled and dried), 5g; Egg, 50g; Carrot, 10g; Welsh onions, 3g; Chicken (ground meat), 41g; White sugar, 2.8g; Sweet cooking seasoning, 5.7g; Barley-koji miso, 6.4g; Vegetable oil (blended), 2g; Soy sauces (common type), 1g; Salt (common salt), 0.4g; Mixed pepper, 0.3g.

Intakes of nutrients, dietary fibers and arsenic:

Energy (kcal)	Proteins (g)	Lipids (g)	Carbohydrates (g)	Dietary fibers (g)	Arsenic (μg)
211.7	16.2	11	12.2	3	83.0

4. Tsukune :

Chicken (ground meat), 46g; Hijiki (boiled and dried), 5g; Carrot, 5g; Onions, 4g; Egg, 8g; Soy sauces (common type), 5.2g; White sugar, 2g; Sweetened shochu by rice koji (Mirin), 6.4g; Potato starch, 6g; Vegetable oil (blended), 3g.

Intakes of nutrients, dietary fibers and arsenic:

Energy (kcal)	Proteins (g)	Lipids (g)	Carbohydrates (g)	Dietary fibers (g)	Arsenic (μg)
173.1	11.5	7.7	13.9	2.4	47.0

5. Hijiki rice :

Cooked paddy rice (well-milled rice), 175g; Salt (common salt), 1g; Sake, 3g; Hijiki (boiled and dried), 5g; Niboshi extract, 2g; Soy sauces (common type), 10g; White sugar, 7g; Sweetened shochu by rice koji (Mirin), 4g; Roasted sesame seeds, 3g.

Intakes of nutrients, dietary fibers and arsenic:

Energy (kcal)	Proteins (g)	Lipids (g)	Carbohydrates (g)	Dietary fibers (g)	Arsenic (μg)
365.8	6.3	2.2	78	3.1	138.0

6. Bihunn :

Rice noodles, 40g; Hijiki (boiled and dried), 5g; Pork, medium type breeds (picnic shoulder), 39g; Carrot, 18g; Black gram sprouts, 30g; Green sweet pepper, 20g; Soy sauces (common type), 4g; Salt (common salt), 0.3g; Black pepper, 0.3g; Sake, 4g; Vegetable oil (blended), 10g.

Intakes of nutrients, dietary fibers and arsenic:

Energy (kcal)	Proteins (g)	Lipids (g)	Carbohydrates (g)	Dietary fibers (g)	Arsenic (μg)
368.0	11.6	17.4	39	4	49.0

7. Fukusani :

Hijiki (boiled and dried), 5g; Fried thin slices of pressed tofu (Abura-age), 17g; Edible burdock, 15g; Carrot, 14g; Chicken (ground meat), 21g; Regular tofu (Momen-tofu), 85g; Vegetable oil (blended), 3g; White sugar, 8g; Soy sauces (light color type), 7.5g; Niboshi extract, 3g.

Intakes of nutrients, dietary fibers and arsenic:

Energy (kcal)	Proteins (g)	Lipids (g)	Carbohydrates (g)	Dietary fibers (g)	Arsenic (μg)
246.1	14.5	14	16.7	4	577.0

Hijiki diets prepared for the daily lunch are described.
The ingestion amounts of nutrients, dietary fibers and minerals were estimated as described in the text. The content of arsenic in the Hijiki was determined by neutron activation analysis as described in the text. All the amounts of arsenic compounds were expressed as the weight of the element arsenic.
Hijiki (boiled and dried), was the commercial-processed product. Before cooking, the dried hijiki was soaked in water, and the soaking water was discarded.

were filtered through a microfilter and applied on the former column to separate arsenobetaine (***AB***), trimethylarsine oxide, arsenocholine and tetramethylarsonium ion, and eluted at a flow rate of 1.0 ml per minute with 0.1 M pyridine-formic acid buffer (pH 3.1). Another aliquot of the sample solution was applied on the latter column to separate arsenite (***As(III)***), arsenate, methylarsonic acid (***MMAA***), and dimethylarsinic acid (***DMAA***), and eluted at a flow rate of 1.5 ml per minute with 0.02M phosphate buffer (pH 6.8). At the outlet of the columns, a concentric type A nebulizer was connected. Arsenic compounds in the eluates were monitored at m/z 75 with a HP 4500 ICP mass spectrometer of Yokogawa Analytical Co., Tokyo, Japan.

