Original Article *J. Clin. Biochem. Nutr.*, **43**, 175–184, November 2008

The Effect of the Dried-Bonito Broth on Blood Pressure, 8-Hydroxydeoxyguanosine (8-OHdG), an Oxidative Stress Marker, and Emotional States in Elderly Subjects

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Received 30 October, 2007; Accepted 26 May, 2008

Summary **Dried-bonito broth (DBB, *katsuo-bushi dashi*) is commonly used in Japanese cuisine, and is also used as a traditional remedy for recovery from fatigue and improvement of blood circulation. To clarify the effect of DBB on blood pressure, oxidative stress and emotional states, a randomized crossover human trial was performed. Twenty-seven elderly Japanese subjects ingested DBB or water for one month. Measurement of blood pressure and urinary 8-hydroxydeoxyguanosine (8-OHdG) and evaluation of emotional states were performed before and after the ingestion periods. The changes in systolic blood pressure (SBP) during DBB ingestion was significantly lower than that during water ingestion ($p = 0.037$). Urinary 8-OHdG significantly decreased during DBB ingestion ($p = 0.0002$). Evaluation of emotional states indicated that composure significantly improved during DBB ingestion ($p = 0.034$). These results suggest that the daily ingestion of DBB lower SBP, reduce urinary 8-OHdG and might improve emotional states in elderly subjects.**

Key Words**: Dried-bonito, katsuo-bushi, blood pressure, 8-hydroxydeoxyguanosine, emotional states**

Introduction

Bonito (skipjack tuna; *Katsuwonus pelamis*) is one of the most popular varieties of tuna and is known as *katsuo* in Japan. Approximately 20% of the total amount of bonito caught in the world is consumed in Japan. Bonito is smoked and dried to make *katsuo-bushi* (dried-bonito), which is an important ingredient in *dashi* (Japanese fish broth). Dried-bonito broth (*katsuo-bushi dashi*) is commonly employed as the base of Japanese cuisine due to its special flavor. Furthermore, dried-bonito broth is considered to be a nutritional supplement that promotes recovery from fatigue and colds, and is used, for example, in *Kachu-yu* (soup containing dried-bonito and soy paste) in Okinawa and in *Cha-bushi* (soup made with dried-bonito, soy paste and green tea) in Kagoshima.

In order to evaluate the physiological function of dried-bonito broth, we investigated its influence on fatigue. In a previous study by Murakami, effects of bonito extract on spontaneous motility in mice after gait on a rolling vehicle were measured by infrared sensor after gait on a rolling vehicle, and bonito extract was found to increase spontaneous motility [*1*]. Additionally, in our previous experiment

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using mice, swimming time in a pool was measured, and it was confirmed that bonito extract prolonged the duration of swimming [*2*]. From the findings that the administration of bonito extract could also recover the ATP/AMP ratio in liver [*1*] or ATP level in muscle [*2*], it has been suggested that bonito extract has an anti-fatigue effect.

Furthermore, several studies have reported improvements in mood states including fatigue due to the ingestion of dried-bonito broth in randomized placebo-controlled human trials. In our previous study, the Profile of Mood States (POMS) test and questionnaire survey before and after daily ingestion of dried-bonito broth for 1 week was performed. It was found that dried-bonito broth improved mood states, especially tension-anxiety, and improved concentration significantly ($p<0.05$) [*3*]. Additionally, in a study on middle-aged human subjects, the POMS test and a questionnaire survey on mood were performed before and after the daily ingestion (2 weeks) of miso (soy paste) soup containing dried-bonito broth, and the soup was found to improve mood states [*4*]. POMS scores for depression were significantly improved ($p<0.05$) in total subjects. Furthermore, in subjects with fatigue symptoms, POMS scores for tension-anxiety and total mood disturbance (TMD) were significantly improved ($p<0.05$), and the questionnaire survey indicated that tiredness and ocular fatigue scores were significantly improved ($p<0.05$) by the ingestion of dried-bonito broth. Ishizaki *et al.* [*4*] reported that the action of dried-bonito broth was more effective in subjects who felt tired. Furthermore, in a study on adult subjects with high fatigue scores, the POMS test and Uchida-Kraepelin test (simple calculation test) were performed during the daily ingestion (4 weeks) of dried-bonito broth. It was reported that dried-bonito broth improved mood states such as vigor, fatigue and TMD, and improved task performance on the calculation test [*5*]. These previous studies suggest that the ingestion of dried-bonito broth improves mood states such as tension-anxiety, fatigue, vigor and depression.

