Seaweed intake and risk of cardiovascular disease: the Japan Public Health Center based Prospective (JPHC) Study

著者（英）	Utako Murai, Kazumasa YAMAGISHI, Mizuki Sata, Yoshihiro Kokubo, Isao Saito, Hiroshi Yatsuya, Junko Ishihara, Manami Inoue, Norie Sawada, Hiroyasu Iso, Shoichiro Tsugane, JPHC Study Group
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Utako Murai[1], Kazumasa Yamagishi[1], Mizuki Sata[1,2], Yoshihiro Kokubo[3], Isao Saito[4], Hiroshi Yatsuya[5], Junko Ishihara[6], Manami Inoue[7], Norie Sawada[7], Hiroyasu Iso[1,8], Shoichiro Tsugane[7], for the JPHC Study Group

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1 Department of Public Health Medicine, Faculty of Medicine, and Health Services Research and Development Center, University of Tsukuba, Tsukuba, Japan.

2 Department of Preventive Medicine and Public Health, School of Medicine, Keio University, Tokyo, Japan.

3 Department of Preventive Medicine, National Cerebral and Cardiovascular Center, Suita, Japan.

4 Department of Public Health and Epidemiology, Faculty of Medicine, Oita University, Yufu, Japan.

5 Department of Public Health, Fujita Health University School of Medicine, Toyoake, Japan.

6 Department of Food and Life Science, School of Life and Environmental Science, Azabu University, Sagamihara, Japan.

7 Epidemiology and Prevention Division, Research Center for Cancer Prevention and

Screening, National Cancer Center, Tokyo, Japan.

8 Public Health, Department of Social Medicine, Osaka University Graduate School of Medicine, Suita, Japan.

Corresponding author: Kazumasa Yamagishi, MD, PhD

Department of Public Health Medicine, Faculty of Medicine, University of Tsukuba, Tennodai 1-1-1, Tsukuba, Japan, 305-8575

Telephone number: +81 29 853 2695

Fax number: +81 29 853 2695

E-mail address: yamagishi.kazumas.ge@u.tsukuba.ac.jp

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Short running head: Seaweed intake and incident cardiovascular disease

Abbreviations used: PHC = public health center, FFQ = food frequency questionnaire, HR = hazard ratio, CI = confidence interval

ABSTRACT

Background: The minerals, vitamins, soluble dietary fibers, and flavonoids of seaweed are protective for prevent cardiovascular diseases. However, the association between seaweed intake and risk of cardiovascular disease has not been established.

Objectives: We examined the dietary intake of seaweed and its impact upon stroke and ischemic heart disease risk among a Japanese study population.

Design: We surveyed 40,707 men and 45,406 women from 2 large cohorts (age range: 40–69 years). Seaweed intake was determined by food frequency questionnaire at baseline (1990–1994). Incidences of stroke and ischemic heart disease were ascertained until the end of 2009 (Cohort I) or 2012 (Cohort II). Sex-specific cardiovascular disease hazard ratios (95% confidence intervals) were estimated using Cox proportional hazard models after stratification by area and adjustment for cardiovascular risk and dietary factors.

Results: During 1,493,232 person-years of follow-up, 4,777 strokes (2,863 ischemic stroke, 1361 intraparenchymal hemorrhages and 531 subarachnoid hemorrhages) and 1,204 ischemic heart disease cases were identified. Among men, the multivariable hazard ratios for almost daily consumption versus almost no consumption of seaweed were only seen in ischemic heart disease: 0.76 (0.58, 0.99; *P* for trend = 0.04) and total cardiovascular diseases: 0.88 (0.78, 1.00; *P* for trend = 0.08). Among women, such inverse associations were 0.56 (0.36, 0.85; *P* for trend = 0.01) for ischemic heart disease, and 0.89 (0.76, 1.05; *P* for trend = 0.10) for total cardiovascular diseases. No significant associations were observed between seaweed intake and risk of total stroke or stroke types among either men or women.

