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Effects of three major amino acids found in Japanese broth on glucose metabolism and gastric emptying

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ABSTRACT

Objectives: To our knowledge, the effect of the broth of dried kelp and dried bonito, *dashi,* on glucose metabolism and digestion has rarely been studied. Based on the component analysis of three actual broths served in traditional restaurants, a chemically synthesized broth with three free amino acids (histidine, glutamate, aspartate) and salt was prepared to investigate their effect on glucose metabolism, glucose-dependent insulinotropic polypeptide (GIP), and glucagon-like peptide 1 (GLP-1) secretion, and digestion.

Methods: In study 1, seven healthy individuals were enrolled in a four-period crossover study. Participants drank or ate hot water, synthesized broth, hot water with rice, and synthesized broth with rice. Plasma glucose, serum insulin, plasma glucagon, plasma GIP, and plasma GLP-1 were measured at baseline and after ingestion. In study 2, 6 of the 7 individuals ingested rice steamed with ^{13}C-labeled sodium acetate with hot water or synthesized broth to estimate gastric emptying by the ^{13}C-labeled acetate breath test in a two-period crossover trial.

Results: Ingesting water or synthesized broth alone elicited no change in plasma glucose or serum insulin levels. Ingesting synthesized broth with rice resulted in a rapid rise in plasma glucose and GLP-1 ($P = 0.01$ and 0.02, respectively) in an early postprandial phase compared with that by ingesting water with rice, but the area under the curve of those showed no significant differences. Ingesting synthesized broth with rice resulted in a significantly higher gastric emptying coefficient than that after rice with water ($P = 0.03$).

Conclusions: Three amino acids and sodium chloride corresponding to those found in actual broth promoted gastric emptying and led to a rapid response of plasma glucose. Our findings suggest that ingestion of the broth of dried kelp and dried bonito may improve gastric motility.

Introduction

There is a wealth of evidence supporting the healthy effects of the Mediterranean diet and the dietary approaches to stop hypertension (DASH) diet. The Japanese diet is thought to be a healthy diet, but evidence of any health benefits is limited [1]. The four basic components of the typical Japanese meal are rice, soup, side dishes, and pickles [2]. Japanese broth (*dashi*) is a soup that Japanese people drink on a daily basis [2]. *Dashi* is prepared by boiling dried foods such as kelp and bonito for a very short time [3], and contains amino acids that confer savory [2,3], umami taste to meals [2–4]. It is distinctive that *dashi* contains almost no animal fat or gelatin [2], and so has virtually no calories. It has a clear, transparent color [4]. Dried bonito broths are reported to relieve nervousness [5], increase peripheral blood flow [6], and decrease oxidative stress [7]. However, the ingredients responsible for these beneficial effects remain unclear.

As a preliminary study, three different Japanese broths, all extracted from dried kelp and dried bonito, were analyzed. The broths were provided by three traditional Japanese restaurants

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in Kyoto. These broths were prepared in the same way as usually served in the restaurants. Surprisingly, component analysis revealed that these three actual broths made with only natural materials contained almost the same composition ratio of amino acid and the same amount of salt. These concentrations remained unchanged after hydrolysis, indicating that the major components of the broths are amino acids and that the peptides contained, if any, are negligible. The procedures used to prepare these broths support these results, as the time at boiling temperature is too short and the temperature too low to extract ingredients other than amino acids.

Monosodium L-glutamate (MSG) is one of the main amino acids contributing to the umami taste, and is generally found in Japanese broth. MSG stimulates secretion of digestive enzymes [8–12], insulin [13], and glucagon-like peptide 1 (GLP-1) [14]. In many previous studies, the dosage of MSG administered has been about 10 times higher than usually found in one actual Japanese meal [8,15,16]. Moreover, effects of MSG on glucose metabolism have been adduced by simply adding MSG to 75 g glucose or a liquid meal [14–17].

In this study, three amino acids and salt acted as the major components of dried kelp and dried bonito broth; we investigated the effects on glucose metabolism, glucose-dependent insulinotropic polypeptide (GIP), GLP-1 secretion, and digestion using a concentration similar to those found in actual broths by a chemically reproduced broth. Additionally, using boiled rice as a staple food rather than only glucose or liquid meals, examinations on the effects of the broth under conditions closer to those in actual meal were achieved.

Methods

Participants

Experiment 1 used seven healthy individuals (four men and three women; mean ± SD) ages 32.8 ± 5.2 y (range 28–39) and body mass index (BMI) 20.5 ± 1.6 kg/m^2 (range 18.1–22.8 kg/m^2), having no history of abdominal surgery, and taking no regular medications. *Helicobacter pylori* infection was assessed by immunoglobulin (Ig)G antibodies (SRL, Inc., Tokyo Japan). The six individuals (three men and three women; mean ± SD age 33.3 ± 5.5 y and BMI 20.2 ± 1.6 kg/m^2) who participated in study 1 also participated in study 2. One man withdrew from the study.

The protocol (UMIN registration UMIN000022051, UMIN000022052) was approved by Kyoto University Graduate School and Faculty of Medicine, Ethics Committee, and all participants gave written informed consent. The study was conducted at Kyoto University Hospital according to the principles of the Declaration of Helsinki.

Study 1

The participants underwent four different ingestion trials: the first with 200 mL hot water; the second with 200 mL synthesized broth; the third with 200 mL hot water with 100 g white rice; and the fourth with 200 mL synthesized broth with 100 g white rice, on different days separated by weekly intervals. Participants were required to refrain from alcohol and exercise, and were told not to consume anything except water after 2100 the day before trials. A catheter was inserted into an antecubital vein, and all trials started at 0900. Blood samples were drawn before (0 min) and after (10, 20, 30, 45, 60, 75, 90, 120 min) ingestion.

The synthesized broth was made by dissolving L-histidine (186 mg), MSG (240 mg), sodium L-aspartate (153 mg), and sodium chloride (1167 mg) in hot water (200 mL). The concentrations of amino acids and sodium were determined according to the component analysis of the broth of traditional Japanese restaurant "A" in Kyoto (Supplementary Table 1). The hot water and synthesized broth were kept at 60°C. In the trials using liquid only, participants were instructed to drink within 3 min. In the trials using white rice, participants were instructed to take the liquid and white rice alternately, and to finish eating within 5 min.

Study 2

The participants underwent two trials: one with 200 mL of hot water plus 100 g white rice and the other with 200 mL synthesized broth plus 100 g white rice on different days separated by a 1-wk interval. The white rice was labeled by preparation at a ratio of 100 g steamed rice and 200 mg of ^{13}C-labeled sodium acetate and by steaming with ^{13}C-labeled sodium acetate (Cambridge Isotope Laboratories Inc., Tewksbury, MA, USA) [18]. They were required to refrain from alcohol and exercise, and not to consume anything except water after 2100 the day before trials. End-tidal breath samples were collected before (0 min) and after (every 5 min for the first 30 min, then at 40, 50, 60, 75, 90, and 120 min) the ingestion.

