Ingestion of Dried-bonito Broth Ameliorates Blood Fluidity in Humans

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To elucidate a physiological function of dried-bonito broth (DBB) on blood fluidity and oxidative stress, we performed a randomized double-blind placebo-controlled study in twenty-four healthy adult subjects. The subjects ingested DBB or a placebo for four weeks, and blood fluidity and oxidative stress were measured before and after ingestion. Blood fluidity was measured using a microchannel array flow analyzer by the passage time of 100 µl of heparinized whole blood through the microchannel array, while oxidative stress was evaluated as a level of derivative of reactive oxygen metabolites (d-ROMs) by a free radical analysis system (FRAS). DBB ingestion significantly shortened the blood passage time from 55.4 ± 3.4 to 47.6 ± 2.0 sec (mean ± SEM, $p < 0.05$), while no significant change was observed in the placebo group (52.4 ± 3.4 to 51.4 ± 2.6 sec, mean ± SEM) indicating that DBB ameliorated blood fluidity. The level of d-ROMs, known as a biomarker of oxidative stress, significantly decreased after DBB ingestion from 337.2 ± 18.5 to 316.5 ± 12.9 Carrotelli units (Carr. U.) (mean ± SEM, $p < 0.05$), suggesting that DBB reduced oxidative stress. Among subjects with a d-ROMs score > 320, regarded as being in a state of oxidative stress, changes in blood fluidity tended to correlate with changes in d-ROMs score ($\rho = 0.55$, $p = 0.06$), showing that blood fluidity may have improved in subjects whose oxidative stress was markedly decreased. These results also showed a possibility that DBB ingestion improved blood fluidity by decreasing oxidative stress. In previous studies, daily DBB ingestion improved various fatigue-related symptoms, so we investigated the effect of DBB on fatigue-related symptoms via a questionnaire survey in the present study. The result of this survey showed that symptoms of shoulder stiffness and visual fatigue were improved only in the DBB group ($p < 0.05$, $p < 0.1$, respectively). Insufficient blood circulation is considered to lead to the development of shoulder stiffness, visual fatigue, and other fatigue-related symptoms. Based on these findings, we considered that dietary intake of DBB may improve blood fluidity by reducing oxidative stress and thus might protect against fatigue.

Key words —— dried-bonito broth, blood fluidity, oxidative stress, visual fatigue, shoulder stiffness, human

INTRODUCTION

Capillary vessels are responsible for constant oxygen and carbon dioxide gas exchange and nutrient transport, enabling the life-support of all tissues.[1)] Insufficient blood circulation has been found to lead to the development of various pathogeneses such as shoulder stiffness, neck pain, poor circulation, and dark skin circles.[2–4)] Furthurmore, impaired microvascular perfusion could contribute to the development and progression of diseases such as high blood pressure, arteriosclerosis, and thrombus syndrome. Thus, the improvement of blood fluidity is important for preventing these pathogeneses and may be important in decreasing fatigue-related subjective symptoms.

Beef and chicken bouillons have been commonly used worldwide as a flavoring base in many cuisines. In Japan, dried-bonito broth (DBB), which has a specific taste and flavor, has been more frequently used than beef and chicken bouillons for soup stock. Dried bonito and DBB have been traditionally considered nutritional supplements that promote recovery from fatigue. Studies have confirmed that DBB shows various physiological functions in both animals and humans. Animal stud-

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ies showed that DBB administration aided recovery from physical fatigue,[5] while in human studies, the daily ingestion of DBB improved mood states[6] and subjective symptoms of visual fatigue.[7–9] In addition, DBB was shown to have an improving effect on the skin of subjects whose skin tends to be dry and rough by maintaining moisture levels in the skin.[10] However, even though DBB has been found to have these physiological effects, an understanding of why its effectiveness is so wide-ranging has remained elusive.