Conclusions: Seaweed intake was inversely associated with risk of ischemic heart disease.

Keywords: epidemiology, follow-up study, ischemic heart disease, risk factor, diet

INTRODUCTION

Seaweed, outside of industrial food processing, is not widely eaten in the West but is broadly consumed in East Asian countries. It contains healthy components such as high levels of potassium, carotenoid, and dietary fiber (1, 2), and the most commonly used seaweeds in food preparation include the following species: brown seaweeds (*Undalia pinnatifida* [*Wakame* in Japanese], kelp (*Laminaria* [*Konbu*], *Hizikia fusiformis* [*Hijiki*]), red seaweeds and green seaweeds (*Laver* [*Nori*]). Although seaweed consumption is not a traditional part of the Western diet, the globalization of cuisine and an increased proportion of Asian immigrants may work to increase future consumption in Western countries.

Previous studies showed that seaweed components, such as carotenoids, peptides, and fiber were related to lower levels of serum total cholesterol, blood pressure, body weight and blood glucose in both animals (3–8) and humans (9–14). As the Japan Public Health Center-based Prospective (JPHC) Study showed that dietary fiber was associated inversely with risk of cardiovascular disease (15), seaweed intake may be prophylactic in this regard.

However, an association between seaweed intake and the incidence of cardiovascular disease has not been established. The aim of the present study was therefore to determine any associations between dietary intake of seaweed and risks of total stroke, stroke types (ischemic stroke, intraparenchymal hemorrhage and subarachnoid hemorrhage), ischemic heart disease (myocardial infarction and sudden cardiac death) and total cardiovascular diseases among Japanese men and women. As the Japanese are a unique population which consumes extremely high amounts of seaweeds compared to the rest of the world (2), any beneficial impact of seaweed may be more easily detected.

SUBJECTS AND METHODS

Study population

The JPHC study consists of two subcohorts based on public health center (PHC) areas; Cohort I (started in 1990, five PHC areas, participants aged 40–59 years) and Cohort II (started in 1993, six PHC areas, participants aged 40–69 years). The study design has been described in detail previously (16). The JPHC study was approved by the institutional review boards of the National Cancer Center, Osaka University and the University of Tsukuba.

The JPHC Study is an ongoing cohort study comprising a community-based sample of 140,420 Japanese participants (68,722 men and 71,698 women) that follows up on incidences of cancer and cardiovascular diseases. However, participants in two PHC areas (Tokyo and Osaka) were excluded from the present study because the incidence of cardiovascular disease was unable to be followed up in these two communities, leaving 116,896 remaining participants as eligible for the follow-up study. Participants were asked to complete self-administered questionnaires about their lifestyles and medical histories. Informed consent was obtained before questionnaire completion or was sometimes obtained from community leaders instead of individuals as this was common practice for informed consent in Japan at that time.

Baseline questionnaire

A self-administered questionnaire included demographic characteristics, medical history, physical activity level, smoking, drinking, and dietary habits. This questionnaire, including a food frequency questionnaire (FFQ), was distributed to all eligible study participants in 1990 for Cohort I and in 1993–94 for Cohort II. The FFQ included 44 food items for Cohort I and 52 food items for Cohort II. Both cohort FFQs included an item about typical seaweed intake and contained four or five frequency categories for seaweeds (*Wakame*, *Konbu*, *Nori* and more), ranging from “almost never,” “1–2 days/week,” “3–4 days/week” to “almost daily,” in

Cohort I and from “rarely,” “occasionally,” “1–2 days/week,” “3–4 days/week” to “almost daily,” in Cohort II. For analysis, we combined the data from both cohorts, classifying the participants into four groups according to their frequencies of seaweed intake: “almost no consumption (almost never, rarely or occasionally)”, “1–2 days/week”, “3–4 days /week”, and “almost daily consumption.” Validation of the FFQ was done by comparing the frequency of seaweed intake from FFQ to that from dietary records over seven consecutive days in each of four seasons (Spring, Summer, Fall and Winter). The Spearman’s correlation coefficients between the frequency of seaweed intake based on the FFQ and dietary records among subsamples of 122 men and 125 women in Cohort I and 176 men and 178 women in Cohort II were moderate: 0.37 and 0.33, respectively, in Cohort I (17) and 0.27 and 0.40, respectively, in Cohort II (unpublished data).

