Role of Alginate in the Mechanism by which Brown Seaweed *Saccharina japonica* Intake Alleviates an Increase in Blood Pressure in 2-Kidney, 1-Clip Renovascular Hypertensive Rats

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Abstract

Background: The intake of *Saccharina japonica* (SJ), a widely consumed brown seaweed, has been reported to decrease blood pressure (BP) in hypertensive rats. It has been suggested that this effect is related to an increase in fecal sodium excretion (SE) by alginate (Alg) to the gastrointestinal tract; however, the mechanism is still unclear. This study investigated how different seaweeds with different amounts of Alg suppressed BP increase and enhanced fecal SE in 2-kidney, 1-clip renovascular hypertensive (2K1C) rats given the seaweed diets.

Methods: Rats with 2K1C or sham operation were fed a normal-/high-salt diet with some kinds of seaweeds (5.0%, w/w) or SJ extract with different Alg contents for 6 weeks. I measured systolic BP every week and mean arterial pressure at the end, and measured the total and molecular weights of Alg in each seaweed. Then, I evaluated the relationship of the Alg amount in each seaweed with the suppression of BP increase in 2K1C rats. Finally, urinary and fecal SE for 24 h was measured.

Results: The intake of SJ, SJ extract, *Saccharina ochotensis* (SO) blades and SO roots suppressed BP increase in 2K1C rats, but the strength was not proportional to the amounts of Alg contained in the seaweeds. Although SJ intake increased fecal SE in 2K1C rats fed a high-salt diet, the fecal SE was much less than urinary SE.

Conclusion: The sodium excretion in feces by Alg in SJ may not be one of the major mechanisms by which SJ intake attenuates hypertension in 2K1C rats.

List of Abbreviations

Alg, alginate

AngII, angiotensin II

ANOVA, analysis of variance

BP, blood pressure

BW, body weight

CTL, a control diet

DASH diet, the Dietary Approaches to Stop Hypertension diet

DOCA, deoxycorticosterone acetate

HS, high-salt

SJ, *Saccharina japonica*

SJE, *Saccharina japonica* extracts

SO, *Saccharina ochotensis*

MAP, mean arterial pressure

Mw, molecular weight

NS, normal-salt

SBP, systolic blood pressure

SD, Sprague-Dawley

SE, standard error

SHAM, sham-operated control

SHR, spontaneously hypertensive rat

UP, *Undaria pinnatifida*

2K1C, 2-kidney, 1-clip renovascular hypertension

Introduction

Hypertension is often accompanied by diabetes, dyslipidemia and obesity and promotes arteriosclerosis, which contributes to the development of cardiovascular diseases.[1,2] Therefore, preventing from hypertension is crucial for inhibiting the onset of the diseases. According to recent studies, the risk of cardiovascular diseases is increased by an inappropriate lifestyle such as unbalanced diets including excessive intake of dietary salt and saturated fatty acid, and lack of exercise and stress.[3–6] Lifestyle modifications, including dietary changes, exercise and avoiding stress, were the major non-pharmacological approaches to hypertension prevention.[7–9] In particular, low-salt intake plays a significant role in alleviating high blood pressure (BP) development and progression in humans.[10–12] A meta-analysis of prospective cohort studies revealed that the group with the lowest average sodium intake (3–5 g/day) has the lowest risk of cardiovascular events and death.[13] In addition to restricting dietary salt intake, the Dietary Approaches to Stop Hypertension (DASH) diet was adopted as one of the strategies to prevent hypertension. It recommended the consumption of fruits, vegetables, grains, low-fat dairy products and foods rich in potassium, magnesium, calcium and phosphorus like seaweeds.[14,15] Further, a previous study demonstrated that the combination of sodium intake reduction and DASH diet adoption was more effective in preventing and treating

hypertension.[16] Indeed, according to the Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC Study) and the Japan Public Health Center-Based Prospective (JPHC) Study,[17–19] there was an inverse association between seaweed intake and cardiovascular mortality among Japanese men and women. As well, a cross-sectional study found that seaweed consumption in childhood can prevent cardiovascular disease in adulthood.[20] Furthermore, interventional studies and animal experiments have demonstrated that seaweed intake may lower BP in healthy children,[21] hypertensive elderly patients,[22] and hypertensive rats.[23–27]

Saccharina japonica (SJ), a widely consumed brown seaweed, has traditionally been consumed in Asian countries. Some studies reported seaweeds to have various bioactive effects, such as anticoagulant,[28] antiviral,[29] antioxidant,[29,30] anti-inflammatory,[31] and anti-obesity activities,[31–33] lipid metabolism improvement,[32] and gut microbiota alteration.[32,33] It was reported that chronic intake of diets containing 5% (w/w) SJ attenuates BP increase for 7 weeks from a very young age in spontaneously hypertensive rats (SHR) and 5 weeks in young Wistar rats fed with 1.5% saline solution.[23,24] SJ contains numerous kinds of minerals, carotenoid fucoxanthin and dietary fibers, such as alginate (Alg), fucoidan and laminarin. According to research findings, these components exhibit a variety of physiological functions. In this study, I focused on

Alg, which is the most abundant dietary fiber in SJ. Alg in SJ is considered to be involved in the antihypertensive effects of SJ intake. Alg is a naturally occurring polyuronic acid composed of two conformational isomer residues, namely, β-D-mannuronic acid (M) and α-L-guluronic acid (G), linked by β-1,4-glycosidic bonds. Alg polymer blocks have three different types: MM, GG and MG. The M/G ratio and the composition of the blocks vary by species, parts and producing area on seaweeds.[34] The higher the percentage of GG, the greater the degree of binding to divalent ions and the viscoelasticity of the gel.[35] The variety of biological activities in Alg include antitumor activities,[36] antioxidant activities,[37–42] mucosal protective effects,[43,44] pepsin activity reduction,[45] anti-inflammation,[37,38,40,46,47] low lipid levels,[47] antihypertension,[40,48–50] anti-obesity,[51,52] excretion of harmful heavy metals and micro-minerals in feces,[53,54] and gut microbiota alteration.[47,55] Among others, BP increase induced by an intake of 1.0% NaCl drinking water was attenuated by a 1.0% NaAlg diet consumed for 20 days in Wistar rats.[24] Additionally, administration of potassium Alg (KAlg) to SHR for 21 days[56] and to deoxycorticosterone acetate (DOCA)-salt rats for 30 days[57] inhibited BP increase in a dose-dependent manner. Furthermore, according to previous reports,[58,59] intake of 4.0% or 8.0% NaAlg oligosaccharide diet for 7 weeks attenuated BP increase in a dose-dependent manner. A study demonstrated that in Dahl-salt rats fed a high-salt diet, the

use of continuous subcutaneous osmotic micropump of Alg oligosaccharide administration (60 mg/day) almost eliminated hypertension.[60]

Renovascular hypertension is a prevalent type of secondary hypertension in humans.[61] The 2-kidney, 1-clip renovascular hypertension (2K1C) animal model was created by activating the renin-angiotensin-aldosterone system.[62,63] Increased levels of angiotensin II led to sympathicotonia, oxidative stress and alterations in sodium and water balance.[64–66] It was previously observed that pressure natriuresis and diuresis enhance sodium and water reabsorption in 2K1C rats. Our previous study demonstrated that the BP of 2K1C rats fed an SJ diet was significantly decreased compared with that of 2K1C rats fed a control diet.[67,68] However, to our knowledge, the detailed mechanism by which SJ intake inhibits BP increase is currently unclear.

