Effects of Undaria pinnatifida (Wakame) on postprandial glycaemia: in vitro and in vivo studies

Rieko Mitamura (mitamura@fujijoshi.ac.jp)
Fuji Women's University
Keiko Yoshinaga
Riken Vitamin Co

Article

Keywords: Undaria pinnatifida , wakame , glucose release rate , postprandial blood glucose

Posted Date: July 27th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1675303/v2

Abstract

Brown seaweeds, such as *Undaria pinnatifida* (wakame), contain sodium alginate, which can affect postprandial blood glucose levels. We previously reported the effects of wakame on postprandial glycaemia in humans; however, the underlying mechanism remains unclear. We aimed to investigate the mechanism underlying the influence of ingested wakame on postprandial blood glucose levels. Glucose release rate of wakame extracts was measured using an *in vitro* cell-free digestion model. The *in vivo* study was conducted using a crossover method, wherein healthy young adult participants were given 150 g of rice with or without wakame (2 g or 4 g dried wakame). The glucose release rates of all wakame extracts were significantly lower than that of the control. The soluble fraction significantly inhibited the release of glucose. In the *in vivo* study, blood glucose levels 15 min after ingestion were significantly lower after consuming rice with 2 g of wakame than when consuming rice alone. Moreover, the ingestion of 4 g of wakame resulted in significantly lower blood glucose levels after meal consumption. These results suggest that the highly viscous soluble fraction of wakame might increases the viscosity of the gastrointestinal contents and delays glucose absorption.

Introduction

The traditional Japanese diet consists of cooked rice, miso soup, and three side dishes that typically contain fish, meat, beans, vegetables, and seaweed. Compared with the Western diet, the Japanese diet is rich in plant-based foods, including dietary fibre, which is non-digestible in the upper gastrointestinal tract and has health benefits [1]. Dietary fibre is a heterogeneous food compound with different structures and physical properties. Water-soluble viscous fibres, such as β-glucan, guar gum, and alginate, may improve the glycaemic response. High viscosity induces delayed gastric emptying and absorption of glucose from the lumen of the small intestine [2-4]. Inhibitors of key enzymes, such as α-amylase and α-glucosidase, decrease the rate of glucose absorption from the small intestine, and some inhibitors are derived from seaweeds [5,6]. A meta-analysis study reported that dietary fibre improved fasting blood glucose levels in patients with type 2 diabetes [7]. However, the effect on fasting blood glucose levels differed depending on the type of dietary fibre used.

Seaweeds are a good source of water-soluble viscous dietary fibres, which might influence glucose uptake. Wakame, i.e., the Japanese name for brown seaweed *Undaria pinnatifida*, is an edible seaweed that is a popular in traditional food in Japan. Alginates are present in brown seaweeds generally as sodium and calcium salts, and wakame also contains alginate. The sodium alginate is formed a gel within the gastrointestinal tract, and may suppress appetite control and glucose absorption by reducing gastric emptying [2, 8-10].

Some studies have shown that ingestion of seaweed or seaweed extract influences postprandial glycaemic control, which is related to slow gastric emptying and delayed glucose absorption [11-14]. However, differences have been reported because of the species, location of seaweed, and extraction methods. Our previous clinical trials indicated that compared to rice intake alone, blood glucose and

insulin levels in the early postprandial stage were effectively reduced when wakame was consumed with rice [15]. This study demonstrates that the acute consumption of wakame containing 1.4 g of fibre reduced glycaemic and insulinemic responses; however, the underlying mechanism is unclear. Although the cell wall of wakame contains fucoidan, alginate, and cellulose, viscous dietary fibre such as alginate, which is abundant in wakame, might improve postprandial glycaemia [16]. However, its effects on the inhibition of carbohydrate digestion are poorly understood. To our knowledge, no study has investigated the effects of wakame extracts on the glucose release rate in the artificial upper gastrointestinal tract. Therefore, we aimed to investigate the mechanism underlying the influence of ingested wakame on postprandial blood glucose levels. Alginate is mainly present in brown algae, such as wakame, and we hypothesized that alginate would be an important component for controlling postprandial blood glucose. We conducted *in vitro* cell-free studies to determine the inhibitory activity of wakame extract on the glucose release rate and *in vivo* studies to determine the acute effects of wakame on postprandial blood glucose levels among young Japanese women.