For the mixture of authentic arsenic compounds was used the Standard Urine Sample of Japan National Institute of Environmental Sciences

Results

Intakes of nutrients and dietary fibers

The amounts of food ingestion recorded by the individuals are shown as the average values of the nutrients in Table 2-a. The daily intakes of energy, proteins, lipids, carbohydrates, and dietary fibers were not significantly different between the periods with and without Hijiki.

Intakes of minerals

The intakes of minerals were generally similar between the two periods, but those of Fe and As tended to increase in the Hijiki diet period as shown in Table 2-b.

Table 2 Intakes of nutrients and dietary fibers **(a)** and minerals **(b)**.

2-a Intakes of nutrients and dietary-fibers

Diets	Energy (kcal)	Protein (g)	Lipids (g)	Carbohydrates (g)	Dietary fibers (g)
Without Hijiki-diets*	1,682 ±238.3	63.1 ±12.2	50 ±15.2	235.5 ±44.1	12.4 ±2.1
With Hijiki-diets**	1,593 ±231.9	58.9 ±7.8	46.3 ±8.5	227.4 ±38.6	12.9 ±2.0

2-b Intakes of minerals

Diets	Na (mg)	K (mg)	Ca (mg)	Mg (mg)	P (mg)
Without Hijiki-diets*	2,218 ±562.8	1,989 ±12.1	496 ±197.3	203 ±20.8	931 ±183.5
With Hijiki-diets**	2,556 ±699.4	2,156 ±264.5	486 ±220.1	222 ±2.3	815 ±174.8

Diets	Fe (mg)	Zn (mg)	Cu (mg)	Mn (g)	As (μg)
Without Hijiki-diets*	6.8 ±1.2	7.2 ±1.2	1 ± 0.1	2.53 ±1.4	474 ±270.5
With Hijiki-diets**	8.3 ±0.8	6.5 ±0.6	0.9 ±0.2	2.65 ±0.4	502 ± 1.89

The daily ingested amounts of the nutrients and dietary-fibers were estimated from the recorded dietary surveys as described in the text. The values were the means ± SD (n=7).
* : Average for 3 days. ** : Average for 7 days.

Arsenic contents in Hijiki

The average concentration of arsenic in the Hijiki samples used for preparing the diets was 8.8 ppm on the basis of dry weight. This concentration ranks rather low among commercial samples of Hijiki obtainable on the market.

Urinary amounts of arsenic compounds

The arsenic compounds excreted into the urine were analyzed for three persons (**A**, **B** and **C**), and expressed as μg of the element arsenic (***As***) per day of respective arsenic compounds (Table 3). In the period without Hijiki, ***As(III)*** showed the highest amounts of about 90 μg of ***As*** in **A** and **B**, and it was about 28 μg of ***As*** in **C**. During the period with Hijiki, the arsenic components excreted in the urine differed between before and after starting the Hijiki diet. In two subjects (**B** and **C**), the urinary ***As(III)*** content in the Hijiki period markedly decreased in comparison with the period without Hijiki.

Urinary concentrations of arsenic compounds

The arsenic concentration was expressed as ppb, the weight of the element arsenic in the arsenic compounds per unit urine volume (Table 4). The subjects **A** and **B** excreted 120 ppb of ***As(III)*** and 60 to 70 ppb of ***AB*** (arsenobetaine) before starting the Hijiki diet. On the other hand, the subject **C** excreted only 35 ppb of ***As(III)*** and less than 25 ppb of ***AB*** before starting the Hijiki diet. During the second period with Hijiki, the concentration of ***As(III)*** decreased in **B** and **C**, but it increased in **A**. When the ratio of the concentrations of ***DMAA*** to ***AB*** was calculated, it increased in the Hijiki period in all the the three subjects.

Percentage compositions of arsenic compounds in human urine

The respective arsenic compounds were expressed as their percentages of their total weights of the element arsenic (Table 5).

Table 3 Amounts of arsenic compounds in human urine.