In addition, Japanese folk wisdom also suggests that dried-bonito broth is effective for improving blood circulation. Recently, Nozawa *et al.* reported that the single ingestion [*6*] and the chronic ingestion [*7*] of dried-bonito broth increased the peripheral blood flow in the dorsal region of hand. They suggested the possibility that the vasodilation was induced by both of single ingestion and chronic ingestion of dried-bonito broth. Furthermore, it was reported that dried-bonito broth relaxed contractions of rat aorta preparations induced by norepinephrine [*8*]. This result also suggests that dried-bonito broth is able to induce vasodilation.

On the other hand, many studies have been performed on the effect of the enzymatic digests of dried-bonito on blood pressure. The digest of dried-bonito obtained by thermolysin digestion was reported to decrease blood pressure in spontaneously hypertensive rats (SHR) [*9*], and this action has been confirmed in human clinical trials [*10*]. The anti-hypertensive action of the thermolysin digest is reported due to the angiotensin converting enzyme (ACE)-inhibiting peptide such as Ile-Tyr, Ile-Trp-His-His-Thr, Ala-Leu-Pro-His-Ala, Phe-Gln-Pro, Ile-Val-Gly-Arg-Pro-Arg-His-Gln-Gly, Leu-Lys-Pro-Asn-Met, and so on [*11*]. These previous studies suggest the possibility that dried-bonito broth obtained by water extraction also has an anti-hypertensive action. However, few studies have been performed on the anti-hypertensive effects of dried-bonito broth obtained by water extraction, and only a few reports have been done on the anti-hypertensive effects of a single ingestion of dried-bonito broth on spontaneously hypertensive rats (SHR) [*12*]. In addition, the anti-hypertensive effect of the daily ingestion of dried-bonito broth has not been clarified, nor has the anti-hypertensive effect been investigated in human trials.

Previous studies have reported that oxidative state is related to blood pressure. It has also been reported that superoxide production is increased in a model hypertensive rat, SHRSP [*13*]. Furthermore, the hydroxyl peroxide contents in hypertensive subjects are reported to be higher than those in normotensive subjects [*14*]. In addition, it has been reported that a lower antioxidant status and higher oxidative stress are associated with hypertension [*15*]. These previous observations suggest that oxidative status is associated with hypertension.

Previously, we investigated the influence of the ingestion of dried-bonito broth on blood pressure and an oxidative stress marker, urinary 8-hydroxydeoxyguanosine (8-OHdG), and reported that ingestion of dried-bonito broth lowered systolic blood pressure and reduced urinary 8-OHdG in elderly Japanese inpatients [*16*]. In the present study, in order to clarify the effect of dried-bonito broth on blood pressure, and to clarify the correlation between the effect on blood pressure and that on 8-OHdG, we re-analyzed the data for blood pressure and 8-OHdG. In addition, to clarify the mechanism of the action, ACE-inhibiting activity of dried-bonito broth and its enzymatic digest was investigated. Furthermore, since it is known that elderly inpatients tend to feel fatigue and have mental stress, dried-bonito broth was expected to improve their emotional states. Therefore, the effect on the emotional states was also investigated.

Materials and Methods

Study design

An intervention study was performed with a cross-over design using random grouping. The study was approved by the Fukuoka Women's University Epidemiologic Study Ethics Examination Association, following the spirit of the Helsinki Declaration regarding medical research involving human subjects.

Subjects

The subjects were elderly inpatients at a hospital or nursing health service facility who were able to drink samples during the examination period and who agreed to participate in this research. The subjects totaled 31 individuals (8 men, 23 women). The age of the male and female subjects were 82.4 ± 10.6 and 83.9 ± 7.9 years old, respectively. The subjects ate meals provided by their in-patient facilities during the experimental period, and no significant changes were observed in their daily living activities or in any alcohol drinking or smoking habits.