Measurements

At all time points, blood samples for measurement of plasma glucose, serum insulin, and C-peptide were collected into tubes containing sodium fluoride (NaF) and EDTA and tubes containing blood coagulation accelerant, respectively. At 0, 10, 20, 30, 60, and 120 min, blood samples for GIP, GLP-1, and glucagon were collected into tubes containing dipeptidyl peptidase-4 inhibitor (BD P800; Becton Dickinson, Franklin Lakes, NJ, USA). Blood samples were centrifuged (3000*g*, 20 min, 4°C), and the collected plasma and serum samples were stored at −80°C until analysis.

Plasma glucose, serum insulin, C-peptide, and glucagon (Mercodia Glucagon ELISA Kit, Mercodia, Uppsala, Sweden) were measured at SRL, Inc., Tokyo Japan. Total GLP-1 was measured by human total GLP-1 (version 2) assay kit (K150 JVC-1; Mesoscale Discovery, Gaithersburg, MD, USA); total GIP was measured by human GIP (total) enzyme-linked immunosorbent assay (EZHGIP-54 K; Merck Millipore, Darmstadt, Germany).

End-tidal breath samples were collected into exhalation bags (PYLORI-BAG20; Otuska Electronics Company, Osaka, Japan). Gastric emptying rate was determined by mathematical modeling, based on changes of the ratio of $^{13}CO_2/^{12}CO_2$ in breath samples measured by an infrared spectral analyzer (POCone, Otsuka Electronics Company). Integrated software (Gastric empting parameter software, Star medical, Tokyo) was used to calculate the adjusted ratio of $^{13}CO_2$ to $^{12}CO_2$ for the time period, % dose/h; the total excretion of $^{13}CO_2$ in the breath during 120 min, cumulative % dose/h; the time when half of the total intake of $^{13}CO_2$ is excreted in the breath, half gastric emptying time, $T_{1/2}$; the time when the excretion rate of $^{13}CO_2$ in the breath is maximum, maximum $^{13}CO_2$ excretion time, and T_{lag}; the gastric emptying coefficient (GEC), according to Ghoos's formula [19–21].

Statistical analyses

All data are presented as mean + SD. Plasma glucose, serum insulin, and C-peptide in the trials of synthesized broth or rice with synthesized broth were compared with those in the trials of water or rice with water, respectively. Plasma glucagon, total GIP, total GLP-1, $^{13}CO_2$ % dose/h, cumulative % dose/h, $T_{1/2}$, T_{lag}, and GEC in the trial with rice and synthesized broth were compared to those in the trial with rice and water. Plasma glucose, serum insulin, C-peptide, plasma glucagon, total GIP, total GLP-1, and $^{13}CO_2$ % dose/h were analyzed with a repeated-measures two-factor (time and meal) analysis of variance (ANOVA). If significant differences were indicated by ANOVA ($P < 0.05$), post hoc one-tailed paired *t* tests with Bonferroni correction were used to determine pairwise differences. The area under the curve (AUC) was calculated above zero. AUC, cumulative % dose/h, $T_{1/2}$, T_{lag}, and GEC were tested by pairwise comparisons using Wilcoxson's signed rank test. Two-tailed $P < 0.05$ was considered significant unless stated otherwise. Statistical analyses were performed with JMP Pro version 13.0.0 (SAS Institute Inc., Cary, NC, USA).

Results

Analysis of three broths from dried kelp and dried bonito

The concentrations of three free amino acids (histidine, glutamate, aspartate) were 10- to 12-fold higher compared with 15 other free amino acids in all three broths (Supplementary Table 1).

Plasma glucose, serum insulin, and C-peptide responses

After water or synthesized broth only ingestion, plasma glucose levels and serum insulin levels showed no change (Fig. 1A, B). After ingestion of rice with water or synthesized broth, plasma glucose levels peaked at 30 min, and showed significant difference in ANOVA ($P = 0.01$). At 20 min, plasma glucose levels after rice with synthesized broth were significantly higher than those after

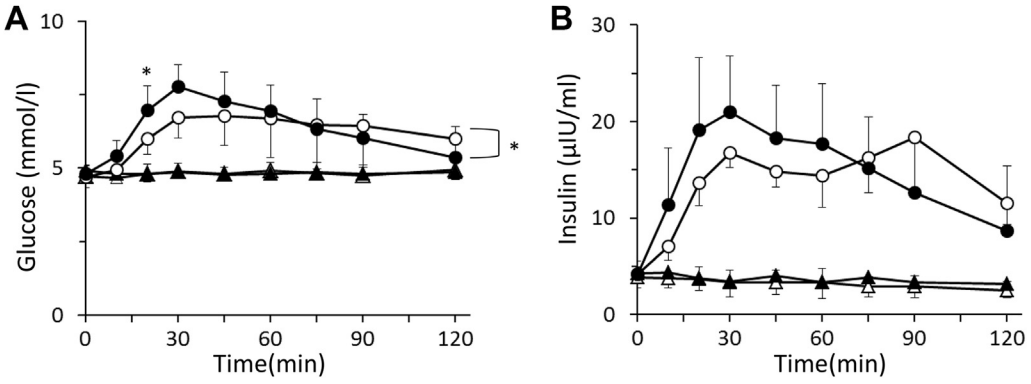

Fig. 1. Postprandial responses of plasma glucose and serum insulin. The levels of glucose (A) and insulin (B) during water + rice (○), synthesized broth + rice (●), water only (△), and synthesized broth only (▲) trials. Values are mean ± SD represented by vertical bars. If significant, differences were indicated by repeated-measures two-factor analysis of variance (*$P < 0.05$), post hoc one-tailed paired *t* tests with Bonferroni correction were used to determine pairwise differences. *$P < 0.05$ for water + rice versus synthesized broth + rice.

rice with water (6.96 ± 0.83 versus 6.00 ± 0.53 mmol/L, $P = 0.045$), but the AUC showed no significant difference (777 ± 88.1 versus 751 ± 96.6 mmol·L·min^{-1}; $P = 0.57$). The shapes of the curves were different, with a faster increase after the synthesized broth. Serum insulin levels peaked at 90 and 30 min after rice with water and rice with synthesized broth, respectively. However, insulin levels showed no significant differences in ANOVA. In the late postprandial period, both glucose and insulin levels after rice with synthesized broth tended to be lower than after rice with water. Time course curves of C-peptide levels were similar to those of serum insulin (Fig. 2A), and showed no significant differences in ANOVA. AUC of insulin and C-peptide showed no significant differences (1767 ± 533 versus 1704 ± 703 μIU·mL·min^{-1}, $P = 0.68$; 339 ± 78.4 versus 313 ± 89.5 ng·mL·min^{-1}, $P = 0.57$, respectively).