Materials And Methods

Preparation of wakame extracts

The alginate extraction process from wakame is based on the conversion of alginic acid from the cell wall into alginate salts [17, 18]. Dried wakame, manufactured by Riken Food Co., Ltd. (Tokyo, Japan), was suspended in ethanol (20% w/w) and stirred at room temperature for 45 min, and the residue was filtered. The ethanol extraction was repeated once at room temperature for 40 min. After filtration, centrifugal dehydration (H-110A, Kokusan Co., Ltd., Saitama, Japan), drying at 80 °C for 120 min, and powdering, a low-sodium wakame powder was obtained. Low-sodium and low-fat wakame powder was prepared using the following procedures. The dried wakame was powdered and suspended in water for 15 min. After swelling, 750 mL of acetone was added, and the mixture was stirred for 30 min and filtered. The acetone extraction was repeated twice, and after filtration and vacuum (VOS-301SD, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) drying at 65 °C for 5 h, low-sodium and low-fat wakame powder was obtained. The soluble and insoluble fractions obtained from dried wakame were as follows: the low-sodium and low-fat wakame powder was suspended in water and 0.4% w/w trisodium citrate dihydrate, and stirred for 60 min. After centrifugation (Avanti HP-20XP, Beckman Coulter Co., Ltd., Tokyo, Japan) for 20 min at 12,000 ×g, the supernatant and the precipitate were obtained. Water was added to the precipitate, which was stirred for 30 min and centrifuged for 20 min at 12,000 ×g. This water extraction was repeated twice, and all supernatants were concentrated under reduced pressure. Ethanol (83% w/w) was added, stirred overnight at room temperature, and centrifuged for 20 min at 12,000 ×g. 600 mL of acetone was added to the precipitate; the mixture was stirred at room temperature for 5 h and filtered. Additional 500 mL of acetone was added, and the mixture was stirred at room temperature overnight, followed by filtration and vacuum drying at 65 °C for 7 h. After dissolution in water, the solution was concentrated under reduced pressure. Ethanol (85% w/w) was added to the concentrate, stirred overnight at room temperature, and centrifuged for 15 min at 12,000 ×g. 600 mL of acetone was added to the precipitate, and the mixture

was stirred at room temperature for 5 h. After filtration, the precipitate was vacuum dried at 65 °C for 8 hours. It was used as the water-soluble fraction containing alginate. After solubilizing the alginic acid in the wakame powder, the precipitate was used as an insoluble fraction. Extraction yield was as follow; 75% low-sodium powder, 68% low-sodium and low-fat powder, 22% insoluble fraction, and 35% soluble fraction. Commercial alginate (Kikkoman Biochemifa Company, Japan) was used as the reference.

In vitro cell-free experiments

Glucose release rate

The wakame extract had a final concentration of 6 g/L. The glucose release rate was determined using *in vitro* cell-free artificial digestion. The cooked white rice was crushed, decomposed by several enzymes, and the free glucose concentration was measured. Briefly, 25 mL of ion-exchanged water was added to the rice and wakame extracts and the mixture was crushed. Three millilitres of 0.3% pepsin with 1 N hydrochloric acid was added, and the mixture was incubated at 37 °C for 30 min. After neutralization, 2.5 mL of 0.3% pancreatin with 0.5% invertase were added and the mixture was incubated at 37 °C for 20 min or 16 h. The supernatant obtained from each sample was analysed using the crude enzyme α-glucosidase obtained from rat intestinal acetone powder (Sigma-Aldrich, St Louis, MO, USA). The powder was dissolved in 50 mM phosphate buffer (pH 6.9), sonicated in an ice bath for 20 min, and centrifuged at 1,500 ×g for 10 min at 4 °C. The crude enzyme supernatant was added to 100 µL of the 20 min or 16 h sample with 1.9 mL of phosphate buffer and incubated at 37 °C for 40 min. The glucose concentration of each sample was measured using a commercial kit (glucose C-II test Wako, FUJIFILM Wako Pure Chemical Co., Osaka, Japan). The solutions were analysed using a spectrophotometer at 505 nm and the absorbance values were recorded. All samples were run eight times. The glucose release rate was calculated as follows: % glucose release = (20 min glucose concentration / 16 h glucose concentration) ×100.

Measurement of α-glucosidase activity

The wakame extract had a final concentration of 3 g/L. α-Glucosidase was determined by measuring glucose production from the maltose solution. The crude enzyme of α-glucosidase was obtained from rat intestinal acetone powder using the same method as that of the glucose release rate. The following solutions were mixed and pre-incubated at 37 °C for 5 min: 2.0 mL of 250 mM maltose solution, 2.4 mL of ion-exchanged water, and 500 µL of wakame extract. Next, 100 µL of crude enzyme was added and incubated at 37 °C for 40 min. The reaction was terminated by adding 5 mL of 200 mM sodium carbonate solution. Water was added as a control instead of wakame extract. For the blank solution, the reaction was stopped immediately after adding the enzyme solution. For the positive control, 1 mg/mL of acarbose was used instead of wakame extract. The amount of glucose was measured in the same manner as the glucose- releasing rate. Inhibition ratios were calculated as follows: % inhibition = (1 – A sample/A control) ×100.