Test samples

The test sample used was prepared as follows. Fifty parts (wt) of commercial dried-bonito broth, called "*Hondzukuri ichiban-dashi* (dried-bonito)" (Ajinomoto Co., Inc., Tokyo, Japan), 0.7 parts (wt) of soy sauce and 49.3 parts (wt) of tap water were mixed and packed. The NaCl concentration was 0.3%. The nutritional element values of the test food (125 ml) are as follows. Energy 41.8 kJ, protein 2.3 g, lipid 0.1 g, carbohydrate 0.1 g, sodium 77 mg, and potassium 106 mg. Tap water was used as the control sample. Both the test and control samples were packaged in 125 ml packs.

Experimental schedule

The trial was performed with a randomized cross-over design. The subjects were divided into two random groups (groups A and B). During the first one-month experimental period, the group A subjects ingested two packs of the test sample per day (one at lunch and one at supper) in addition to their regular diet, while those in group B ingested two packs of the control sample per day in addition to their regular diet. A one-month washout period was followed by a second one-month experimental period in which the groups were reversed.

Measurement of blood pressure and urinary 8-OHdG contents

Blood pressure was measured before and after the ingestion periods. Using a mercury sphygmomanometer, blood pressure was measured on the morning before the medication in a sitting position after an approximately 5 min rest. Blood pressure was measured twice or more times, and an average value was used for analyses. The measurement was performed at the same time (10:30–11:00 am.). Before and after the ingestion of the test or control samples, spot urine samples were collected. The collection of urine was performed at the same time (10:30–11:00 am.). The concentration of 8-OHdG was determined using a competitive enzyme-linked immunosorbent assay (ELISA) kit (high sensitive 8-OHdG Check, Japan Institute for the Control of Aging, Shizuoka, Japan) according to the manufacturer's instructions, and the values were adjusted by creatinine concentration.

Measurement of blood biochemical parameters

Blood samples were collected at the same time (10:30–11:00 am. or 14:00–14:30) at least 2 h after the meal. Plasma glucose concentration was analyzed by enzymatic method using glucose dehydrogenase. Hemoglobin A1c levels were determined by a latex cohesion method. Total cholesterol contents were measured by an enzyme assay using cholesterol dehydrogenase. High density lipoprotein (HDL)-cholesterol contents were analyzed by a direct method. Triglyceride was analyzed by enzyme assay (glycerol kinase-glycerophosphate oxidase method). C-reactive protein concentration was determined by a latex cohesion and immunological method.

Evaluation of emotional states

Emotional states were evaluated by medical doctors and nurses with a 4-point scale before and after the ingestion period. The questionnaire was constructed by slight modification of the method reported by Matsuda *et al.* [*17*]. It included the following items: "complain of a fatigue", "is able to exercise", "get nervous", "get irritated", "is able to make decisions" and "is able to communicate". These behaviors were rated as 3, frequently; 2, occasionally; 1, rarely; 0, not at all.

Measurement of ACE-inhibiting activity

ACE-inhibiting activity was measured using hippuryl-histidyl-leucine (HHL) as substrates with the method of Wu *et al.* [*18*]. Each assay was done in 33.5 µl of a reaction mixture containing 1.5 mM HHL, 1.0 milliunit of ACE, 30 mM NaCl, and 100 mM borate buffer (pH 8.3). The reaction was performed at 37°C for 1 h, and the hippuric acid produced was quantified by HPLC using the method of Wu *et al.* [*18*]. The IC_{50} value was the concentration that resulted in a 50% ACE inhibition in the reaction mixture. In order to investigate the ACE-inhibiting activity of dried-bonito broth after digestion in the stomach and intestines, a pepsin and pancreatin digest of dried-bonito broth was prepared, and the activity was measured. For pepsin digestion, to 2.5 ml of dried-bonito broth, 5 ml of pepsin solution (pH 1.2) containing 16 mg porcine pepsin (Sigma Co., Inc.) and 10 mg NaCl were added, and the enzymatic reaction was performed at 37°C for 1 h. For pancreatin digestion, to 2.5 ml of dried-bonito broth, 5 mL of pancreatin solution containing 14 mg porcine pancreatin (Sigma Co., Inc.) and 75 mg sodium bicarbonate were added, and an enzymatic reaction was performed at 37°C for 1 h. The reaction was stopped by holding the mixture at 95°C for 5 min.