Stroke and ischemic heart disease registry

The nine PHC areas were comprised of 78 total hospitals, which were core hospitals capable of treating cardiovascular disease events. Physicians in the hospitals, PHCs, or research physician-epidemiologists (all blinded to patient lifestyle data) reviewed the medical records of cohort participants at each hospital and extracted clinical information, including brain images, electrocardiographs, and enzyme test results, onto cohort-specific registration forms. To confirm the fatal cardiovascular disease events, we used the information on death certificates. All death certificates were forwarded to the PHC in the area of residency, and the mortality data was sent to the Ministry of Health, Welfare and Labor to be coded for national vital statistics. As registration of deaths is required by the Family Registration Law in Japan, we were assured that all relevant causes of deaths were recorded.

Stroke was confirmed according to the criteria of the National Survey of Stroke (18), which requires the presence of focal neurological deficits of sudden or rapid onset lasting at least 24 hours or until death. Stroke was classified into ischemic strokes, intraparenchymal hemorrhages, and subarachnoid hemorrhages. As participating hospitals were equipped with computed tomography and/or magnetic resonance imaging scans, imaging was available for 97% of registered stroke events.

Ischemic heart disease was defined as myocardial infarction and sudden cardiac death. Myocardial infarction was confirmed in the medical records according to the criteria of the Monitoring Trends and Determinants of Cardiovascular Disease (MONICA) project (19), which requires typical chest pain and evidence from electrocardiography and/or cardiac enzyme levels. For those cases with typical prolonged chest pain (≥20 min) but not confirmed by electrocardiography or cardiac enzyme tests, a tentative myocardial infarction diagnosis was made and these were included in myocardial infarction cases. Deaths within 28 days of the onset of myocardial infarction and sudden cardiac deaths were regarded as fatal coronary events while sudden cardiac death was defined as a death of unknown origin that occurred within 1 hour of the event onset. The primary outcome of this study is incident cardiovascular diseases (total stroke, stroke types and ischemic heart disease) and both fatal and nonfatal cases were included.

Total cardiovascular disease was defined as total stroke, stroke types and ischemic heart disease, whichever occurred first, during the follow-up.

Statistical analysis

Follow-ups were started from the completion of the questionnaire and ended on the dates of death, emigration, incident stroke/ischemic heart disease events, or the end of either 2009 (for Cohort I) or 2012 (for Cohort II), whichever came first.

Within the pool of 116,896 participants, we excluded those who were lost to, or refused follow-up (n=240), leaving a total of 116,656 people from the two cohorts (Cohorts I and II) who were eligible for participation in the present study. Of these, 94,275 participants (81%) completed the baseline questionnaires, including information on seaweed intake. In addition, we excluded persons who had histories of stroke, angina pectoris, myocardial infarction or cancer at the time of the baseline questionnaires (n=3,630). We further excluded persons with a total energy intake of less than 2.5 or more than the 97.5 percentile (n=4,532). Ultimately, 86,113 people (40,707 men and 45,406 women) were included in the present study.

Sex-specific, age-adjusted mean values and prevalence of selected factors were calculated and compared among the frequencies of seaweed intake using linear or logistic regression analyses. The person-years of follow-up for each participant were calculated from the baseline to the first endpoint which was defined as a cardiovascular event, a death, a move from the community, or the end of the follow-up period (2009 for Cohort I and 2012 for Cohort II).

