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14. ABSTRACT The purpose of this research is to investigate whether eating brown seaweed (*Undaria pinnatifida*) can influence breast cancer risk. Brown seaweeds are popular in Japan, where the incidence of breast cancer is about 1/6 the rate of that reported for American women. In several animal studies of diet and cancer, adding seaweed to the normal diet resulted in longer healthy lives. In particular, we will examine cell surface binding characteristics and protein expression associated with the consumption of dietary seaweeds by women without breast cancer, women with estrogen receptor negative breast cancer, and women with estrogen receptor positive breast cancer.All subjects have completed the study, and all samples have been analyzed for the endpoints definedin the study. Additionally, samples have been analyzed for urokinase and urine as been included in the proteomic study. Papers on iodine content in commercially available seaweeds has been published, as has a review of the health effects of seaweeds and a paper describing the bioavailability of seaweed iodine in brown seaweeds. The analyses of the proteomics data and the flow cytometry data were completed in April and May, and preparation of manuscripts is in process.

15. SUBJECT TERMS
seaweed, breast cancer, prevention

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INTRODUCTION

Breast cancer is the second leading cause of cancer among American women. Survival rates at 5 years average 87%, decreasing to only 52% at 20 years. About 40,000 American women die of breast cancer each year[1]. There is an urgent need for new treatments for metastatic breast cancer and chemoprevention that can be used to prevent breast cancer recurrence. Interestingly, there is dramatic variation in breast cancer rates, even among industrialized nations. Epidemiologic studies done in Japan in the 1980s, before Westernized diets were common, reported that Japanese women had 1/3 the rate of premenopausal breast cancer and 1/9 the rate of postmenopausal breast cancer [2]. In addition, when a Japanese woman developed breast cancer, she was more likely to survive at least five years than a woman diagnosed with breast cancer in the United States [3]. The purpose of this study was to investigate whether dietary seaweed supplementation could be safely given to American women (as measured by iodine metabolism, thyroid hormone regulation), and whether or not it would directly affect immune cell populations (T lymphocytes, monocytes, natural killer cells, B cells) and their cell surface markers (CXCR4 and CCR5). We also wanted to investigate whether seaweed would be associated with differences in serum and urinary protein expression, with supporting data on urokinase that could inform our interpretation of the proteomic data.

BODY

Epidemiologic studies comparing breast cancer rates among Japanese women in Japan and American women in the US are supportive that dietary factors could be critical to understanding breast cancer rates. In vitro work using seaweed extracts have shown high antitumor activity. In vivo work using rats and mice have demonstrated that seaweed, both as part of a regular diet, as an extract in drinking water, and as extracts which were injected into tumor bearing rats, have all confirmed that something in seaweed inhibits cancer formation and can cause tumor remission/tumor rejection in tumor bearing animals.

Although little is known about relative breast cancer risk and seaweed intake among humans, a small body of research, both *in vivo* and *in vitro*, suggests seaweed may be useful in breast cancer prevention. Seaweeds are specifically used to treat tumors in Traditional Chinese Medicine and Japanese folk medicine. On a population level, those people for whom seaweed is a regular part of their diet, most notably in Japan, have dramatically lower rates of hormone sensitive cancers, both of the breast and prostate [4-6].

No clinical studies of breast cancer and seaweed have yet been done, however, in a large prospective dietary study 21,852 Japanese nurses in Japan, investigators reported after 9 years of follow-up, that high intake of miso (a seaweed-rich broth flavored with fermented soybean paste) soup was the food most closely associated with the lowest breast cancer risk[7]. This is particularly interesting since an *in vivo* study compared dietary seaweed water extract to powdered seaweed and to injected seaweed extract, and reported that dietary seaweed water extract was the most effective against induced tumors [8, 9]. Women who had three or more bowls of miso soup each day had about half the rate of breast cancer (RR 0.51; 95% Confidence Interval 0.32 to 0.83). On average, seaweed intake in Japan is estimated between 7 and 10 g/d dry weight[10]. The bioavailability of the seaweed iodine to humans has been reported [11-13], and we have therefore chosen a low-iodine seaweed for this study. On average, *Undaria pinnatifida* contains 50µ iodine/g. 5 grams of *Undaria* will provide an additional 250µ iodine/d, well under the 1,000 µ iodine/d that is considered the maximum tolerated dose of iodine/d.

This research project was begun in 1999, at the University of Massachusetts. However, only the initial work on seaweed toxicity was completed before the PI moved to the University of South Carolina. This coincided with the necessity of obtaining Army IRB approval, and although numerous renditions of the grant have now been made, and tentative Army IRB approval was given in August 2003, the study was not officially approved, and further changes have been made to the study design. The Memorandum for Record was completed, and we enrolled the 15 required subjects, all of whom have completed the study.

Based on the findings of significant changes in cell surface binding characteristics associated with dietary seaweed, we are conducting flow cytometry to analyze changes. These cell surface binding sites are particularly important in breast cancer metastases, CXCR4 and CCR5 [14], and changes in CD36 binding to CD11+ monocytes are associated with angiogenesis[15]. CXCR4 appears to act as a homing signal for metastatic breast cancer cells, binding exclusively to stromal derived factor-1, a cytokine found most abundantly in the liver, lung, and bone, all preferred sites for breast cancer metastases. The role of CCR5 is less well understood in breast cancer metastasis, but is also considered crucial in breast cancer[16]. We will use standard flow cytometry to identify the relative binding site densities and in-depth proteonomics to indicate which proteins are involved in both binding site activation and responses to dietary seaweed. Based on concurrent work on breast cancer and CXCR4 and CCR5 changes on CD4

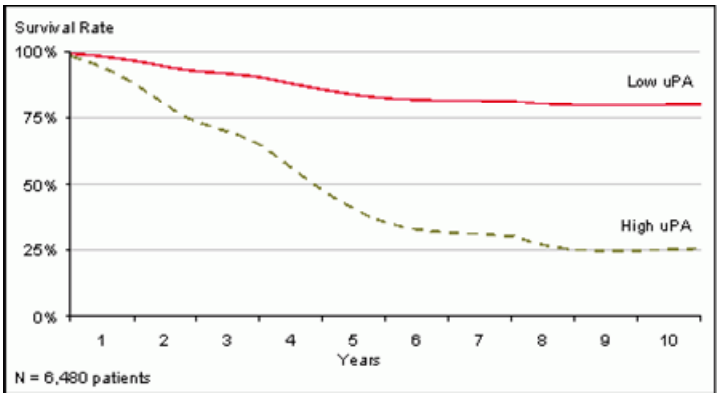
and CD8 cells associated with dietary seaweed as measured by flow cytometry, we will focus on serum T lymphocytes. CD36 is a marker of monocyte, and alterations in this binding site are associated with decreased angiogenesis [15].

Profiling of serum proteins using surface enhanced laser desorption/ionization time of flight (SLEDI-TOF) mass spectrometry has become increasingly specific and can now identify with high sensitivity and specificity cancer types, including breast cancer based on the specific signature of proteomic serum biomarkers. Recent studies (reviewed by Laronga[17]) have shown that using SLEDI-TOF can differentiate between BrCa1 carriers and healthy controls (13/15 women with BrCa1 compared to one of the 15 non-carriers), 14/16 patients with breast cancer even 6-9 months following treatment for breast cancer, compared to healthy controls, and sentinel lymph node positive (22/27) patients from sentinel lymph node negative (55/71) patients. SELDI ProteinChip® technology is the primary proteomic platform technology for the NCI Early Detection Research Network (EDRN) study of early detection serum biomarkers of prostate cancer (e.g., review by Grizzle et al.[18]; other prostate diseases (e.g., review by Fung[19], Semmes[20]), ovarian cancer [21] and. In addition, SELDI ProteinChip® technology has been used to identify changes in serum protein expression with the addition of novel foods, like green tea, to the diet [22].

The purpose of this research is to investigate whether consuming brown seaweed (*Undaria pinnatifida*) can change lymphocyte populations, surface binding sites on CD4 and CD8 cells, and alter serum protein expressions. Specifically we will study CXCR4 and CCR5 cytokine receptor sites, both known to be important in determining location of breast cancer metastases. To minimize the variation with menstrual cycle phase, and to concentrate on the age group with the highest risk of breast cancer, we will focus on postmenopausal women. Based on our *in vitro* studies showing that seaweed extract has a dose dependent inhibitory effect on estrogen receptor negative (ER-), but no effect on estrogen receptor positive (ER+) breast cancer cells, we anticipate that estrogen receptor status will be an important variable in our study. Our blinded, crossover study design will serve to address the issues of any carry-over effect of seaweed after cessation of seaweed intake.

In a second study done at the University of Massachusetts on the bioavailability of seaweed iodine has now been published in the *Journal of Medicinal Food*. Our conclusions were that although 5 grams/day of seaweed (*Alaria esculenta*) provided approximately 500 ug/d of iodine and was approximately the average daily consumption in Japan, the statistically significant increase in thyroid stimulating hormone was small and not biologically important. All clinical values remained within normal limits. However, to be safe, we changed seaweeds to utilize the lower iodine levels (approximately 50 ug/g) in *Undaria pinnatifida*, so that in the current study the seaweed provided an additional 250 ug/day of iodine.

Two endpoints that were added to the study as it progressed were an analysis of urinary urokinase. To do this, we analyzed both serum and urine using an ELIZA kit (RnD Systems). This was based on the observations of many breast cancer researchers who have reported that high urokinase levels indicate high cancer cell driven proteolysis of the extracellular matrix in preparation for metastatic activity. The figure below shows the importance of urokinase levels for women with breast cancer.



Tissue Levels of Urokinase are Independently Predictive of Breast Cancer Recurrence

Several studies of the antitumor activity of seaweed have reported an effect of fucoidan, the unique sulfated polysaccharide found only in brown seaweeds, on urokinase activity [23, 24]. We were therefore intrigued by the possibility that at least part of the antitumor effects of brown seaweeds might be due to modulation of urokinase, and that dietary supplementation could alter urinary excretion of urokinase.

A second added study was to include urine samples as well as serum samples in the proteomics analysis. This provides additional information about how dietary seaweeds modulate protein expression.

KEY RESEARCH ACCOMPLISHMENTS

1. Sociodemographic characteristics of 15 subjects who completed the study.

			Disease Status		
		All	Control	ER+	ER-
Age		59.4 ± 4.6	59.8 ± 3.2	60.4 ± 4.5	58 ± 6.3
Ethnicity					
	European American	9	3	5	1
	African American	6	2	0	4
BMI*		30 ± 7.1	34 ± 5.4	24 ± 4.6	32 ± 7.5
Education					
	High School	2	1	0	1
	Some college	5	1	2	2
	College graduate	5	2	2	1
	Additional graduate courses	3	1	1	1
Family income					
	Not reported	2			
	<$50,000	4			
	$50,000-100,000	6			
	>$100,000	3			
Employed fulltime (percent)		8 (65%)			
Married or living as married		11 (73%)			
Living alone		4 (27%)			
Recreational exercise(> 1 hrs/wk		8 (65%)	1	3	4
Reproductive history					
Ave number live births		1.6 ± 1.6	1.7 ± 1.5	1.8 ± 0.8	1.2 ± 1.8
Cancer history					
Family member (mother, sister) has breast cancer		5	1	1	3
Treatment for breast cancer					
	surgery			5	5
	radiation			3	4
	chemotherapy			1	5
Years since dx BC				8.6 ± 6.1	4 ± 2.3

cancer					
FSH at recruitment (postmenopausal = 23-116.3 mIU/mL)		69.4 ± 21.1	57.4 ± 9.7	72.5 ± 30.7	78.3 ± 15.1
Concurrent medicat for thyroid and blood pressure					
	Blood pressure		2	1	3
	Thyroid		3	0	2

* Body Mass Index (BMI) = Kg/m2 (Weight in Kilograms / Surface Area in Square Meters)

2. Change lymphocyte populations, surface binding sites on CD4 and CD8 cells

No changes were detected for percentage of T-lymphocytes that were CD3 positive for CD4 (helper cells), CD8 (suppressor cells), or for percentage CD4 cells of all lymphocytes. Nor did we detect changes in percent natural killer cells and B cells. In addition, there were no changes in CXCR4 or CCR5 expression on any lymphocyte population. We also found no change in monocyte population, either by percentage, or by those that expressed CD11.

3. Changes in serum protein expression

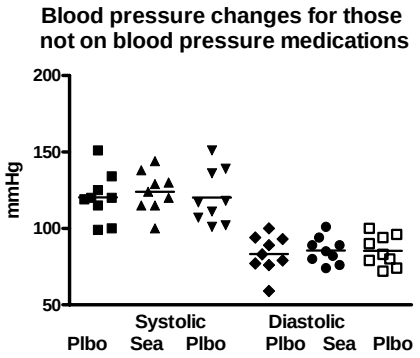
Serum was fractionated by anion exchange and fractions were tested on 3 array surfaces. Statistical analysis was performed on Ciphergen Express software with both paired and unpaired analysis. Controls were either included or excluded from analysis to normalize for breast cancer incidence. No significant peaks (p-value <0.05) were seen between the week 2 and week 4 time points. However, upon comparison of the week 4 and week 6 time points, 2 protein peaks were significantly different in the unpaired analysis and 3 were significantly different between the paired analyses. 1 protein peak, at 4413 Da (p=0.008, week 4 intensity = 1.991, week 6 intensity = 3.041), was significantly different in both the paired and unpaired analysis.