To investigate whether daily DBB ingestion improved blood fluidity, we used a microchannel array flow analyzer (MC-FAN), which enabled us to take into account erythrocytes deformability, platelet aggregation, and leukocyte adhesiveness. The time required for a specified volume of blood to pass through microchannels that simulate human blood vessels is measured to evaluate blood fluidity by this system,[11–13] which is widely used for the evaluation of the physiological function of foods and supplements[14–17] and in disease models.[18,19] The fatigue-related subjective symptoms were also evaluated by a questionnaire survey during the test diet ingestion period.

Erythrocytes form the largest group of cells in the blood, and they are highly deformable. The membrane plays an important role in maintaining the flexibility for normal erythrocyte deformability, which was reported to be damaged by reactive oxygen.[20,21] Oxidized erythrocytes would be more prone to form aggregates and increase the viscosity of blood flow in the microcirculation.[16,22] Since the antioxidative effect is considered to be a factor in improving blood fluidity, we investigated derivatives of reactive oxygen metabolites (d-ROMs) using a free radical analysis system.[23–25]

In the present study, we measured blood fluidity, the level of oxidative stress marker before and after the ingestion period, and the change of subjective symptoms assessed by a daily questionnaire each night. Based on the results obtained, we discuss why DBB is effective to multiple symptoms.

MATERIALS AND METHODS

Study Design and Protocol —— This study was conducted in accordance with the Declaration of Helsinki, and was approved by the ethics committee of the institutional review board of CPCC Co., Ltd. (Tokyo, Japan) Written informed consent was obtained from each subject participating in the study. A randomized double-blind placebo-controlled study was performed in twenty-four healthy adult subjects aged 42.0 ± 2.0 (mean $\pm$ SEM) years. Each group was comprised of seven male and five female volunteers. Subjects were screened before the start of this study and randomly divided into two groups so that there were no differences in blood fluidity measured by MC-FAN. The subjects ingested the DBB or placebo for four weeks, and blood fluidity and oxidative stress were measured before and after the ingestion period. The subjects filled in a questionnaire during the study period. The study protocol is outlined in Fig. 1.

Test Diet —— The compositions of the test diets, DBB, and the placebo are shown in Table 1. Commercial dried-bonito broth, named "*Hondzukuri ichiban-dashi*" (Ajinomoto Co., Inc., Tokyo Japan),

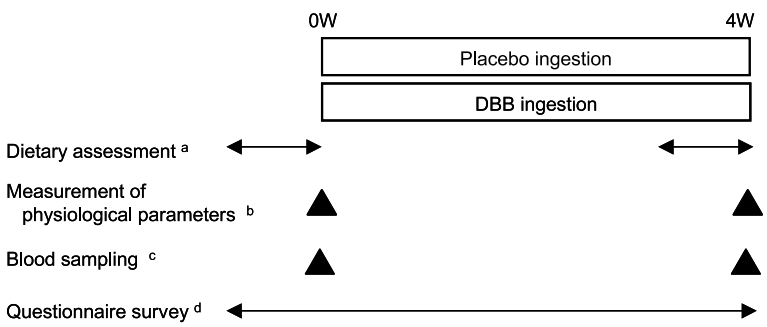

Fig. 1. Study Protocol

a; All meals were recorded beginning three days before blood testing. The subjects also filled in a questionnaire that contained questions regarding their daily appetite, food and water intake, urination, and defecation. b; Body weight, percentage body fat, and blood pressure were measured before and after test diet ingestion. c; Blood testing was performed before and after the ingestion period to evaluate the blood fluidity and oxidative stress marker. d; Each night, the subjects filled in a questionnaire that asked about subjective symptoms (frequency of feeling fatigue, anxiety and stress, visual fatigue, shoulder stiffness, skin condition), beginning three days before initiation of the study and continuing until completion of the study.

Table 1. Nutrient Compositions of the Test Diets (Per 100 ml)

		Placebo[a)]	DBB[b)]
Energy	(kcal)	4.2	16
Protein	(g)	—	3.6
Lipid	(g)	—	0.1
Carbohydrate	(g)	0.3	0.1
Ash content	(g)	0.3	0.9
Water content	(g)	99.6	95.3
Sodium	(mg)	120	123

a) Placebo: Dried-bonito flavor, caramel, and sodium chloride were dissolved in water. *b*) DBB: Dried-bonito broth; —: Not calculated because the value was lower than 0.1 g/100 g.