Past studies demonstrated that the intake of Alg has an antihypertensive effect on hypertensive rat models because it excretes sodium in feces.[48,55,69] Therefore, it is assumed that the suppression of BP increase by seaweed intake in hypertensive rat models is also related to the sodium excretion in feces by Alg in SJ. Then, seaweed with high Alg content may have a stronger antihypertensive effect than seaweed with low Alg content. However, in our preliminary study, the intake of SJ extract (SJE; “Kombu-Dashi” in Japanese) produced using the typical extraction method inhibited BP increase in 2K1C

rats by no less than the intake of SJ. The amount of Alg in the SJ was much less than the original amount of SJ from which the SJE was extracted. The observation questioned the theory that the suppression of BP increase by seaweed intake is due to the sodium excretion in feces by Alg in seaweed.

To verify this theory, I investigated: 1) whether the prevention of hypertension in 2K1C rats fed a seaweed diet is dependent on the amount of Alg in the seaweed and 2) whether an increase of fecal sodium excretion by the binding of sodium ion to Alg in the gastrointestinal tract can be a major mechanism by which SJ intake attenuates hypertension in 2K1C rats. This study examined the role of Alg on the mechanism of hypertension alleviation in 2K1C rats by observing whether and how BP increase was suppressed by an intake of seaweeds containing different amounts of Alg in 2K1C rats, as well as fecal sodium excretion in 2K1C rats fed an SJ diet.

Methods

Animals and treatment

Four-week-old male Sprague-Dawley (SD) rats obtained from Japan SLC Co., Ltd. (Shizuoka, Japan) were housed in individual cages in temperature- and moisture-controlled rooms (21 ± 1°C, 60 ± 10%) equipped with artificial lighting set on a 12-h day/night cycle. They had free access to standard rat chow (CE-2, CLEA Japan, Inc., Tokyo, Japan) and water for 2 weeks. Protocol approval was obtained from the Animal Experiment Committee of Kobe Women's University (A106, A120, A128, A138, A200, A207 and A230).

To induce 2K1C model of renovascular hypertension, the rats were anesthetized with a mixture of 0.15 mg/kg medetomidine hydrochloride, 2 mg/kg midazolam and 2.5 mg/kg butorphanol injected intraperitoneally. Attached to the rats was a silver clip with an internal diameter of 0.254 mm on the left renal artery as previously described.[70–72] Meanwhile, a sham control group (SHAM) of rats received a similar surgical intervention, except that the silver clip was not attached. After surgery, both the SHAM and 2K1C rats started receiving control diet or experimental diets for 6 weeks.

Diets

Four species of brown seaweed, *Saccharina japonica* (SJ; “Ma-kombu” in Japanese), *Saccharina ochotensis* (SO; “Rishiri-kombu”), *Undaria pinnatifida* (UP; “Wakame”) and its sporophyll (UPS; “Mekabu”), were harvested in Japan. Two parts of SO were used: the blades (SO-B) and the roots (SO-R). These species were freeze-dried and powderized using a mixer mill for the experiments. I tested how the intake of these seaweeds with different Alg contents alleviated BP increase in 2K1C rats. SJE was produced using a typical extraction method, traditionally used for the preparation of Japanese cuisine. Thirty grams of SJ was placed in a beaker with 1,000 mL of water and allowed to stand for 30 min. After soaking, SJ in water was set over heat and simmered for 7 min until the water was almost boiling. Then, SJ was removed from the water, and the obtained extracts were allowed to cool. To prepare for the experiment, the SJ extract (SJE) was freeze-dried and powderized. The amount of SJE used in the diet was the same as that extracted from SJ contained in the corresponding SJ diet.

The experimental diets were mixed with the seaweed and control diet (5:95, w/w). For the control diet, I used a standard diet (CE-2) in all the experiments, except for the experiment in which the effects of high-salt diet were observed. In this experiment, a purified normal-salt (NS; 0.7% NaCl) standard diet (AIN93G, CLEA Japan, Inc., Tokyo,

Japan) or a purified high-salt (HS; 6.0% NaCl) standard diet (AIN93G) was used as a control diet to adjust the salt content in each diet. The experiments were also performed adjusting the salt contents and energy intake to be equal among the groups.

Protocol observing the effect of diets with seaweeds on BP and sodium excretion

After surgery, the 2K1C rats started receiving a control diet with or without 5.0% (w/w) seaweed (SJ, SJE, SO-B, SO-R, UP or UPS) via pair feeding for 6 weeks. The SBP and body weight (BW) were measured every week, as described below. The BW was not significantly different among the groups throughout each experiment. At the end of the experiment, the mean arterial pressure (MAP) was measured under anesthesia as described above.

Urine and feces were collected for 24 h through a simple metabolic cage to measure the urinary and fecal sodium excretion for 5–6 weeks after surgery. The urine was filtered, and the feces were burned to ashes in a 550℃ furnace and extracted in the HCl solution. The sodium levels of the urine and feces were measured using a polarized Zeeman atomic absorption spectrophotometer (Hitachi Z-2010; Hitachi, Ltd., Tokyo, Japan).

Blood Pressure Measurements

SBP was evaluated as described previously.[71,72] and measured using a tail-cuff method utilizing the MK-1030 NIBP monitor (Muromachi Kikai Co., Ltd., Tokyo, Japan) in conscious animals every week. Before taking the measurements, the rats were restrained and placed in a chamber under 38℃ and maintained for 10 min to easily detect arterial pulsation in the tail in order to stabilize their SBP. SBP was measured 10 times consecutively using the average value for evaluation.

For the MAP measurement, the method previously described was used.[71,72] The left femoral artery was catheterized with a polyethylene tubing (PE-10; Becton Dickinson, Sparks, MD) attached to a pressure transducer and recorder. The MAP was continuously monitored using a PowerLab System with a signal amplifier (AD Instruments, Belle Vista, Australia) hooked up to a BP transducer (AR611G; Nihon Kohden Corp., Tokyo, Japan). The data used was collected during the last 3 min of a 10-min stabilization period.

Abstration of Alg from brown seaweed

Alg was abstracted from brown seaweed using high temperature alkaline extraction described by Davis et al.[73] with slight modifications. One gram of dried seaweed powder was treated twice with mild depigmentation and defatting in 30 mL of

acetone for 1 h at room temperature. Supernatant was eliminated by centrifugation at 10,000 rpm for 15 min at 20℃. The residue was treated with 30 mL of 0.1 M HCl at 60℃ for 2 h under constant stirring to eliminate the supernatant by centrifugation at 10,000 rpm for 15 min at 20℃. Then, it was washed with distilled water and treated with 30 mL of 3.0% Na_2CO_3 at 60℃ for 2 h under constant stirring to solubilize the Alg in sodium salt form. The supernatant was eliminated after centrifugation at 10,000 rpm for 15 min at 20℃. The residues were quantified Alg concentrations using the carbazole sulfate method and precipitated twice with the volume of absolute ethanol. Some of the precipitate was recovered by centrifugation at 3,000 rpm for 10 min at 20℃, and suspended in distilled water. The Alg in it was purified by second precipitation with absolute ethanol. The other of the precipitate was resuspended in distilled water and dialyzed and freeze-dried to yield Alg powder. The Alg powder was used to measure the average molecular weight (Mw) and viscosity of Alg.

