Consumption of Dried-bonito Broth Acutely Increases Peripheral Blood Flow in Humans

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We investigated the acute effect of a single dose of dried-bonito broth (DBB) on peripheral blood flow using a laser Doppler blood flow meter. In a randomized, double-blind, crossover, placebo-controlled study, 19 healthy female subjects ingested DBB (4900 mg) or placebo. The peripheral blood flow was measured before ingestion and at 5, 10, 15, 20, 25, 30, 45, and 60 min after ingestion of the test diet. Blood flow significantly increased after DBB ingestion, and the area under the blood flow-time curves calculated up to 60 min ($AUC_{0–60}$) for the ingestion with DBB was significantly higher than that for the placebo ingestion ($p < 0.001$). Following consumption of lower dose of DBB (2450 mg), an increase in peripheral blood flow was observed and that the $AUC_{0–60}$ after subjects consumed DBB was significantly higher than that after they consumed the placebo ($p < 0.01$). The mean $AUC_{0–60}$ for the treatment with 4900 mg DBB was about 2 times that for 2450 mg DBB, suggesting that an orally administered single dose of DBB might have an acute dose-dependent effect on peripheral blood flow.

Key words —— dried-bonito broth, microcirculation, human study, peripheral blood flow

INTRODUCTION

Bonito (skipjack tuna, *Katsuwonus pelamis*) is a medium-sized fish in the mackerel family, known as *katsuo* in Japan. It is smoked and dried to make dried bonito, *katsuobushi*, which is an important ingredient in *dashi* (Japanese fish stock). Dried-bonito broth (kastuobushi-dashi) has a specific taste and flavor,[1,2] and has been used extensively in Japanese cuisine. Dried-bonito and dried-bonito broth (DBB) have also been traditionally considered as nutritional supplements that promote recovery from fatigue. DBB has been confirmed to show various physiological functions. Animal studies showed that DBB administration aided recovery from physical fatigue.[3] Among human studies, it was reported that the daily ingestion of DBB improved the mood state.[4] DBB was also reported to possibly improve subjective symptoms of visual fatigue.[5,6] In addition, consumption of 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.[7]

According to traditional Chinese medicine, certain foods have physiological effects of warming or cooling the body such as ginger and persimmon.[8] It is known that vasodilation and vasoconstriction of the peripheral blood vessels regulate body heat radiation. Effect of ginger or Japanese persimmon on peripheral blood flow was practically investigated and discovered to warm and cool the body respectively, as has been traditionally mentioned in Chinese medical theory.[9,10] Peripheral blood circulation plays an important role in maintaining organ function due to the supply between blood and tissues of oxygen and nutrients, in addition to its role in regulating body heat radiation; both roles help maintain the integrity of the human body.[11] Insufficient blood circulation has been found to lead to the development of various symptoms considered to be related to fatigue such as shoulder stiffness and neck pain.[12] Furthermore, impaired microvascular perfusion can cause the development and progression of diseases such as hypertension, arteriosclerosis, and thrombus syndrome. In view of the four categories (hot, warm, cool, and cold groups) in Chinese medical theory, bonito belongs to the warm group.[7] Taken together with the function of DBB we have already reported and Chinese medical theory, DBB has a possibility to affect the blood flow. We therefore investigated whether ingestion of DBB increases human peripheral blood flow using a laser Doppler blood flow meter in this study.

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MATERIALS AND METHODS

Study Design and Subjects —— The study was conducted in accordance with the Declaration of Helsinki; the study was approved by the ethics committee of Kobe Gakuin University, and written informed consent was obtained from each subject participating in it. A randomized, double-blind, placebo-controlled crossover study was performed in 19 female subjects aged 18–22 years old. Subjects were randomly assigned to either the placebo diet group or the active diet group. Crossover was performed after a washout period of 14 days. The subjects ingested the test diet, and peripheral cutaneous blood flow measurement was performed before ingestion and at 5, 10, 15, 20, 25, 30, 45, and 60 min after ingestion. In Experiment 2, half-amounts (dry-matter basis) of the active or placebo diet from Experiment 1 were ingested to investigate the possible dose dependence of the blood flow changes. Experiment 2 was performed 1 month after Experiment 1 measurements by the same subjects.