Analytical grade chemical reagents were used in the *in vitro* experiments. Ethanol, acetone, trisodium citrate dihydrate, hydrochloric acid, sodium carbonate, pepsin, pancreatin, maltose, and acarbose were obtained from FUJIFILM Wako Pure Chemical Co., (Osaka, Japan).

In vivo experiments

Effects of wakame on postprandial glycaemia in humans

Healthy young Japanese women were recruited from April 21, 2016 to Jun 26, 2019, if they had untreated type 2 diabetes with fasting blood glucose levels between 70 and 110 mg/dL at a preliminary examination. The exclusion criteria were as follows: fasting blood glucose levels >125 mg/dL, excessive alcohol intake, possible allergy to seaweed, use of any medicine for any clinical treatment of disease, and taking oral drugs periodically or supplements that could affect blood glucose levels. This study was conducted in accordance with the Declaration of Helsinki and with the approval of the Fuji Women's University Ethics Committee (registration; April 19, 2016 and May 30, 2018) and the protocol was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN000047854, 25/05/2022).

An open-label, randomised, two-period, crossover design was used. Subjects were randomly allocated (1:1) to one of two sequences using a computer-generated randomisation list prepared by us. The participants were asked to fast and drink only plain water until their first blood collection. They were also instructed to consume normal quantities of food, exercise as usual, and abstain from excessive eating, drinking, and exercise during the study period. Data was collected in the laboratory of Fuji Women's University. The first study included 12 healthy women aged 25 ± 2.4 years with a body mass index of 19.4 ± 0.5 kg/m^2. The participants underwent baseline blood collection and ingested 150 g of aseptically packaged rice (Sato Foods Co., Ltd., Niigata, Japan) and wakame soup or soup without wakame. The soup containing wakame was made a using commercial product (WAKAME SOUP) manufactured by Riken Food Co., Ltd., (Tokyo, Japan). This soup contained 0.7 g of fibre, 0.9 g of protein, 0.7 g of lipids, and 2.2 g of carbohydrates. Soup without a wakame was made using the same soup, and only the wakame was absent. In the second study, subjects were 16 healthy women aged 22 ± 0.7 years with a body mass index of 20.2 ± 0.36 kg/m^2. The subjects ingested 150 g of packed rice, with or without a wakame salad. We used 4 g of dried wakame (FUERU WAKAME-CHAN® Sanriku), manufactured by Riken Food Co., Ltd., by soaking in water, draining, and squeezing out excess water. The 4 g of dried wakame contained 1.4 g of fibre, 0.8 g of protein, 0.2 g of lipids, and 1.6 g of carbohydrates. In the third study, subjects were 19 healthy women aged 22 ± 0.9 years with a body mass index of 20.3 ± 0.34 kg/m^2. The subjects ingested 150 g of packed rice and curry, with or without wakame salad like that in the second study. Curry is a commercial product manufactured by House Foods Group Inc. (Tokyo, Japan), containing 3.9 g of protein, 5.9 g of lipids, and 19.3 g of carbohydrates. In all studies, blood glucose levels

were measured 15, 30, 45, 60, 90, and 120 min after consuming the test meal using a simple blood glucose meter (Freestyle-freedom-lite or Freestyle-libre, Abbott Japan LLC, Tokyo, Japan).

Statistical analysis

Data are shown as the means ± standard error of the mean. Statistical differences were determined using Duncan's multiple range test for the *in vitro* study and Wilcoxon signed-rank test for between-group comparisons in the *in vivo* study. Statistical analyses were conducted using IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA). Two-sided significance level was set at < 0.05.

Results

In vitro cell-free experiments

Inhibitory effects of wakame extracts on glucose releasing rate and α-glucosidase activity

As shown in Table 1, wakame powder, wakame extracts, and commercial sodium alginate inhibited the glucose release rate compared with the control. In particular, the soluble fraction showed stronger inhibition of glucose release than the other fractions. The water-soluble fraction was presumed to contain sodium alginate and, its viscosity was measured using commercial alginate as reference. It was confirmed that the viscosity of the soluble fraction (86.3 mPa/s, 0.2%) was much higher than that of commercial alginate (8.5 mPa/s, 0.2%). However, inhibition of α-glucosidase activity was not observed in any of the wakame extracts used in this study.

Table 1. Glucose release rate in wakame extracts

	Glucose (mg/dL) 20 mins	Glucose (mg/dL) < 16 hours	Glucose release rate (%)
Rice (control)	58±1.1 a	86±0.6	69±1.2 a
Rice + low-sodium powder	47±1.4 b	84±1.8	55±1.0 b
Rice + low-sodium and low-fat powder	46±0.8 b	88±1.4	53±1.1 b
Rice + insoluble fraction	48±1.3 b	87±1.8	55±0.7 b
Rice + soluble fraction	38±0.6 c	85±1.3	45±1.5 c
Rice + commercial alginate	46±0.4 b	88±1.7	53±1.7 b

Data represent the average of n 8 and with different superscript letters are significantly different using Duncan's multiple range test at $p < 0.05$.