Table 2. Nutrient Composition of DBB

Nutrient		Amount
Protein (include peptides)	(mg/ml)	20.6
Free amino acid	(mg/ml)	10.4
taurine	(mg/ml)	1.2
histidine	(mg/ml)	6.4
anserine	(mg/ml)	0.9
Organic acid		
lactic acid	(mg/ml)	8.2
Minerals		
iron	(μg/ml)	2.0
magnesium	(μg/ml)	243.3
sodium	(mg/ml)	2.3
potasium	(mg/ml)	1.4
calcium	(μg/ml)	36.7
Creatine	(mg/ml)	0.9
Creatinine	(mg/ml)	1.2

produced via a hot-water extraction process from dried bonito, was used as an active dietary supplement. The nutrient composition of DBB is shown in Table 2. The placebo consisted of dried-bonito flavor, caramel, and sodium chloride, and was prepared so that the two test diets were indistinguishable. The subjects ingested 125 ml of the diet every morning in addition to their regular diet for four weeks.

Dietary Assessment —— All meals were recorded for three days before blood testing (Fig. 1). According to questionnaire, intake of energy, carbohydrate, protein and fat were calculated from the diet records using calculation software (Excel Eiyo-kun, Kenpakusha, Tokyo, Japan). Furthermore, the intake of rice, cereal, fish, meat, egg, pulse, milk, potato, vegetable and fruits were evaluated. The subjects also filled in a questionnaire that asked about their daily appetite, food and water intake, urination, and defecation.

Measurement of Physiological Parameters —— Body weight, percentage body fat (InBody 3.2, Biospace Inc., Seoul, Korea), and blood pressure (Digital blood measurement TM-2655P, A&D Co., Ltd., Tokyo, Japan) were measured before and after test diet ingestion (Fig. 1).

Measurement of Blood Fluidity —— After blood testing, we measured blood fluidity with a MC-FAN using the method described by Kikuchi *et al.* (Fig. 1).[11, 12, 26)] Blood samples were collected in heparinized tubes for anticoagulation in the morning. Blood was then forced to flow through microchannels (Bloody 7–7, 7854 flow channels, 7 μm width chip) and measured by an MC-FAN KH-7 model (Hitachi Haramachi Electronics Co., Ltd., Ibaraki, Japan). The passage time of each blood sample was measured twice, and the mean value was regarded as the blood passage time, which was an index of blood fluidity.

Measurement of Oxidative Stress Marker —— Blood testing was performed, ultracentrifuged and evaluated photometrically by using a free radical analysis system (FRAS, Wismerll Co., Ltd., Italy; Fig. 1). The d-ROMs were measured to monitor oxidative stress in serum.[23–25, 27)] The d-ROMs score reflects the level of hydroperoxides of lipids, proteins, amino acids, and nucleic acids oxidized by free radicals, and is known as an oxidative injury index. The results of the d-ROMs test were expressed in arbitrary units called Carratelli units (Carr. U.), where 1 Carr. U. corresponds to 0.08 mg/100 ml H_2O_2.[28)]

Questionnaire on Subjective Symptoms —— Each night, the subjects filled in a questionnaire on subjective symptoms, beginning three days before initiation of the study and continuing until completion of the study (Fig. 1). In the questionnaire, items related to fatigue such as frequency of feeling fatigue, anxiety and stress, visual fatigue, shoulder stiffness and skin condition were included.