Measurement of Peripheral Blood Flow —— Peripheral blood flow was measured in the dorsal region of the right hand using a laser Doppler blood-flow imaging system (Peri Scan PIMII, PERIMED Co., Järfälla, Sweden). This system mainly measures blood flow in capillary blood vessels located at a maximum depth of about 0.5 mm from the skin surface. After resting on a chair, blood flow was measured in a quiet room with the temperature kept at around 23 degrees centigrade. The measurement of each individual was always performed in each day at the same time to avoid circadian variations.

Test Diet —— Experiment 1: Commercial DBB, "*Hondzukuri ichiban-dashi*" (Ajinomoto Co., Inc., Tokyo, Japan), produced via a hot-water extraction process from dried-bonito, was used as the active diet. The placebo diet consisted of dried-bonito flavor, caramel, sodium chloride, and dextrin, and was prepared so that subjects could not distinguish between the test diets and received an equal calorie intake. The compositions of the test diets, DBB and the placebo, are shown in Table 1. The subjects ingested 125 ml of the diet kept at room temperature.

Experiment 2: Active diet and placebo diet used in Experiment 1 were diluted 50% with water, and half-amounts of DBB or the placebo were prepared as 1/2 DBB or 1/2 placebo. The subjects ingested 125 ml of the diet kept at room temperature.

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

		DBB[a)]	Placebo[b)]
Energy	(kcal)	14.0	16.0
Protein	(g)	3.6	—
Lipid	(g)	—	—
Carbohydrate	(g)	—	4.0
Ash content	(g)	0.8	0.3
Water content	(g)	96.1	96.0
Sodium	(mg)	102	120

[a)]DBB: Dried-bonito broth, *Hondzukuri-ichibandashi* (Ajinomoto Co., Inc.). [b)]Placebo: Dried-bonito flavor, caramel, sodium chloride, and dextrin were dissolved in water. —: Not calculated because the value was lower than 0.1 g/100 g.

Statistical Analysis —— All values are expressed as means ± standard errors of the mean (SEM). One-way Analysis of variance with Dunnett post-hoc test was used to compare between different times and time zero on peripheral blood flow. Areas under the blood flow-time curves were calculated up to 60 min ($AUC_{0–60}$) after ingestion. A paired Student's *t*-test was used for differences in $AUC_{0–60}$ values during DBB and placebo ingestion. Differences with $p < 0.05$ were considered significant. SPSS 13.0J for Windows (SPSS Inc., Chicago, IL, U.S.A.) was employed for analysis.

RESULTS

Experiment 1

All nineteen subjects completed the study. Time course changes in blood flow on ingesting DBB and the placebo are shown in Fig. 1(A). An increase in peripheral blood flow was observed after consumption of DBB, while blood flow did not change after consumption of the placebo. The AUC was calculated up to 60 min after administration according to the trapezoidal method. The mean $AUC_{0–60}$ for the 4900 mg DBB ingestion was higher than that for the placebo ingestion, and the difference was significant [Fig. 1(B), $p < 0.001$].