Characteristics of Alg obtained from seaweeds

The average Mw of Alg extracted from brown seaweed was determined by size-exclusion chromatography. High-performance liquid chromatography (HPLC) (JASCO LC Net II/ADC, Nihon-Bunko Co., Tokyo, Japan) was employed for analysis using a

column of TSKgel G5000PW (7.8 i.d. × 600 mm, Tosoh Co., Tokyo, Japan) and a refractive index detector (RI-1530, Nihon-Bunko Co., Tokyo, Japan). The mobile phase consisted of 0.2M sodium phosphate buffer (pH 6.8) dissolved in ultrapure water and filtrated with an Omnipore membrane filter with a pore size of 0.45 μm (Merck Millipore JVWP04700, Billerica, MA, USA) under reduced pressure. The eluent was pumped at a flow rate of 1.0 mL/min, with a volume injection of 50 μL. The analyses were conducted at room temperature. The samples were dissolved to 10 mg/mL in ultrapure water and filtered through a 0.45-μm cellulose acetate membrane (DISMIC filter; ADVANTEC, Toyo Roshi Kaisha, Ltd., Tokyo, Japan) to remove dust particles. Pullulan P-100 ($Mw=11.2 \times 10^4$), P-200 ($Mw =21.2 \times 10^4$), and P-800 ($Mw =78.8 \times 10^4$) (Showa Denko K. K., Tokyo, Japan) were used as the molecular standards. I evaluated the relationship of the extent of suppression of BP increase in 2K1C rats to not only the amount of Alg but also that corrected by the average Mw.

It was reported that the average Mw and the viscosity of Alg have a significant correlation.[74–76] Actually, our data on the seaweeds in this study demonstrated the correlation ($R = 0.90$, $P < 0.05$, Table 1). Therefore, I used viscosity to Alg as well as the average Mw for correcting the amount of Alg to evaluate the relationship of it to BP increase suppression. After the Alg powder extracted from seaweeds was dissolved in

ultrapure water to a concentration of 0.5% (w/w), the viscosity of Alg was determined using a Brookfield viscometer (LVT; Brookfield Engineering Laboratories, Inc., Middleboro, MA) at 30 rpm with spindle no. 2.

Statistical Analysis

Data were expressed as mean ± standard error (SE). The results were evaluated via analysis of variance (ANOVA), followed by Tukey's correction for multiple comparisons. Spearman's rank correlation coefficients were evaluated for the association between two variables. The statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS) version 23 (IBM Co., Chicago, IL). A P value < 0.05 was considered significant.

Results

Effects of dietary SJ and SJE intake on SBP in 2K1C rats

The SBP was significantly higher in 2K1C rats fed a CTL diet (2K1C-CTL) than in SHAM rats fed a CTL diet (SHAM-CTL) throughout the experimental period ($P < 0.01$; Figure 1). The SBP in 2K1C rats fed a SJ diet (2K1C-SJ) was significantly lower than that in 2K1C-CTL throughout the experimental period ($P < 0.05$). The MAP was significantly higher in 2K1C-CTL than in SHAM-CTL (152 ± 3 vs 136 ± 4 mmHg; $P < 0.01$). The MAP was lower in 2K1C-SJ than in 2K1C-CTL at marginal significance (145 ± 5 vs 152 ± 3 mmHg; $P = 0.05$). These findings, as well as the data of 2K1C-CTL and -SJ groups fed a purified diet, presented in Figure 2, confirmed that SJ intake attenuates BP increase in 2K1C rats.

Figure 2 shows that the SJE diet and SJ diet significantly decreased the SBP compared with the control diet in 2K1C rats throughout the experimental period ($P < 0.001$ for SJE, $P < 0.01$ for SJ). It also indicates that the SBP in 2K1C-CTL similarly had a significant increase compared with SHAM-CTL ($P < 0.001$). The carbazole sulfate method indicated that the Alg contained in an original SJ and in SJE extracted from the same amount of the SJ was 243.4 ± 15.9 and 48.7 ± 5.5 (mg/g dry weight), respectively. Thus, SJE was contained Alg only 20% as much as Alg in the original SJ. Put together,

intake of SJ and SJE was suggested to attenuate hypertension in 2K1C rats, regardless of the amounts of Alg contained in the diet.

Effects of seaweed intake with different amounts of Alg on SBP in 2K1C rats

The SBP was significantly higher in 2K1C-CTL than in SHAM-CTL ($P < 0.001$) and significantly lower in 2K1C-SO-B and 2K1C-SO-R than in 2K1C-CTL throughout the experimental period ($P < 0.001$, each; Figure 3). 2K1C-SO-B exhibited a more significant reduction in SBP compared with 2K1C-SO-R throughout the experimental period ($P < 0.05$). As can be seen from the Table 1, SO-B contained less Alg than SO-R. However, SO-B decreased BP more compared with SO-R.

As shown in Figure 4, the SBP was significantly higher in 2K1C-CTL than in SHAM-CTL throughout the experimental period ($P < 0.001$); moreover, neither 2K1C-UP nor 2K1C-UPS demonstrated any significant difference in the SBP compared with 2K1C-CTL ($P = 0.99$ for UP, $P = 0.83$ for UPS). While UP contained more Alg than SJ and UPS contained less Alg than SJ (Table 1), neither UP nor UPS attenuated hypertension in 2K1C rats. These observations suggested that the effects of seaweed diets on hypertension are not dependent on the Alg contained in them.

Correlation between the Alg concentration in seaweed and changes in SBP in 2K1C rats fed the seaweed diet.

The Table 1 presents the concentration, average Mw and viscosities of Alg in each seaweed and diet. As described in Methods, the viscosity was significantly correlated with average Mw ($P < 0.05$). Figure 5(a) demonstrated that the Alg concentrations were not associated with SBP in 2K1C rats fed seaweed diets. Also, the Alg concentrations corrected for neither the average Mw nor the viscosities were associated with the decrements in SBP by the diets in 2K1C rats (Figures 5(b) and 5(c)). These findings indicated that the effects of seaweed diets on hypertension are independent of the amount of Alg contained in the unit amount of seaweeds.

Effects of dietary SJ intake on SBP and fecal sodium excretion in 2K1C rats fed a high-salt diet.

Even in the case of a high-salt diet, the SBP was significantly higher in 2K1C-HS-CTL than in SHAM-HS-CTL ($P < 0.001$; Figure 6(a)). Significant differences were already observed after a week and maintained throughout the study. The SBP in 2K1C-HS-SJ was significantly lower than that in 2K1C-HS-CTL throughout the experimental period ($P < 0.05$). Conversely, there was no significant difference in the SBP between

SHAM-NS-CTL and SHAM-HS-CTL ($P = 0.99$) or between SHAM-HS-CTL and SHAM-HS-SJ ($P = 0.21$). The MAP in 2K1C-HS-CTL was significantly higher than that in SHAM-HS-CTL (153 ± 2 vs 132 ± 4 mmHg; $P < 0.01$). The MAP in 2K1C-HS-SJ was significantly lower than that in 2K1C-HS-CTL (140 ± 5 vs 153 ± 2 mmHg, $P < 0.05$; Figure 6(b)). No significant differences were observed in the MAP between SHAM-NS-CTL and SHAM-HS-CTL ($P = 0.54$), between SHAM-HS-CTL and SHAM-HS-SJ ($P = 0.92$) or between SHAM-HS-SJ and 2K1C-HS-SJ ($P = 0.30$). These findings indicated that SJ intake attenuates hypertension in 2K1C rats even when fed a high-salt diet.