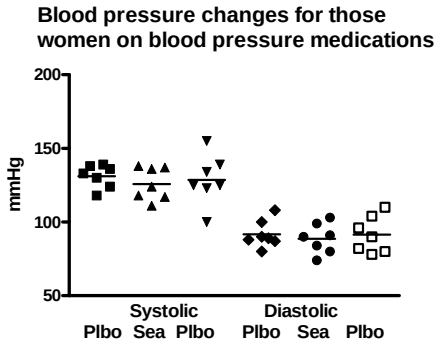
4. Changes in urine protein expression

Samples from fifteen women were obtained before kelp treatment (week 2), during treatment (week 4), and after treatment (week 6). Urine was analyzed in duplicate on weak cation exchange chips (CM10) and statistical analysis performed on Ciphergen Express software. We compared all combinations of two time points to obtain clusters and detect peaks with significant differences in mean intensity (P-value< 0.05). These analyses were performed as paired and unpaired tests. For the paired test, each person at the two time points constituted a pair. Of particular interest were two peaks which were significantly reduced (P-value <0.05), both in the paired and unpaired statistical analysis, during treatment (week 4) when compared to before or after treatment time points (week 2 or week 6).

5. Clinical measurements: Blood pressure

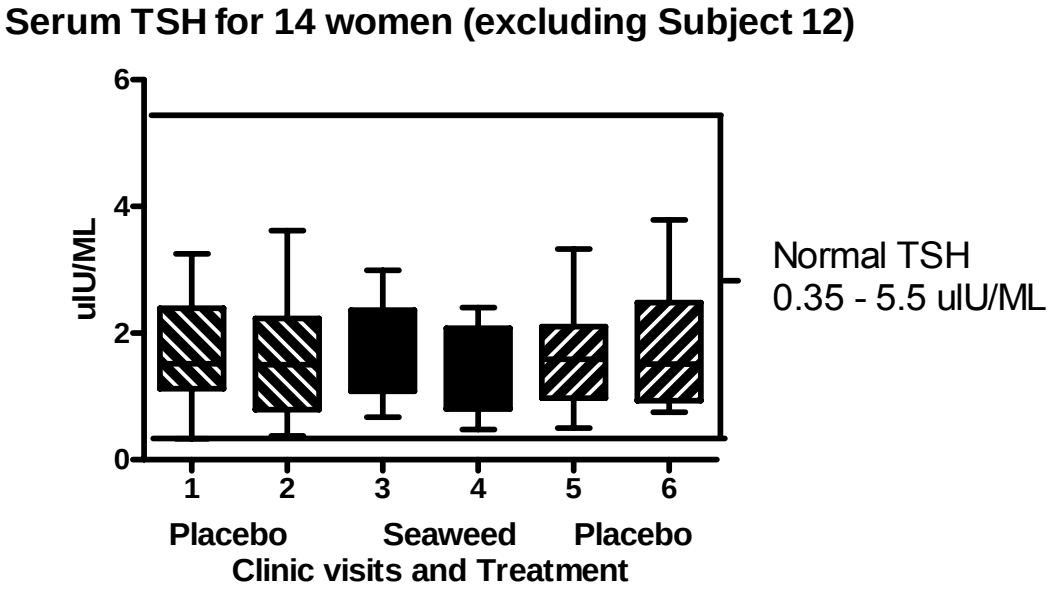
In this study, seaweed was not associated with changes in blood pressure, although like other studies of seaweed supplementation of subjects who were already on medication for hypertension [25], there was a small decrease in systolic blood pressure with seaweed (decrease from 131.1 mmHg to 125.9 mmHg which increased to 128.7 mmHg on the second month on placebo) and diastolic blood pressure (91.7 mmHg decreased to 88.7 mmHg on seaweed which increased to 91.4 mmHg in the following month on placebo). Although these changes were small, and the effects were non-significant (p=0.3), there were only seven women in this group.



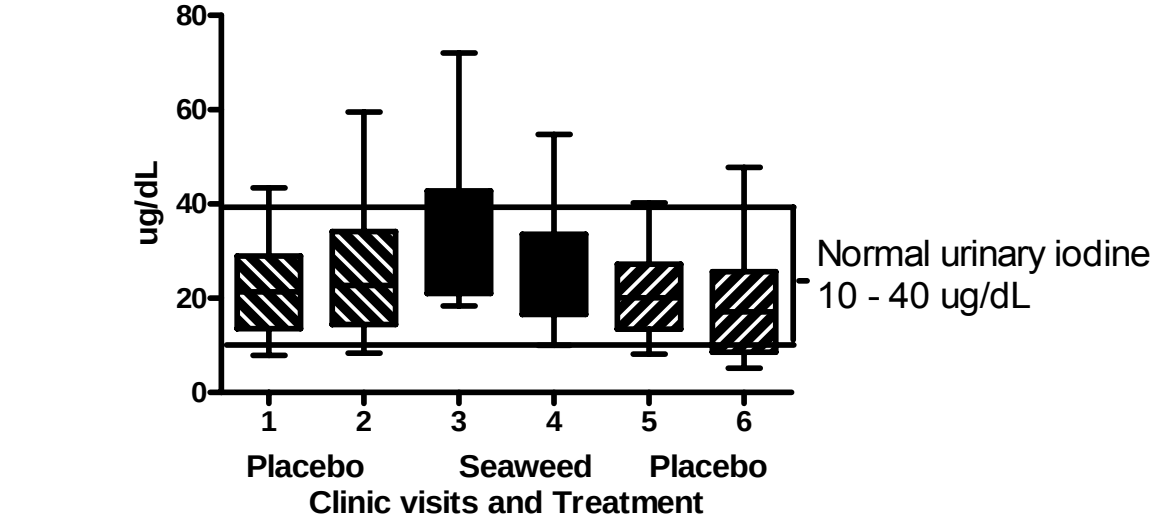


6. Changes in Thyroid Stimulating Hormone (TSH) and urinary excretion of iodine.

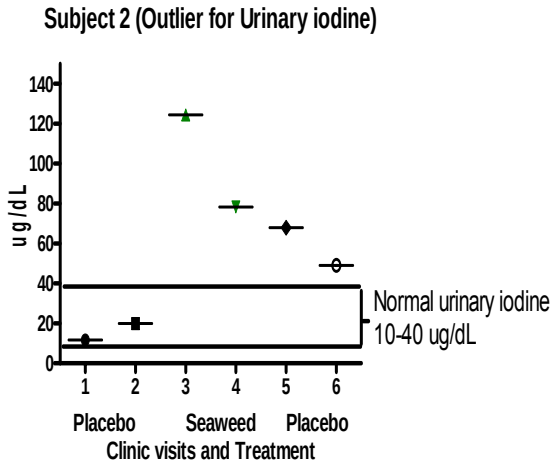
The bioavailability of the seaweed iodine to humans has been reported [11-13]. In this study of low-iodine seaweed, for 15 of the women, TSH values remained within normal range and did not change with the addition of approximately 250 ug/day of additional iodine from seaweed. One of the women (subject 12) was taking Synthyroid, and here TSH values began high, decreased on seaweed, and further decreased on placebo. This was thought to be the result of a previous thyroid condition that was not being controlled by medications.

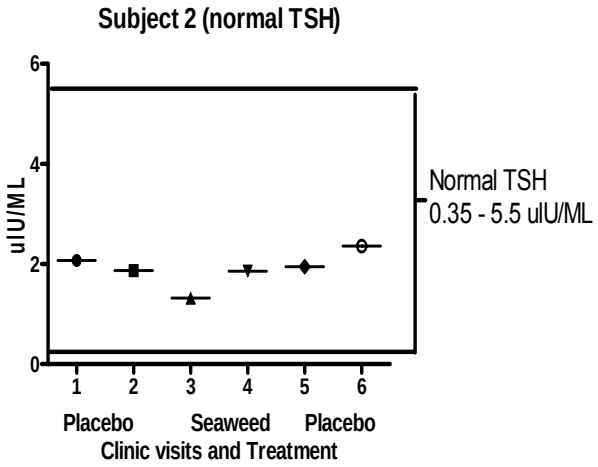


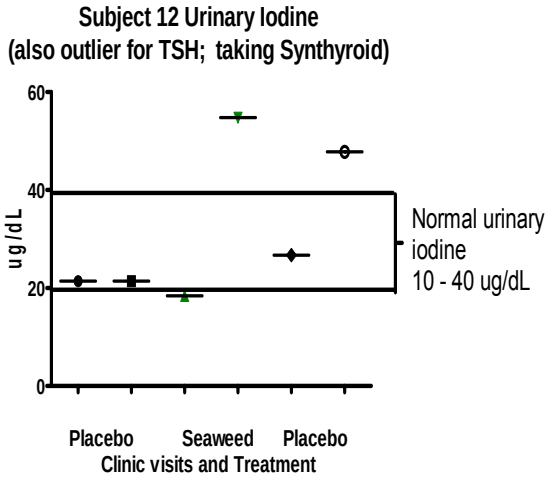
Urinary excretion of iodine increased after the first two weeks on seaweed ($p = 0.05$), but then returned to baseline after the second two weeks of seaweed. This suggests that the women accommodated the extra iodine after the initial two weeks.

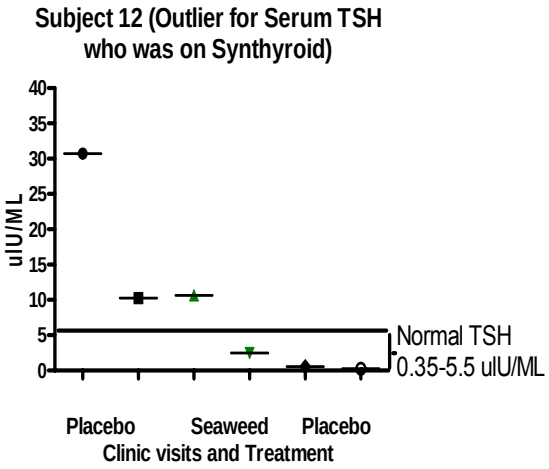


One woman (Subject 2) had a large increase in urinary iodine with seaweed, and this persisted even after a month on placebo. Since 90% of dietary iodine is cleared from the body within 48 hours ([13], it seems unlikely that the seaweed supplement was the cause of this persistent increase in urinary iodine more than 30 days after the cessation of the exposure to seaweed. For this woman, TSH levels remained normal.







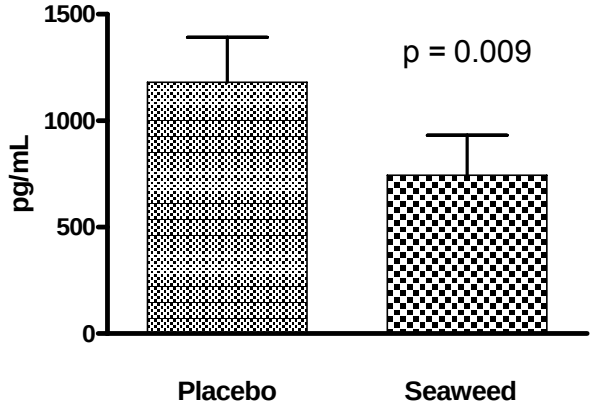


7. Changes in urinary urokinase levels

Exploratory analysis of serum and urine samples for urokinase receptor (uPAR) were conducted to provide another reference endpoint for comparison with the protein expression assays, and as a likely target for seaweed activity[24]. Serum uPAR did not vary by treatment; however urinary excretion of uPAR did vary. Levels were significantly lower ($p= 0.009$) for women who had been

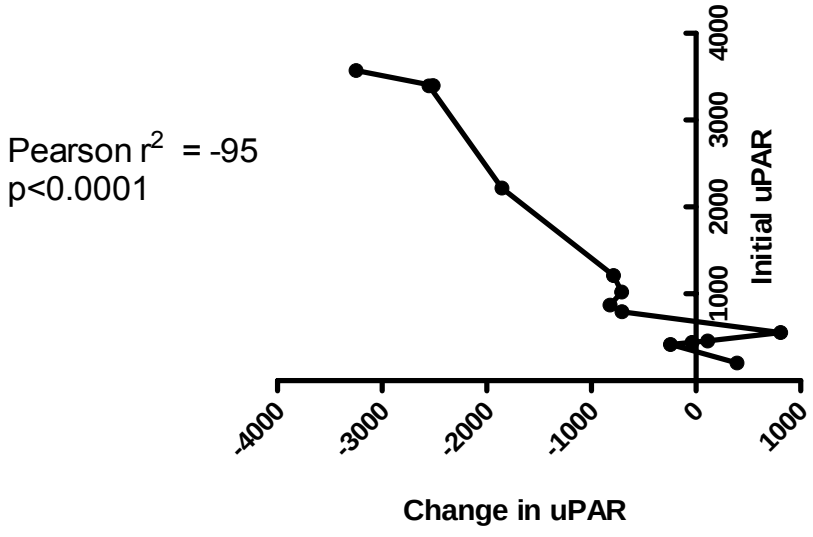
treated for breast cancer but were disease free at the time of enrollment in the study. Levels did not change for women who had never had breast cancer.

Comparison of Urinary Urokinase Levels for 10 Women who Had Been Treated for Breast Cancer Compared by Placebo and Seaweed Treatment



There also appeared to be a highly significant relationship between initial uPAR levels and the absolute change in uPAR with seaweed. This would be consistent with findings of others who have reported a protective effect on normal cells but an anti-tumorogenic effect on cancer cells and cancer-cell activity [9, 26].