Experiment 2

Among the nineteen subjects, three subjects failed to ingest the 1/2 placebo diet; thus, analysis was performed on data from only sixteen subjects. Time course changes in blood flow upon ingesting the 1/2 DBB and 1/2 placebo are shown in Fig. 2(A). Following consumption of DBB, an increase in peripheral blood flow was observed, while blood flow did not change after the placebo inges-

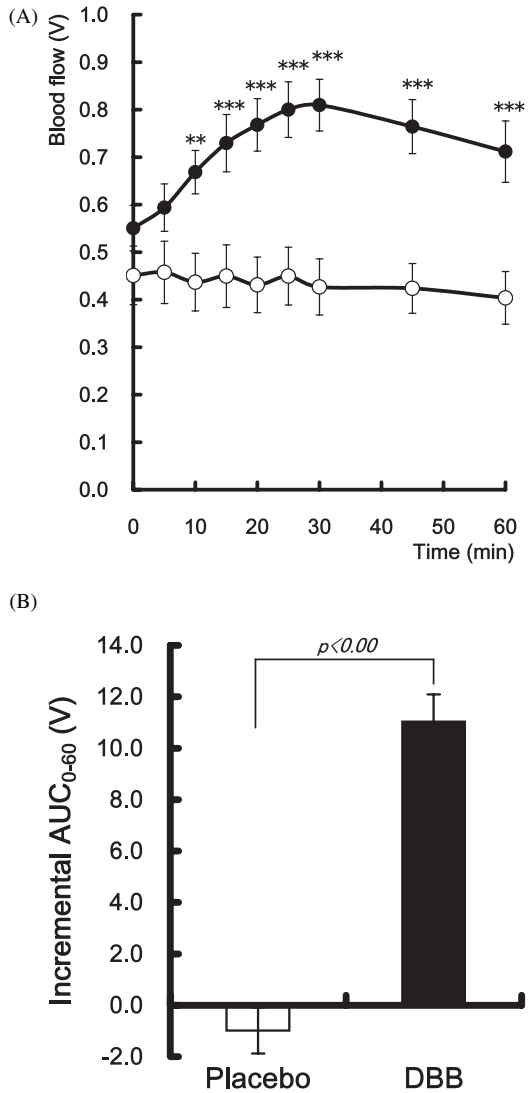

Fig. 1. Effect of DBB and placebo on peripheral blood flow.

(A) Time course of blood flow after test diet ingestion: DBB (●), placebo (○). Values are means ± S.E. (n = 19). $^{**}p < 0.01$, $^{***}p < 0.001$ *vs*. time zero during DBB ingestion. (B) Incremental area under the blood flow-time curves for 60 min ($AUC_{0–60}$) during DBB and placebo ingestion. $p < 0.001$ *vs*. placebo ingestion.

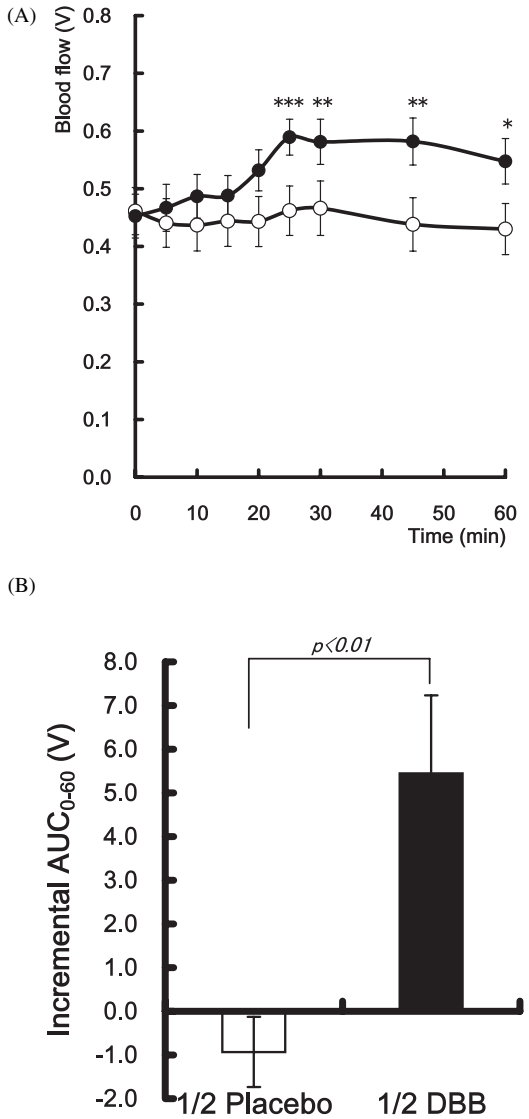

Fig. 2. Effect of 1/2 DBB and 1/2 placebo on peripheal blood flow.