Statistical analysis

The statistical package SAS version 8.02 (SAS Institute,

Inc., Cary, NC) was used for data analysis. All values are expressed as means ± standard deviation. Background data in the two groups (group A and group B) were compared by Student's *t* test. For analysis of the emotional states, comparisons between before and after ingestion periods and comparison between samples were performed using Wilcoxon signed-rank test. Comparisons of blood pressure and urinary 8-OHdG contents between before and after ingestion periods were performed using a paired *t* test (two-tailed). Statistical analysis of the changed value in blood pressure and urinary 8-OHdG contents was performed using analysis of variance (ANOVA) with four factors (samples, period, group and subject). All results were considered significant at $p<0.05$.

Results

Background of the subjects

In this study, data from subjects who changed their medication ($n = 1$) was omitted. Furthermore, urinary 8-OHdG could not be measured in 3 subjects because the sufficient amount of urine could not be collected from these subjects. Therefore, data from 27 subjects was analyzed. The characteristics of the subjects ($n = 27$) was indicated in Table 1. Table 1 also revealed the background data of the subjects in A group and B group. Mean age, height, weight and initial blood pressure showed no significant difference between group A and group B. In addition, results of blood chemical analysis such as glucose, hemoglobin A1c, total cholesterol, HDL-cholesterol, triglyceride and C-reactive protein showed no significant differences in both groups. These results suggest that subjects in the two groups were balanced. Table 1 indicates the type of diseases and anti-hypertensive medications of the 27 subjects. The results show that many of the subjects ($n = 17$, 63.0%) take anti-hypertensive medications, especially calcium channel blockers ($n = 16$, 59.3%). The type of disease and anti-hypertensive medication were almost similar in group A and B.

Change in blood pressure

Table 2 shows the changes in blood pressure during the ingestion period. The results obtained by 4-way ANOVA indicate that the systolic blood pressure (SBP) significantly lowered during the ingestion of dried-bonito broth compared to water ingestion ($p = 0.037$). On the other hand, there was no significant difference in the changes in diastolic blood pressure (DBP) ($p = 0.180$).

Change in urinary 8-OHdG contents

To examine the effect of dried-bonito broth intake on oxidative state, the urinary 8-OHdG content, an oxidative stress marker, was measured before and after each ingestion period. When dried-bonito broth was ingested, the urinary 8-OHdG content significantly decreased ($p = 0.0002$) during the ingestion period, while no significant changes were observed when water was ingested (Table 3).

Changes in emotional states

Table 4 shows the changes in the emotional states during the ingestion period. The results indicate that the score for "get nervous" significantly decreased during the ingestion of dried-bonito broth ($p = 0.034$). The score for "complain of fatigue" tended to be lower after ingestion of dried-bonito broth than after water ingestion ($p = 0.068$). Furthermore, in items such "is able to make decisions" and "is able to communicate", the score after ingestion of dried-bonito broth tended to be higher than that after water ingestion ($p = 0.059$, $p = 0.084$, respectively).

Changes in blood chemical parameters

Table 5 shows the changes in blood chemistry which are reported to affect the oxidative states during the ingestion period. The results obtained by 4-way ANOVA indicate that the changes in the content of glucose, hemoglobin A1c, total cholesterol, HDL-cholesterol, triglyceride, and C-reactive protein did not show significant difference between samples ($p>0.05$).

ACE-inhibiting activities of dried-bonito broth and the enzymatic digests

Table 6 showed ACE-inhibiting activities of dried-bonito broth and the enzymatic digests. The IC_{50} of the dried-bonito broth was 1015 μg/ml. The IC_{50} of the pepsin digest and pancreatin digest of dried-bonito broth was 428 μg/ml and 870 μg/ml, respectively. The IC_{50} of the digest obtained by both of the pepsin and pancreatin digestion was 564 μg/ml.