In vivo experiments

Effects of wakame on postprandial glycemia in humans

In the first study, the subjects ingested 150 g of rice and wakame soup, which contained 0.6 g of fibre, or soup without wakame. The blood glucose profiles and incremental area under the curve (iAUC) of 0 –120 min of subjects are shown in Fig. 1. Compared with soup intake without wakame, soup addition of wakame was associated with significantly lower ($p < 0.05$) postprandial glucose concentrations at 15 min, but the iAUC0 –120 did not change in this situation. In the second study, the subjects ingested rice with or without wakame salad, which contained 1.4 g of fibre. The blood glucose profiles, shown in Fig. 2, were also significantly lower ($p < 0.05$) 15, 30, and 45 min after wakame salad ingestion. The iAUC0 –120 min also did not change depending on whether the wakame salad was used. Compared with the ingestion of wakame soup in the first study, ingestion of wakame salad resulted in lower postprandial blood glucose levels in the second study not only 15 min, but also at 30 and 45 min after meals. In the third study, the subjects ingested 150 g of packed rice and curry, with or without wakame salad; compared with the control, ingestion of wakame salad significantly lowered ($p < 0.05$) the blood glucose concentration at 30 –90 min and the iAUC0 –120 min (Fig. 3).

figure 1

figure 2

figure 3

Discussion

This *in vivo* study demonstrated that the intake of wakame combined with 150 g of white rice significantly reduced the postprandial blood glucose response in the early phase (Fig. 1,2). The *in vitro* study also showed that wakame extract, which contains sodium alginate, inhibited the glucose release rate (Table 1). In particular, the soluble fraction showed stronger inhibition of glucose release than the other fractions. The soluble fraction (86.3 mPa/s, 0.2%) has much higher viscous than that of commercial alginate (8.5 mPa/s, 0.2%), and this high viscosity could inhibit glucose release. Soluble viscous fibres generally affect carbohydrate metabolism in the intestine because of the delay in gastric emptying [2-4]. Alginate may slow down the glucose absorption by increase digestive fluid viscosity and form a gel within the gastrointestinal tract, and decrease gastric emptying rate [8-10]. The effect of alginate consumption on gastric emptying rate is still unclear, however alginate-based beverage which contains 15.0 g alginate in 500 ml reduced blood glucose response in healthy subjects [8]. Alginate

represent the main polysaccharide of wakame and polysaccharide content of wakame is 35.2% of dry mass [9]. Since the concentration of alginate in wakame is low, effects of wakame on postprandial glycemia in human was limited to the early phase. One previous study demonstrated the consumption of mekabu, the *sporophylls* of *Undaria pinnatifida* and more viscous than wakame, reduced postprandial glucose in the early phase [11]. Our results also demonstrated that the amount of viscous dietary fibre contained in the wakame salad (1.4 g) was higher than that contained in the wakame soup (0.7 g) might be affect to reduce postprandial glucose response. The property of the water-soluble fraction of wakame could delay gastric emptying and glucose absorption in the small intestine, and reduce the postprandial blood glucose response in the early phase, depending on the amount of dietary fibre. However, wakame extracts did not inhibit α-glucosidase and thus, did not change total glucose absorption, which may explain why iAUC0 –120 was not affected in the first and second studies. Phenolic compounds and fucoidans are characterised as α-glucosidase inhibitors [6,12,19]. Cho *et al.* reported that wakame polysaccharides act as α-glucosidase inhibitors only when the sulphation degree increases [20]. Moreover, compared with other seaweeds, wakame has low water-soluble dietary fibre and water-soluble polyphenols; therefore, its α-glucosidase inhibitory activity is weak [12]. Similar results were obtained in this study.

The third *in vivo* study demonstrated that ingestion of wakame salad with rice and curry significantly lowered glucose absorption in the early phase along with total glucose absorption and affected iAUC0 –120 (Fig. 3). We used the commercial curry, which contained 3.9 g of protein, 5.9 g of lipids, and 19.3 g of carbohydrates, in the third study. Meal fat delays gastric emptying and amino acids stimulate insulin secretion [21,22]. In this study, ingestion of wakame salad along with ingestion of curry, which contained protein and lipid, could delayed gastric emptying and stimulated insulin secretion; synergistic effects with wakame salad may have been observed. Further research is needed to clarify in that detail.