A			**B**			**C**		
	Hijiki-diets			Hijiki-diets			Hijiki-diets	
	Before	*After*		*Before*	*After*		*Before*	*After*
	(μg)	(μg)		(μg)	(μg)		(μg)	(μg)
As (III)	88.92	145.4	***As (III)***	96.79	0.00	***As (III)***	27.55	19.93
MMAA	0.00	4.29	***MMAA***	0.00	0.00	***MMAA***	0.00	0.90
DMAA	12.98	46.62	***DMAA***	4.37	47.61	***DMAA***	20.92	18.16
AB	45.81	67.59	***AB***	53.15	9.26	***AB***	17.78	12.66
Total	147.71	263.9	Total	147.71	56.87	Total	66.25	51.65

The experimental design is described in Fig. 1. The urine samples were collected for 24 hours before and after starting the Hijiki diet (designated as *Before* and *After*). The sample of *After* is the urine of the 7th day after starting the Hijiki diet. Measured aliquots of the urine samples were stored in a deep-freezer until analysis. **A**, **B** and **C** represent three individual subjects.

The arsenic content was expressed as total amount of the element arsenic (μg) in the respective arsenic compounds excreted in the urine per day.

Table 4 Concentration of arsenic compounds in human urine (ppb).

A			**B**			**C**		
	Hijiki-diets			Hijiki-diets			Hijiki-diets	
	Before	*After*		*Before*	*After*		*Before*	*After*
	(ppb)	(ppb)		(ppb)	(ppb)		(ppb)	(ppb)
As (III)	114.00	149.90	***As (III)***	125.70	0.00	***As (III)***	34.87	20.13
MMAA	0.00	4.42	***MMAA***	0.00	0.00	***MMAA***	0.00	0.91
DMAA	16.64	48.06	***DMAA***	5.68	51.75	***DMAA***	26.48	18.34
AB	58.73	69.68	***AB***	69.02	10.07	***AB***	22.51	12.79

The experimental conditions are described in Table 3, and the arsenic compounds were expressed as ppb of element arsenic in the arsenic compounds per unit urine volume.

Table 5 Percentage compositions of arsenic compounds in human urine (%).

A			**B**			**C**		
	Hijiki-diets			Hijiki-diets			Hijiki-diets	
	Before	*After*		*Before*	*After*		*Before*	*After*
	(%)	(%)		(%)	(%)		(%)	(%)
As (III)	60.2	55.1	***As (III)***	62.7	0.0	***As (III)***	41.6	38.6
MMAA	0.0	1.6	***MMAA***	0.0	0.0	***MMAA***	0.0	1.7
DMAA	8.8	17.7	***DMAA***	2.8	83.7	***DMAA***	31.6	35.2
AB	31.0	25.6	***AB***	34.4	16.3	***AB***	26.8	24.5
Total	100.0	100.0	Total	100.0	100.0	Total	66.25	100.0

The experimental conditions are described in Table 3, and the percentage compositions are expressed as their percentages of the element arsenic in the total.

Discussion

Although the average value of total arsenic intakes in the Hijiki diet period was higher than that in the period without Hijiki, these values were not significantly different from each other. Therefore, the composition of the arsenic compounds excreted into the urine might not reflect the compositions of the arsenic compounds in Hijiki much more than it reflects the effect of the Hijiki diet on arsenic metabolism.

The percentage compositions of the respective arsenic compounds (Table 5) may be more explainable. For example, the higher ratios of the concentration of ***DMAA*** to that of ***As (III)*** compared with the other components in the Hijiki diet period suggest that the Hijiki diet ingestion may promote excretion of ***DMAA*** into the urine.

In the urine of rats fed Hijiki diets[4], the arsenic compounds showed different compositions from those without Hijiki diets, owing to some changes in the arsenic metabolism as discussed in the previous report[4] : the ratio of the ***DMAA*** to ***As(III)***, which was higher in the urine after ingestion of Hijiki diets, suggests a promoting effect of Hijiki on methylation of arsenic compounds. To obtain clearer data, cancellation of the individual differences should be attained.

The seaweed has been used as a traditional Japanese food, and it has been recognized as a good source for minerals as well as for beneficial dietary fibers[8, 9, 10]. It has often been reported that Hijiki contains rather higher amounts of arsenics[11, 12]. However, the arsenic contents in the fresh plants of Hijiki could be largely reduced during the processes of preparing commercial products, i.e., drying after harvest, boiling in water and drying before packaging. Moreover, during cooking, the commercial dried Hijiki is usually washed and soaked in water and the soaking water is discarded. The valuable merit of Hijiki as a food stuff could be focused on the promotion effect of Hijiki on methylation of arsenics, leading to detoxification of arsenic compounds in the living body[4].

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