Discussion

The present study investigated the effect of the daily ingestion of dried-bonito broth (*katsuo-bushi dashi*) for one month on blood pressure in the elderly. The results showed that the ingestion of dried-bonito broth lowered SBP. However, the ingestion of dried-bonito broth did not affect the DBP. In the elderly, the decrease in aortic elasticity caused by the progression of arteriosclerosis results in increased systolic arterial pressure and pulse pressure. Since the increase of pulse pressure causes the reduction of DBP, the DBP tends to be lower in the elderly. Increased systolic arterial pressure and pulse pressure are considered risk factors for cardiovascular disease [*19*, *20*]. The results from meta-analyses of epidemiological studies have indicated that the risk of cardiovascular disease increases with the increase in blood pressure when SBP is more than 115 mmHg or DBP is more than 75 mmHg at any age [*21*]. Moreover, SBP was

Table 1. Background data of the subjects (n = 27) in the two groups

	Total (n = 27)		A group (n = 15)		B group (n = 12)		p[a]
Gender	Male 8, Female 19		Male 3, Female 12		Male 5, Female 7		
Age	83.3 ± 8.8		81.4 ± 8.2		85.8 ± 9.4		0.210
Physiological data							
Height (cm)	148.9 ± 8.9		147.3 ± 10.0		150.9 ± 7.2		0.302
Weight (kg)	48.4 ± 10.0		47.5 ± 9.1		49.5 ± 11.5		0.618
SBP(mmHg)	127.4 ± 17.1		126.0 ± 17.1		129.2 ± 17.6		0.641
DBP(mmHg)	71.1 ± 8.4		71.6 ± 7.5		70.5 ± 9.6		0.741
Blood chemistry							
Glucose (mg/dl)	127.2 ± 36.2		128.8 ± 32.4		125.2 ± 41.8		0.807
HemoglobinA1c(%)	5.1 ± 0.6		5.0 ± 0.5		5.2 ± 0.8		0.387
Total cholesterol (mg/dl)	192.9 ± 45.9		186.2 ± 41.7		201.2 ± 51.2		0.422
HDL-cholesterol (mg/dl)	53.5 ± 12.8		53.6 ± 10.5		53.4 ± 15.7		0.973
Triglyceride (mg/dl)	91.1 ± 26.8		88.7 ± 26.0		94.0 ± 28.7		0.626
C-reactive protein (mg/dl)	0.24 ± 0.27		0.21 ± 0.28		0.26 ± 0.26		0.658
Type of Diseases	Number	Ratio (%)	Number	Ratio (%)	Number	Ratio (%)	
Disorder of circulatory system	14	51.9	8	53.3	6	50.0	
Disorder of cerebral nervous system	15	55.6	8	53.3	7	58.3	
Disorder of endocrine system	3	11.1	2	13.3	1	8.3	
Disorder of digestive system	4	14.8	4	26.7	0	0.0	
Disorder of respiratory system	4	14.8	3	20.0	1	8.3	
Cancer	4	14.8	2	13.3	2	16.7	
Disorder of sensory system	4	14.8	2	13.3	2	16.7	
Disorder of urinary system	3	11.1	2	13.3	1	8.3	
Others	5	18.5	4	26.7	1	8.3	
Anti-hypertensive medication	17	63.0	8	53.3	9	75.0	
Calcium channel blockers	16	59.3	8	53.3	8	66.6	
ACE inhibitors	1	3.7	1	6.7	0	0.0	
α-Blockers	2	7.4	2	13.3	0	0.0	
AT-II inhibitors	3	11.1	2	13.3	1	8.3	
Diuretics	3	11.1	2	13.3	1	8.3	
Smoking Habit							
Current smokers (%)	1	3.7	1	6.7	0	0.0	
Past smokers (%)	9	33.3	5	33.3	4	33.3	

[a]p value obtaibned by Student's t-test

Abbreviations. SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high density lipoprotein; ACE, angiotensin converting enzyme; AT-II, angiotensin-II.

Table 2. Change in blood pressure during the ingestion of dried-bonito broth and water

	Ingested sample	Before ingestion	After ingestion	Changed value	p[a]
SBP (mmHg)	Control	123.5 ± 13.5	126.7 ± 17.7	3.3 ± 15.9	0.037
	Dried-bonito broth	128.1 ± 16.2	121.6 ± 20.0	−6.4 ± 17.1	
DBP (mmHg)	Control	67.6 ± 9.4	69.0 ± 11.9	1.4 ± 9.8	0.191
	Dried-bonito broth	70.0 ± 8.0	68.1 ± 8.1	−1.9 ± 10.0	