Statistical Analysis —— All values were presented as the mean ± standard error of the mean (mean ± SEM). Dietary assessment, physiological parameters, blood fluidity and oxidative stress were analyzed using a paired *t* test to compare data before and after the 4-week period of ingestion. The relation between the change of blood fluidity and that of oxidative stress marker was analyzed by Spearman's correlation coefficient test. Regarding the questionnaire results, replies related to subjective symptoms were summed per week in each test diet group, and changes between week 1 and other

weeks were compared. Frequencies were analyzed by the Wilcoxon rank sum test. All data analysis were conducted with the StatView software package (StatView 5 for SAS Institute, Inc., Cary, NC, U.S.A.). $p < 0.05$ was regarded as significant.

RESULTS

All twenty-four subjects completed the study and were included in the analysis.

Results of Dietary Assessment

The results of measuring the the intake of energy, carbohydrate, protein and fat are shown in Table 3. The amounts of daily intake before blood testing showed no significant differences between before and 4-week after ingestion period (Table 3). We have also analyzed the amounts of daily intake of rice, cereals, fish, meat, egg, pulse, milk, potato, vegetable, and fruits which can be calculated by the soft used, and found that there were no significant differences between before and after ingestion period (data not shown). As a result of questionnaire analysis by Wilcoxon rank sum test, we found that there were no significant changes in the frequency of the daily appetite, food and water intake (data not shown). These results indicated that the subjects' dietary habit has not changed during the study.

Results of Physiological Parameters

There were no significant changes in the physiological parameters of body weight, percentage body fat, and blood pressure before and after the ingestion of placebo or DBB (Table 4).

Table 3. Results of Daily Intake of Energy, Carbohydrate, Protein, and Fat Before Blood Testing (Per Day)

	Placebo	DBB
Energy intake (kcal)		
before	2047.4 ± 167.9	1932.8 ± 126.8
after	1938.9 ± 114.3	1893.1 ± 117.6
Carbohydrate (g)		
before	259.3 ± 25.1	235.1 ± 17.3
after	255.1 ± 19.0	256.9 ± 21.0
Protein (g)		
before	77.5 ± 5.7	78.2 ± 5.4
after	70.1 ± 3.4	70.8 ± 5.8
Fat (g)		
before	68.7 ± 6.6	67.7 ± 6.8
after	65.9 ± 5.1	59.7 ± 4.3

Values are mean ± SEM ($n = 12$).

Effects on Blood Fluidity

The passage time of 100 μl of whole blood and the time points when 25, 50, and 75 μl of the blood sample passed were measured before and after the ingestion period in the placebo and DBB groups. Measurement for one subject in the placebo group failed, so analysis was performed on the data from the eleven remaining subjects. In the placebo group, no significant changes were observed in any passage time during the ingestion period (Table 5). In contrast, the 50-μl passage time tended to be shorter after DBB ingestion compared to that before ingestion ($p < 0.1$; Table 5), and the 75-μl and 100-μl passage times were significantly shortened in the DBB group ($p < 0.05$; Table 5).

Table 4. Results of Physiological Parameters Before and After Ingestion

	Placebo	DBB
Body weight (kg)		
before	65.3 ± 4.2	66.5 ± 4.3
after	65.5 ± 4.2	66.8 ± 4.4
Percentage body fat (%)		
before	22.4 ± 2.9	25.3 ± 1.6
after	22.5 ± 2.9	25.1 ± 1.8
Systolic blood pressure (mmHg)		
before	118.5 ± 6.2	113.3 ± 5.2
after	121.4 ± 6.0	114.8 ± 5.2
Diastolic blood pressure (mmHg)		
before	74.7 ± 4.2	72.6 ± 4.5
after	78.6 ± 3.7	75.2 ± 4.2

Values are mean ± SEM ($n = 12$).