Sex-specific, area (nine PHCs) -stratified and age (continuous) -adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated using Cox proportional hazard models. We confirmed the proportional hazards assumption according to the frequency of seaweed intake by time interactions, and found that the assumption was not violated. For seaweed intake categories, dummy variables were created, and the almost no consumption group was regarded as the reference. We further adjusted for body mass index (quintile), histories of hypertension and diabetes mellitus, including treatment (yes or no), treatment for hypercholesterolemia (yes or no), leisure-time physical activity (no, 1–3 days/month, 1–2 days/week, 3–4 days/week and almost every day), smoking status (never, ex-smoker and current smoker), alcohol intake (0, 1–149, 150–299, 300–449 and 450 g/week or more), quartiles of total energy intake, dietary intakes of vegetables, fruits, red meat, processed meat, fish, soy, green tea and sodium. The food intakes were estimated by the Japan Food Table, 5th version. Trend tests were performed by allocating scores of 0, 1.5, 3.5 and 6 for each seaweed intake groups.

The socio-economic status may be an important confounding factor, but we did not have information except for education levels in subcohort (Cohort I). Therefore, we additionally performed subgroup analyses in Cohort I adjusting for education levels (junior high school or less, high school, and university or higher education).

We used SAS version 9.4 software (SAS Institute Inc, Cary, NC) for the analyses. All probability values for statistical tests were two-tailed, and values where $p<0.05$ were regarded as statistically significant.

RESULTS

The baseline survey revealed that those respondents who consumed seaweeds frequently tended to consume more energy-adjusted dietary intakes of vegetables, fruits, red and processed meat, fish, soy and sodium, and as well as have lower proportions of current smokers among both men and women.

During 1,493,232 person-years of follow-up in 86,113 men and women, 5,873 (3,611 in men and 2,262 in women) incident cases of total cardiovascular disease, including 2,863 (1,805 in men and 1,058 in women) ischemic strokes, 1,361 (805 in men and 556 in women) intraparenchymal hemorrhages, 531 (180 in men and 351 in women) subarachnoid hemorrhages, 1,204 (884 in men and 320 in women) ischemic heart disease events were identified. Total seaweed intake by the participants ranged from a mean value of 0.3 g/day in the almost no consumption to 4.7 g/day in the almost daily consumption groups among men and 0.2 g/day to 3.9 g/day, respectively, among women (**Table 1**).

Seaweed intake was inversely associated with risk of ischemic heart disease among men and women. The multivariable HRs (95%CIs) for the almost daily consumption versus the almost no consumption groups were 0.76 (0.58, 0.99; *P* for trend=0.04) for ischemic heart disease and 0.88 (0.78, 1.00; *P* for trend=0.08) for total cardiovascular disease among men (**Table 2**). The multivariable HRs (95% CIs) among women were 0.56 (0.36, 0.85; *P* for trend=0.01) for ischemic heart disease and 0.89 (0.76, 1.05; *P* for trend=0.10) for total cardiovascular diseases (**Table 3**).

To examine any potential reverse causation for the frequency of seaweed intake and risk of cardiovascular disease, we performed the same analyses but excluded early events (≤5 years from the baseline). These associations did not materially alter any outcomes among either men or women (data not shown). When we further adjusted for education levels in this subgroup (Cohort I, n=39,176), these associations were not materially altered for any outcomes among either men or women (data not shown).

DISCUSSION

In this large, prospective study of middle-aged Japanese men and women, we found an inverse association between seaweed intake and risk of ischemic heart disease that was more pronounced among women. To our knowledge, this is the first study to show an association between seaweed intake and risk of incident cardiovascular disease. Another previous Japanese cohort study showed a significant inverse association between the frequency of seaweed intake and age-adjusted mortality from stroke (not ischemic heart disease) among women, but not among men (20). In that study, however, the adjustment for potential confounding factors was not conducted and the risk of cardiovascular disease mortality, instead of incidence, was examined.