Plasma glucagon responses

Plasma glucagon levels peaked at 10 min after rice with water or synthesized broth and then decreased rapidly (Fig. 2B). No significant differences were observed in postprandial responses.

Plasma GIP and GLP-1 responses

Plasma GIP levels peaked at 30 min after rice with water or synthesized broth, and no significant differences were observed (Fig. 3A). Plasma GLP-1 levels, however, showed significant difference in ANOVA ($P = 0.02$). Plasma GLP-1 levels after rice with synthesized broth were significantly higher than those after rice with water at 20 min (26.3 ± 6.05 versus 16 ± 6.8 pg/mL; $P = 0.042$; Fig. 3B), but no significant difference was observed in AUC (2093 ± 304 versus 1983 ± 610 pg·mL·min^{-1}; $P = 0.37$).

Gastric emptying

The $^{13}CO_2$ excretion rate (% dose/h; Fig. 4) and total excretion of $^{13}CO_2$ (cumulative % dose) 120 min after rice with synthesized broth, which meant AUC, tended to be higher than after rice with water, but showed no significant differences (38.9 ± 4.71 versus 35.4 ± 5.35; $P = 0.074$; Table 1). $T_{1/2}$ and T_{lag} showed no significant differences. However, GEC after rice with synthesized broth was significantly higher than that after rice with water (4.68 ± 0.19 versus 4.51 ± 0.25; $P = 0.032$). GEC is one of the parameters of gastric emptying, and was inversely and strongly correlated with half emptying time by scintigraphy [19].

Discussion

Study 1 demonstrated that ingestion of chemically synthesized broth of dried kelp and dried bonito with rice elicited a rapid response of plasma glucose, the hypothesis being that gastric emptying was enhanced; study 2 confirmed that gastric emptying measured by ^{13}C-labeled sodium acetate was promoted in the early postprandial period after ingestion of the synthesized broth.

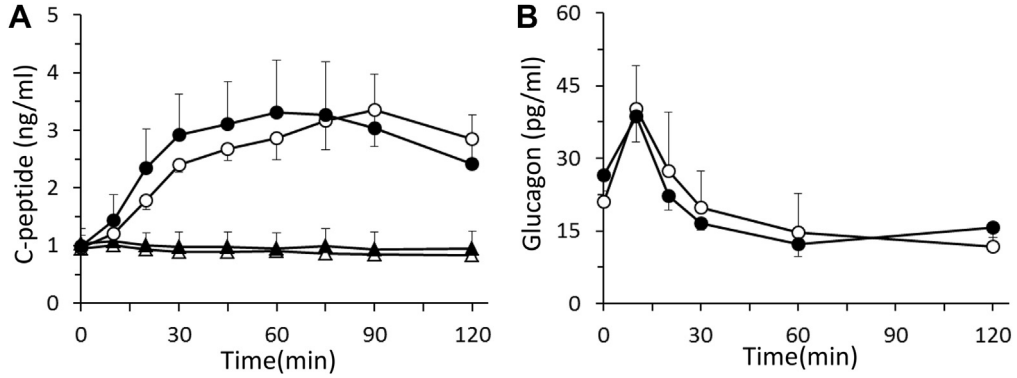

Fig. 2. Postprandial responses of C-peptide and glucagon. The levels of C-peptide (A) and glucagon (B) during water + rice (○), synthesized broth + rice (●), water only (△), synthesized broth only (▲) trials. Values are mean ± SD represented by vertical bars. No significant differences were observed between water + rice and synthesized broth + rice by repeated-measures two-factor analysis of variance.

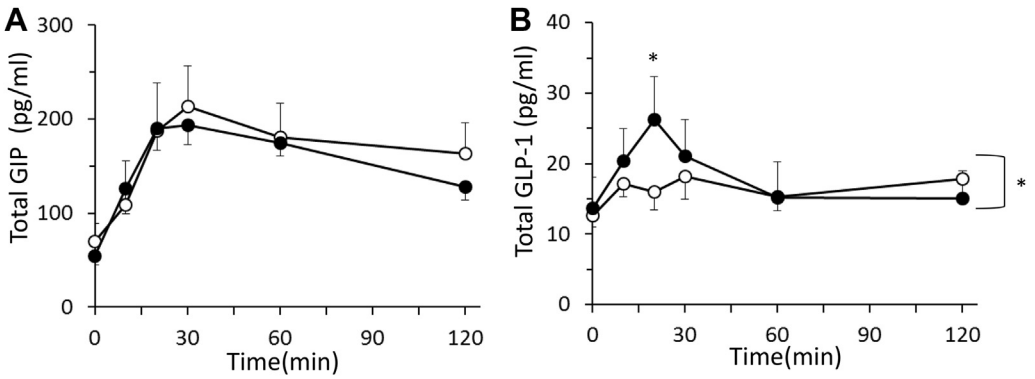

Fig. 3. Postprandial responses of GIP and GLP-1. The levels of total GIP (A), total GLP-1 (B) during water + rice (○) and synthesized broth + rice (●) trials. Values are mean ± SD represented by vertical bars. If significant differences were indicated by repeated-measures measures two-factor analysis of variance (*$P < 0.05$), post hoc one-tailed paired *t* tests with Bonferroni correction were used to determine pairwise differences. GIP, glucose-dependent insulinotropic polypeptide; GLP, glucagon-like peptide. *$P < 0.05$ for water + rice versus synthesized broth + rice.

The synthesized broth used in the present study was composed of three amino acids, MSG, sodium L-aspartate, and L-histidine, based on analysis of typical, traditional Japanese broth. The combination of rice, the staple food in Japan, and Japanese broth is a basic Japanese dietary pattern in daily life [4]. The present study demonstrated the effect of Japanese *dashi* using a readily reproducible loading of 100 g rice and chemically synthesized broth composed of the three major amino acids and sodium as contained in typical Japanese broths.