Table 5. Results of Blood Passage Time and d-ROMs Score before and after Ingestion

		Placebo	DBB	
Blood passage time (sec)				
25 μl	before	10.1 ± 0.5	10.3 ± 0.4	
	after	10.5 ± 0.4	9.9 ± 0.3	
50 μl	before	22.1 ± 1.4	22.4 ± 1.0	#
	after	22.4 ± 0.9	20.9 ± 0.6	#
75 μl	before	37.7 ± 3.9	37.0 ± 1.9	*
	after	35.7 ± 1.5	33.3 ± 1.1	*
100 μl	before	52.4 ± 3.4	55.4 ± 3.4	*
	after	51.4 ± 2.6	47.6 ± 2.0	*
d-ROMs score (Carr. U.)				
	before	321.5 ± 17.1	337.2 ± 18.5	*
.	after	306.6 ± 13.5	316.5 ± 12.9	*

The passage times of 25, 50, 75, and 100 μl of whole blood, and derivatives of reactive oxygen metabolites (d-ROMs) are shown. Values are mean ± SEM ($n = 11$–12). $^{*}p < 0.05$ *vs.* before ingestion. $^{\#}p < 0.1$ *vs.* before ingestion.

Table 6. Changes in Subjective Symptoms after Placebo or DBB Ingestion (Number of Replies Related to Frequency of Feeling Fatigue)

	Placebo				DBB			
	1 week	2 weeks	3 weeks	4 weeks	1 week	2 weeks	3 weeks	4 weeks
Very frequently	2	0	5	0	1	0	3	0
Frequently	24	24	28	18	24	14	16	25
Moderate	40	47	38	43	50	55	43	48
Rarely	17	12	12	19	9	14	22	11
Not at all	1	1	1	4	0	1	0	0

The frequency of "felt fatigue" was not changed significantly in week 2, 3, and 4 compared to week 1.

Table 7. Changes in Subjective Symptoms after Placebo or DBB Ingestion (Number of Replies Related to Anxiety and Stress)

	Placebo				DBB			
	1 week	2 weeks	3 weeks	4 weeks	1 week	2 weeks	3 weeks	4 weeks
Very frequently	0	0	2	0	0	1	2	0
Frequently	3	6	10	7	10	5	18	7
Moderate	56	60	52	52	61	61	53	55
Rarely	23	14	17	18	13	17	10	22
Not at all	2	4	3	7	0	0	1	0

The frequency of "felt anxiety and stress" was not changed significantly in week 2, 3, and 4 compared to week 1.

Table 8. Changes in Subjective Symptoms after Placebo or DBB Ingestion (Number of Replies Related to Skin Condition)

	Placebo				DBB			
	1 week	2 weeks	3 weeks	4 weeks	1 week	2 weeks	3 weeks	4 weeks
Very well	0	0	0	3	0	0	0	0
Well	7	3	1	11	8	6	9	8
Moderate	67	75	67	61	73	70	61	73
Bad	10	6	8	7	3	7	12	3
Very bad	0	0	8	2	0	1	2	0

The frequency of "skin condition" was not changed significantly in week 2, 3, and 4 compared to week 1.

Effects on Oxidative Stress Marker

Table 5 shows the results of measurement of a free radical oxidative injury index, d-ROMs. The d-ROMs value significantly decreased after DBB ingestion compared to before ingestion ($p < 0.05$), but no significant change was noted in the placebo group.

Effects on Subjective Symptoms

Changes in the replies were analyzed in weeks 1 to 4, and no significant changes were noted in the frequency of feeling fatigue, anxiety and stress, and skin condition in either test diet group (Tables 6–8).

In the replies related to "visual fatigue" and "shoulder stiffness," changes were noted only in the DBB group (Tables 9 and 10). No change was noted in the frequencies of replies related to visual fatigue in the placebo group. In the DBB group, the frequency of "felt visual fatigue frequently" tended to decrease in week 4 of DBB ingestion compared to week 1 ($p < 0.1$; Table 9). Similarly, no significant time-course changes were noted in the frequency of the reply related to shoulder stiffness in the placebo group, while the frequency of "felt shoulder stiffness very frequently" significantly decreased in week 3 compared to week 1 in the DBB group ($p < 0.05$; Table 10).