The traditional Japanese diet could be associated with an extremely low rate of cardiovascular disease. A prospective cohort study demonstrated that Japanese dietary patterns, which are highly correlated with seaweed, soybean products, fish, vegetables, fruit, and green tea consumption, are associated with a low risk of cardiovascular disease mortality [23]. However, recently, there have been changes in the diet of the Japanese population. The intake of plant-based foods has declined, and the Western-style diet, which is rich in lipids, has increased [24]. Obesity, metabolic syndrome, and type 2 diabetes have become major health problems in Japan owing of this dietary trend. Seaweeds are an important component of the daily diet in Japan, and there is substantial evidence regarding the health benefits of seaweed-derived food products, including a decreased risk of diabetes mellitus [25-27]. One of the most common seaweeds in Japan is the wakame. In a previous report, we demonstrated that wakame ingestion reduces postprandial glucose and insulin concentrations in healthy adults. In the early postprandial stage (0–30 min), blood glucose and insulin levels were significantly lower following wakame intake than after rice intake alone [15]. The brown algae of seaweed are rich in soluble viscous fibres, such as alginates. Torsdottir *et al.* [28] demonstrated that acute ingestion of 5 g sodium alginate with a lipid meal reduced postprandial glucose and insulin concentrations in patients with type 2 diabetes and that this correlated with delayed

gastric emptying on scintigraphy. Our results are also consistent with those of previous studies and show that wakame could represent a functional food with specific anti-hyperglycaemic properties in humans.

The effect of wakame intake on postprandial glycemia is might to be due to alginate in the water-soluble fraction. However, it is still unclear whether the wakame delay the gastric emptying rate. Further *in vitro* Caco cells and/or *in vivo* studies will be needed to determine the physiological mechanisms of wakame.

Conclusion

Our findings showed that wakame intake effectively reduced postprandial glucose concentrations in healthy young women. Compared to rice intake alone, blood glucose levels in the early postprandial stage were significantly decreased when wakame was consumed with rice. The viscosity of the water-soluble wakame extract could reduce the postprandial glucose concentration by inhibiting the glucose release rate. Thus, our results indicate that wakame may be used as a functional food item with specific hypoglycaemic properties.

Declarations

Data Availability

The datasets analysed during the current study are available from the corresponding author on reasonable request.

Acknowledgements

We would like to thank Editage (www. editage.com) for English language editing.

Author contributions

All authors contributed to the study conception and design. Material preparation was performed by Keiko Yoshinaga, data collection and analysis were performed by Rieko Mitamura. The first draft of the manuscript was written by Rieko Mitamura and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Competing interests

RM has no conflicts of interest to declare, KY is an employee of Riken Vitamin Co., Ltd.

Consent to Participate

Written informed consent was obtained from the participants.

Consent to Publish

The participant has consented to the submission of the case report to the journal.

References

1. Suzuki, N. *et al*. Characteristics of the Japanese diet described in epidemiologic publication: A qualitative systematic review. *J. Nutr. Sci. Vitaminol. (Tokyo)* **64, 129-137** (2018).
2. Müller, M., Canfora, E. E. & Blaak, E. E. Gastrointestinal Transit Time, Glucose Homeostasis and Metabolic Health: Modulation by Dietary Fibers. *Nutrients.* **10, 275**; 10.3390/nu10030275 (2018).
3. Yu, K., Ke, M. Y., Li, W. H., Zhang, S. Q. & Fang, X. C. The impact of soluble dietary fibre on gastric emptying, postprandial bold glucose and insulin in patients with type 2 diabetes. *Asia Pac. J. Clin. Nutr.* **23, 210-218** (2014).
4. Jenkins, D. J. A. *et al.* Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity. *Br. Med. J.* **1, 1392-1394** (1978).
5. Okimura, T., Jiang, Z., Liang, Y., Yamaguchi, K. & Oda, T. Suppressive effect of ascophyllan HS on postprandial blood sugar level through the inhibition of α-glucosidase and stimulation of glucagon-like peptide-1 (GLP-1) secretion. *Int. J. Biol. Macromol.* **125, 453-458** (2019).
6. Attjioui, M. *et al*. Comparison of edible brown algae extracts for the inhibition of intestinal carbohydrate digestive enzymes involved in glucose release from the diet. *J. Nutr. Sci.* **10, 1-10** (2021).
7. Post, R. E., MainousIII, A. G., King, D. E. & Simpson, K. N. Dietary fiber for the treatment of type 2 diabetes mellitus: A meta-analysis. *J. Am. Board. Fam. Med.* **25, 16-23** (2012).
8. Jensen, M. G., Kristensen, M., Belza, A., Kundsen, J. C. & Astrup, A. Acute effect of alginate-based preload on satiety feelings, energy intake, and gastric emptying rate in healthy subjects. *Obesity.* **20, 1851-1858** (2012).
9. Gabbia, D. & Martin, S. D. Brown seaweeds for the management of metabolic syndrome and associated diseases. *Molecules*. **25, 4182**; 10.3390/molecules25184182 (2020).
10. Xing, M. *et al*. Advances in research on the bioactivity of alginate oligosaccharides. *Mar Drugs*. **18, 144**; 10.3390/md18030144 (2020).
11. Tanemura, Y. *et al*. Effects of the intake of *Undaria pinnatifida* (Wakame) and its sporophylls (Mekabu) on postprandial glucose and insulin metabolism. *J. Med. Invest.* **61, 291-297** (2014).
12. Moreira, A. R. S. *et al*. Effect of *Undaria Pinnatifida, Himanthalia elongate* and *Porphyra umbilicalis* on *in vitro* α-glucosidase activity and glucose diffusion. *Nutr. Hosp.* **29, 1434-1446** (2014).
13. Kato, T. *et al*. Rondomized, double-blind, crossover clinical trial of the effect of calcium alginate in noodles on postprandial blood glucose level. *Biol. Pharm. Bull.* **41, 1367-1371** (2018).
14. Kim, M. S., Kim, J. Y., Choi, W. H. & Lee, S. S. Effects of seaweed supplementation on blood glucose concentration, lipid profile, and antioxidant enzyme activities in patients with type 2 diabetes mellitus. *Nutr. Res. Practice.* **2, 62-67** (2008).