One possible mechanism for the protective effect of seaweed against the risk of ischemic cardiovascular disease is a lipid-lowering effect as shown in a previous study of apolipoprotein E-deficient mice where fucoidan contained in seaweed regulated the metabolism of serum lipids by increasing lipoprotein lipase activity (21), reducing blood levels of triglycerides and low-density lipoprotein cholesterol. Furthermore, the supplementation of a high-fat diet with 5% fucoidan for 12 weeks attenuated the development of atherosclerosis and plaque formation compared to a high-fat diet alone (21). Another possible mechanism is a blood pressure lowering effect. Peptides isolated from *wakame* have inhibitory activity for the angiotensin-1-converting enzyme, leading to decreases in blood pressure in spontaneously hypertensive rats (6). In humans, a randomized controlled trial showed that diastolic blood pressure levels in hypertensive patients decreased by 8 mmHg after treatment with 5 g/day *wakame* powder for 8 weeks, whereas the decrease in the non-treated group was not significant (9). In addition, a double-blinded crossover trial using a 4-week supplement of 12 or 24g/day, but not 6g/day seaweed fiber showed that untreated mild hypertensive patients saw a significant decrease in mean blood pressure compared with the placebo group (11). Accordingly, another study in a spontaneously hypertensive rat model using 1% NaCl supplementation in drinking water showed a 4-fold increase in fecal sodium excretion in the intervention group fed on 10% alginic acid (the main soluble fiber of seaweed) compared with a control group fed on 10% kaolin (22). Ikeda et al. (4) reported that blood pressure levels in stroke-prone spontaneously hypertensive rats given 0.5% NaCl-supplemented drinking water and *wakame* powder did not differ with groups given cellulose or kaolin control supplements. However, the mean life-span of the *wakame* powder group was significantly extended compared to controls (77 days for w*akame* group vs. 62 days for the control group). In that study, neuronal cell injury after 4-day hypoxic insult was attenuated in a dose-dependent fashion by the addition of 152 or 1520 nmol/L fucoxanthin isolated from seaweeds, suggesting that *wakame* may have a beneficial effect on stroke independent of blood pressure-lowering effect (4). In the present study, the adjustment for history of hypertension and treatment of hypercholesterolemia did not alter the results. These variables, however, were not minute so that the residual confounding may remain.

Seaweed intake may be a marker of healthy dietary patterns. A previous principal component analysis run in the JPHC Study identified that seaweed intake consisted of a ‘prudent’ (healthy) dietary pattern that involved vegetables, fruit, potatoes, soy products, mushrooms, seaweed, fish, and green tea (23). Among these foods, potatoes and mushrooms were not adjusted in our analysis, because these intakes were not confounded for the association with the risk of ischemic heart disease in our cohort (data not shown). Furthermore, when we adjusted for modified DASH dietary score (including vegetables, fruits, red and processed meat intake, dairy instead of low-fat dairy, soy instead of nuts and legumes, sodium, and sweetened beverages), the results did not change materially (data not shown). Therefore, these results support that seaweed may lower the risk of cardiovascular disease independent of healthy dietary pattern.

Some kinds of seaweed, particularly *Hizikia fusiformis* (*Hijiki*), are rich in inorganic arsenic, which has been suggested to be associated with increased risk of cardiovascular disease (24). Potential mechanisms for arsenic-related atherosclerosis include endothelial dysfunction, smooth-muscle proliferation, and enhanced platelet aggregation (25). However, we did not find any adverse effect of seaweed on cardiovascular diseases suggesting that adverse impact of arsenic, if any, may be overwhelmed by the beneficial effect of seaweed as discussed above. Furthermore, inorganic arsenic in *Hijiki* could be removed by soaking in water and boiling when cooking (26, 27).