Free glutamate is the most abundant amino acid contained in dietary protein [11]. Oral intake of MSG promotes umami taste, and activates the efferent discharges in the vagal gastric and pancreatic nerves in rats [22] and stimulates salivary secretion in healthy adults [11,23]. MSG increases the firing rate of afferent fibers of the gastric branches of the vagus nerve in rats, but the other 19 amino acids and sodium chloride do not [24]. In animal studies, MSG alone stimulates pepsin and gastric acids [8,9]. Glutamate receptors are found in the mouth as well as in the stomach [25,26]. MSG promotes gastric exocrine secretion by direct action on gastric mucosa in dogs, which is mediated by the vagus nerve [8]. The effect of MSG on gastric exocrine secretion depends on the characteristics of coapplied nutrients. MSG promotes gastric exocrine secretion when administered with amino acids, but has no effect when administered with only carbohydrate in dogs [10]. Zai et al. [17] reported that MSG accelerates gastric emptying when added to casein-calcium with dextrin liquid meal, but has no significant effect on gastric emptying when added to dextrin liquid meal alone in humans. Protein included in the rice in the present study may have played some role when synthesized broth promoted gastric emptying.

High concentration MSG solution (3.57%) stimulates insulin secretion by itself without any effects on glucose levels [13]. However, when added to 75 g glucose, the same concentration of MSG either has no significant effect on both glucose and insulin levels compared with placebo [15] or significantly reduces glucose levels without any significant difference in insulin levels [16]. Glucose levels after ingestion of high-dosage MSG with 75 g glucose are inconsistent [15,16]. In some previous studies, about 0.5% MSG solution was used as the concentration included in meals [14,17,27,28]. In the present study, a low concentration of MSG (0.12%) and the other two amino acids in synthesized broth did not stimulate insulin secretion, whereas the addition of a low concentration of these three amino acids and sodium chloride solution to rice led to a rapid response of glucose and insulin levels. To our knowledge, there has been no other study using a similar, low concentration of MSG.

GLP-1 is secreted by intestinal endocrine L cells located mainly in the ileum and colon. GLP-1 secretion usually shows a biphasic pattern: An early phase of rapidly rising GLP-1 15 to

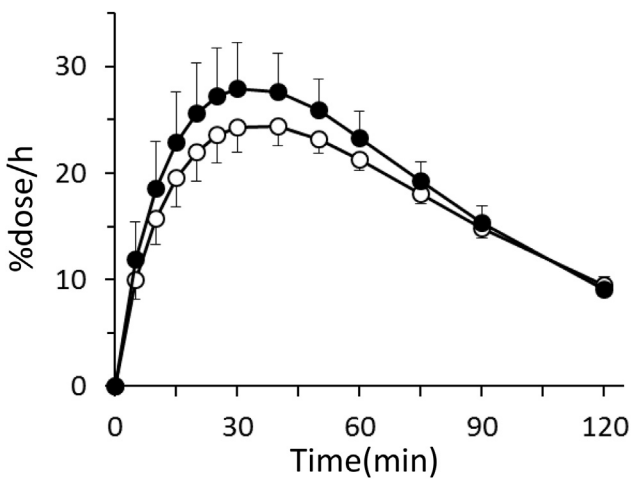

Fig. 4. Postprandial gastric emptying. Curves of $^{13}CO_2$ excretion (% dose/h) during water + rice (○) and synthesized broth + rice (●) trials. Values are mean ± SD represented by vertical bars. No significant differences were indicated by measures two-factor analysis of variance ($P < 0.05$).

Table 1
Total excretion of $^{13}CO_2$ and gastric emptying parameters

	Water + rice, mean ± SD	Synthesized broth + rice, mean ± SD	*P* value*
Total excretion of $^{13}CO_2$† (cumulative % dose)	35.4 ± 5.35	38.9 ± 4.71	0.07
$T_{1/2\ ex}$ (h)‡	1.18 ± 0.18	1.12 ± 0.09	0.26
$T_{lag\ ex}$ (h)§	0.63 ± 0.17	0.56 ± 0.08	0.19
GEC‖	4.51 ± 0.25	4.68 ± 0.19	0.03¶

GEC, gastric emptying coefficient; $T_{1/2}$, half gastric emptying time; T_{lag}, maximum $^{13}CO_2$ excretion time

* *P* values are derived by Wilcoxson's signed rank test.

† Total excretion of $^{13}CO_2$ in the breath during 120 min.

‡ The time when half of the total intake of ^{13}C is excreted in the breath.

§ The time when the excretion rate of $^{13}CO_2$ in the breath is maximum.

‖ A parameter of gastric emptying, inversely correlated with the half emptying time by scintigraphy.

¶ $P < 0.05$ for water + rice versus synthesized broth + rice.

30 min after meal and a late phase of gradually rising GLP-1 90 to 120 min after a meal [29,30]. It is thought that the early phase of GLP-1 secretion induced through vagus nerve activity; the late phase is induced by direct stimulus of nutrients. Hosaka et al. [14] reported that MSG stimulated the early phase of GLP-1 secretion after a lipid-containing liquid meal. The secretion pattern was similar to that of after rice with synthesized broth in the present study. GLP-1 inhibits gastric emptying when kept at continuously high levels [31,32]. In the present study, the GLP-1 level was significantly higher only at 20 min, and tended to be as low as about one-third of the reported level required to inhibit gastric emptying [31]. GIP is secreted by intestinal endocrine K cells, and is stimulated by lipids and carbohydrates [33]. In this study, we found that the GIP level was increased after rice loading, but with no significant difference between rice with water or broth.

Patients with functional dyspepsia (FD) have chronic symptoms thought to originate from the gastroduodenal region; these include postprandial distress syndrome (early satiation or postprandial fullness) and epigastric pain syndrome (pain or burning in the epigastrium) in the absence of any organic, systemic, or metabolic disease that is likely to explain the symptoms [34]. Delayed gastric emptying is one of the pathophysiologic mechanisms of FD [35–37]. Two meta-analyses reported that gastroprokinetic drugs significantly improve the symptoms of dyspepsia [38,39]. For management of patients with FD, it is commonly recommended that lifestyle improvement (stopping smoking and/or alcohol) and dietary therapy (eating small, low-fat meals) is helpful, but convincing evidence is lacking [34]. A clinically interesting finding of the present study is that the common broth in the Japanese diet may be a dietary option for patients with FD to alleviate delayed gastric emptying and promote digestion. Further studies to demonstrate the beneficial effect of broth in patients with FD are required.

Limitations of the present study were mainly that the effects of the chemically synthesized broth used in this study may not precisely reflect the effects of actual broth. *Dashi* contains nucleotides, organic acids, and a small amount of carbohydrates and minerals in addition to amino acids and sodium chloride. Additionally, although the synthesized broth in the present study was found to promote gastric emptying, this effect could have been somewhat affected by protein included in the rice. Also, in actual dining conditions, the gastric emptying rate will vary according to the main and side dishes chosen.