As can be seen from Figure 6(c), the 24-h urinary sodium excretion in the SHAM group was increased by a high-salt diet intake ($P < 0.001$). The urinary sodium excretion in SHAM-HS-SJ was significantly lower than that in SHAM-HS-CTL ($P < 0.05$). No significant difference was observed in the urinary sodium excretion between SHAM-HS-CTL and 2K1C-HS-CTL ($P = 0.11$) or between 2K1C-HS-CTL and 2K1C-HS-SJ ($P = 0.21$). Contrarily, the urinary sodium excretion in 2K1C-HS-SJ increased compared with that in SHAM-HS-SJ ($P < 0.05$). As can be seen from Figure 6(d), a high-salt diet induced an increase in fecal sodium excretion compared with a normal-salt diet in SHAM rats ($P < 0.05$). In SHAM rats, SJ intake did not significantly change the fecal sodium excretion ($P = 0.15$). Nevertheless, 2K1C-HS-SJ exhibited a significant increase

in fecal sodium excretion compared with 2K1C-HS-CTL ($P < 0.01$). These findings indicated that SJ intake increases fecal sodium excretion in 2K1C rats fed a high-salt diet. However, the amounts of sodium excretion by feces were much less than those by urine: the increment in fecal sodium excretion by SJ was no more than 0.5% of the total excretion by urine and feces.

Discussion

Chronic intake of 5.0% (w/w) SJ diet for 7 weeks was reported to attenuate the BP increase in SHR at 3–10 weeks of age,[23] whereas intake for 28 days attenuated the BP increase in 5–9-week-old Wistar rats fed with 1.5% saline solution.[24] This study demonstrated that the BP of 2K1C rats fed an SJ diet decreased compared with that of 2K1C rats fed a control diet (Figures 1, 2 and 6). These findings indicated that SJ intake may attenuate hypertension in 2K1C rats together with SHR and high-salt-induced hypertensive rat models.

Previous studies demonstrated that the effect of Alg on the increase in sodium excretion in feces was involved in the clampdown of BP increase by SJ intake.[48,55,69] The administration of sodium Alg decreased BP in a dose-dependent manner.[55–58,69] Based on these findings, I assumed that the intake of SJE, which includes small amounts of Alg, would have a minimal inhibitory effect on BP increase. However, the study demonstrated that, contrary to the expectations, the increase in the BP of 2K1C rats was alleviated by SJE intake, which contained approximately 20% of the Alg content of SJ (Figure 2). Therefore, I found that both SJ and SJE have a preventive effect on hypertension regardless of the amount of Alg contained in 2K1C rats. SJ equivalent of daily food intake is too much to be consumed by humans. However, the amount of SJE can be easily mixed

with meals of humans. These results may have important implication for the antihypertensive effect of SJE. Thus, SJE, that is “Kombu Dashi”; one of typical “Dashi”, may help prevent renovascular hypertension in humans through routine consumption of food such as Japanese food “Washoku” which is frequently seasoned with “Dashi”. However, the problem of the excessive amount of iodine contained in SJ and SJE[77–79] should be solved before putting into practice. Therefore, it is very beneficial to develop a cooking method which retains the active ingredients and removes iodine as much as possible from SJ and/or SJE. After the development, I may be able to utilize the effects of SJ/SJE easily through meals to prevent hypertension. That is why I need to explore the mechanism by which SJ attenuates hypertension and the active ingredients in SJ in the mechanism.

This study also investigated the extent to which seaweeds with different Alg contents suppressed the BP increase in 2K1C rats in order to examine the role of Alg in the mechanism of hypertension alleviation in the rats. Since the roots of SJ have more Alg content than the leaves,[80] I compared the degree of suppression of BP increase between the intake of the roots, SO-R, and that of the blades, SO-B, in 2K1C rats. I found that the degree of suppression was higher in SO-B than in SO-R, contrary to the case of Alg contents. In addition, UP was reported to contain more Alg than UPS.[81] Previous

studies have demonstrated that intake of UP or UPS reduced BP increase in SHR[25] and patients with hypertension,[22] but not in Wistar rats fed with 1.5% saline solution or stroke-prone spontaneously hypertensive rats.[24,82] In a previous study, the administration of angiotensin-converting enzyme (ACE) inhibitory peptide, isolated from the hot water extract of UP, suppressed the BP increase in SHR.[83] In the study, neither UP nor UPS intake exhibited any significant inhibitory effects on the BP increase in 2K1C rats, although both contain Alg.

From the above results, the amount of Alg in each of the seaweed, namely, SJ, SO, UP and UPS, was not correlated with the suppression of BP increase by an intake of each seaweed diet in 2K1C rats (Figure 5(a)). Since the average Mw of Alg contained in each seaweed could be different, I need to compare to the amount of Alg in a given amount of seaweeds due to adjusting in the Mw and/or viscosity of Alg. The differences in the amount of Alg adjusted for them in seaweed may impact sodium absorption in gastrointestinal tract; that is, the more the amount of Alg adjusted for them is, the more Alg absorbs salt, which may result in increased amounts of excreted sodium into feces. I examined whether the amount of Alg adjusted for its average Mw or its viscosity in the unit amount of seaweed was related to BP increase suppression by seaweed diet intake in 2K1C rats. However, no relationship was found between each adjusted amount of Alg in

the unit amount of seaweed and the suppression of BP increase (Figures 5(b) and 5(c)). Therefore, I concluded that the attenuation of hypertension by seaweed intake in 2K1C rats does not depend on the amount nor the Alg contents adjusted for its average Mw or its viscosity in the seaweed.

Several studies indicated that Alg intake increased fecal sodium excretion and decreased BP in hypertensive rat models, suggesting that Alg adsorbs sodium ion in the gastrointestinal tract and inhibits the uptake of dietary sodium in the body.[48,55,69] A study has reported that a diet with 7.0% *Laminaria digitata*, which belongs to the same genus of seaweed as SJ, increased fecal sodium excretion in Wistar rats fed a 2.0% cholesterol-enriched diet.[84] In the present study, SJ intake significantly increased fecal sodium excretion and suppressed BP increase in 2K1C rats fed a high-salt diet (Figure 6). The excretion of sodium ions in feces might be involved not only in the mechanism of the effect of chronic Alg intake in hypertension alleviation but also in the mechanism of the effects of the intake of SJ containing Alg. In this study, nevertheless, the amount of sodium ion excreted in feces was about one-sixtieth compared with the amount of sodium excreted in urine (Figures 6(c) and 6(d)). These findings indicated that the contribution of sodium excretion through feces by SJ was very limited in reducing sodium absorption into the whole body. These mean that an increase in fecal sodium excretion by Alg may

not be the major mechanism by which SJ intake attenuates hypertension in 2K1C rats. This finding provides new evidence against the previous theory that an intake of Alg contained in seaweed significantly suppresses BP increase or prevents hypertension via excreting salt into feces by absorbing salt to Alg in intestine.

Some of the previous studies have reported that BP increased[85–89] and others have reported that BP remained unchanged[90–97] when some kinds of normotensive rats were fed a high-salt diet or provided with sodium chloride in a drinking water, not considering species differences. In the present study, the high-salt diet did not significantly increase BP compared with the normal-salt diet in a normotensive model of SHAM rats (Figures 6(a) and 6(b)). Moreover, SJ intake did not decrease BP in SHAM rats when fed a high-salt diet. These findings indicated that SJ intake has an antihypertensive effect on the hypertensive rat models but no effect on BP increase suppression in the normotensive rat models, unlike many kinds of antihypertensive drugs that suppress BP increase in both hypertensive and normotensive rat models. As shown in Figure 6(c), urinary sodium excretion was decreased in SHAM rats fed a high-salt diet with SJ compared with SHAM rats fed a high-salt diet. Thus, SJ intake did not reduce BP in SHAM rats fed a high-salt diet, maybe through the effect of maintaining BP by facilitating sodium absorption in kidney of SHAM rats against reducing BP by SJ intake,

although further studies are needed to certify it.