(A) Time course of blood flow after test diet ingestion: 1/2 DBB (●), 1/2 placebo (○). Values are means ± S.E. (n = 16). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs*. time zero during DBB ingestion. (B) Incremental $AUC_{0–60}$ during DBB and placebo ingestion. $p < 0.01$ *vs*. placebo ingestion.

tion. The $AUC_{0–60}$ after subjects consumed 1/2 DBB was higher than that after they consumed 1/2 placebo [Fig. 2(B), $p < 0.01$].

DISCUSSION

The results of this study indicate that the ingestion of DBB acutely increases the peripheral blood flow [Fig. 1(A)], as measured by a laser Doppler blood-flow imaging system. There was a significant increase in the $AUC_{0–60}$ during DBB ingestion, suggesting that the blood vessels were vasodilated due to DBB intake [Fig. 1(B)]. Following the consumption of a half-amount of DBB, a significant increase was also found in peripheral blood flow and its $AUC_{0–60}$ [Fig. 2(A), (B)]. The mean $AUC_{0–60}$ for the 4900 mg DBB ingestion was about 2 times that for 2450 mg DBB, indicating that an orally administered single dose of DBB might have an acute dose-dependent effect on peripheral blood flow. Increasing blood flow may result in smoother blood supply to the periphery, and more effective functioning of capillary blood vessels. Microcirculation is important for thermoregulation, nutrient and oxy-

gen supply, such that sufficient blood circulation has been found to lead to recover from various symptoms related to fatigue,[11,12] and to affect skin condition and appearance.[13,14] DBB has been investigated and discovered to improve physical fatigue,[3] visual fatigue,[5,6] and skin condition.[7] From the results observed in this study, DBB may exhibits these functions due to improve peripheral blood circulation.

The mechanism underlying why DBB increased blood flow has not yet been understood; however, there is evidence from an *in vitro* study to show vasodilatory activity of DBB. Namely, DBB was found to affect vascular smooth muscle contractions through the activation of α-adrenergic receptors rather than through the entry of extracellular calcium ions using rat thoracic aortas without endothelia.[15] Ingestion of DBB was possibly involved in direct action on vascular smooth muscle, expanding the vascular diameter, and thereby increasing blood flow.

Another hypothesis for increasing blood flow during DBB ingestion involves nitric oxide, a key vasodilator and a putative factor for vasodilation. It has been known that the production of vascular endothelial nitric oxide is induced by the ingestion of some food components such as red wine or cacao polyphenol[16] and cassis anthocyanin,[17] and these components have also been reported to show antioxidative actions. A single dose and long term ingestion of cocoa enriched flavanols increased peripheral blood flow in human skin.[13,18] Endothelium-dependent vasodilation was reported to be preserved through the actions of some antioxidants.[19] DBB characteristically contains abundant histidine and anserine, and these compounds are also reported to have antioxidative actions *in vitro*.[20] DBB was reported to decrease the level of derivatives of reactive oxygen metabolites (d-ROMs), known as a biomarker of oxidative stress.[21,22] Since DBB exhibits antioxidative actions *in vivo*, it is possible that the increased blood flow is caused by endothelium-dependent vasodilation due to its antioxidative property.

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 physical and mental fatigue. To our knowledge, this is the first study to investigate the physiological effects of acute DBB ingestion. We found that single-dose DBB ingestion might have an acute dose-dependent effect on peripheral blood flow in healthy subjects. In future studies, it would be of great interest to determine what component of DBB affects the increase of peripheral blood flow, and through what mechanisms.

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