15. Yoshinaga, K. & Mitamura, R. Effects of *Undaria pinnatifida* (Wakame) on postprandial glycemia and insulin levels in humans: a randomized crossover trial. *Plant Foods Hum. Nutr.* **74, 461-467** (2019).
16. Paxman, J. R., Richardson, J. C., Dettmar, P.W. & Corfe, B. M. Alginate reduces the increased uptake of cholesterol and glucose in overweight male subjects: a pilot study. *Nutr. Res.* **28, 501-505** (2008).
17. Montes, L., Gisbert, M., Hinojosa, I., Sineiro, J. & Moreira, R. Impact of drying on the sodium alginate obtained after polyphenols ultrasound-assisted extraction from *Ascophyllum nodosum* seaweeds. *Carbohyde. Polym.* **272, 118455**; 10.1016/j.carbpol.2021.118455 (2021).
18. Florez-Fernandez, N., Dominguez, H. & Torres, M. D. Functional features of alginates recovered from *Himanthalia elongate* using subcritical water extraction. *Molecules.* **26, 4726**; 10.3390/molecules26164726 (2021)
19. Nowicka, P., Wojdyło, A. & Laskowski, P. Inhibitory potential against digestive enzymes linked to obesity and type 2 diabetes and content of bioactive compounds in 20 cultivars of the peach fruit grown in Poland. *Plant Foods Hum. Nutr.* **73, 314-320** (2018).
20. Cho, M., Han, J. H. & You, S. Inhibitory effects of fucan sulfates on enzymatic hydrolysis of starch. *Food Sci. Technol.* **44, 1164-1171** (2011).
21. Stacher, G. *et al.* Fat preload delays gastric emptying: reversal by cisapride. *Br. J. Clin. Pharmac.* **30**, **839-845** (1990).
22. Zhang, T. & Li, C. Mechanisms of amino acid-stimulated insulin secretion in congential hyperinsulinism. *Acta. Biochim. Biophys. Sin.* **45**, **36-43** (2013).
23. Shimazu, T. *et al*. (2007) Dietary patterns and cardiovascular disease mortality in Japan: a prospective cohort study. *Int. J. Epidemiol.* **36**, **600-609** (2007).
24. Murakami, K., Barbara, M., Livingstone, E. & Sasaki, S. Thirteen-Year Trends in Dietary Patterns among Japanese Adults in the National Health and Nutrition Survey 2003 2015: Continuous Westernization of the Japanese Diet. *Nutrients.* **10, 994**; 10.3390/nu10080994 (2018)
25. Wells, M. L. *et al*. Algae as nutritional and functional food sources: revisiting our understanding. *J. Appl. Phycol.* **29, 949-982** (2016).
26. Lee, H. J., Kim, H. C., Vitek, L. & Nam, C. M. Algae consumption and risk of type 2 diabetes: Korean National Health and Nutrition Examination Survey in 2005. *J. Nutr. Sci. Vitaminol. (Tokyo)* **56**, **13-18** (2010).
27. Maeda, H., Yamamoto, R., Hirao, K. & Tochikubo, O. Effects of agar (kanten) diet on obese patients with impaired glucose tolerance and type 2 diabetes. *Diabetes Obes. Metab.* **7, 40-46** (2005).
28. Torsdottir, I., Alpsten, M., Holm, G., Sandberg, A. S. & Tölli, J. A small dose of soluble alginate-fiber affects postprandial glycemia and gastric emptying in humans with diabetes. *J. Nutr.* **121, 795-799** (1991).