Mean ± SD

[a]p for samples obtained by four-way ANOVA

Abbreviations. SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 3. Change in urinary 8-OHdG during the ingestion of dried-bonito broth and water

Ingested sample	Before ingestion	After ingestion	Changed value	p^a
Control	12.6 ± 6.6	12.4 ± 8.1	−0.2 ± 6.8	0.124
Dried-bonito broth	12.1 ± 5.1	9.7 ± 4.5***	−2.4 ± 2.9	

Mean ± SD

[a]*p* for samples obtained by four-way ANOVA

***Significantly different from the data before ingestion at $p<0.001$ (paired *t* test).

Table 4. Change in emotional states during the ingestion of dried-bonito broth and water

	Ingested sample	Before ingestion	After ingestion	
Complain of fatigue	Control	1.15 ± 0.86	1.26 ± 0.90	$p = 0.068$
	Dried-bonito broth	0.96 ± 0.65	1.00 ± 0.78	
Is able to exercise	Control	2.67 ± 0.62	2.74 ± 0.53	
	Dried-bonito broth	2.59 ± 0.93	2.85 ± 0.36	
Get nervous	Control	0.67 ± 0.96	0.63 ± 1.04	
	Dried-bonito broth	0.85 ± 0.95	0.56 ± 0.97*	
Get irritated	Control	0.93 ± 1.00	1.00 ± 1.07	
	Dried-bonito broth	0.89 ± 1.05	0.85 ± 1.06	
Is able to decide	Control	2.22 ± 0.85	2.30 ± 0.78	$p = 0.059$
	Dried-bonito broth	2.37 ± 0.74	2.48 ± 0.70	
Is able to communicate	Control	1.93 ± 0.96	1.85 ± 0.91	$p = 0.084$
	Dried-bonito broth	1.93 ± 0.92	2.07 ± 0.92	

Mean ± SD

*Significantly different from the data before ingestion at $p<0.05$ (Wilcoxon signed rank test).

Table 5. Change in the blood biochemical parameters during the ingestion of dried-bonito broth and water

	Ingested sample	Before ingestion	After ingestion	Change	p^a
Glucose (mg/dl)	Control	127.6 ± 31.9	138.1 ± 41.1	10.6 ± 38.9	0.857
	Dried-bonito broth	127.9 ± 36.5	138.5 ± 37.7	10.7 ± 38.3	
Hemoglobin A1c (%)	Control	5.1 ± 0.6	4.9 ± 0.6	−0.2 ± 0.3	0.142
	Dried-bonito broth	5.1 ± 0.5	5.0 ± 0.5	0.0 ± 0.2	
Total cholesterol (mg/dl)	Control	194.6 ± 48.4	193.9 ± 49.6	−0.7 ± 12.4	0.744
	Dried-bonito broth	190.8 ± 41.2	190.9 ± 45.7	0.1 ± 17.8	
HDL-cholesterol (mg/dl)	Control	53.6 ± 13.8	52.1 ± 13.2	−1.4 ± 6.5	0.948
	Dried-bonito broth	53.6 ± 12.5	52.0 ± 11.9	−1.6 ± 4.1	
Triglyceride (mg/dl)	Control	94.8 ± 33.1	95.1 ± 38.7	0.4 ± 22.5	0.756
	Dried-bonito broth	94.9 ± 31.2	93.7 ± 39.2	−1.2 ± 23.3	
C-reactive protein (mg/dl)	Control	0.29 ± 0.32	0.31 ± 0.58	0.02 ± 0.66	0.645
	Dried-bonito broth	0.21 ± 0.25	0.32 ± 0.55	0.11 ± 0.48	

Mean ± SD

[a]*p* for samples obtained by four-way ANOVA

Abbreviations. HDL, high density lipoprotein.

Table 6. ACE inhibiting activity (IC_{50}) of dried-bonito broth and the enzymatic digests of dried-bonito broth

Enzymes used	IC_{50} (μg/ml)
Untreated	1015
Pepsin	428
Pancreatin	870
Pepsin + Pancreatin	564

positively correlated with the risk of cardiovascular system disease and the appearance rate of cerebral infarction [*22*, *23*]. These previous studies reported that a 1–2 mmHg decrease in blood pressure improves both the appearance rate of disease and the mortality rates of apoplexy and cardiac infarction. Therefore, the SBP lowering action of dried-bonito broth (changed value; -6.4 ± 17.1 mmHg) is suggested to be effective for reducing the risk of cardiovascular system disease.