The strengths of the present study included its large sample size, a population-based prospective design, a high follow-up rate, the use of validated FFQ and cardiovascular incident data, and the extensive adjustment for potentially important confounding factors, including physical activity level, smoking status, alcohol intake, and other food items. Japanese are a unique population who consume an extremely high amount of seaweeds, which allowed us to perform associative studies of a large distribution of seaweed intake with incident cardiovascular disease. As seaweed in non-Asian countries is mostly used in industrial food processing, this type of study could not be performed in Western populations.

Several limitations in the present study warrant discussion. First, although validated by dietary records, the use of an FFQ inevitably leads to seaweed intake misclassification. The low correlation coefficients between the frequency of seaweed intake (based on FFQ) and dietary records suggest that the self-reported intake is subject to measurement errors. However, as any misclassification was likely to be random concerning the outcome, this was not thought to impact the results greatly. Second, we could not negate the possibility of residual confounding by unmeasured variables such as socio-economic status. When we further adjusted for education levels (junior high school or less, high school, and university or higher education) in the subgroup, there were no substantial changes in the results for either men or women (not shown in Table).

In conclusion, seaweed intake was significantly and inversely associated with risk of ischemic heart disease. Our results suggest that a high seaweed intake may have a beneficial effect on cardiovascular disease among middle-aged men and women.

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Conflict of Interest: none declared.

The author's responsibilities were as follows: UM: designed the analysis plan; UM, KY and HI: wrote the manuscript and had primary responsibility for the final content; UM: performed statistical analysis; MI, NS and ST: contributed to the acquisition of data; and all authors: contributed to the interpretation of data, contributed to revising the manuscript critically of the manuscript. None of the authors reported a conflict of interest related to the study.

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Table 1. Baseline cardiovascular risk factors and selected dietary variables among 40,707 men and 45,406 women according to the frequency of seaweed intake[1]

	Men					Women				
	Frequency of seaweed intake									
	Almost no consumption	1-2 days/week	3-4 days/week	Almost daily consumption	*P* for difference	Almost no consumption	1-2 days/week	3-4 days/week	Almost daily consumption	*P* for difference
Number at risk	9,033	14,067	11,826	5,781		6,537	13,351	16,197	9,321	
Age at baseline, mean	52.7±8.7	51.0±7.7	51.7±7.7	52.6±7.6	0.07	54.2±8.9	51.3±7.9	51.9±7.6	52.4±7.5	<0.001
Body mass index, kg/m^2	23.5±3.0	23.5±2.9	23.5±2.8	23.4±2.9	0.002	23.6±3.4	23.5±3.1	23.5±3.2	23.5±3.1	0.009
Treatment for hypertension, %	18.1±39.2	18.3±38.0	18.5±38.8	19.2±40.0	0.02	17.8±40.2	17.6±37.2	17.4±37.6	17.8±38.6	0.24
Treatment for hypercholesterolemia, %	1.0±10.3	1.1±10.3	1.6±12.4	1.6±12.6	<0.001	1.1±12.0	2.0±13.4	2.2±14.7	2.3±15.3	<0.001
Treatment for diabetes mellitus, %	6.4±24.8	5.9±23.1	6.4±24.5	6.9±25.8	0.05	3.3±18.8	3.0±16.6	2.9±16.7	2.8±16.7	0.29
Current smokers, %	56.7±49.6	52.0±50.0	51.1±50.0	48.9±50.0	<0.001	8.0±26.6	6.0±30.3	4.8±21.6	4.1±19.8	<0.001
Alcohol intake ≥450 ml/week, %	24.8±43.1	21.6±41.1	22.2±41.6	22.0±41.7	0.002	4.8±21.5	3.5±18.4	2.7±16.1	3.3±17.7	0.04