Conclusion

Three amino acids (histidine, glutamate, aspartate) were identified as major components of traditional Japanese broth. A low concentration of three amino acids and sodium chloride that closely parallel those in broth of dried kelp and dried bonito promote gastric emptying and lead to a rapid response of plasma glucose in healthy adults. These ingredients are not specific to dried bonito or dried kelp. Similar composition may be found in other broth, such as vegetable broth. The present study shed light on the function of clear broth, which may have some healthy effects.

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Appendix

Supplemental Table 1
Analyses of three different Japanese broths

Restaurant	A	B	C
Energy (kcal/100g)	7	7	7
Sodium (mg/100g)	255	245	250
Free amino acid (mg/100g)			
Arginine	2	3	3
Lysine	7	5	7
Histidine	94	43	98
Phenylalanine	2	3	3
Tyrosine	—	1	1
Leucine	4	4	4
Isoleucine	2	2	3
Methionine	—	—	—
Valine	3	3	3
Alanine	7	6	6
Glycine	2	2	2
Proline	4	4	4
Glutamic acid	93	56	55
Serine	2	3	3
Threonine	2	2	2
Aspartic acid	61	37	44
Tryptophan	—	—	—
Cystine	—	—	—

All of them were extracted from dried kelp and dried bonito, and were kindly provided by three traditional Japanese restaurants (A-C) in Kyoto. These three Japanese broths contained almost the same amount of salt and the same composition ratio of amino acid. The concentrations of three free amino acids (histidine, glutamate, aspartate) were 10- to 12-fold higher compared to 15 other free amino acids in all three broths.

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f)日本語表記

題名；味噌汁の摂取頻度は一般住民横断調査において胃食道逆流症症状の軽減と関連する：ながはま 0 次予防コホート

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要旨；

背景　食習慣や生活習慣は上腹部症状に影響すると考えられているが、特定の食品の習慣的摂取が酸逆流や運動不全の症状にどう関与しているかについて一般人口集団で検討した研究は乏しい。我々の先行研究にて、だし中の 3 種の遊離アミノ酸が胃排出を促進することが示されたため、だしと味噌から成る味噌汁の摂取頻度と上腹部症状の程度の関連を調べることとした。

方法　一般集団における味噌汁の摂取頻度と酸逆流・運動不全症状との関連について、横断的研究を行った。食習慣は自己記入式質問票、上腹部症状は FSSG 質問票で収集した。味噌汁摂取頻度と FSSG・酸逆流・運動不全スコアの関連の解析のために、年齢・性別・BMI・その他の食習慣・喫煙・飲酒・不適切な食行動を調整して一般化線型モデルのあてはめを行った。

結果　9364 人が解析対象となった。傾向検定では味噌汁摂取の頻度が高いほど FSSG スコアが低いことが示された（P <0.001）。一般化線形モデルでは毎日味噌汁を摂取することが年齢・性別・BMI・その他の食習慣・喫煙・飲酒・不適切な食行動とは独立して FSSG スコア、酸逆流スコアおよび運動不全スコアの低さと関連していた（推定値= -0.46, -0.22, -0.27; %CI = -0.83 – -0.12; -0.38 – -0.07; -0.47– -0.08）。

結論　毎日味噌汁を摂取することは、上腹部症状の少なさと関連した。

Summary

Background Dietary habits and lifestyles are considered to affect the frequency of epigastric symptoms. In our previous study, we found that three amino acids in Japanese broth promoted gastric emptying. We hypothesized that the higher consumption of *miso* soup which was mainly composed of Japanese broth and *miso* paste would be associated with a lower frequency of epigastric symptoms.

Methods We conducted a cross sectional study of the association between frequency of *miso* soup intake and reflux or dyspepsia symptoms in a general Japanese population. Sixteen items of dietary habits were assessed using a self–reported questionnaire, and epigastric symptoms were evaluated using the Frequency Scale for Symptoms of Gastroesophageal Reflux Disease (FSSG). We fitted generalized linear models to analyze the association between *miso* soup intake and FSSG, reflux, or dyspepsia scores adjusted by age, sex, body mass index (BMI), other 15 dietary habits, smoking, drinking alcohol, and unfavorable dietary behaviors.

Results A total of 9364 subjects were included in the analysis. Trend analysis revealed that higher frequency of *miso* soup intake was associated with lower FSSG scores ($P < 0.001$). In a generalized linear model, daily intake of *miso* soup was associated with lower FSSG, reflux, and dyspepsia scores independent of age, sex, BMI, other 15 dietary habits,

smoking, drinking alcohol, and unfavorable dietary behaviors (estimate = -0.46, -0.22, and -0.27, respectively; 95% CI = -0.83, -0.12; -0.38, -0.07; and -0.47, -0.08, respectively).

Conclusion Dairy intake of *miso* soup was associated with lower epigastric symptoms.

Keywords: Japanese diet; *miso* soup; gastric emptying; Frequency Scale for Symptoms of Gastroesophageal Reflex Disease (FSSG); dietary habit

Introduction

Upper gastrointestinal disorders, such as gastroesophageal reflux disease (GERD) and functional dyspepsia (FD), are common throughout the world. The prevalence of GERD symptoms in Asia has increased to around 20% (*1-4*), and the prevalence of FD is approximately 10% to 20% of the general population in Japan and worldwide (*5*). The symptoms of GERD and FD have some overlap, and are associated with a poor health-related quality of life (*6-9*).

Both dietary and lifestyle habits are generally considered to affect the frequency of reflux or dyspepsia symptoms, and patients with GERD or FD are advised to refrain from certain food and lifestyle choices (*10, 11*). For example, unfavorable dietary behaviors are reported to be associated with GERD symptoms (*12, 13*). Fat intake provokes reflux symptoms in patients with GERD or FD (*14-17*). Tobacco, chocolate, carbonated beverages, and a postprandial right lateral decubitus position decrease pressure on the lower esophageal sphincter in patients with GERD (*18*). These studies, however, mainly included patients with GERD, and few population-based studies have evaluated the role of dietary behaviors in GERD or FD symptoms (*11, 18, 19*).

The Japanese dietary pattern is composed of the dietary staple, side dishes, and soup (*20, 21*). *Miso* soup is the most basic soup of Japanese daily meals, and it is made

with *miso* paste dissolved in Japanese broth and various ingredients such as vegetable, mushroom, seaweed, or *tofu*, depending on regional and seasonal recipes (*21*). A bowl of *miso* soup usually contains a tablespoon of *miso* paste that has about 32 kcal of energy, 2.1g of protein, 1.1g of fat, and 3.5g of carbohydrate (http://www.mext.go.jp/en/policy/science_technology/policy/title01/detail01/1374030.htm), and the salt content of *miso* soup is 0.5–1.2% (*22*). Japanese broth contains amino acids, but has virtually no calories (*23*). The nutrients contained in *miso* soup can differ between the ingredients used, basically, however, *miso* soup has low fat and low energy. The influence of the consumption of *miso* soup on salt intake and blood pressure are often studied (*22*, *24*, *25*). But, there is no study to focus on the relationship between frequency of *miso* soup intake and frequency of epigastric symptoms. In our previous study, we found that levels of three amino acids (histidine, glutamate, and aspartate) and sodium chloride that closely parallel those in Japanese broth promote gastric emptying (*23*). We hypothesized that higher consumption of *miso* soup would be associated with a lower frequency of reflux or dyspepsia symptoms.