Some studies have reported that urinary sodium excretion remained unchanged in 2K1C rats,[98] whereas others have reported an increase[99,100] or decrease[101] in urinary sodium excretion when compared with that in SHAM rats. In the present study, no significant differences were observed in the urinary sodium excretion between SHAM rats and 2K1C rats (Figure 6(c)). 2K1C rats exhibited increased activity of the RAAS and an initial transitory increase in daily water intake.[102] I considered that it may be due to the influence of an increase in water intake on the urinary sodium excretion in 2K1C rats because the rats were allowed free access to tap water in this study.

I also measured the viscosity of the diets digested using the pepsin-pancreatin enzyme method by Boisen and Fernández et al. [103] with some modifications, in order to explore the relationship between an overall condition of each diet assumed after digestion in the intestine and the effect on BP (Figure 7 (a)). Briefly, each diet was digested with pepsin/HCl to simulate the gastric conditions, followed by a digestion with pancreatic juice to simulate intestinal digestion. As a result, the artificially-digested diet viscosity was inversely correlated with the suppression of BP increase in 2K1C rats with statistical significance (Figure 7 (b)). This preliminary observation might lead to elucidation of the mechanism by which SJ attenuates BP increase in 2K1C rats. Seaweed contains dietary

fiber, including not only Alg but also fucoidan, laminarin and pectin, which may increase the viscosity of the seaweed. Since the dietary fiber is reported to have antioxidant and immunomodulatory activities,[104] they may be involved in the prevention from hypertension by seaweed intake. Further investigation is required to reveal the involvement of dietary fiber including Alg as a whole, as well as of dietary fiber other than Alg, contained in seaweed, in the suppression of BP increase in rats with renovascular hypertension. In addition, the dietary fiber may effect on BP through the intestinal mucosa and changes of the gut microbiota. Some of gut microbiota ferment dietary fiber and produce short chain fatty acids,[47,50,55,105] which may induce vasodilation and lower BP through activation of cell surface G-protein coupled receptors, like GPR41 and GPR43.[106] Therefore, it is necessary to observe the signaling to BP regulation through direct actions on the receptors.

This study has some limitations. First, although I investigated the relationship of the amount of Alg contained in seaweeds with the effect of suppressing BP increase, not all Alg contained in seaweeds may have effects on BP, but a part of Alg must be effective after digestion. Therefore, our consideration was made on the assumption that the proportion of Alg acting on BP is almost constant independent of the type of seaweed which contains the Alg. Especially, SO-B and SO-R are corrected from a species of

seaweed, and so are UP and UPS. Even if some of the seaweeds have the different proportion, the conclusion of this study, on the basis of multiple other grounds, would not change. However, I need to verify this assumption in the next study, in order to investigate the mechanism in detail by which SJ attenuates BP increase in hypertension. In addition, it is reported the intake of potassium has a diuretic effect and an antihypertensive effect.[107–110] Previous studies reported that potassium Alg intake increased urinary sodium excretion in the DOCA–salt hypertensive rat models.[57] Therefore, further research is needed to confirm the role of potassium in SJ. Moreover, it is necessary to investigate the largeness of the effects of Alg with different Mw in the gastrointestinal tract. These studies are our next step and are important to further elucidate the mechanisms of the preventive effects against hypertension by SJ intake.

In summary, the study has demonstrated that an increase in fecal sodium excretion by the binding of sodium ion to Alg in the gastrointestinal tract may not be a major mechanism by which SJ intake attenuates hypertension in 2K1C rats. This is because 1) the amounts of Alg in a seaweed diet were not related to the suppression of BP increase in 2K1C rats fed the seaweed diets; 2) the intake of SJE, which contains Alg only 20% as much as Alg in SJ, suppressed BP increase to an extent similar to that of the SJ intake in 2K1C rats; and 3) the amount of sodium ion excreted in feces was much less

compared with that excreted in urine in 2K1C rats fed a high-salt diet with SJ. These findings may help elucidate the mechanism of the antihypertensive effect of SJ/SJE intake.

Conclusion

The sodium excretion in feces by Alg in SJ may not be one of the major mechanisms by which SJ intake attenuates hypertension in 2K1C rats.

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Table

Table 1. Characteristics of Alg extracted from brown seaweed, such as its concentration, average molecular weights and viscosity.

Dietary group	Alg concentration (mg/g dry weight)	The average M_w of Alg (kDa)	The viscosity of Alg solutions (mPa·s)
SJ	219.8 ± 5.6	113.70	7.6 ± 0.7
SO-B	216.8 ± 12.8 $^{\dagger\dagger\dagger, \ddagger\ddagger\ddagger, \S\S\S}$	119.70	7.8 ± 0.4 ††
SO-R	272.3 ± 2.8 $^{***, \ddagger\ddagger\ddagger, \S\S\S}$	137.69	11.0 ± 0.5 **
UP	387.5 ± 10.0 $^{***, \dagger\dagger\dagger, \S\S\S}$	129.20	9.2 ± 0.2
UPS	150.1 ± 0.9 $^{***, \dagger\dagger\dagger, \ddagger\ddagger\ddagger}$	118.02	8.0 ± 0.5 ††

Values are expressed as mean ± SE, n = 3. One-way ANOVA for the Alg concentration and viscosity of Alg solutions: $P < 0.001$ for diet (CTL *vs* SJ *vs* SO-B *vs* SO-R *vs* UP *vs* UPS). $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs* SJ, $^{\dagger\dagger}P < 0.01$ and $^{\dagger\dagger\dagger}P < 0.001$ *vs* SO-R, $^{\ddagger\ddagger\ddagger}P < 0.001$ *vs* UP, $^{\S\S\S}P < 0.001$ *vs* UPS. There were no significant differences in the average M_w of Alg among each dietary group. The viscosity of Alg solutions was significantly correlated with the average M_w ($R = 0.90$, $P < 0.05$). Abbreviations: Alg, alginate; M_w, molecular weight; SJ, *Saccharina japonica*; SO-B, blades of *Saccharina ochotensis*; SO-R, roots of *Saccharina ochotensis*; UP, *Undaria pinnatifida*; UPS, sporophyll of *Undaria pinnatifida*.

Figures

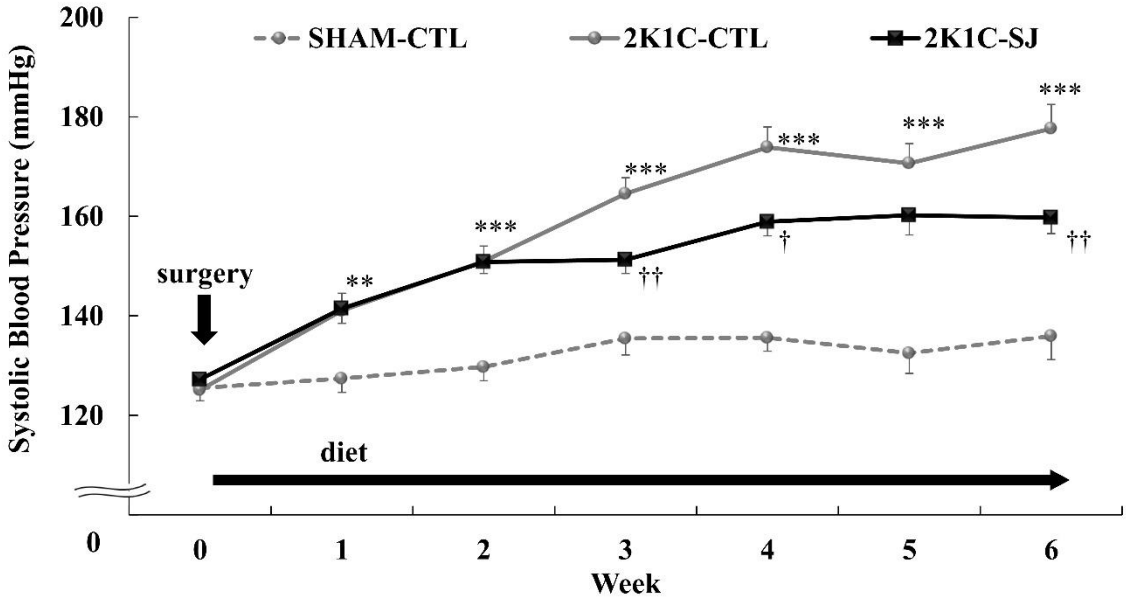

Figure 1. Systolic blood pressure obtained using a tail-cuff method in SHAM or 2K1C rats fed a CTL or SJ for 6 weeks.