Correlation of initial uPAR and absolute change in uPAR with seaweed



REPORTABLE OUTCOMES:

Manuscripts:

Submitted:

Teas J. Public Health and Seaweed. Submitted to **J. Applied Phycology**.

In Press:

J. Helen Fitton, **Jane Teas**. Marine algae and polysaccharides with therapeutic applications. Chpt 14. In: Marine Nutraceuticals and Functional Foods. Chpt 14. 343-363. In Press. Taylor and Francis, publ. In Press.

Publications:

Teas J, Braverman LE, Kurzer MS, Pino S, Hurley TG, Hebert JR. Seaweed and Soy: Companion Foods in Asian Cuisine and Their Effects on Thyroid Function in American Women. **J. Medicinal Food**. 10(1):90-100, 2007.

Teas J, Pino S, Critchley A, Braverman LE. Variability of iodine content in common commercially available edible seaweeds. **Thyroid** 2004;14(10) 836-841.

Teas J. Dietary Brown Seaweeds and Human Health Effects. In: World Seaweed Resources (CD). Ed: Critchley, Alan T , Masao Ohno and Danilo Largo. Publisher Expert Centre for Taxonomic Identification, Univ. Amsterdam. 2006.

Conference Abstract submitted:

Jane Teas, Qixia Zhang, Valerie Kennedy, Dawen Xie. An integrative model for dietary seaweed inhibition of breast cancer Mechanisms & Models of Cancer Meeting. August 8-12, 2007. Salk Institute, La Jolla CA

Presentations

Teas J. Public Health and Seaweed. **International Seaweed Symposium**, Kobe, Japan March 26-30, 2007.

Teas J, Kurzer M, Hurley T, Sepkovic D, Longcope C, Hebert J. Seaweed, soy, and estrogen metabolism in healthy postmenopausal American women. **American Association for Cancer Research** Annual Meeting, Washington DC, April 1-4, 2006.

Jane Teas, Sam Pino, Thomas G. Hurley, Alan Critchley, Lewis E. Braverman Effect of Seaweed Ingestion on Thyroid Function in Postmenopausal Women (P3-675). **Endocrinology Society** Annual Meeting, Philadelphia, PN June 2003.

Funding applied for and awarded

Dietary Algae as a Modulator of Breast Cancer Metastases: An exploratory Grant to Document Proof of Principle (**Principal Investigator: Jane Teas).** Cancer Prevention and Cancer Control (Department of Defense Award to encourage collaboration between the Medical University of South Carolina and the University of South Carolina). Awarded December 2003.

Dietary Algae and Breast Cancer. University of South Carolina preliminary grant to be used in application for NIH funding of a Cancer Complementary and Alternative Medicine Center. (**Principal Investigator: Jane Teas**). Awarded May 2004.

Funding applied for:

Changes associated with dietary algae in poor-prognosis breast cancer patients. Project within the University of South Carolina SPORE submission. **P.I.: Jane Teas, Ph.D.**

Cinical intervention for metabolic syndrome. Project within the University of South Carolina EX02 submission. **PI: Jane Teas**

CONCLUSIONS

Using *Undaria pinnatifida,* one of the most popular dietary seaweeds consumed in Japan, appeared to be well tolerated in the 15 healthy postmenopausal women, 10 of whom had been treated for breast cancer. Changes in thyroid stimulating hormone and urinary excretion of iodine were non-significant for 14 of the women. One woman with an underlying thyroid dysfunction was abnormal at baseline and over the period of the study became normal and then subnormal. A second woman had significant increases in urinary iodine during seaweed supplementation, and this increase persisted for a month after exposure to seaweed ended. As 90% of dietary iodine is excreted within 48 hours, it seems likely the woman had some other source of dietary iodine that continued for the duration of the study.

Small changes were noted in blood pressure for the seven women on hypertensive medication. More studies will be designed to test for this effect.

No changes were noted in immune cell populations or in the expression of CXCR4 and CCR5. Future studies will focus on flow cytometry antibodies that bind to urokinase and urokinase receptors.

Our main findings were significant alterations in protein expression in both serum and urine, with two peaks identified for future study. The second main finding was significant change in urinary urokinase receptor levels for women who had been treated for breast cancer. These changes were most noted for women with initial high levels of uPAR. As urokinase is one of the most important proteolytic enzyme receptors involved in breast cancer metastases, and high levels are predictive of shortened survival from breast cancer, these results will be further investigated.

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APPENDICES

Manuscripts
Teas J, Braverman LE, Kurzer MS, Pino S, Hurley TG, Hebert JR. Seaweed and Soy: Companion Foods in Asian Cuisine and Their Effects on Thyroid Function in American Women. **J. Medicinal Food**. 10(1):90-100, 2007.

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Poster Presentation
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Seaweed and Public Health

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Abstract

Seaweeds are commonly eaten in many parts of Asia. People living in Japan have the longest life expectances in the world, mostly due to reduced heart disease, diabetes, and many forms of cancer. Dietary intakes of fish and shellfish in Japan are the highest in the world, but seaweeds may also play an important role. The use of seaweeds for the treatment of hard swellings thought to be indicative of cancer, have been included in ancient Egyptian medical texts, Traditional Chinese Medicine, Ayurvedic medical texts, and by traditional healers in cultures living near the sea. In this review, epidemiologic information about seaweed intake and specific cancers is presented. New directions for clarifying the importance of seaweed in the diet include better definitions of amounts of seaweed actually consumed, the need to categorize how seaweeds are eaten, whether as dried or rehydrated, identification of processing methods which could decrease bioactive components, and attention to the specific kinds of seaweeds, parts of seaweeds, and reproductive status of the seaweeds need to be addressed.

Key words

Functional food, breast cancer, seaweed

The use of seaweed to treat breast cancer was first described in the Smith Papyrus in Case 45 (Breasted, 1930). Written in 1700 BC, the text is thought to have been a record of medical practices some as early as 2640 BC, during the reign of Imhotep. In 1956, Loeser cited this use in a Letter to the Editor in Lancet, hypothesizing that the critical component was the iodine in the seaweed(Loeser, 1956). The association between iodine deficiency and breast cancer incidence was true in many regions of the world, including the Great Lakes region of the US. However, adding iodized salt did not eradicate the higher breast cancer incidence, and thus, other components of seaweed may be more critical for breast cancer prevention.

In Asia, dietary seaweeds have traditionally been held as important foods for maintaining a healthy life, and in particular, to treat cancer. Seaweeds in particular are regarded as important foods for maintaining a healthy life, (Tsuchiya, 1969), and have been used by various medical traditions: Japanese (Itoh et al., 1995), Filipino, Chinese, and other Asian folk medicine (Hoppe, 1979), ancient Egyptian medicine (the Ebers Papyrus) (Loeser, 1956), Roman medicine (Abdussalam, 1990), Greek medicine (Hoppe, 1979), Traditional Chinese Medicine (Bensky and Gamble, 1993; Wood, 1974), and Traditional Indian medicine (Ayurvedia) (Misra and Sinha, 1979). The uses have ranged from treating goiter to treating hard masses and cancer (Dai-zhao, 1994), gastric distress(Arasaki and Arasaki, 1983), and skin and other health problems (Arasaki and Arasaki, 1983; Chapman, 1970; Tseng, 2001; Tseng, 1946).

People living in Japan customarily eat seaweed as part of their daily diet, and possibly as a consequence have the longest life expectances in the world. This is mostly due to reduced incidence of diabetes, heart disease (Yamori et al., 2001), many cancers including lung(Wynder et al., 1992), breast(Kelsey, 1993), and other sites (Tominaga, 1985) These differences are evident for both primary disease diagnosis and recurrence/mortality. Wynder (Wynder et al., 1992) noted that although more Japanese men smoke, fewer developed lung cancer than did men in the US, and the Japanese risk of dying from lung cancer was also lower.

The epidemiologic evidence for seaweed as a chemopreventive food is very compelling. Although daily seaweed consumption is not uniform, even in Japan, people in Okinawa consume more seaweed and have the lowest cancer incidence, mortality, and longest life spans (Okai et al., 1997). Using data from the 1970s, predating widespread westernization of the Japanese diet, Japanese women had one third the rate of pre-menopausal breast cancer and one ninth the rate of postmenopausal breast cancer(Reddy et al., 1980). When a Japanese woman developed breast cancer, she was more likely to survive at least five years longer than a woman diagnosed with breast cancer in the United States (Morrison et al., 1973; Wynder et al., 1963), and this seems to continue even after Japanese women immigrate to the US (Kanemori and Prygrocki, 2005a; Pineda et al., 2001). In a more recent study, Asian women who were born in the US have 60% higher risk of developing breast cancer than Asian women born in Asia (Ziegler et al., 1993). This difference is even more striking when recent immigrants from Asia are compared to those who have been in the US for 10 or more years: their increased risk of developing breast cancer is 80% higher than Asian women who have lived in the US for less than 10 years. This argues strongly for an effect of environment and probably diet. Many food habits change with migration, including increased meat and milk, decreased vegetables, decreased rice, fish, and especially decreased dietary seaweed. In addition, when Japanese women do develop breast cancer, they have a very low rate of recurrence (0.67% of 504 women after 6 years follow-up at Kuakini Hospital, in Hawaii, a National Surgical Adjuvant Breast and Bowel Project study site since 1979) (Kanemori and Prygrocki, 2005b), consistent with the 3.7% recurrence rate among a study of 1561 Japanese women treated in Japan followed for an average of 77 months(Ohsumi et al., 2003), and in contrast to a 7-10% recurrent rate in European and US studies (Ohsumi et al., 2003).

Seaweed intake is rarely included in cancer and dietary assessment, but there have been five epidemiologic studies done in Japan that have included frequency estimates of seaweed intake. Primarily investigating the possibility that seaweed could be associated with the high incidence of stomach cancer seen in Japan, these studies have compared seaweed intake and aerodigestive cancers. Four of the studies have found significant reductions in cancer risk associated with increasing seaweed consumption. Eating seaweed two or more times/week showed a highly significant reduction in risk ($p < 0.0001$) (Hoshiyama and Takafumi, 1992)of both single and multiple stomach cancers, a significant reduction in esophageal cancer (Nakachi et al., 1988), a significant reduction in rectal cancer ($p = 0.01$) and in two studies of colon cancer, one study reported that higher intake of seaweed was associated with a significant reduction in risk ($p < 0.01$) (Hoshiyama et al., 1993) whereas the second study reported a non-significant reduction in colon cancer ($p=0.59$) (Hoshiyama et al., 2000). A difference in definition of seaweed intake frequency distinguished the two studies, with ingestion 5 or more times a week being highly protective, but two or more times being only weakly protective. These two studies give some estimate of a dose response relationship between seaweed and cancer protection.

No epidemiologic studies of breast caner in Japan have specifically investigated seaweed consumption. However, three studies have examined the role of miso soup and breast cancer. Since miso soup is a hot water extract of seaweed with a tablespoon or less of miso and usually a few vegetables added it is very suggestive that seaweed and seaweed soup consumption may help explain the lower breast cancer rates of women in Japan. This is particularly interesting since two *in vivo* rat studies of seaweed extract used as drinking water found that it was highly effective against DMBA-induced tumors (Funahashi et al., 2001; Yamamoto et al., 1986)In a large prospective dietary study 21,852 Japanese nurses in Japan, investigators reported after 9 years of follow-up, that high intake of miso (fermented soybean paste) soup was the food most closely associated with the lowest breast cancer risk (Yamamoto et al., 2003). Women who had miso soup three or more times per day had about half the rate of breast cancer (RR 0.51; 95% Confidence Interval 0.32 to 0.83). Two other epidemiologic studies of diet and breast cancer in Japan also reported lower rates of breast cancer were associated with increased miso soup consumption (Hirayama, 1990; Key et al., 1999).

Only one study has reported a possible detrimental effect of higher seaweed intake and health. Omura found a dose response increase in hemorrhagic stroke and seaweed intake. Interestingly, in other industrialized countries, ischemic (clot-related) stroke is most common, whereas in Japan hemorrhagic stroke is most common. Even in this study, seaweed was protective against stroke when 6 g/d or less was consumed, but very slightly increased risk of stroke (relative risk of 1.1), when more than 6 g/day was consumed.

Omura's use of g/d, compared to other studies which used frequency of eating seaweed per week, gives a more precise estimate of the effective dose of seaweed. It is somewhat contradictory that Omura recorded g/d, but the other studies compared eating seaweed less than once a week, 2 to 5 times/week, and more than 5 times per week. Since these studies were all done in Japan, where seaweed consumption is a daily food for most people, it is hard to know what was measured by these studies. However, they all give a general idea that more seaweed is associated with greater health, and that 6 g/d might be slightly disadvantageous.

Cell culture work with seaweed extracts have demonstrated inhibition of tumor cells beginning in 1974(Yamamoto et al., 1974), but the specific putative component of seaweed has yet to be identified. Several candidates have been proposed, including the structural polysaccharides of seaweeds(both the

major insoluble fiber (alginate), fucoidan, a relatively minor sulfated polysaccharide cell wall component, the storage polysaccharide laminarin (a β1-3 glucan which is a soluble fiber) constituting up to 36% of the dry weight of seaweed, the brown seaweed-specific carotenoid fucoxanthin, phlorotannins (cell wall constituent, chemical antiherbivore defense and UV protection for seaweed), and indirect effects of antioxidant activity, anti inflammatory activity, intestinal flora modification(Kuda et al., 2005), and the relatively unusual omega 3 fatty acid (18:4-3). All of these components/activities of seaweed have been isolated and tested for direct tumor inhibition, antiangiogenic and proapoptotic properties. Based on epidemiologic data, people who frequently eat seaweed in Japan and Korea have significantly lower rates of many kinds of cancer (Tominaga and Kuroishi, 1997), and for Japanese women who do develop cancer, tend to live longer with fewer recurrences (Ohsumi et al., 2003).