Correlation Analysis

To investigate the relationship between improvement in blood fluidity and decreased oxidative stress, we analyzed the correlations between the

Table 9. Changes in Subjective Symptoms after Placebo or DBB Ingestion (Number of Replies Related to Visual Fatigue)

	Placebo				DBB			
	1 week	2 weeks	3 weeks	4 weeks	1 week	2 weeks	3 weeks	4 weeks[a)]
Very frequently	8	7	8	8	0	0	3	0
Frequently	21	16	24	8	34	23	15	16
Moderate	35	48	44	49	34	43	51	54
Rarely	20	13	7	16	14	17	15	14
Not at all	0	0	1	3	2	1	0	0

a) The frequency of "felt visual fatigue" tended to decrease in week 4 compared to week 1 ($p < 0.1$).

Table 10. Changes in Subjective Symptoms after Placebo or DBB Ingestion (Number of Replies Related to Shoulder Stiffness)

	Placebo				DBB			
	1 week	2 weeks	3 weeks	4 weeks	1 week	2 weeks	3 weeks[a)]	4 weeks
Very frequently	11	10	12	9	13	10	2	4
Frequently	15	15	22	11	24	23	22	28
Moderate	46	52	39	47	40	47	54	40
Rarely	12	7	10	14	7	4	6	12
Not at all	0	0	1	3	0	0	0	0

a) The frequency of "felt severe shoulder stiffness" significantly decreased in week 3 compared to week 1 ($p < 0.05$).

changes in blood fluidity and changes in d-ROM score during DBB and placebo ingestion. Among subjects with a d-ROMs score > 320, regarded as being in a state of oxidative stress, changes in blood fluidity tended to correlate with changes in d-ROMs score ($\rho = 0.55$, $p = 0.06$, $n = 13$), showing that blood fluidity may have improved in subjects whose oxidative stress was markedly decreased.

DISCUSSION

Peripheral blood circulation plays an important role in maintaining organ function by facilitating the exchange of oxygen and nutrients between blood and tissues. The diameter of capillary vessels varies among tissues, with a mean of 6 μm, while the diameters of red and white blood cells are 8 μm and 10–25 μm, respectively, indicating that blood cells have to deform to pass through capillary blood vessels.[1)] Erythrocytes form the largest group of cells in the blood, and they are highly deformable. The membrane plays an important role in maintaining the flexibility for normal erythrocyte deformability, however, oxidative damage to the erythrocyte membrane has been suggested to induce impairment in these cells' physiological functions, including deformability.[20,21,29,30)] Diet and general health status are closely related to the morphological and rheological characteristics of erythrocytes.[19,28)] In this study, daily DBB ingestion significantly decreased the passage time of the specified volume of blood through the microchannels that simulate the human blood capillaries, suggesting that DBB ingestion improved blood fluidity (Table 5). Therefore, DBB intake may improve microcirculation. It was found that single-dose DBB ingestion had an acute dose-dependent effect on peripheral blood flow in healthy subjects.[31)] Although there is a difference between the ingestion period, single ingestion or 4-weeks daily ingestion, DBB single ingestion might increase peripheral blood flow due to its effect of improving peripheral blood fluidity.

Erythrocytes oxidized via reactive oxygen species would be more prone to form aggregates and increase the viscosity of the bloodstream, and reactive oxygen species might impair blood flow in the microcirculation.[31)] The d-ROMs score, known as a biomarker of oxidative stress, was measured before and after the test diet ingestion. The d-ROMs score significantly decreased during DBB ingestion (Table 5). It decreased in the placebo group, however, its change was not statistically significant and the reason why placebo ingestion decrease the value is uncertain. Therefore, we should draw conclusions from this result with caution. In our previous studies, DBB was shown to decrease the urinary amount of 8-OHdG which is known as