Values are expressed as mean ± SE, n = 12–18. Three-way ANOVA: $P < 0.001$ for time, animal (SHAM *vs* 2K1C), diet (CTL *vs* SJ), time × animal and time × diet. $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs* SHAM-CTL, $^{\dagger}P < 0.05$ and $^{\dagger\dagger}P < 0.01$ *vs* 2K1C-CTL. Abbreviations: SHAM, sham-operated control rats; 2K1C, 2-kidney, 1-clip hypertensive rats; CTL, a control diet; SJ, a diet with *Saccharina japonica*; SE, standard error; ANOVA, analysis of variance.

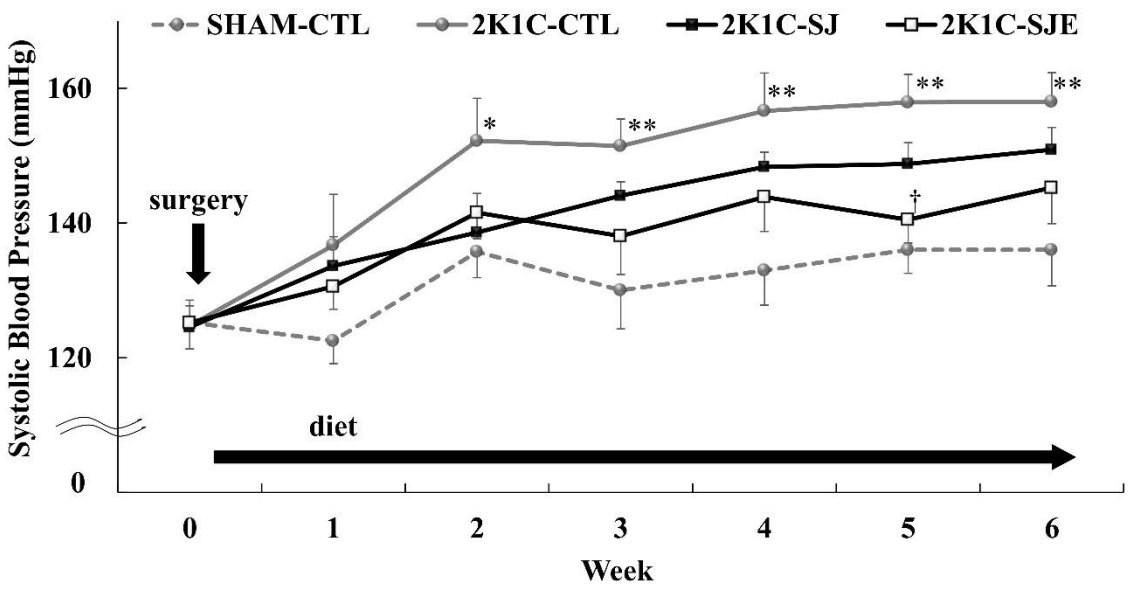

Figure 2. Systolic blood pressure obtained using a tail-cuff method in SHAM or 2K1C rats fed a CTL, SJ or SJE for 6 weeks.

Values are expressed as mean ± SE, n = 5. Three-way ANOVA: $P < 0.001$ for time, animal (SHAM *vs* 2K1C) and diet (CTL *vs* SJ *vs* SJE). Two-way ANOVA: $P < 0.01$ for 2K1C-CTL *vs* 2K1C-SJ and $P < 0.001$ for 2K1C-CTL *vs* 2K1C-SJE. $^{*}P < 0.05$ and $^{**}P < 0.01$ *vs* SHAM-CTL, $^{\dagger}P < 0.05$ *vs* 2K1C-CTL. Abbreviations: SJE, a diet with *Saccharina japonica* extract. See legend of Figure 1 for the other abbreviations.

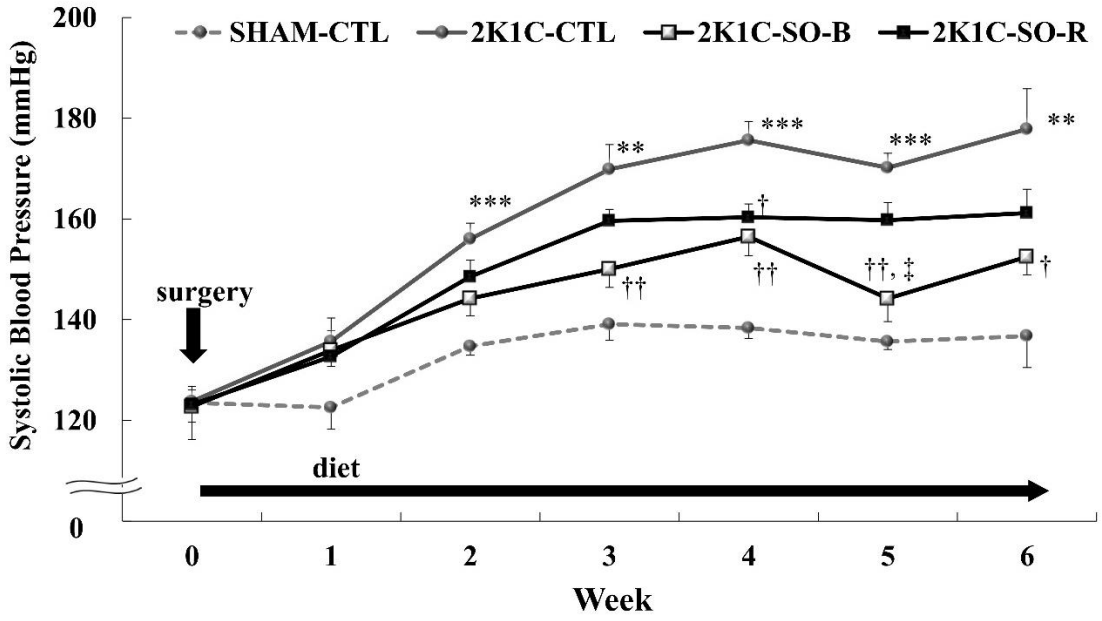

Figure 3. Systolic blood pressure obtained using a tail-cuff method in SHAM or 2K1C rats fed a CTL, SO-B or SO-R for 6 weeks.

Values are expressed as mean ± SE, n = 5–7. Three-way ANOVA: $P < 0.001$ for time, animal (SHAM *vs* 2K1C), diet (CTL *vs* SO-B *vs* SO-R) and time × animal, and $P < 0.05$ for time × diet. Two-way ANOVA: $P < 0.001$ for 2K1C-CTL *vs* 2K1C-SO-B and 2K1C-CTL *vs* 2K1C-SO-R, and $P < 0.05$ for 2K1C-SO-B *vs* 2K1C-SO-R. $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs* SHAM-CTL, $^{\dagger}P < 0.05$ and $^{\dagger\dagger}P < 0.01$ *vs* 2K1C-CTL, $^{\ddagger}P < 0.05$ *vs* 2K1C-SO-R. Abbreviations: SO-B, a diet with blades of *Saccharina ochotensis*; SO-R, a diet with roots of *Saccharina ochotensis*. See legend of Figure 1 for the other abbreviations.