To date, no clinical trials using seaweed has been conducted, although a range of animal studies (Aroma et al., 2002; Connolly et al., 1999; Combed et al., 1987; Dias et al., 2005; Funahashi et al., 1999; Funahashi et al., 2001; Furusawa and Furusawa, 1985; Furusawa and Furusawa, 1988; Furusawa and Furusawa, 1989; Furusawa and Furusawa, 1990; Furusawa et al., 1991; Hu et al., 2004; Iizima-Mizui et al., 1985; Ito and Sugiura, 1976; Itoh et al., 1995; Itoh et al., 1993; Jolles et al., 1963; Koyanagi et al., 2003; Liu et al., 2000; Maruyama et al., 1987; Maruyama et al., 2003; Matsueda et al., 1982; Mayer and Panick, 1984; Miao et al., 1999; Nagasawa et al., 1989; Nagumo et al., 1988; Noda et al., 1990; Ohigashi et al., 1992; Okai et al., 1997; Okuzumi et al., 1993; Rossitto, 1958; Suzuki et al., 1980; Takahashi et al., 2000; Tanaka et al., 1984; Teas et al., 1984{Usui, 1980 #2211; Yamamoto and Maruyama, 1985; Yamamoto et al., 1987; Yamamoto et al., 1986; Yamamoto et al., 1984; Yamamoto et al., 1982)} and cell culture studies (Aisa et al., 2005; Asai et al., 2004; Chenieux et al., 1980; Chida and Yamamoto, 1987; Chui et al., 2004; Depix et al., 1998; Dias et al., 2005; Ellouali et al., 1993; Fujihara and Nagumo, 1992; Fujihara and Nagumo, 1993; Furusawa and Furusawa, 1989; Harada et al., 1997; Haroun-Bouhedja et al., 2002; Hata et al., 2000; Hosokawa et al., 2004; Hosokawa et al., 1999; Hu et al., 2004; Kaeffer et al., 1999; Kosovel et al., 1988; Kotake-Nara et al., 2005a; Kotake-Nara et al., 2005b; Kotake-Nara et al., 2001; Koyanagi et al., 2003; Matsueda et al., 1982; Miao et al., 1999)Numata, 1991 #2188}(Okai et al., 1993; Okai et al., 1997; Okai et al., 1994; Okuzumi et al., 1990; Park et al., 2002; Reddy et al., 1984; Riou et al., 1996; Rocha et al., 2001; Sekine et al., 1995; Sekiya et al., 2005; Soeda et al., 1994; Son et al., 2003; Stevan, 2001; Suzuki et al., 1980; Takahashi et al., 2000; Tang et al., 2004; Tissot and Daniel, 2003; Wessels et al., 1999; Yamamoto et al., 1974; Yamamoto et al., 1981; Yamamoto et al., 1977) (Zhaoqian et al., 1991) (Zhuang et al., 1995)exist, all of which confirm the non-toxic effects of seaweed on normal cells and in healthy animals, and cytotoxic effects against cancer cells and tumors.

Given the plausibility of dietary seaweed as an important part of the Japanese cuisine, future research should include more detailed information about kinds of seaweed and preparation methods. For instance, many kinds of red, brown, and green seaweeds are consumed in Japan. The difficulty in estimating amounts of seaweed is highlighted by the many ways in which seaweed is eaten. Dried *Porphyra* (nori) seaweed is eaten as sheets around fish and rice, and as a condiment. Other seaweeds are usually rehydrated and eaten as vegetables. Seaweed-based soup stock may be a rich source of seaweed bioactive molecules, but the actual presence of seaweed at the time of consumption may be missing. On the other hand, seaweeds soaked in soy sauce and spices and cooked for several hours may loose their bioactive molecules.

Additionally, the reproductive status of the seaweed consumed may be important. Studies in rats and cell culture have identified at least the reproductive sporophyll (mekabu) of *Undaria* as being particularly active against cancer (Funahashi et al., 1999; Maruyama et al., 2003; Sekiya et al., 2005).

Dietary seaweed is not without its potential problems. First is the issue of iodine content (Teas et al., 2004), which ranges from 30 ug/g in *Sargassum*, to well over 6,000 ug/g in some *Laminaria* species. In a normal Japanese population where seaweed is cooked, much of the iodine is aerosolized and would not be a health risk. However, for non-seaweed eating populations, a nutritional supplement in pill form would deliver the whole amount of iodine found in the seaweed. Considering that WHO has set an upper limit of 1,000 ug/d, it could be difficult to approximate the 4-7 g/d of seaweed consumed in Japan without jeopardizing thyroid health. The low iodine seaweeds of *Sargassum* and *Undaria* are the only commonly consumed seaweeds that could be safely used for nutriceuticals without some form of de-iodination.

Dietary seaweed, either as a food or as a nutritional supplement, holds great promise for improving health, and possibly decreasing breast cancer incidence. However, before exact recommendations can be made, more information is needed about the differential effects of various species of seaweed, the impact of processing, specific information about actual quantities of dry versus wet weight of the normal diet, and the impact of preparation on iodine content.

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14 Marine Algae and Polysaccharides with Therapeutic Applications

J. Helen Fitton and Jane Teas

CONTENTS

14.1 INTRODUCTION

Human use of marine macroalgae and microalgae for both food and medicine has taken place the world over for millennia [1]. Archaeological studies in South America revealed that marine algae were being used as "medicine" in approximately 12000 BC [2]. Ancient texts suggest that seaweeds were used for ailments as diverse as snakebite, lung diseases, and gout.

Algae and algal extracts are used in present-day medicine, and play a signifi cant part in "ethnic" medicine. Therapeutically active agents found in marine algae have untapped potential in functional foods, nutraceuticals, and pharmaceuticals. Noteworthy activity includes profound antiviral properties, lipidlowering activity, and anticancer activity. Interestingly, many of these effects can be observed with oral intake.

Most of the world's annual seaweed harvest is used to produce the algal hydrocolloids alginate, agar, and carrageenan. These bulk commodities are used largely as viscosity-modifying agents in foods and pharmaceuticals.

This short review describes the therapeutic benefi ts of marine algae from both historic and present-day perspectives. Macroalgae are the focus of our discussions, with some references to the more commonly ingested microalgae.

14.2 THE COMMERCIAL USES OF MARINE ALGAE

There are thousands of species of marine algae, which represent over 90% of all marine plants. They are classifi ed into major groups: brown (Phaeophyta), red (Rhodophyta), or green (Chlorophyta), determined by their pigment contents [3]. Algae may be macrophytes (seaweeds) or microalgae such as *Spirulina* (*Arthrospira platensis*). Marine algae thrive in well-lit, relatively shallow areas of the sea, where they can photosynthesize and absorb nutrients. The growth rates and composition are dependent upon environmental factors. Perennial macroalgae such as *Macrocystis* are some of the largest plants on Earth. Macroalgae chelate minerals and are often rich in trace elements and iodine.

Seaweeds are a rich source of polysaccharides that are used largely as bulk commodities in food and pharmaceutical industries. The main commercial phycocolloids are agar, alginate, and carrageenan. The production and uses of these are well covered in the FAO documents prepared by Dennis McHugh and colleagues [4]. At the end of the last century, the annual seaweed harvest was above 2 million tonnes (dry weight) with a value in excess of US$ 6 billion with more than 8 million tonnes of wet seaweed used annually. Of the 221 species of seaweed in use, 145 species are for food and 101 species for phycocolloid production [5]. Microalgae that tolerate high salt conditions, surviving in estuarine areas and salt-laden lakes, may also be considered "marine algae." Both *Spirulina* (*Arthrospira platensis*) and *Dunaliella salina* are cultured. The latter yields high concentration of vitamin A and lipids, whereas the former is cultivated largely for use as a health food [6]. *Spirulina* is also harvested from naturally occurring sources for food use [7].

14.3 TYPES OF POLYSACCHARIDES FOUND IN MARINE ALGAE

The structure characteristics of some well-known polysaccharides are detailed in Table 14.1. Agar is extracted from the "agarophytes," such as *Gelidium*, *Gracilaria*, and *Phyllophora* by soaking them in water to remove foreign matter, and then heating with water, which causes the agar to dissolve in the water. After fi ltration and cooling, a 1% agar gel is formed. This is broken up, washed to remove soluble salts, and the water is removed from the gel, either by a freeze–thaw process or by squeezing it out using pressure. The product is dried in hot air, and then milled [4].

Similarly, carrageenan can be obtained by hot-water extraction, although this method is relatively expensive compared to a less refi ned version popular today. In the latter method, the seaweed is treated with alkali and water, leaving the carrageenan and other insoluble matter behind. The insoluble residue is carrageenan and about 15% cellulose, which is sold as semirefi ned carrageenan.

The fi rst source of carrageenan was *Chondrus crispus*, or "Irish moss" (often used in traditional set milk puddings). Today, most carrageenan is extracted from *Kappaphycus alvarezii* (also called *Eucheuma cottonii*) and *Eucheuma denticulatum*. Some South American species used are *Gigartina skottsbergii*, *Sarcothalia crispata,* and *Mazzaella laminaroides* [4].

Carrageenan has three major commercially useful fractions, kappa, lambda, and iota carrageenan. It is possible to fi nd both agarans and carrageenans in the same alga [8]. Each species yields a blend of fractions. *C. crispus* yields a mixture of kappa and lambda, *Kappaphycus* yields mainly kappa, whereas *E. denticulatum* yields mainly iota [4].

The characteristics of the different carrageenans are as follows:

Iota forms clear, freeze–thaw stable gels with calcium salts.

Kappa forms strong, opaque gels with potassium salts.

Lambda forms no gels, but creates viscosity suitable for suspensions.

Alginate or "algin" is extracted from brown macroalgae such as *Ascophyllum*, *Durvillaea*, *Ecklonia*, *Laminaria*, *Lessonia,* and *Macrocystis.* Sodium alginate is produced using alkali extraction technique. The brown seaweed source material is treated with cross-linking agent to immobilize polyphenol fractions. The material is then acid rinsed to eliminate fucoidan and laminarin fractions and then alkali treated, which turns the alginates into a gelatinous mass. This material is then treated with acid to extract it from the cellulosic mass. Sodium alginate, and smaller quantities of alginic acid and the calcium, ammonium, and potassium salts, and an ester, propylene glycol alginate are also produced. Recent publications by Hernadez-Carmona et al. [9,10] detail the process for extraction.

Polysaccharides with sulfate hemiester groups attached to sugar units are found in the form of "fucoidan" in Phaeophyceae, as "galactans" (agar and carrageenans) in Rhodophyceae, and as "arabinogalactans" with lesser amounts of other sugars in Chlorophyceae. The fucoidans encompass three different

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TABLE 14.1
Types of Polysaccharides Found in Marine Algae

Extract	Polysaccharide	Components	Sugars	Sulfate	Isolated From	Uses (Other Than Food)
Agar	Agarose (60%)	d-galactose, 3,6 l-anhydro-galactose			No Rhodophyta e.g., *Gracilaria, Phyllophora*	Bacterial culture media,
	Agaropectin (40%)	d-galactose, 3,6 l-anhydro-galactose				wound-healing absorbents, bulk-forming laxative
Alginic acid		d-guluronic acid				
Carrageenan	Kappa	3,6-anhydro-α-d-galactose		Yes 3.5–9.7% sulfate	Rhodophyta *Chondrus crispus Gigartina*, *Furcellaria Eucheuma cottonii*	Antiviral topical agent
	Lambda	Mostly d-galactose 3,6-anhydro-α-d-galactose, small amount of l-galactose		35% ester sulfate		
	Iota	3,6-anhydro-α-d-galactose				
Alginic acid	Blocky or dispersed	mannuronic and guluronic acid polymers	Guluronic and mannuronic acids		No Phaeophycaea, *Durvillea*, *Macrocystis*	Wound dressing Drug delivery Dental mold making

Fucoidan	Fucose rich branched polysaccharide	Fucose rich Up to 38% ester sulfate	Phaeophyceae e.g., *Laminaria digitata*, *Fucus vesiculosis*	Functional food, nutraceutical
Furcelleran	Carrageenan-type polysaccharide	43–46% d-galactose, 30% 3,6,anhydro d-galactose, some xylose 20% ester sulfate	*Furcellaria fastigiata*	Approved as a food additive, as for carrageenan
Ascophyllan	Also called glucuronoxylofucan sulfate	25% fucose, 26% xylose, 19% sodium uronate 13% sulfate	*Ascophyllyum nododum*	As for fucoidan
Laminarin	Beta glucan	1,3 linked Glucose	Yes	Phaeophyceae	No commercial use
Polyuronides from green algae	Highly branched polysaccharides Structure unit 4-O-β-d-glucuronosyll-rhamnose present in all the mucilages from different species	d-glucuronic acid, l-rhamnose, d-xylose, d-glucose 20% ester sulfate on rhamnose, xylose	*Ulva lactuca*, *Enteromorpha compressa*, and *Spongomorpha arcta*	Nutraceuticals

subclasses of sulfated fucose–rich polysaccharides according to their origin: fucoidans, ascophyllans, and sargassan [11].
Investigation of the sulfated polysaccharides from green algae reveals watersoluble polysaccharides composed of a variety of sugars in variable molar ratios.