Figures

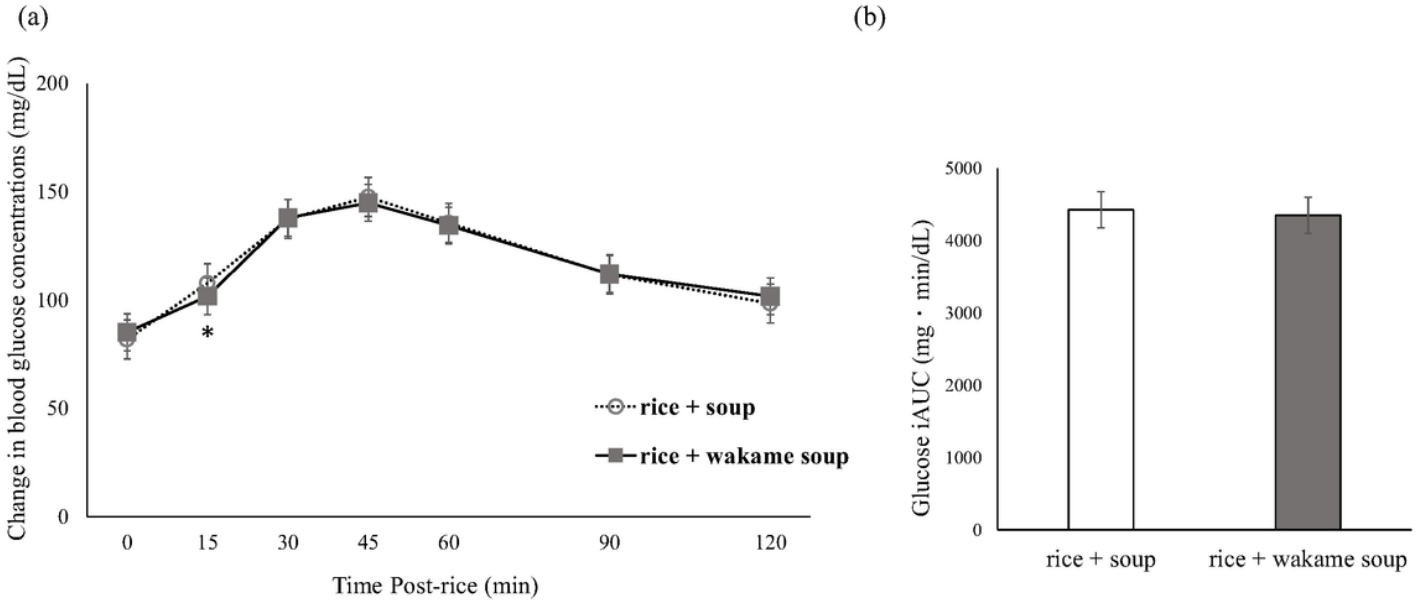

Figure 1.

Figure 1

Reduction of postprandial blood glucose response in healthy young women after consuming rice with or without wakame soup (the first study)

a. Change in blood glucose concentration from baseline over 120 min. **b.** The incremental area under the blood concentration-time curve for glycaemia over 0 to 120 min. Values are shown as means ± standard error of the mean. Significant difference by inter-group comparison: *$p < 0.05$ (Wilcoxon signed-rank test).