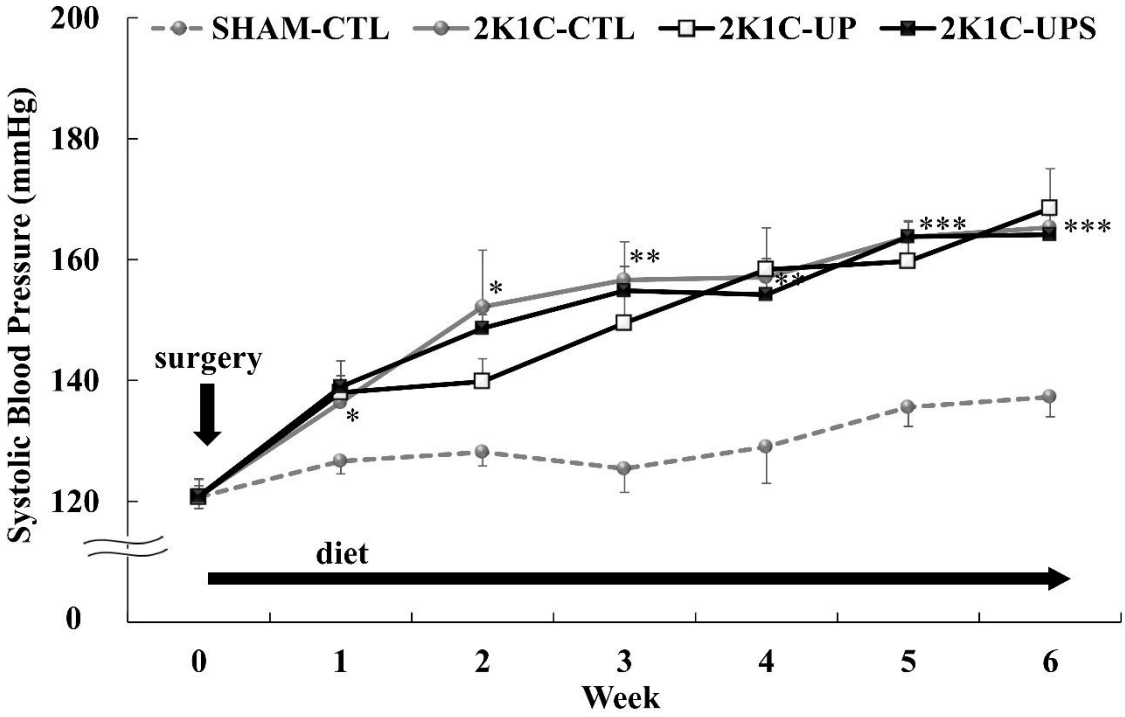

Figure 4. Systolic blood pressure obtained using a tail-cuff method in SHAM or 2K1C rats fed a CTL, UP or UPS for 6 weeks.

Values are expressed as mean ± SE, n = 7–9. Three-way ANOVA: $P < 0.001$ for time and animal (SHAM *vs* 2K1C) and $P < 0.01$ for time × animal. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs* SHAM-CTL. Abbreviations: UP, a diet with *Undaria pinnatifida*; UPS, a diet with sporophyll of *Undaria pinnatifida*. See legend of Figure 1 for the other abbreviations.

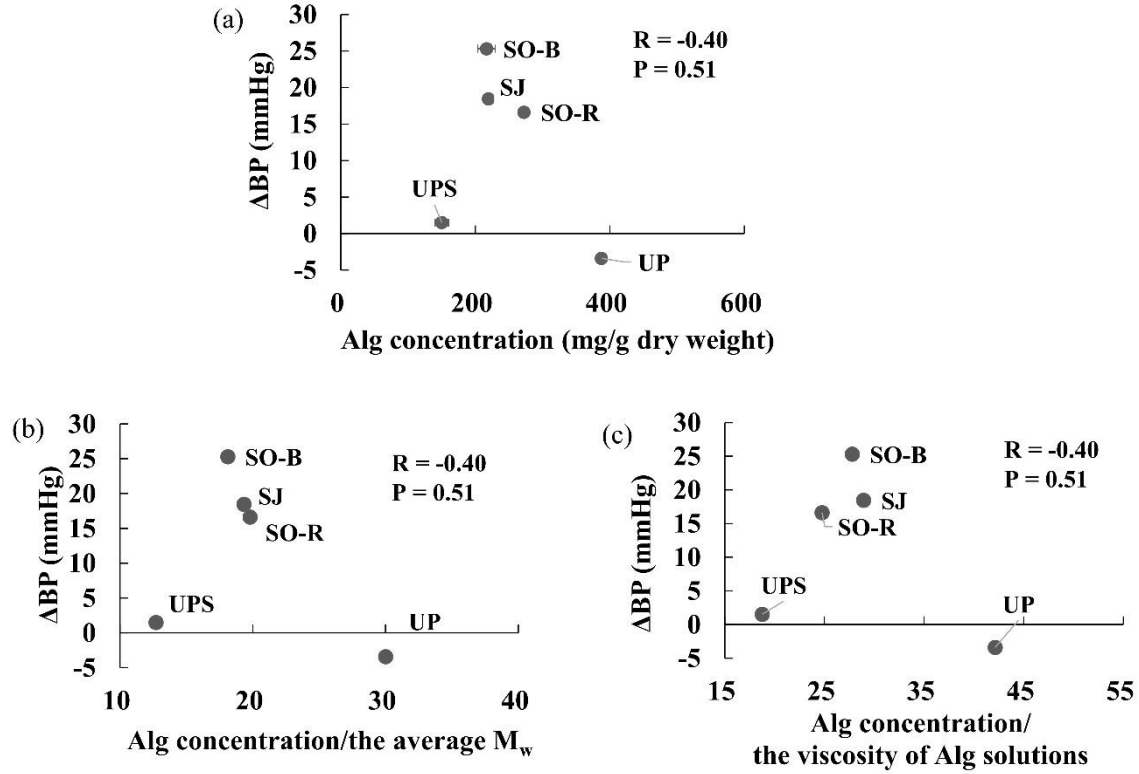

Figure 5. Correlation between ΔBP and Alg concentration (a), Alg concentration per the average M_w of Alg (b) or Alg concentration per the viscosity of Alg solutions (c).

No significant relationships were found in all scatter diagrams. Alg concentration, the average Mw of Alg, and the viscosity of Alg solutions are of seaweed itself. ΔBP is on rats fed each seaweed diet. Abbreviations: BP, blood pressure; ΔBP, amount of suppressed BP increase for each group fed a brown seaweed diet; Alg, alginate; M_w, molecular weight; SJ, *Saccharina japonica*; SO-B, blades of *Saccharina ochotensis*; SO-R, roots of *Saccharina ochotensis*; UP, *Undaria pinnatifida*; UPS, sporophyll of *Undaria pinnatifida*.