14.4 HISTORY OF ALGAE IN MEDICINAL APPLICATIONS

The earliest records of the use of marine algae in medicine were uncovered by Jack Rossen and Tom Dillehay at Monte Verde in Chile, a site that dates back to approximately 12000 BC. Four species were found at the site: *Sargassum*, *Gracilaria*, *Porphyra*, and *Durvillea*. *Sargassum* was found wrapped around a plant called boldo, which has hallucinogenic qualities. These sargassum_boldo packets were apparently chewed into mouth-shaped "cuds," and were found in a wishbone-shaped healer's hut [2]. *Sargassum* must have been transported from some distance away, as it grows much further to the north.
Traditional use of algae as food and medicine by indigenous people is well recognized. North American native people prize *Porphyra* for its medicinal food value [1,12]. Coastal harvesting and transport of macroalgae inland for food use occurs today in South- and North America, preventing iodine defi ciency. *Porphyra abbottae* is a culturally important species used by the First Peoples of coastal British Colombia, Canada. It is said to be a "health food" and used to alleviate indigestion or heartburn, as a laxative and can be used as an antiseptic poultice for a deep cut or swelling, or even a broken collarbone [12].
The UNESCO courier reports on the present-day Chilean harvest: "Near Temuco and Chiloé, in the central regions of Chile and in the very heart of Araucana, they gather 'cochayuyo' and 'luche' (*Durvillaea antarctica* and *Porphyra*), with which they make bread and cakes, or cook with mutton" [1,13]. Island populations in the Caribbean enjoy "healthy" or reputedly aphrodisiac milk–based seaweed drinks. Brie Cocos [14] noted that in Belize, boiled *Euchuema isoforme* is used to make a much prized concoction with condensed milk, nutmeg, cinnamon, and a dash of brandy or rum.
In Northern Europe, Ireland, and the United Kingdom, marine algae has long been valued as a food for people and livestock, and sometimes used "medicinally." Home-produced remedies include cough medicines and strengthening milk–based jellies made from *Chondrus crispus* (Irish moss). In Victorian times, macroalgaebased remedies were popular as dieting aids. Various orally ingestible concoctions of brown algae were also used to treat arthritis and rheumatism, and topical applications of "seapod liniment" or bruised algae were recommended as poultices for scrofula (mycobacterium infection of the skin), sprains and bruises, and embrocations for "the limbs of rickety children" [15]. Today, brown algae are still included in supplements designed to aid weight loss [16]. Many of the medicinal applications described by Mrs. Grieve in the 1901 classic "A modern herbal" were probably developed from earlier texts, such as Gerard's *On the History of Plants,* published in 1633 [17]. This in turn, borrowed from ancient texts such as that by Pliny the elder who describes uses for macroalgae [18]. In Book XXVI, Chapter 66,

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The structure characteristics of some well-known polysaccharides are detailed in Table 14.1. Agar is extracted from the "agarophytes," such as *Gelidium*, *Gracilaria*, and *Phyllophora* by soaking them in water to remove foreign matter, and then heating with water, which causes the agar to dissolve in the water. After filtration and cooling, a 1% agar gel is formed. This is broken up, washed to remove soluble salts, and the water is removed from the gel, either by a freeze–thaw process or by squeezing it out using pressure. The product is dried in hot air, and then milled [4].

Similarly, carrageenan can be obtained by hot-water extraction, although this method is relatively expensive compared to a less refined version popular today. In the latter method, the seaweed is treated with alkali and water, leaving the carrageenan and other insoluble matter behind. The insoluble residue is carrageenan and about 15% cellulose, which is sold as semirefined carrageenan.

The first source of carrageenan was *Chondrus crispus*, or "Irish moss" (often used in traditional set milk puddings). Today, most carrageenan is extracted from *Kappaphycus alvarezii* (also called *Eucheuma cottonii*) and *Eucheuma denticulatum*. Some South American species used are *Gigartina skottsbergii*, *Sarcothalia crispata,* and *Mazzaella laminaroides* [4].

Carrageenan has three major commercially useful fractions, kappa, lambda, and iota carrageenan. It is possible to find both agarans and carrageenans in the same alga [8]. Each species yields a blend of fractions. *C. crispus* yields a mixture of kappa and lambda, *Kappaphycus* yields mainly kappa, whereas *E. denticulatum* yields mainly iota [4].

The characteristics of the different carrageenans are as follows:

Iota forms clear, freeze–thaw stable gels with calcium salts.

Kappa forms strong, opaque gels with potassium salts.

Lambda forms no gels, but creates viscosity suitable for suspensions.

Alginate or "algin" is extracted from brown macroalgae such as *Ascophyllum*, *Durvillaea*, *Ecklonia*, *Laminaria*, *Lessonia,* and *Macrocystis.* Sodium alginate is produced using alkali extraction technique. The brown seaweed source material is treated with cross-linking agent to immobilize polyphenol fractions. The material is then acid rinsed to eliminate fucoidan and laminarin fractions and then alkali treated, which turns the alginates into a gelatinous mass. This material is then treated with acid to extract it from the cellulosic mass. Sodium alginate, and smaller quantities of alginic acid and the calcium, ammonium, and potassium salts, and an ester, propylene glycol alginate are also produced. Recent publications by Hernadez-Carmona et al. [9,10] detail the process for extraction.

Polysaccharides with sulfate hemiester groups attached to sugar units are found in the form of "fucoidan" in Phaeophyceae, as "galactans" (agar and carrageenans) in Rhodophyceae, and as "arabinogalactans" with lesser amounts of other sugars in Chlorophyceae. The fucoidans encompass three different

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TABLE 14.1
Types of Polysaccharides Found in Marine Algae

Extract	Polysaccharide	Components	Sugars	Sulfate	Isolated From	Uses (Other Than Food)
Agar	Agarose (60%)	d-galactose, 3,6 l-anhydro-galactose	No	Rhodophyta e.g., *Gracilaria, Phyllophora*	Bacterial culture media,	
	Agaropectin (40%)	d-galactose, 3,6 l-anhydro-galactose d-guluronic acid	Yes	3.5–9.7% sulfate		wound-healing absorbents, bulk-ulk-forming laxative
Carrageenan	Kappa	3,6-anhydro-α-d-galactose 24% alkali stable ester sulfate		Rhodophyta *Chondrus crispus Gigartina*, *Furcellaria Eucheuma cottonii*		Antiviral topical agent
	Lambda	Mostly d-galactose 3,6-anhydro-α-d-galactose, small amount of l-galactose		35% ester sulfate		
	Iota	3,6-anhydro-α-d-galactose				
Alginic acid	Blocky or dispersed	mannuronic and guluronic acid polymers	Guluronic and mannuronic acids	No Phaeophycaea, *Durvillea*, *Macrocystis*		Wound dressing Drug delivery Dental mold making

Fucoidan Fucose rich branched polysaccharide Fucose rich Up to 38% ester sulfate Phaeophyceae e.g., *Laminaria digitata*, *Fucus vesiculosis* Functional food, nutraceutical
Furcelleran Carrageenan-type polysaccharide 43–46% d-galactose, 30% 3,6,anhydro d-galactose, some xylose 20% ester sulfate *Furcellaria fastigiata* Approved as a food additive, as for carrageenan
Ascophyllan Also called glucuronoxylofucan sulfate 25% fucose, 26% xylose, 19% sodium uronate 13% sulfate *Ascophyllyum nododum* As for fucoidan
Laminarin Beta glucan 1,3 linked Glucose Yes Phaeophyceae No commercial use
Polyuronides from green algae Highly branched polysaccharides Structure unit 4-O-β-d-glucuronosyll-rhamnose present in all the mucilages from different species d-glucuronic acid, l-rhamnose, d-xylose, d-glucose 20% ester sulfate on rhamnose, xylose *Ulva lactuca*, *Enteromorpha compressa*, and *Spongomorpha arcta* Nutraceuticals

subclasses of sulfated fucose–rich polysaccharides according to their origin: fucoidans, ascophyllans, and sargassan [11].
Investigation of the sulfated polysaccharides from green algae reveals watersoluble polysaccharides composed of a variety of sugars in variable molar ratios.

14.4 HISTORY OF ALGAE IN MEDICINAL APPLICATIONS

The earliest records of the use of marine algae in medicine were uncovered by Jack Rossen and Tom Dillehay at Monte Verde in Chile, a site that dates back to approximately 12000 BC. Four species were found at the site: *Sargassum*, *Gracilaria*, *Porphyra*, and *Durvillea*. *Sargassum* was found wrapped around a plant called boldo, which has hallucinogenic qualities. These sargassum_boldo packets were apparently chewed into mouth-shaped "cuds," and were found in a wishbone-shaped healer's hut [2]. *Sargassum* must have been transported from some distance away, as it grows much further to the north.
Traditional use of algae as food and medicine by indigenous people is well recognized. North American native people prize *Porphyra* for its medicinal food value [1,12]. Coastal harvesting and transport of macroalgae inland for food use occurs today in South- and North America, preventing iodine defi ciency. *Porphyra abbottae* is a culturally important species used by the First Peoples of coastal British Colombia, Canada. It is said to be a "health food" and used to alleviate indigestion or heartburn, as a laxative and can be used as an antiseptic poultice for a deep cut or swelling, or even a broken collarbone [12].
The UNESCO courier reports on the present-day Chilean harvest: "Near Temuco and Chiloé, in the central regions of Chile and in the very heart of Araucana, they gather 'cochayuyo' and 'luche' (*Durvillaea antarctica* and *Porphyra*), with which they make bread and cakes, or cook with mutton" [1,13]. Island populations in the Caribbean enjoy "healthy" or reputedly aphrodisiac milk–based seaweed drinks. Brie Cocos [14] noted that in Belize, boiled *Euchuema isoforme* is used to make a much prized concoction with condensed milk, nutmeg, cinnamon, and a dash of brandy or rum.
In Northern Europe, Ireland, and the United Kingdom, marine algae has long been valued as a food for people and livestock, and sometimes used "medicinally." Home-produced remedies include cough medicines and strengthening milk–based jellies made from *Chondrus crispus* (Irish moss). In Victorian times, macroalgaebased remedies were popular as dieting aids. Various orally ingestible concoctions of brown algae were also used to treat arthritis and rheumatism, and topical applications of "seapod liniment" or bruised algae were recommended as poultices for scrofula (mycobacterium infection of the skin), sprains and bruises, and embrocations for "the limbs of rickety children" [15]. Today, brown algae are still included in supplements designed to aid weight loss [16]. Many of the medicinal applications described by Mrs. Grieve in the 1901 classic "A modern herbal" were probably developed from earlier texts, such as Gerard's *On the History of Plants,* published in 1633 [17]. This in turn, borrowed from ancient texts such as that by Pliny the elder who describes uses for macroalgae [18]. In Book XXVI, Chapter 66,

he discusses a seaweed treatment for gout: *But it is the phycos thalassion, or sea-weed, more particularly, that is so excellent a remedy for the gout . . . Used before it becomes dry, it is effi cacious as a topical application not only for gout, but for all diseases of the joints. There are three kinds of it; one with a broad leaf, another with a longer leaf of a reddish hue, and a third with a crisped leaf, and used in Crete for dyeing cloths. All these kinds have similar properties; and we fi nd Nicander prescribing them in wine as an antidote to the venom of serpents even.*
Pliny's reference to the Greek philosopher Nicander's treatise "Theriaca" is a curious echo of recent research indicating the potential of fucoidans as snakebite enzyme inhibitors [19]. He also mentions red seaweed as a treatment for the sting of a scorpion. Interestingly, there is traditional use of seaweed for snakebite by the Guguyalanji tribe in Northern Queensland, Australia [20].
Throughout the texts of Grieve, Gerard, and Pliny, there are references to the use of particular seaweeds as antiparasitic remedies. These references are to algae such as "Corsican moss" that contain kainic acid, a potent antihelminthic. Today, kainic acid and a related chemical, domoic acid, are extracted from macroalgae for use as neuroexcitory agents [21]. Tseng [22] also refers to the use of similar antihelminthic algae in present-day China.
Antitumor properties of seaweeds are also mentioned in Gerard and Pliny. Gerard comments: "Lungwoort (probably Fucus) is much commended of the learned physitions of our time against the diseases of the lungs, especially for the infl ammations and ulcers of the same, being brought into pouder and drunke with water." These observations correlate with today's research fi ndings in animals, in which tumor arrest or delay is seen with oral, i.v., or i.p delivery of macroalgal extracts [23–28].
In traditional Chinese medicine, diet is considered necessary for the prevention of disease, and superior to treatment. Kelp (large brown marine algae) is considered to be a "Han" or cold food, in a system that considers foods to cold, hot, neutral, or strengthening [29]. There are many Chinese medicines that include macroalgae. An example is "concoction of the Jade fl ask" (a reference to the shape of the neck in goiter), which includes *Ecklonia* and *Sargassum*. The use of macroalgae in traditional medicines is common in present-day China [22].
Another traditional community that still makes use of seaweed are the Maori people of New Zealand. They used *Durvillea* (Rimu) and *Porphyra* (karengo) as treatments for goiter prevention and as laxatives, in addition to food purposes. The tender tips of *Durvillea*, roasted, were considered useful as antiparasitic agents. *The author has been told of the use, to good effect, of a large blade of kelp to wrap around a Maori child suffering from burns* [30].