In this study, we investigated the associations between frequency of *miso* soup intake and reflux and dyspepsia symptoms in a general Japanese population using cross-sectional data obtained in the Nagahama Prospective Cohort for Comprehensive Human

Bioscience study (the Nagahama study).

Material and methods

Study subjects

Study subjects were participants of the baseline survey in the Nagahama Study. The Nagahama Study is an ongoing community-based cohort study conducted by the Kyoto University Graduate School of Medicine and Nagahama City. The participants are members of the general population living in Nagahama City, a rural city of 125,000 inhabitants in Shiga prefecture located in central Japan, aged 30 to 74 years, recruited from 2008 to 2010. Among the total of 9764 study participants, subjects who did not complete the Frequency Scale for Symptoms of Gastroesophageal Reflux Disease (FSSG) (n = 1), who were pregnant (n = 42), who received GERD treatment (n = 105) or who reported a medical history of upper gastrointestinal cancer (n = 71) or Helicobacter pylori infection (n = 181) were excluded from this study (Figure 1). The Ethics Committee of Kyoto University Graduate School and Faculty of Medicine, the Ethical Review Board of the Nagahama Study, and the Nagahama Municipal Review Board of Personal Information Protection approved all study procedures.

Data collection

At the baseline survey in the Nagahama Study, physical and biochemical parameters were measured. Data regarding medical history; medications; epigastric symptoms; smoking status; and dietary habits, including alcohol consumption, were obtained using a self-reported questionnaire. An individual who consumed alcohol more than 4 days/w was defined as a frequent drinker (*12*).

Epigastric symptoms

Epigastric symptoms were evaluated using the FSSG, a well-established and widely used questionnaire in Japan for evaluating the symptoms of GERD (*26*) and the response to treatment of GERD (*16*) or FD (*27*). The FSSG comprises 12 questions: 7 questions (# 1, 4, 6, 7, 9, 10, and 12) about acid-reflux related symptoms (reflux score) and 5 questions (# 2, 3, 5, 8, and 11) about dyspepsia symptoms (dyspepsia score), which were scored to indicate the frequency of symptoms, as follows: never = 0, occasionally = 1, sometimes = 2, often = 3, and always = 4 (*26*) (Supplemental table 1). At a cutoff score of 8 points, the FSSG shows 62% sensitivity, 59% specificity, and 60% accuracy for an endoscopic diagnosis of GERD (*26*).

Dietary habits

Dietary habits were assessed using a simple 16-item questionnaire about the frequency of intake of 1) meat dishes; 2) fish dishes; 3) *tofu* (soy bean curd) dishes (or soy bean dishes) ; 4) egg dishes; 5) milk; 6) vegetable dishes; 7) fruits; 8) deep-fried foods; 9) cakes or Japanese confectioneries, 10) juice or isotonic drinks; 11) junk foods; 12) sweets like candies and chocolates; 13) *miso* (fermented soybean paste) soup; 14) pickles; 15) ham, sausage, or *kamaboko* (boiled fish paste)*;* and 16) frozen foods or precooked foods. Subjects answered each item by choosing one of the four options: “less than once per week”, “two to three times per week”, “four to five times per week”, and “every day”.

Unfavorable dietary behaviors

Dietary behaviors which are supposed to be closely correlated with GERD symptoms (*12, 13*) were assessed by the following four questions that are used in the standard health checkup program performed by the Japanese government: 1. Do you have dinner within 2 h before going to bed more than 3 days a week? 2. Do you snack after dinner more than 3 days a week? 3 Do you have a habit of eating rapidly? 4. Do you skip breakfast more than 3 days a week? Subjects answered each item by choosing “yes” or “no”. A score of one was assigned to each “yes” response.

169

170 **Statistical Analysis**

171 Values are expressed as percentage (%), or mean (standard deviation (SD)) or
172 95% confidence interval (CI). From our preliminary check, we found that FSSG scores
173 were asymmetrically distributed, and that an exponential distribution was fit well. In the
174 analysis of FSSG, reflux, and dyspepsia scores, we adopted generalized linear models
175 with identity link and exponential distribution including 16 dietary habits, age, sex, BMI,
176 smoking, drinking alcohol, and unfavorable dietary behaviors as independent variables.

177 For trend testing, we assigned variables to each category of dietary habits as
178 follows: less than once per week = 1, two to three times per week = 2, four to five times
179 per week = 3, every day = 4. Three dummy variables corresponding to each category of
180 dietary habit frequency with 0 and 1 as possible values were then constructed. “Less than
181 once per week” category was selected as the reference category. Sex was coded as male
182 = 1, female = 0.

183 A two-tailed $P < 0.05$ was considered statistically significant. All statistical
184 analyses were performed using JMP Pro version 13.0.0 (2016 SAS Institute).

185

Results

A total of 9364 subjects were included in the analysis (Figure 1). Approximately one-third of the subjects were male (Table 1). Mean age (SD) was 53.4 (13.4) years old. Among the subjects, 201 subjects (2.1%) reported a medical history of GERD, and 2049 subjects (21.9%) had FSSG scores ≥ 8. The mean FSSG score was 4.5, and its 95% CI was 4.5 - 4.7. The mean FSSG scores, reflux scores, and dyspepsia scores decreased with an increase in *miso* soup intake (Table 2). Differences in the mean scores between the "less than once per week" and "every day" were 1.1, 0.3, and 0.8, respectively. The mean FSSG scores, reflux scores, and dyspepsia scores by sex and frequency of each of the other dietary habits are shown in Supplemental Table 2.

Trend analysis revealed an inverse association between FSSG scores and the frequency of *miso* soup intake ($P_{trend} < 0.001$; Figure 2). The same association was observed in the analysis in which FSSG scores were separated into reflux and dyspepsia scores (Figure 2).

In a generalized linear model with FSSG scores as the dependent variable, male sex, age, and everyday intake of *miso* soup were significantly associated with lower FSSG scores (Table 3). From all independent variables, only the results of frequency of *miso* soup and major adjusted variables are shown in Tables 3-5. The full models including

other adjusted variables (15 dietary habits, smoking, drinking alcohol, and unfavorable dietary behaviors) are shown in Supplemental tables 3-5. With reflux scores as the dependent variable, male sex, and everyday intake of *miso* soup were significantly associated with lower reflux scores, and age and BMI were significantly associated with higher reflux scores (Table 4). With dyspepsia scores as the dependent variable, male sex, age, BMI and everyday intake of *miso* soup were all significantly associated with lower dyspepsia scores (Table 5).