The present study was performed on elderly inpatients at a hospital or a nursing health service facility, all of whom were taking some kind of medication (Table 1). Therefore, the data of subjects whose medications had changed during the experimental periods was omitted. It is possible that the medication affected the action of dried-bonito broth. However, because this study was performed with a cross-over design and the medications were not changed during the experimental period, it was considered likely that the difference between the action of the dried-bonito broth and that of water could be detected if the broth was effective. From the result that the change in systolic blood pressure (SBP) during the ingestion of dried-bonito broth (-6.4 ± 17.1 mmHg) is significantly lower than that during water ingestion (3.3 ± 15.9 mmHg) ($p = 0.037$), it is considered that the dried-bonito broth is effective to lower SBP in elderly subjects.

Previously, many studies have been done on the anti-hypertensive action of enzymatic digests obtained from dried-bonito. Especially, the thermolysin digest has been reported to have a strong ACE-inhibiting activity *in vitro* [*11*] and also an anti-hypertensive action against animals [*9*] and hypertensive human subjects [*10*]. The ACE-inhibiting activity (IC_{50}) of the thermolysin digest from dried-bonito is reported to be 58.3 μg/ml [*10*]. Furthermore, it has been reported that enzymatic digests of the hot water extract of dried-bonito obtained by the action of mammalian digestive enzymes (pepsin, trypsin and chymotrypsin) have weak ACE-inhibiting activities (IC_{50} : from 123 to 362 μg/ml) [*11*]. These results suggest the possibility that dried-bonito broth has an ACE-inhibiting activity and then lowers the blood pressure. The present results indicate that the IC_{50} of dried-bonito broth is 1015 μg/ml. Its reaction with digestive enzymes, particularly pepsin, increased the ACE-inhibiting activity, suggesting the possibility that the enzymatic digest of dried-bonito broth obtained from pepsin has an anti-hypertensive action. However, the ACE-inhibiting activity of the pepsin digest (IC_{50}: 428 μg/ml) is much weaker than that of the thermolysin digest (IC_{50}: 58.3 μg/ml). These results suggest that the blood pressure-lowering action of the dried-bonito broth is partly due to its ACE-inhibiting activity. However, it is also considered that there are some other mechanisms in the action of dried-bonito broth.

The urinary 8-OHdG contents were significantly decreased by the ingestion of dried-bonito broth (Table 3). This result suggests that dried-bonito broth may have an anti-oxidative effect *in vivo* and that the decrease in 8-OHdG is related to the decrease in blood pressure. In previous studies, it was indicated that oxidative stress is closely related to hypertension, and that the oxidative state is higher in subjects with hypertension than in normotensive subjects. Several studies have been performed to examine the relationship between blood pressure and urinary 8-OHdG as an oxidative stress marker. 8-OHdG, an oxidative product of DNA, was used as the oxidative stress marker. Recent studies have shown that the urinary 8-OHdG levels in hypertensive rat models, stroke-prone spontaneously hypertensive rats (SHRSP) [*24*] and Dahl salt-sensitive hypertensive rats [*25*], were higher than in normal rats. Furthermore, it has been reported that reactive oxygen species or 8-OHdG contents in hypertensive subjects were higher than those in normotensive subjects [*26*]. It has also been reported that a type of anti-hypertensive medicine (T-type calcium channel blocker) decreased the urinary 8-OHdG content in a human trial [*27*]. From the previous studies and the present one, it is shown that the ingestion of dried-bonito broth not only reduces the SBP but also the oxidative stress marker. Although it is still unclear if the increase of the oxidative product is a consequence of hypertension or a cause of it, since it has been reported that antioxidants such as tempol (superoxide dismutase mimics) [*28*] or alpha-tocopherol [*29*] can lower the blood pressure in hypertensive model rats, it is possible that the SBP lowering action of the dried-bonito broth is partly due to its anti-oxidative action. Furthermore, since the dried-bonito broth has a weak ACE-inhibiting activity, and since several ACE inhibitors have been reported to have antioxidative action *in vivo* [*30–32*], the antioxidative action of dried-bonito broth was suggested due to that ACE-inhibiting action.