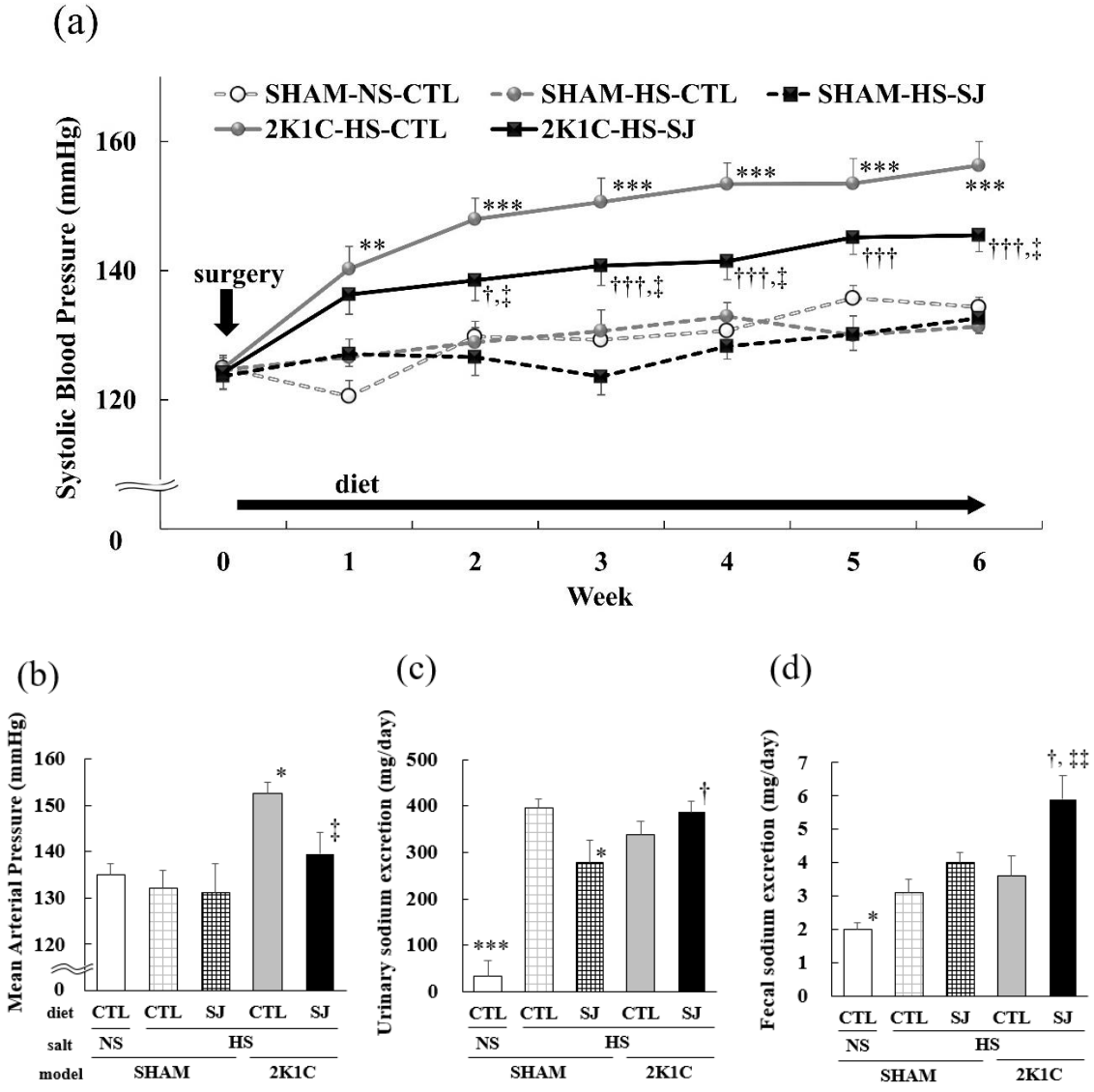

Figure 6. Systolic blood pressure obtained using a tail-cuff method for 6 weeks (a), mean arterial pressure under anesthesia at the end of the protocol (b), and urinary (c) and fecal (d) sodium excretion in SHAM or 2K1C rats fed a CTL or SJ diet with NS or HS.

Values are expressed as mean ± SE, n = 6–15. (a) Three-way ANOVA: $P < 0.001$ for time, animal (SHAM *vs* 2K1C), diet (CTL *vs* SJ), salt (NS *vs* HS) and time × animal,

and $P < 0.01$ for animal × diet. Two-way ANOVA between 2K1C-HS-CTL and 2K1C-HS-SJ: $P < 0.001$ for time and diet. (b) Three-way ANOVA: $P < 0.001$ for animal (SHAM *vs* 2K1C). (c) Three-way ANOVA: $P < 0.001$ for salt (NS *vs* HS), $P < 0.01$ for animal (SHAM *vs* 2K1C) × diet (CTL *vs* SJ). (d) Three-way ANOVA: $P < 0.01$ for animal and diet. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ vs SHAM-HS-CTL, $^{\dagger}P < 0.05$ and $^{\dagger\dagger\dagger}P < 0.001$ vs SHAM-HS-SJ, $^{\ddagger}P < 0.05$ and $^{\ddagger\ddagger}P < 0.01$ vs 2K1C-HS-CTL. Abbreviations: NS, normal-salt diet; HS, high-salt diet. See legend of Figure 1 for the other abbreviations.

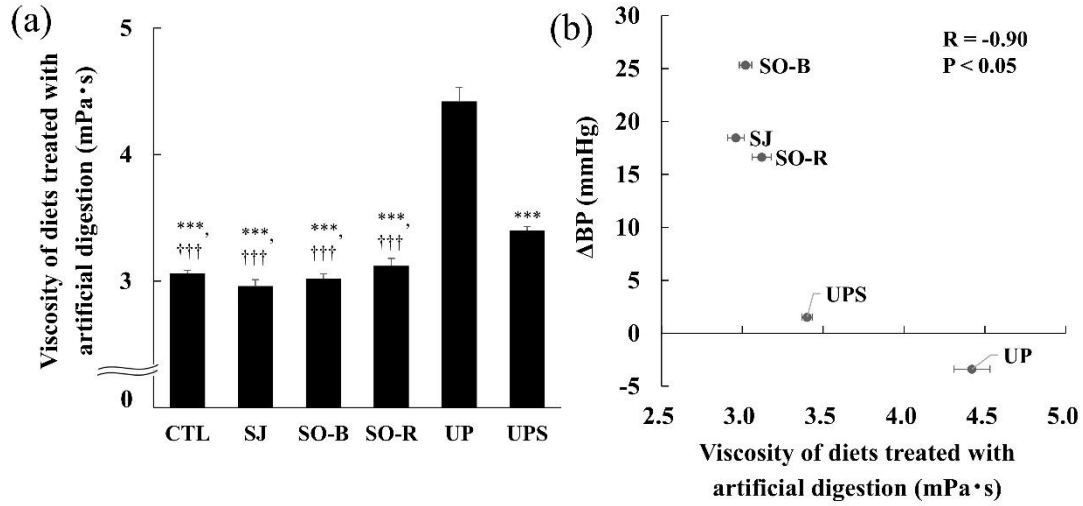

Figure 7. Viscosity of diets treated with artificial digestion (a), and their correlation to the suppression of BP increase by taking them (b).

(a) Values are expressed as mean ± SE, n = 5. One-way ANOVA: $P < 0.001$ for diet (CTL *vs* SJ *vs* SO-B *vs* SO-R *vs* UP *vs* UPS)). $^{***}P < 0.001$ *vs* UP, $^{†††}P < 0.001$ *vs* UPS. **(b)** A P value < 0.05 was considered statistically significant. Abbreviations: BP, blood pressure; ΔBP, amount of suppressed BP increase for each group fed a brown seaweed diet; SE, standard error; ANOVA, analysis of variance; CTL, a control diet; Alg, alginate; M_w, molecular weight; SJ, a diet with *Saccharina japonica*; SO-B, a diet with blades of *Saccharina ochotensis*; SO-R, a diet with roots of *Saccharina ochotensis*; UP, a diet with *Undaria pinnatifida*; UPS, a diet with sporophyll of *Undaria pinnatifida*.

Publication

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