Discussion

The present findings suggest that a higher frequency intake of *miso* soup is associated with fewer epigastric symptoms in the general population. The difference in the mean FSSG scores between “less than once per week” intake of *miso* soup and “every day” intake of *miso* soup was 1.1, which corresponds the difference of the frequency between “occasionally” and “never” of one epigastric symptom. This inverse trend was evident in the FSSG score, and similar trends were observed both in reflux and dyspepsia symptoms. In a generalized linear model, daily intake of *miso* soup was associated with lower FSSG, reflux, and dyspepsia scores independently of sex, age, BMI, other 15 dietary habits, and unfavorable dietary behaviors.

These results are consistent with our previous findings that levels of three amino acids (histidine, glutamate, and aspartate) and sodium chloride corresponding to those found in Japanese broth promote gastric emptying(*28*). Promoted gastric emptying is a possible mechanism for less reflux and less dyspepsia. Glutamate accelerates gastric emptying when added to a liquid meal with protein, but has no significant effect on gastric emptying when added to water or a liquid meal with only carbohydrates in humans (*29*). Based on the fact that *miso* soup comprises both Japanese broth and *miso* paste, which contains soybean protein, *miso* soup is likely to promote gastric emptying.

In addition, some studies suggest that soybean-derived products ameliorate gastroparesis (*30*) and decrease regurgitation (*31*).

In the present study, male sex was associated with lower FSSG, reflux, and dyspepsia scores. The same sex differences have been reported in previous Japanese studies (*13, 32, 33*). Younger age was associated with higher FSSG scores in the present study. Previous studies among Japanese have also demonstrated that younger subjects have higher FSSG scores in the general population (*13, 32*). When reflux and dyspepsia symptoms were analyzed separately, younger age was associated with fewer reflux symptoms, and more dyspepsia symptoms. BMI was not associated with lower FSSG scores. In a separate analysis of reflux scores and dyspepsia scores, however, BMI was associated with more reflux symptom and fewer dyspepsia symptoms.

GERD, which is mainly based on reflux symptoms, has a multifactorial pathogenesis, including the presence of excess gastric acid (*34*), hiatal hernia (*35*), lower esophageal sphincter (LES) dysfunction (*36, 37*) and esophageal motility dysfunction (*38*). Aging is a major risk factor for GERD (*39*). The age-related increase of GERD is mainly caused by the higher prevalence of hiatal hernia (*35*) and esophageal motility dysfunction (*38*). Increased BMI is also associated with reduced esophageal body contractility (*36*) and impaired LES function (*36, 37*), and causes erosive

esophagitis (*4, 40*). Our results that aging and increased BMI were associated with higher reflux scores were in accordance with these data.

The pathogenesis of FD, which is mainly based on dyspepsia symptoms, is more heterogeneous. Psychological distress, particularly anxiety, is associated with FD (*41*). A disturbance of gastric physiologic factors, such as slow gastric emptying, failure of the gastric fundus to relax after a meal, or gastric hypersensitivity with distension of the stomach are also involved in FD (*42*). Several population-based studies demonstrated that FD is more common in younger subjects (*43, 44*). There have been no studies that revealed any association between BMI and FD. Our results which revealed an association of aging with lower dyspepsia scores was consistent with previous data (*43, 44*).

A limitation of the current study is that endoscopic assessment to diagnose esophagitis was not performed. However, we focused on the frequency of the epigastric symptoms with or without endoscopic esophagitis, and found significant associations between *miso* soup intake and epigastric symptoms. Because of the limitation of a cross-sectional study, the results of the current study do not mean that miso soup intake has any preventive or therapeutic effects on GERD. Further longitudinal research is required to clarify the protective effect of *miso* soup against deterioration of epigastric

symptoms. One strength of the current study is the simple questionnaire for dietary assessment. Study subjects readily answered the question regarding the frequency of intake of *miso* soup per week, even those that do not cook for themselves.

In conclusion, daily intake of *miso* soup was associated with the lower FSSG, reflux, and dyspepsia scores in a Japanese community-based cohort population.

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the paper. N. I. had primary responsibility for final content. All authors read and approved the final manuscript.

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Conflicts of interest

None of the authors has any conflicts of interest related to this study.

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Table 1 Characteristics of subjects

Factors	All (n = 9364)
Male, *n* (%)	3048 (32.6)
Age, *y*, (SD)	53.4 (13.4)
BMI, kg/m^2, (SD)	22.3 (3.3)
Current smoker, *n* (%)	1369 (14.6)
Frequent drinker, *n* (%)	2123 (22.7)
FSSG scores, (95% CI)	4.6 (4.5 – 4.7)
Reflux scores, (95% CI)	2.1 (2.0 – 2.1)
Dyspepsia scores, (95% CI)	2.5 (2.5 – 2.6)
8 points or more, n (%)	2049 (21.9)
Medication, *n* (%)	
Steroid	63 (0.7)
Antithrombotic drugs	370 (4.0)
NSAIDs	307 (3.3)
Antihypertensive drugs	1560 (16.7)
Medical history, *n* (%)	
GERD	201 (2.1)

Continuous variables are described as mean (SD or 95% CI), and categorical variables are expressed as numbers (%)

An individual who consumed alcohol more than 4 times per week was defined as a frequent drinker.

CI, confidence interval; GERD, gastroesophageal reflux disease; NSAIDS, non-steroidal anti-inflammatory drugs; SD, standard deviation

Table 2　Mean FSSG scores, reflux scores, and dyspepsia scores by the frequency of intake of *miso* soup

Miso soup	n	FSSG scores (95% CI)	Reflux scores (95% CI)	Dyspepsia scores (95% CI)
≤1/w	1081	5.3 (5.0 – 5.6)	2.3 (2.2 – 2.4)	3.0 (2.8 – 3.2)
2-3/w	2510	4.9 (4.7 – 5.1)	2.2 (2.1 – 2.2)	2.7 (2.6 – 2.8)
4-5/w	2524	4.5 (4.3 – 4.7)	2.0 (2.0 – 2.1)	2.5 (2.4 – 2.6)
Every day	3249	4.2 (4.0 – 4.3)	2.0 (1.9 – 2.0)	2.2 (2.1 – 2.3)

CI, confidence interval; FSSG, Frequency Scale for Symptoms of Gastroesophageal Reflux Disease

Continuous variables are described as mean (95% CI).