14.5 CONTEMPORARY USE OF MACROALGAE IN MEDICINE

Whole macroalgae are only used in two applications in conventional medicine. Iodine supplementation using dried kelp granules is sometimes suggested for patients with suspected defi ciencies. Iodine levels vary widely between macroalgae, and there are some potential risks in ingesting high doses of kelps,

although the commonly eaten *Porphyra* and *Undaria* are relatively low in iodine [31]. The second application is in surgery. Specially prepared stipes from *Laminaria* are used to dilate the cervix in gynaecological procedures. This long-used procedure is still in vogue, as its slow swelling does not damage the cervix [32]. Radiation medicine has made use of macroalgae to inhibit radioactive iodine uptake, and assist in the chelation and elimination of radioisotopes [33]. Sodium alginate from *Sargassum siliquastrum* was a potent agent for reducing strontium absorption in clinical tests, when added to bread at 6% level, while alginate syrup was more suitable for emergencies because of its rapid action [34].

Agars are commonly used as microbiological culture mediums for identifying infectious agents, and also in proprietary laxatives. Alginates are also used in constipation remedies. The hydrocolloids act as bulking agents, and may stimulate mucus production. In a rat model, carrageenan and sodium alginate, but not cellulose, increased colonic mucus [35]. Alginic acid is also a component of medicines designed to block acid refl ux. Alginic acid forms a water-swollen raft on the top of the stomach contents, preventing acidic erosion of the oesophagus [36]. Tonneson reviewed the uses of purifi ed alginates. More than 200 different alginate grades and a number of alginate salts are available [37]. Controlled-release drug applications use alginate, because it gels differentially, depending on pH. At low pH, such as in the stomach, alginic acid forms a high-viscosity “acid gel.” Ionotropic gels are formed when alginate gels in the presence of calcium ions. These gels create a diffusion barrier to encapsulated drugs, extending delivery times. Calcium alginate dressings are used in wound-dressing applications for absorbing wound fl uids. They are less painful to change, and popular for use in ulcers [38]. A niche application for *Corallina offi cinalis*, a calcifi ed species once used in Europe as a vermifuge, is found in bone-defect fi lling materials, sold as “Algipore biomaterial” [39].

14.6 DRUGS IN DEVELOPMENT FROM MARINE ALGAE

There are a variety of smaller molecules found in some marine algae that have useful pharmacological properties. These molecules are then synthesized and tested for their safety and effi cacy as drugs in a clinical setting. Examples include the depsipeptide Kahalalide F, which was discovered in the green marine alga *Bryopsis* and has clinically assessed anticancer properties [40]. Furanones from *Delisea pulchra* have disrupting effects on the accumulation of bacterial biofi lms, and are being investigated for their ability to inhibit the buildup of *Pseudomonas* lung infections [41]. Other examples of such druglike molecules have been discovered in marine algae [42].

14.7 DISEASE INCIDENCE IN ALGAL CONSUMERS

The populations of Japan and Korea consume macroalgae every day, averaging 6.6 g_day in 1995 in Korea [43]. Cancer has an overall lower incidence in Japan. Breast cancer is typically 10 times less prevalent in postmenopausal Japanese

women than in Western women, and dietary macroalgae have been implicated as a potential protective agent [44,45]. Macroalgae are also part of the macrobiotic diet, which may have some benefi ts to cancer sufferers [46].

Realizing the potential value of dietary algae as antiviral agents, we recently proposed algal consumption as one unifying characteristic of countries with anomalously low rates of HIV [47]. In a pilot study in which HIV positive patients ingested 5 g_day for 3 weeks of either *Undaria* or *Spirulina*, viral loads were reduced by up to 76%, and CD4 also rose [48]. Given the large percentage of HIV positive people in the world who will never have access to drugs, dietary supplementation with algae may be a realistic, helpful treatment.

14.8 BIOLOGICAL ACTIVITIES AND APPLICATIONS OF ALGAE

Marine algae possess antiviral and antimicrobial properties in addition to antitumor and immune activity. Sulfated polysaccharide extracts display anticlotting, enzyme inhibitory, and growth factor modulation activity, in addition to being potent selectin blockers.

14.8.1 TUMOR INHIBITION AND IMMUNE MODULATION ACTIVITY

Macroalgae contain several potentially "anticancer" constituents. Whole macroalgae, macroalgae soaked water, and macroalgae extracts, all inhibit tumor development in solid tumors and leukemias in animal models [23–28]. These inhibitory effects are often mediated by either brown marine algae or their acid-soluble polysaccharides, fucoidans. The commonly eaten Japanese Kombu (*Laminaria*), wakame (*Undaria*), and Mozuku (*Cladosiphon*) all have substantial activity, although the mechanism is uncertain. Algal administration seems to enhance innate immunity, which includes an increase in the Th1 cytokine profi le (IFN gamma) and increased NK cell activity [49,50]. Research by Shimuzu demonstrated an overall increase in cytotoxic T cells in mice fed on a high-molecularweight fucoidan ($2–3 \times 10^5$) from *Cladosiphon okamuranus*, at a level of 5% in the diet. In this model, lower-molecular-weight fucoidans from the same source had no effect [51].

Other components that may exert anticancer effects include iodine, which causes apoptosis in cancer cells. Additionally, omega-3 fatty acids such as stearidonic acid and hexadecatetraenoic acid are found in edible marine algae such as *Undaria* and *Ulva* up to 40% of total fatty acids [52]. Algal galactolipids found in *Undaria*, *Laminaria*, *Porhphyra*, and *Cladosiphon* include high concentrations of the telomerase inhibitor sulfoquinovosyldiacylglyceride (SQDG) [53,54]. Fucoxanthins, the carotenoids found in algae, are metabolized to fucoxanthinol and thus may exert anticancer effects [55]. Glutathione, an antioxidant, is a constituent of all macroalgae (*Sargassum thumbergeii* and *Ishige okamurai* contain 1482 and 3082 mg_100) [56]. Fucoidan fractions, alginates, and phloroglucinols isolated from marine algae have enzyme inhibitory properties against hyaluronidase, heparanases, phospholipase A2, and tyrosine kinase, which may also contribute

to the anticancer activity [57–61]. Cyclic and noncyclic nucleotides with potential therapeutic value have been identifi ed in *Porphyra umbilicalis* [62].
There have been a number of human clinical studies involving marine algae. In 1968, a fucoidan preparation known as Algosol T 128 was used to treat leukemia [63]. *Spirulina* ingestion was shown to inhibit oral cancer [64]. Decreased allergic responses were observed with an alginic acid oligosaccharide, which suppressed IgE production by inducing IL-12 production [65]. Similarly, in a mouse model, *Cladosiphon* fucoidan downregulated IL6 (A Th2 cytokine) and ameliorated colitis [66]. Interestingly, algal polysaccharides have immune stimulatory effects on plants. Sulfated oligo-fucan fractions elicited "systemic acquired resistance," and the accumulation of salicylic acid in tobacco plants [67].

14.8.2 Direct Antipathogenic Activity

All marine algae contain sulfated polysaccharides. Carrageenans, fucoidans, and sulfated rhamnogalactans have inhibitory effects on entry of enveloped viruses (such as herpes and HIV) into cells. Some other algal fractions have virucidal and enzyme inhibitory activity, or the ability to inhibit syncytium formation [68–74]. Pilot studies with *Undaria* showed inhibitory effects on herpes infections [75]. Lambda carrageenan preparations (Carraguard$_{tm}$) are being tested as vaginal microbiocides [76]. Antibacterial activity of marine algae is partly attributable to iodine, but some polysaccharides prevent bacterial adhesion. Funoran extracted from *Gloiopeltis furcata* inhibited the adherence of dental plaque [77]. Fucoidan extracts from *Cladosiphon* (Okinawan "Mozuku") inhibit adhesion of the ulcer-causing bacterium *Helicobacter*. Human trials in which subjects consumed 1.5–4.5 mg_kg_day of *Cladosiphon* fucoidan provided relief for nonulcer dyspepsia over 2 weeks [78]. Marine algal lectins provide antibacterial activity against marine *Vibrios* [79], and algal-derived furanones inhibit the ability to form biofi lms, which may be clinically useful in a number of applications [80].

14.8.3 Anti-Inflammatory Activity

Fucoidans are excellent selectin blocking agents, and have been used experimentally to reduce postischemic leukocyte infl ux, or so-called "reperfusion injury" [81]. Fucoidans also have a protective effect on kidney function in animal models, when orally delivered [82]. Carrageenans may be macrophage toxic, and act as infl ammation initiators in animal models [83]. Some carrageenan fractions are also anti-infl ammatory [84]. Nori (*Porphyra*)-fed rats had a higher incidence of submucosal edema than the Konbu (*Laminaria*)-fed rats. However, this may have been related to the higher Na_K ratio, rather than any intrinsic infl ammatory activity in the Nori diet [85].

14.8.4 Hypertension, Serum Lipids, and Sugar Metabolism

Marine algae come close to being an ideal food to reduce the potential for developing ischemic and cardiovascular diseases. They are nutrient dense, with

protein yields exceeding 30% in some cases, containing all essential amino acids, omega-3 lipids and plenty of soluble fi ber [1]. In addition, commonly eaten species contain a variety of lipid-modulating, blood pressure–lowering, and glucose metabolism–modifying components. Ethnic communities benefi t from the continuing tradition of algal consumption, which counteracts the atherogenic qualities of some Western foods [12].

In the 1950s, there was considerable interest in the antitumor and lipidmodifying properties of sulfated polysaccharides from seaweeds. Heparin was known to clear "lipemia," but had undesirable anticoagulant qualities. Besterman and Evans carried out clinical trials with two relatively low sulfate laminarins (0.62 and 0.37 sulfate groups per glucose) produced by the British pharmaceutical company, Boots Ltd., in 12 patients with ishemic heart disease. The laminarins had low anticoagulant activity (1.4 and 1.3 IU_mg) and had low toxicity in guinea pigs in contrast with a high sulfated laminarin. The patients were administered 100 mg i.v. or i.m. Two patients had a fatty meal prior to the injection, and in these patients the visible turbity of the serum was reduced. Only the lower sulfated laminarin was active, and no effects were seen via intramuscular injection [86]. Despite the apparent success of this trial, in which lipemia-clearing activity without the anticoagulant effects was observed, there were no further published trials. Since that time, algae and algal fractions delivered orally as well as i.v. have shown considerable activity in moderating serum lipid levels. Ingestion of carrageenans lowers serum lipid and cholesterol levels [87]. Synergistic effects on serum lipids were observed when *Undaria* and fi sh oils were combined as part of an experimental diet [88]. *Undaria* fucoidan fractions delivered i.v. resulted in rapid clearance of serum lipids [89]. Up to 500 mg_kg polysaccharides from *Ulva pertusa* delivered orally to mice decreased plasma total cholesterol, LDL, triglycerides and markedly increased HDL [90].

Sterols, which have dietary cholesterol modulating activities, are also found in fairly high concentrations in marine algae [91]. The predominant sterol was fucosterol in brown seaweeds (83–97% of total sterol content; 662–2320 µg_g dry weight), and desmosterol in red seaweeds (87–93% of total sterol content; 187–337 µg_g dry weight). Fucosterol has additional antidiabetic properties, which may explain the use of high fucosterol seaweeds in traditional diabetes remedies. Fucosterol from *Pelvetia siliquosa* was administered at 300 mg_kg in epinephrine-induced diabetic rats. It caused an inhibition of blood glucose level and glycogen degradation [92].

Peptide fractions of *Undaria* contain angiotensin converting enzyme (ACE) inhibitory activity [93,94]. The inhibition of development of cerebrovascular diseases in stroke-prone spontaneously hypertensive rats was observed with an *Undaria*-rich diet [95,96]. The polysaccharide components of macroalgae modify the uptake of glucose in the gut [97]. Brown marine macroalgae also contain phloroglucinols such as "eckol" and "dieckol" in *Eisenia bicyclis*, which modify glucose metabolism. These substances demonstrated inhibition of aldose reductase and glycation [98].

Finally, the inhibition of vascular smooth muscle proliferation by fucoidans may assist in the arrest or reversal of atherogenesis. Following binding to the

cell surface, heparins or high MW fucoidan undergo internalization by receptormediated endocytosis and subsequent breakdown. The largest fucoidan fractions remain virtually intact, and have the highest antiproliferative activity [99]. The antimitogenic action of fucoidan differs from that of heparin, being effective even in "heparin-resistant" vascular smooth muscle cells [100].

14.8.5 Effects on Blood

14.8.5.1 Anticoagulants and Antithrombotic Effects

Fucoidans are intensively researched as heparin replacements. Fucoidans are plant sourced (as apposed to porcine gut or lung mucosae for heparin), have good potential antithrombotic qualities, and complement inhibitory qualities. These are well reviewed by Berteau and Blondin [101,102]. One attractive feature of algalderived fucoidan fractions is their potential stability as compared to heparin.

Fucoidans appear to act on specifi c parts of the clotting cascade (antithrombin II or to potentiate heparin cofactor II) in similar ways to endogenous sulfated polysaccharides such as heparan sulfate and dermatan sulfate. The MW, patterns of sulfation, and sugar composition are critical to the activity of the fraction. Oversulfated fucoidan had a potent fi brinolytic (clot dissolving) activity, by stimulating the action of tissue plasminogen activator. It also inhibited hyaluronic acid–mediated enhancement of clot formation [103].