a biomarker of oxidative DNA injury.[32,33] Four-week DBB ingestion decrease the urinary 8-OHdG contents from 12.1 ± 5.1 ng/mg creatinine (CRE) to 9.6 ± 4.5 ng/mg CRE (mean ± S.D., $n = 27$, $p < 0.001$ *vs*. before ingestion), while there was not significant change between before and after ingestion of water (12.6 ± 6.6 to 12.4 ± 8.1 ng/mg CRE, not significant *vs*. before ingestion) in elderly subjects.[34] In addition to that study, we found that two-week DBB ingestion decrease the amounts of 8-OHdG which were excreted for 24 hr in urine from 16.6 ± 0.6 mg to 14.5 ± 0.8 mg (mean ± SEM, $n = 29$, $p = 0.029$ *vs*. before ingestion), while placebo ingestion did not decrease the level (15.0 ± 0.7 mg to 16.1 ± 1.2 mg, mean ± SEM, $n = 29$, not significant *vs*. before ingestion) in healthy female.[35] Since DBB contains abundant histidine and anserine, which are characteristic to DBB (Table 2),[36] and these compounds are also reported to have antioxidative actions *in vitro*,[37] it is possible that the observed effect of DBB is derived from the action of these substances. Taken together with thses results, it is highly likely that DBB exhibits an antioxidative effect *in vivo*. To investigate the relationship between the improvement of blood fluidity and the decrease of oxidative stress, we analyzed the correlations between the changes in the passage with a d-ROMs score > 320 Carr. U., regarded as indicating a state of oxidative stress.[38] Changes in the passage time of 100 μl of whole blood tended to correlate with the changes in the d-ROMs score ($\rho = 0.55$, $p = 0.06$, $n = 13$). This result reflected that blood fluidity improved in subjects whose levels of oxidative stress were markedly decreased. There is one possibility that the improvement of blood fluidity was caused by antioxidative activity of DBB.

DBB ingestion was shown to improve mood states and the subjective symptoms of visual fatigue in the previous studies.[7–9] DBB was also shown to have an improving effect on the skin by maintaining moisture levels in the skin.[10] In the present study, we investigated the effects of DBB on visual fatigue using a questionnaire survey and found that it was observed in only the DBB group (Table 9). This result is consistent with previous observations.[7–9] In addition, we newly found that daily DBB intake reduced shoulder stiffness, as a fatigue-related symptom (Table 10). On the other hand, no significant changes were noted in the frequency of feeling fatigue, anxiety and stress, or skin condition in either test diet group (Tables 6–8). The reason why the effect of DBB on the frequency of feeling fatigue, anxiety and stress, or skin condition was not exhibited in the present study is uncertain, but the method of asking the questions about these effects as well as the subjects were different. In the previous studies, mood states were assessed using the profile of mood states survey,[39,40] known as a sensitive measurement of mood states, which were divided into six mood factors (tension-anxiety, depression-dejection, anger-hostility, vigor, fatigue, and confusion), and DBB intake was found to improve the mood states. In the previous study, we tried evaluating the effect of DBB ingestion on the skin condition, we recruited subjects whose skin tended to be dry and rough, while we did not recruit subjects with a view to their skin condition in this study.

Capillary vessels are responsible for oxygen and carbon dioxide gas exchange, nutrient supply, and waste removal. Insufficient blood circulation is considered to lead to the development of shoulder stiffness,[2] neck pain, poor circulation,[3] and dark skin circles.[4] Furthermore, stress has been reported to be closely related to shoulder pain and back pain.[41] Taken together with the function of DBB we have already reported and its effect on blood fluidity and oxidative stress marker, we have formulated a hypothesis to explain why DBB is effective against fatigue. It is possible that the antioxidative activity of DBB may improve the oxidative condition of blood vessels or blood cells. Improving blood fluidity may have resulted in a smooth blood supply to the periphery, leading to a more effective functioning of capillary blood vessels. Thus, various symptoms related to fatigue were reduced.

DBB, a hot water extract of bonito muscle, has long been the most familiar soup stock in Japan and is also used as a folk remedy for fatigue. We found that four-week DBB ingestion might exhibit antioxidative activity, ameliorate blood fluidity, and improve some fatigue-related symptoms in healthy subjects. A study to determine what component of DBB affects the blood fluidity and the oxidative stress marker, and through what mechanisms, is now in progress.

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