Table 3　A generalized linear model with FSSG scores as the dependent variable

Factors	Estimate	95% CI	*P*-value
Male	-0.567	-0.795 – -0.333	< 0.001*
Age	-0.010	-0.019 – 0.001	0.027*
BMI	-0.026	-0.054 – 0.003	0.080
Miso soup			
2-3/w	-0.146	-0.506 – 0.199	0.413
4-5/w	-0.306	-0.672 – 0.044	0.088
Every day	-0.463	-0.828 – -0.115	0.009*

BMI, body mass index; CI, confidence interval; FSSG, Frequency Scale for Symptoms of Gastroesophageal Reflux Disease

Generalized linear model: $P * < 0.05$

Adjusted for the other 15 dietary habits, smoking, drinking alcohol, and unfavorable dietary behaviors. Estimates were derived from a generalized linear model.

Table 4　A generalized linear model with reflux scores as the dependent variable

Factors	Estimate	95%CI	*P*-value
Male	-0.219	-0.323 – -0.112	< 0.001*
Age	0.013	0.009 – 0.016	< 0.001*
BMI	0.020	0.007 – 0.033	0.003*
Miso soup			
2-3/w	-0.051	-0.207 – 0.099	0.510
4-5/w	-0.152	-0.311 – 0.0002	0.050
Every day	-0.222	-0.382 – -0.068	0.004*

BMI, body mass index; CI, confidence interval

Generalized linear model: $P * < 0.05$

Adjusted for the other 15 dietary habits, smoking, drinking alcohol, and unfavorable dietary behaviors. Adjusted estimates were derived from a generalized linear model.

Table 5　A generalized linear model with dyspepsia scores as the dependent variable

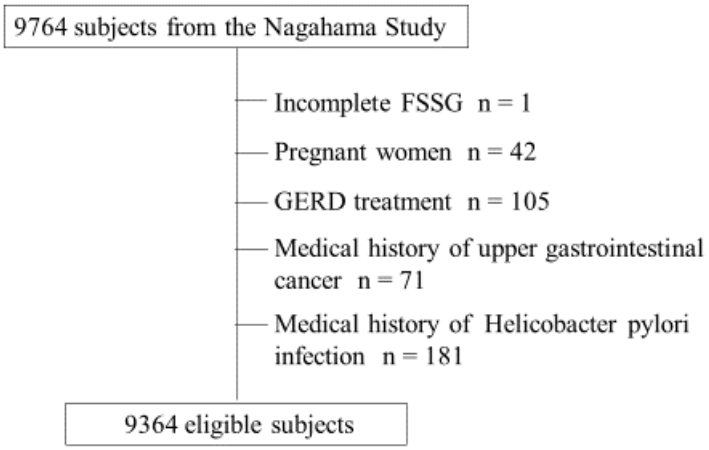
Factors	Estimate	95%CI	*P*-value
Male	-0.323	-0.442 – -0.201	< 0.001*
Age	-0.022	-0.027 – -0.017	< 0.001*
BMI	-0.043	-0.057 – -0.028	< 0.001*
Miso soup			
2-3/w	-0.103	-0.302 – 0.086	0.288
4-5/w	-0.168	-0.368 – 0.023	0.085
Every day	-0.271	-0.468 – -0.084	0.004*

BMI, body mass index; CI, confidence interval

Generalized linear model: $P * < 0.05$

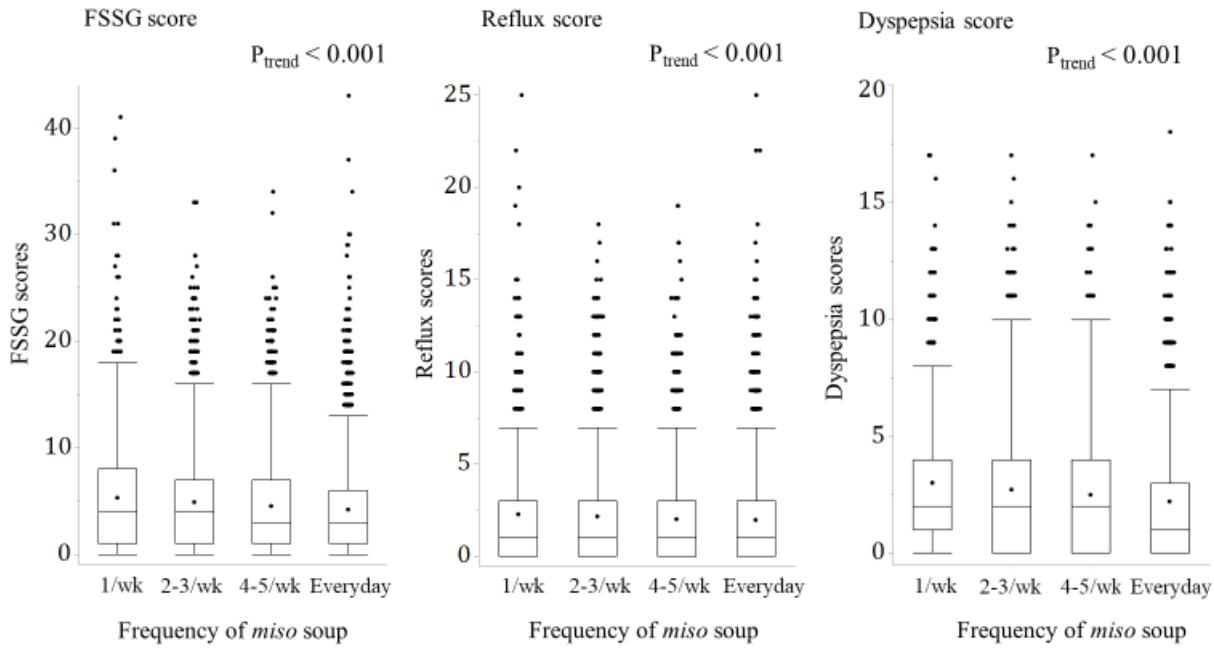
Adjusted for the other 15 dietary habits, smoking, drinking alcohol, and unfavorable dietary behaviors. Adjusted estimates were derived from a generalized linear model.

Figure 1　Flowchart detailing the process of obtaining subjects for analysis



FSSG: Frequency Scale for Symptoms of Gastroesophageal Reflux Disease

Figure 2　FSSG, reflux, and dyspepsia scores for subjects by frequency of intake of *miso* soup



Boxplot; IQR = the 3rd quartile minus the 1rd quartile. Whiskers drawn to the furthest point within 1.5 × IQR from the box.

Points indicate average values. Trend analysis was performed by a generalized linear model.

FSSG, Frequency Scale for Symptoms of Gastroesophageal Reflux Disease