Leisure-time physical activity ≥ 3 times/week, %	8.2±27.9	9.1±28.4	10.1±30.1	11.6±32.4	<0.001	7.8±27.9	7.0±24.9	8.8±28.2	11.2±31.7	<0.001
Dietary intake[2]										
Total energy intake, kcal/day	1,662±479	1,852±499	1,992±500	2,080±502	<0.001	1,072±250	1,213±306	1,326±328	1,425±359	<0.001
Vegetable intake, g/day	58±41	100±47	128±48	158±60	<0.001	73±52	102±60	126±63	154±76	<0.001
Fruit intake, g/day	51±64	68±72	81±82	94±103	<0.001	84±67	97±80	109±85	122±104	<0.001
Red meat intake, g/day	16.5±11.7	21.1±13.8	24.4±16.2	25.2±18.7	<0.001	14.9±9.4	17.0±11.5	18.5±13.2	19.2±16.0	<0.001
Processed meat intake, g/day	2.6±3.1	3.5±3.7	4.1±4.2	4.3±5.0	<0.001	2.9±3.0	3.3±3.5	3.6±3.9	3.8±4.7	<0.001
Fish intake, g/day	50±36	51±33	59±35	67±41	<0.001	39±27	42±26	48±28	54±32	<0.001
Soy intake, g/day	42±26	48±25	58±25	68±25	<0.001	42±25	47±23	54±23	62±23	<0.001
Green tea intake, ml/day	446±360	406±334	411±331	419±332	<0.001	431±328	398±312	409±310	403±306	0.07
Seaweed intake, g/day	0.3±0.1	1.1±0.2	2.4±0.5	4.7±1.1	<0.001	0.2±0.1	0.9±0.2	2.0±0.5	3.9±0.9	<0.001
Sodium intake, g/day	4.1±1.7	4.8±2.0	5.3±2.1	5.7±2.2	<0.001	3.8±1.4	4.2±1.8	4.5±1.9	4.7±2.0	<0.001

[1]Sex-specific, age-adjusted mean (unadjusted standard deviations), or age-adjusted percentages were calculated according to the frequency of seaweed intake using linear or logistic regression analysis.

[2]Energy-adjusted values except for total energy intake.

*P-values for the overall difference using analysis of covariance.

Table 2. Multivariable-adjusted HRs and 95% confidence intervals for risk of incident total cardiovascular diseases, total stroke, stroke types and ischemic heart disease according to the frequency of seaweed intake among 40,707 men[1]

	Frequency of seaweed intake				
	Almost no consumption	1-2 days/week	3-4 days/week	Almost daily consumption	*P* for trend
Number of men at risk	9,033	14,067	11,826	5,781	
Person years	144,764	237,476	199,746	96,497	
Total stroke, n	646	916	813	429	
Model 1	1.00	0.87 (0.78, 0.97)	0.85 (0.76, 0.95)	0.88 (0.77, 0.998)	0.08
Model 2	1.00	0.93 (0.83, 1.04)	0.91 (0.81, 1.03)	0.93 (0.80, 1.07)	0.35
Ischemic stroke, n	434	571	526	274	
Model 1	1.00	0.83 (0.73, 0.95)	0.83 (0.73, 0.96)	0.84 (0.71, 0.98)	0.08
Model 2	1.00	0.89 (0.77, 1.02)	0.91 (0.78, 1.05)	0.91 (0.76, 1.09)	0.53
Intraparenchymal hemorrhage, n	173	274	238	120	
Model 1	1.00	0.95 (0.78, 1.17)	0.93 (0.75, 1.15)	0.94 (0.73, 1.20)	0.59
Model 2	1.00	1.03 (0.83, 1.27)	0.98 (0.78, 1.24)	0.94 (0.71, 1.24)	0.52
Subarachnoid hemorrhage, n	37	65	44	34	
Model 1	1.00	0.89 (0.58, 1.39)	0.66 (0.41, 1.08)	1.04 (0.62, 1.75)	0.90
Model 2	1.00	0.94 (0.59, 1.50)	0.67 (0.40, 1.14)	1.01 (0.56, 1.79)	0.80