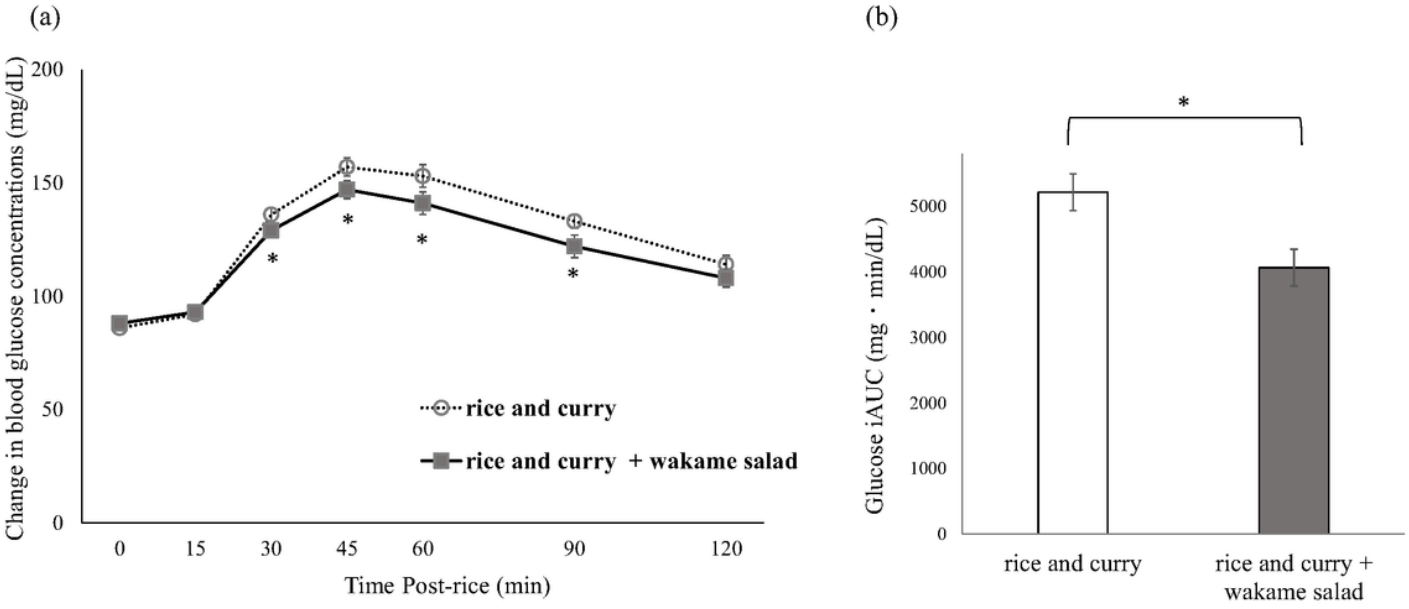

Figure 3.

Figure 2

Reduction of postprandial blood glucose response in healthy young women after consuming rice with or without wakame salad (the second study)

a. Change in blood glucose concentration from baseline over 120 min. **b.** The incremental area under the blood concentration-time curve for glycaemia over 0 to 120 min. Values are shown as means ± standard error of the mean. Significant difference by inter-group comparison: *$p < 0.05$ (Wilcoxon signed-rank test).

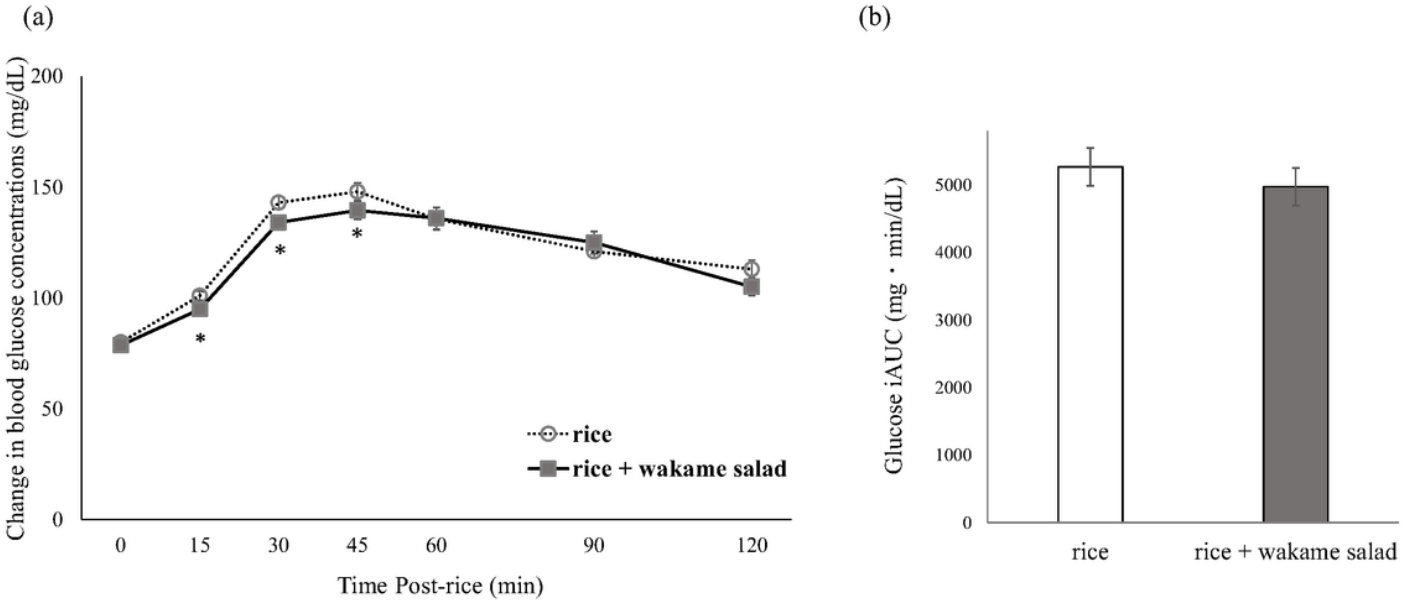

Figure 2.

Figure 3

Reduction of postprandial blood glucose response in healthy young women after consuming rice and curry with or without wakame salad (the third study)

a. Change in blood glucose concentration from baseline over 120 min. **b.** The incremental area under the blood concentration-time curve for glycaemia over 0 to 120 min. Values are shown as means ± standard error of the mean. Significant difference by inter-group comparison: *$p < 0.05$ (Wilcoxon signed-rank test).