14.8.5.2 Stem Cell Modulation

Hemopoetic stem cells (CD34+ cells) give rise to the different lineages of immune cells, and possibly to other tissue cell types. It is sometimes desirable to mobilize greater numbers of these cells from the bone marrow stroma into the peripheral blood for harvesting and later engrafting. A cytokine called GCSF is often used for this purpose, but it had been noted that heparin had some mobilizing effects. In a clinical study, conventional heparin anticoagulation resulted in a 2.49-fold increase in circulating CD34+ HPCs [104]. Unfractionated *Fucus* fucoidan was observed to have a potent, long-lasting mobilizing effect when injected into mice or primates [105,106]. It is thought that heparin and fucoidan mobilize stem cells by displacing growth factor called SDF-1 into the serum, thus creating a chemotracting gradient for CXCR4, the cell surface ligand for SDF-1. This was confi rmed in a study, which demonstrated increased plasma SDF1 levels in an ischemic hindlimb revascularization model using a low-molecular-weight fucoidan fraction [107]. Pilot clinical studies with orally delivered *Undaria fucoidan* showed a large increase in the expression of CXCR4 on CD34+ cells [108].

14.8.6 Uptake and Toxicity of Marine Algal Polysaccharides

Much of this review has considered the immunomodulating, antitumor, and antiviral effects of algal polysaccharides. Although these materials have a high molecular weight, they exert effects after oral administration. It is not known whether absorption or uptake by the gut lymphatic system occurs. However, other

high-molecular-weight sulfated polysaccharides such as chondroitin sulfate are absorbed whole in small amounts in the small intestine [109]. There were no toxicological changes observed in rats given up to 300 mg_kg orally of fucoidan from *Laminaria japonica*. Anticoagulant effects were observed at doses of 900–2500 mg_kg, but no other signs of toxicity were observed. The composition of the fucoidan was fucose 28%, sulfate 29%, fucose:galactose ratio 1:0.24, with a molecular weight of 189,000 [110]. No side effects were reported in human clinical studies with algae [108].

Gut permeability is affected by disease. Tight junctions between gut epithelium break down in a variety of disease states, such as irritable bowel syndrome and viral infections. Posttransplant cytomegalovirus (even subclinical infections)-infected patients demonstrated increased gut permeability [111]. HIV-infected patients who have progressed to AIDS and have diarrhea also have increased bowel permeability [112]. Gut permeability is also different in infants. This presents an easy route for HIV infection in breast-feeding infants of HIV-infected women, but paradoxically, it would also present a route for high-molecular-weight HIV inhibitory substances like fucoidans.

The uptake of high MW substances, such as alginates, by Peyers patches (part of the gut-associated lymphatic system) is now being exploited to develop orally delivered peptide vaccines. Ease of delivery, increased safety, and economic benefi ts make oral delivery of peptide vaccines highly desirable. However, peptides are easily degraded in the gastrointestinal tract, and lack the size to produce an immune response. Commercial vaccine development based on this technology is in progress [113].

14.9 REGULATORY STATUS AND SAFETY

Agar-agar and alginic acid, and its salts were granted the GRAS (generally recognized as safe) status by FDA. Carrageenan, furcelleran, and their salts, as well as propylene glycol alginate are also in the legal food additive list permitted for direct addition to food for human consumption. Some brown and red algae species are also included in FDAs GRAS list. Carrageenan and processed *Eucheuma* seaweed (a form of carrageenan with a higher cellulose content) have a JEFCA recommended group allowable daily intake (ADI) of “not specifi ed” [114].

All marine algae sequester ions readily, and depending on their polysaccharide content, and the local environment, heavy metal loading may occur. Marine algae contain arsenic as the potentially toxic “inorganic arsenic” and a greater part of the possibly benign, “organic arsenic” [115]. The WHO has established a “provisional tolerable weekly intake” of 15 μg inorganic arsenic per week per kilogram of body weight.

Hijiki, a commonly eaten Japanese seaweed, has a naturally high inorganic arsenic content, and is banned in many Western countries [116], and some Porphyra products from China had excessive arsenic levels [117]. The toxicity of seaweed-derived arsenic is, to some extent, tested by animals that survive on seaweed. These include North Ronaldsey sheep [118] that forage almost exclusively

on beachcast *Laminaria digitata*, and a species of deer that has a high intake of seaweed [119].

Excessive iodine intake could potentially induce thyrotoxicosis. In a recent study of 12 different species of macroalgae, iodine content ranged from 16 mcg_g in Nori (*Porphyra tenera*) to over 8165 mcg_g in kelp granules made from *Laminaria digitata*. Relatively low iodine–containing species included *Undaria* and *Sargassum* [31]. The maximum tolerated dose of iodine is 1000 mcg_day.

There have been some concerns about radioactivity in macroalgae in the northern hemisphere after nuclear accidents have occurred. A survey of Canadian, Japanese, and European sources of macroalgae found traces of cesium-137 in a product from Norway and radium-226 was found in a product from Japan [120].

Although marine algae contain sulfated polysaccharides known to inhibit clotting, no reports of clotting inhibition are reported in the literature subsequent to ingestion by humans. Marine algae also contain vitamin K (phylloquinone), which is a "procoagulant." One report exists of a 33-year-old woman eating Nori (*Porphyra*), averaging 18.8 mcg_100 g product, which interfered with postoperative warfarin therapy (an anticlotting agent) [121].

14.10 NEW HORIZONS FOR MARINE ALGAE

As outlined in this review, marine algae have great potential for further development as products in the nutraceutical, functional food, and pharmaceutical markets. Patent activity in this area has increased in the last few years, and several novel products based on macroalgae have entered the market. Red marine algae can be found in several nutraceuticals aimed at ameliorating herpes infections. A particularly novel application is found in "Plaque–Off$_{tm}$," an orally administered macroalgal supplement aimed at decreasing the deposition of calculus, in both people and their pets. Fucoidan extracts are well accepted in Japan and are included in mainstream functional foods such as yogurts and fruit juices. South Pacifi c–derived fucoidan-based liquid preparations are a popular nutraceutical offering in the United States.

The development of new products in the pharmaceutical area will depend on the ability to isolate well-characterized uniform polysaccharides with defi ned activity. Heterogeneous fucoidan preparations, such as that used for selectin blocking in many experimental systems [122], will gradually become less attractive as biological tools.

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Seaweed and Soy: Companion Foods in Asian Cuisine and Their Effects on Thyroid Function in American Women

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ABSTRACT Seaweeds and soy are two commonly eaten foods in Asia. Both have been reported to affect thyroid function, seaweed because of its iodine content and soy because of its goitrogenic effect. Twenty-five healthy postmenopausal women (mean age 58 years) completed a double-blinded randomized crossover study. Ten capsules (5 g/day) of placebo or seaweed (*Alaria esculenta*), providing 475 _g of iodine/day, were consumed daily for 7 weeks. A powdered soy protein isolate (Solae Co., St. Louis, MO), providing 2 mg of isoflavones/kg of body weight, was given daily during the last week of each treatment arm. On average, this provided 141.3 mg of isoflavones/day and 67.5 g of protein/day. Blood samples and 48-hour urine samples were collected before and after each intervention period, and urinary I/C (_g of iodine/g of creatinine) and serum thyroxine, free thyroxine index, total triiodothyronine, and thyroid stimulating hormone (TSH) were measured. Seaweed ingestion increased I/C concentrations (*P* _ .0001) and serum TSH (*P* _ .0001) (1.69 _ 0.22 vs. 2.19 _ 0.22 _U/mL, mean _ SE). Soy supplementation did not affect thyroid end points. Seven weeks of 5 g/day seaweed supplementation was associated with a small but statistically significant increase in TSH. Soy protein isolate supplementation was not associated with changes in serum thyroid hormone concentrations.

KEY WORDS: • *breast cancer* • *chemoprevention* • *diet* • *postmenopausal women* • *seaweed* • *soy* • *thyroid stimulating hormone*

INTRODUCTION

SEAWEEDS ARE PART of many indigenous cuisines around the world, and have been incorporated into some healing therapies, including traditional Chinese medicine, Ayurveda, and modern macrobiotics, as well as many folk medicines. On a population level, those people for whom seaweed is a regular part of their diet, most notably in Japan, have dramatically lower breast cancer and prostate cancer rates.[1–3] Epidemiologic studies done in the 1980s, before Westernized diets were common, found that Japanese women had one-third the rate of premenopausal breast cancer and one-ninth the rate of postmenopausal breast cancer.[4] In addition, when a Japanese woman developed breast cancer, she was more likely to survive at least 5 years longer than women with breast cancer in the United States.[5,6] The histologic type of breast cancer also varies by country, with Japanese women having greater humoral immune responses to the tumors as suggested by the greater degree of lymphocytic invasion of their breast tumors.[5]

No previous intervention studies have combined seaweed and soy, but epidemiologic evidence suggests that this combination could be anticarcinogenic. Consumption of miso soup has been associated with reduced breast cancer rates.[7–9] Miso soup is usually a combination of soy (miso, tofu cubes) and seaweed (as a stock flavoring and as garnish), and sometimes vegetables.

Seaweed is not necessarily a safe food to consume. We have reported elsewhere that commonly consumed seaweeds have a wide range of iodine concentrations, *i.e*., between 16 _g/g and 8,000 _g/g,[10] making iodine an important doselimiting factor in seaweed consumption, particularly for people not accustomed to eating seaweed.[11] The lowest-observed-adverse-effect level, based on increases in serum thyroid stimulating hormone (TSH) in thyroid function challenge tests, is 1,700 _g/day (U.S. and Canadian RDI Committee).[12] However, habituation to high iodine-containing seaweeds appears to be common in Asia, particularly Japan, Korea, and coastal China, where seaweeds are frequently

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eaten and appear to be well tolerated by millions of people. The average seaweed intake in Japan is approximately 4–7 g/day,[13–15] with some estimates as high as 10 g per person per day.[16] It is difficult to quantify the actual amount of seaweed consumed as it is often added as flavoring to noodles, soups, and garnishes and may be served as a snack, salad, or side dish. Based on dietary intake surveys, the average daily iodine intake in these regions is between 500 and 1,000 _g/day (ranging from 200 to 20,000 _g/day), with most of the dietary iodine coming from seaweed consumption.[16] For this study, we chose a low-iodine-containing seaweed (*Alaria esculenta*) to approximate the Japanese average seaweed intake, rather than the approximate iodine intake.

Soyfoods have been suggested as possible human goitrogens.[17] The dose of soy protein in our study (average 67.5 g/day) was based on early estimates of average total soy intake in Japan.[18] More recent dietary studies indicated that the soy protein intake in Japan is actually lower (10 g/day).

The present study determined whether iodine in seaweed was bioavailable and would affect thyroid function, whether a short-term soy protein isolate supplementation would affect thyroid function, and whether consumption of these two foods together would have any discernible effect on thyroid function that might be clinically important. Individual contrasts were created to test three hypotheses: (1) was seaweed different from placebo (seaweed main effect), (2) was soy different from placebo (soy main effect), and (3) was there an effect from combining seaweed and soy that was different from the additive effects of seaweed plus soy (seaweed soy interaction)? Since the greatest difference between U.S. and Japanese breast cancer rates begins at about age 45 years,[19] we studied only postmenopausal women.

SUBJECTS AND METHODS

Study population

The University of Massachusetts Medical School Institutional Review Board approved the study. Consent forms were reviewed verbally, and all participants gave written informed consent.

Participants were a group of Caucasian American women living in central Massachusetts. We specifically recruited healthy postmenopausal women who had been treated for

early breast cancer and women who had never had breast cancer. Women were recruited by word of mouth, by physicians, and through responses to an article in the newspaper. Our inclusion criteria included being postmenopausal (no bleeding for at least 1 year), intact ovaries at the time of menopause, no history of cancer (other than early breast cancer), no thyroid dysfunction or treatment within the previous 5 years, negative thyroid peroxidase antibodies excluding Hashimoto's thyroiditis, no hormone replacement therapy within the previous 3 years, no ulcer medications or lithium-based medications, no gastrointestinal disorders such as Crohn's disease or irritable bowel syndrome, no allergies to seaweed, soy, shellfish, or iodine, no treatment with oral antibiotics, iodine-containing medications, or corticosteroids within the previous 3 months, no diabetes, and no high blood pressure medications. In addition, only nonvegetarian women who consumed soy products fewer than two times per week were eligible. Women agreed to avoid eating soy foods during the study, including soybeans and soy products, as well as sprouts, beans, peas, and lentils, and to restrict alcoholic intake to one or fewer drinks per week. Although vitamin and supplement use was allowed, women were asked to refrain from changing dosage or usage during the study. Use of black cohosh, Dong Quai herbal supplements, or yam cream was an additional reason for ineligibility.

Forty-eight postmenopausal women were recruited. Based on screening blood samples, we excluded 15 additional women, two for abnormal serum TSH values (either _0.4 or _4.5 _U/mL TSH), three for elevated thyroid peroxidase antibodies, three for current or recent thyroid medications, and seven for lack of interest. Thus, 33 women were enrolled in the study and provided baseline data. Subsequently, four women dropped out during the course of the study because of a lack of interest (one), naturopath advice (one), or allergic reaction (two). Of the two who developed allergic reactions to seaweed, one had red itchy eyes, and the other experienced re-activated esophageal reflux. Both conditions resolved spontaneously following cessation of seaweed intake. At the end of the study, two women were excluded because one woman began menstruating again, while the other failed to follow study protocol.