Previous studies have indicated that the hyperglycemia [*33–35*] or hyperlipidemia [*35*, *36*] was related to the oxidative stress in human. In this study, the several blood biochemical parameters which have been reported to be associated with hyperglycemia and hyperlipidemia were investigated. In this trial, since the subjects were elderly inpatients, in order to reduce the strain, fasting blood was not collected. Therefore, contents of plasma glucose and serum

triglyceride were considered to be affected by the intake of meal. However, blood was collected at same time, and the meal was controlled. Therefore, it is possible to compare the data for the investigation on the subjects' background. As shown in Table 1, the initial value in glucose, hemoglobin A1c, total cholesterol, HDL-cholesterol, triglyceride and C-reactive protein did not show significant difference between group A and group B, suggesting that there are no significant difference in the factors that is related to hyperglycemia and hyperlipidemia in both groups. Furthermore, as shown in Table 5, the change in blood glucose, hemoglobin A1c, total cholesterol, HDL-cholesterol, triglyceride and C-reactive protein did not show significant difference between samples, suggesting that the antioxidative action of dried-bonito broth had little correlation with hyperglycemia and hyperlipidemia.

Several previous studies have been done on the *in vitro* anti-oxidative action of dried-bonito broth and the components contained in the broth. It has been reported that dried-bonito broth contains abundant histidine and anserine [*37*], and these compounds are reported to have an anti-oxidative action *in vitro* [*38*]. Therefore, it is possible that the anti-oxidative action is derived from histidine and anserine. Furthermore, Suzuki and Motosugi reported that dried-bonito broth has a strong anti-oxidative action against the oxidation of linoleic acid *in vitro* [*39*]. They also reported that the anti-oxidative capacity increased during the drying and smoking process of dried-bonito due to the increase of phenolic compounds, suggesting that the anti-oxidative action is also derived, at least in part, from such compounds. From these previous studies, histidine, anserine and phenolic compounds are suggested to contribute to the *in vivo* anti-oxidative action of dried-bonito broth. However, the components contributing to the *in vivo* anti-oxidative action need to be clarified.

In this trial, the evaluation of emotional states indicated that the score of "get nervous" significantly decreased during the ingestion of dried-bonito broth ($p = 0.034$), suggesting that ingestion of dried-bonito broth significantly improved composure. The score of "complain of fatigue" tended to be lower than that after water ingestion ($p = 0.068$). Furthermore, in items such as "is able to decide" and "is able to communicate", the score after ingestion of dried-bonito broth tended to be higher than that after water ingestion ($p = 0.059$ and $p = 0.084$, respectively). Since this trial was performed in open-labeled design, although it should be interpreted with caution, it was possible that dried-bonito broth also improve the emotional states in elderly subjects. Recently, oxidative stress has been reported to be associated not only with physical fatigue [*40*], but also with mental fatigue related to the central nervous system [*41*, *42*]. These previous studies suggest that the anti-oxidative effect of dried-bonito broth may contribute to the improvement of emotional states.

Our examination of the effect of dried-bonito broth on blood pressure in the elderly showed that the broth lowers SBP and has an anti-oxidative action. It is now necessary to clarify the mechanism by which the broth reduces blood pressure, and to clarify the active components in dried-bonito broth. Clarification of the mechanism by which it occurs is in progress in our laboratory.

Acknowledgments

The authors wish to thank Dr. N. Sakaguchi, Dr. Y. Kitahara, Ms. K. Mawatari, Ms. M. Ishibashi, Ms. R. Nakano, Ms. I. Akada, Ms. M. Sasaki and Ms. M. Hara of the Nishi-Fukuoka Hospital for their cooperation to this study. The authors would also like to thank Ms. E. Suzuki, Ms. M. Hokumen, Ms. M. Bekki, Ms. M. Matsuo, Ms. A. Fujihira and Ms. M. Ishihara of Fukuoka Women's University for their assistance. The authors would also like to thank Ms. Y. Nozawa, Mr. T. Shimazu and Ms. Y. Satou of Ajinomoto for their technical assistance and helpful discussion. The authors would also like to thank the study participants.

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