Ischemic heart disease, n	220	303	246	115	
Model 1	1.00	0.83 (0.69, 0.997)	0.77 (0.63, 0.94)	0.72 (0.57, 0.91)	0.007
Model 2	1.00	0.87 (0.70, 1.03)	0.79 (0.64, 0.98)	0.76 (0.58, 0.99)	0.04
Total cardiovascular diseases, n	846	1192	1038	535	
Model 1	1.00	0.86 (0.78, 0.95)	0.83 (0.76, 0.92)	0.84 (0.75, 0.95)	0.006
Model 2	1.00	0.91 (0.82, 1.00)	0.88 (0.79, 0.98)	0.88 (0.78, 1.00)	0.08

[1]HRs (95% CIs) were derived from Cox proportional hazards regression models. Model 1 was stratified by area and adjusted for age. Model 2 was adjusted further for body mass index, leisure-time physical activity, smoking status, alcohol intake, histories of hypertension or diabetes mellitus, treatment for hypercholesterolemia, total energy intake, dietary intakes of vegetables, fruits, red meat, processed meat, fish, soy, green tea and salt.

Table 3. Multivariable-adjusted HRs and 95% confidence intervals for risk of incident total cardiovascular diseases, total stroke, stroke types and ischemic heart disease according to the frequency of seaweed intake among 45,406 women[1]

	Frequency of seaweed intake				
	Almost no consumption	1-2 days/week	3-4 days/week	Almost daily consumption	*P* for trend
Number of women at risk	6,537	13,351	16,197	9,321	
Person years	113,514	239,797	292,681	168,757	
Total stroke, n	309	567	680	417	
Model 1	1.00	0.96 (0.83, 1.11)	0.88 (0.76, 1.01)	0.88 (0.75, 1.03)	0.05
Model 2	1.00	1.05 (0.90, 1.22)	0.98 (0.84, 1.15)	0.96 (0.81, 1.15)	0.36
Ischemic stroke, n	186	314	348	210	
Model 1	1.00	0.96 (0.79, 1.15)	0.80 (0.66, 0.97)	0.78 (0.63, 0.97)	0.004
Model 2	1.00	1.05 (0.86, 1.28)	0.93 (0.76, 1.15)	0.90 (0.71, 1.14)	0.16
Intraparenchymal hemorrhage, n	73	150	207	126	
Model 1	1.00	1.06 (0.79, 1.42)	1.14 (0.85, 1.51)	1.14 (0.84, 1.55)	0.35
Model 2	1.00	1.16 (0.86, 1.57)	1.29 (0.95, 1.75)	1.25 (0.89, 1.76)	0.23
Subarachnoid hemorrhage, n	48	102	124	77	

Model 1	1.00	0.91 (0.63, 1.30)	0.83 (0.58, 1.18)	0.84 (0.57, 1.24)	0.37
Model 2	1.00	0.94 (0.64, 1.37)	0.81 (0.55, 1.20)	0.78 (0.51, 1.20)	0.18
Ischemic heart disease, n	74	89	109	48	
Model 1	1.00	0.72 (0.53, 0.996)	0.71 (0.52, 0.97)	0.52 (0.36, 0.77)	0.002
Model 2	1.00	0.75 (0.54, 1.05)	0.74 (0.52, 1.04)	0.56 (0.36, 0.85)	0.01
Total cardiovascular diseases, n	376	647	779	460	
Model 1	1.00	0.92 (0.81, 1.05)	0.85 (0.74, 0.97)	0.82 (0.71, 0.95)	0.004
Model 2	1.00	0.99 (0.86, 1.14)	0.94 (0.82, 1.09)	0.89 (0.76, 1.05)	0.10

[1]HRs (95% CIs) were derived from Cox proportional hazards regression models. Model 1 was stratified by area and adjusted for age. Model 2 was adjusted further for body mass index, leisure-time physical activity, smoking status, alcohol intake, histories of hypertension or diabetes mellitus, treatment for hypercholesterolemia, total energy intake, dietary intakes of vegetables, fruits, red meat, processed meat, fish, soy, green tea and salt.