Following publication of studies during the trial on the effects of tamoxifen/roloxifen on thyroid function,[20–22] we excluded the three women who used tamoxifen during the entire study, and the one woman who started taking tamoxifen late in the trial had her last three observations omitted. Her earlier measures were included in the analysis. This left a final study sample of 25 women among whom 10 had a history of early (Stage I or II) breast cancer but were disease free at the time of the study and 15 women who had never been diagnosed with breast cancer.

Study design

The study utilized a randomized, placebo-controlled crossover design. Women were randomized to either 6 weeks of 5 g/day seaweed powder (10 capsules) each

evening with the last meal of the day or 6 weeks of 5 g/day maltodextrose in 10 identical gelatin capsules. For 1 additional week, women received either seaweed capsules or placebo capsules and the high isoflavone powder. To minimize possible effects of season, all women began the study the same week in late October. Samples were collected a total of seven times (blood samples and 48-hour urine collections) throughout the study. A 3-week washout period separated the two arms of the study, and a final 3-week washout period followed the end of the last supplements (Fig. 1). Randomization was done using a computer-generated random number table. In addition, to assure blinded laboratory

analysis, each patient at each clinic visit was assigned a unique ID number.

Seaweed

In order to ensure safe iodine exposure, seaweed samples were collected from seaweed harvesters and health food stores for a preliminary survey of iodine analysis (Fig. 2). For this study, we chose *A. esculenta*, also known as American wakame, a low-iodine (95 _g/g)-containing seaweed. The *A. esculenta* was harvested at an extremely low tide from the subtidal rocks of the Sally Islands, located near Stuben, ME. The blades of *Alaria* were cut by hand, placed in plastic baskets, and transported from the islands to the shore in a separate boat made especially for hauling seaweed, which was towed behind a larger boat. No gasoline or other potentially toxic substances were present in the seaweed barge. Within 1 hour of harvesting, the seaweed fronds were hung on untreated wooden racks to sun-dry. After about 10 hours in the sun, the fronds were gathered in bundles, placed in plastic bags, and stored at ambient room temperature in a dark room until shipping, which was done within 2 months of harvest.

FIG. 1. Study design.

FIG. 2. Comparison of iodine content of common dietary seaweeds.

Encapsulation

The seaweed was shipped by overnight mail to Beehive Botanicals, a subsidiary of Twin Labs (Hayward, WI), where it was tested for mold and fungus and found to be negative. The seaweed was ground and encapsulated into gelatin capsules. No fillers or binders were added to the seaweed powder.

Placebo

Maltrin M100 maltodextrin (Grain Processing Corp., Muscatine, IA) was used for placebo. The daily dose of 5 g/day provided 18 kcal of food energy. We used the same capsules for maltodextrin and the seaweed. No fillers or binders were added to the placebo powder.

Iodine content of finished capsules

Gelatin capsules were used and, when analyzed for iodine content, were found to contain no iodine. The finished seaweed capsules were analyzed, and each capsule contained

47.5 _g of iodine. Thus for our study, we provided an additional 475 _g of iodine/day.

Soy protein

Soy powder [Supro High Protein Nutritious Food Ingredient Powder (with isoflavones)] (lot number G198–8) was provided by Solae Co. (formerly Protein Technologies, Inc., St. Louis, MO). It contained 1.43 mg of total aglycone (unconjugated or free) isoflavone/g of soy powder. We calculated the appropriate dose for each subject based on her weight, so that each woman consumed 2 mg of isoflavones/kg of body weight. On average, each woman was given 67.5 g/day of soy protein. This provided 141.3 mg/day isoflavones, 376 calories, 2.2 g of fat, and 22 g of carbohydrates and supplied 539 g of calcium. Subjects were advised to consume the soy protein isolate as a substitute meal during the 2 weeks of soy supplementation.

Thyroid hormones

Chemiluminometric immunoassays (Chiron Diagnostics, East Walpole, MA) were used to measure serum TSH, triiodothyronine (T3), thyroxine (T4), and T3 resin uptake [thyroid hormone-binding ratio (THBR)]. The free T4 index (FTI) was calculated as the product of THBR _ T4. A chemiluminometric enzyme-linked immunosorbent assay was used to measure anti-thyroid peroxidase antibody (ALPCO, American Laboratory Products Co., Wyndham, NH) and was sensitive to 5 IU/mL. All of these assays and urinary creatinine were measured by the Endocrine-Hypertension Laboratory at the Brigham & Women's Hospital, Boston, MA.

Iodine

Iodine in urine, empty capsules, and finished capsules was analyzed using the ceric-arsenic redox reaction. Samples were analyzed according to standard determination of total iodine protocol as outlined by Benotti *et al*.[23] This used the reduction-oxidation reaction between ceric and arsenite cat-

TABLE 1. DEMOGRAPHIC FACTORS FOR 25 WOMEN

Characteristic	*Breast cancer Number*	*%*	*Disease free Number*	*%*	P *value*
Education					0.58[a]
High school graduate or less	2	20	4	27	
Some college or associate degree	4	40	3	20	
Bachelors degree or more	4	40	8	53	
Ethnicity					
Caucasian American	10	100	15	100	—
Social status					0.36[a]
Living alone	3	30	2	13	
Living with someone	7	70	13	87	
Employment					0.83[a]
Full-time	7	70	9	60	
Part-time	1	10	1	7	
Not working	2	20	5	33	
	Mean	*SD*	*Mean*	*SD*	
Age (years)	58.4	6.1	58.1	8.5	0.93[b]
BMI (kg/m2)	27.2	6.7	26.2	4.4	0.66[b]

This analysis includes 25 women, 10 with a history of breast cancer and 15 with no such history.

[a]By Fischer's exact test.

[b]By *t* test.

alyzed by iodide. The iodine concentration was proportional to its catalytic activity. First, iodine was precipitated with perchloric acid, and the samples were digested with chloric acid. They were then measured spectrometrically at 420 nm (Autoanalyzer, Technicon Instrument, Inc., Tarrytown, NY). Calculations were based on an iodine standard curve. The urine results were calculated as _g of iodine/dL, per g of creatinine, or total urinary iodine/day.

TABLE 2. LIFE-STYLE- AND HEALTH-RELATED FACTORS FOR 25 WOMEN

Characteristic	*Breast cancer Number*	*%*	*Disease free Number*	*%*	P *value*
General health					0.72[a]
Excellent	2	20	5	33	
Very good	6	60	9	60	
Good	2	20	1	7	
Exercise					0.12[a]
Yes	5	50	12	80	
No	5	50	3	20	
Alcohol use[b]					0.67[a]
Yes	4	40	4	27	
No	6	60	11	73	
Multivitamin use					0.69[a]
Yes	6	60	7	47	
No	4	40	8	53	
Herbal supplements use					1.00[a]
Yes	4	40	7	47	
No	6	60	8	53	
Hysterectomy					0.27[a]
Yes	3	30	1	7	
No	7	70	14	93	
Ever pregnant					1.00[a]
Yes	9	90	13	87	
No	1	10	2	13	
Menopausal symptoms					0.24[a]
Yes	6	60	5	33	
No	4	40	10	67	
Self-medication for menopausal symptoms[c]					1.00[a]
Yes	3	30	5	33	
No	7	70	10	67	
Family history of breast cancer					
Yes	2	20	10	67	0.04[a]
No	8	80	5	33	
If yes, first-degree breast cancer					0.45[a]
Not applicable	8	—	5	—	
Yes	1	50	8	80	
No	1	50	2	20	
If first-degree, breast cancer type					1.00[a]
Premenopausal	0	0	1	13	
Postmenopausal	1	100	7	87	
Not applicable	8	—	5	—	
Missing	1	—	2	—	
Mean	*SD*		*Mean*	*SD*	
Age at first pregnancy	24.2	3.4	24.3	3.7	0.24[d]
Age (years) at menopause	48.8	3.5	50.5	2.2	0.15[d]
Social support[e]	17.9	9.9	13.1	9.4	0.24[d]
Number of miscarriages	0.9	1.4	0.4	0.8	0.28[d]

This analysis includes 25 women, 10 with a history of breast cancer and 15 with no such history.

[a]By Fischer's exact test.

[b]One or fewer drinks per week.

[c]Self-medication of menopausal symptoms with dietary herbal supplements, or over-the-counter drugs.

[d]By *t* test.

[e]Social support was defined as the number of friends and relatives a woman was in contact with during a week.

Urine collection

Women collected 48-hour urine specimens in 3-L containers to which 3 g of ascorbic acid powder had been added. Women stored the collection jugs in their refrigerators until they came in for their next clinic visit (within a day of the end of the collection period). After the contents of the jugs were completely mixed, aliquots were taken and stored at _20°C until analysis.

Statistical analyses

Analyses were conducted on 25 women using an intention-to-treat approach. The study sample characteristics are presented using descriptive statistics. To test the main study hypotheses, a repeated-measures analysis of variance was conducted using Proc Mixed in SAS (SAS, Cary, NC).[24] In these models, subject was fit as the repeated factor, while the independent variables treatment group (placebo, seaweed, soy, and seaweed plus soy), treatment arm (treatment followed by placebo or placebo followed by treatment), and disease status (history of breast cancer: yes or no) were fit as independent variables. Individual models were run for seven dependent variables: serum total T3, T4, FTI, TSH, and urinary iodine (24-hour excretion, concentration/dL, and iodine standardized per g of creatinine). Results are presented as both average concentrations (_SD) and least squares means, and the differences are tested using the pdiff option of the Proc Mixed procedure.

RESULTS

The demographic and life-style characteristics of the 25 subjects are presented in Tables 1 and 2. The only significant difference between the women who had been treated for breast cancer and those who had never had breast cancer was in family history. Although we had expected that women with breast cancer would have a stronger family history of breast cancer, that was not the case in our study. Two-thirds of the control women had a family history of breast cancer compared to only 20% of the breast cancer patients (P _ .04), although there was no difference between groups in number of first-degree relatives who had been diagnosed with breast cancer.

There were no differences in thyroid function during seaweed and/or soy ingestion between the women who had been treated for early breast cancer but were disease free compared to women who never had breast cancer.

Table 3 presents average serum measurements of thyroid function and urinary iodine excretion in each treatment group. The only significant effects were that seaweed markedly increased urinary iodine excretion and slightly increased serum TSH (Table 3 and Fig. 3). These seaweed effects were not altered by soy ingestion.

Because the within-subject variation in 24-hour urinary creatinine excretion averaged 15% (median 13%), we were concerned that urine collections may have been incomplete for some subjects. Data were analyzed using urinary iodine/g of creatinine concentration, urinary iodine concentration

(_g/dL), and total 24-hour urinary iodine (I/day) excretion. The statistical results did not vary, suggesting that our ability to estimate treatment effects was not affected by this factor. Soy protein isolate supplementation had no significant effect, and there was no evidence that there was an interaction between seaweed and soy, when administered together. Body mass index (BMI) of the women varied from 18 to 44 kg/m2. We found no treatment-by-BMI interaction, and although there was a marginally significant association between weight and TSH, there was no evidence that it influenced our estimation of the seaweed effect.

DISCUSSION

Our data support our first hypothesis that seaweed contains bioavailable iodine, and that consuming seaweed supplements would affect thyroid function. The changes were small, and although statistically significant for an increase in serum TSH, the values remained well within normal ranges and were unlikely to be physiologically important.

TABLE 3. MEAN VALUES FOR THYROID FUNCTION AND URINARY IODINE EXCRETION BY TREATMENT PERIOD FOR 25 WOMEN

Placebo		*Seaweed*		*Soy*		*Seaweed and soy*	
Thyroid tests	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Serum hormones								
T3 (ng/dL)	124.1	16.5	128.4	16.2	125.6	17.7	125.8	16.6
T4 (_g/dL)	6.6	1.0	6.6	0.9	6.8	1.1	6.7	0.8
FTI	5.7	0.7	5.8	0.7	5.9	0.9	5.9	0.7
TSH (_IU/mL)	1.69	0.95	2.19**	1.23	1.64	1.01	1.94**	1.13
Urinary iodine excretion								
Iodine (_g/day)	265.8	155.8	567.8**	177.8	290.5	190.9	545.6**	136.7
Iodine (_g/g of creatinine)	290.5	147.8	586.9**	177.6	328.5	185.4	571.7	147.8
Iodine (_g/dL)	13.5	7.8	31.5**	12.3	115.0	12.2	28.7	8.2

This analysis includes 25 women, 10 with a history of breast cancer and 15 with no such history. As this was a crossover study, each woman received each treatment, and these values represent the average values in all 25 subjects.
**P _ .01 versus placebo or soy alone.

However, some common dietary seaweeds, especially kelp (*Laminaria*), can contain 40 times as much iodine. The small changes we observed in euthyroid women may not be representative of the effects of high-iodine kelp in the general population.

Soy protein isolate supplementation has been associated with goiter formation in iodine-deficient rats and humans.[17,25] This does not appear to be the case in healthy postmenopausal American women. In our study of iodinereplete subjects, none of the women experienced clinically significant changes in thyroid hormones or urinary excretion of iodine during either of the two 1-week periods of high isoflavone (average 141 mg of total isoflavones/day) soy powder supplementation. Our adjusted dose of isoflavones to body weight (2 mg of isoflavones/kg/day), resulted in an average daily intake of 67.5 g of soy protein, or about six to seven times the daily intake of soy protein in Japan.[26] The previous study by Bruce *et al*.[27] of 38 healthy postmenopausal women in a randomized double-blind placebo-controlled study showed no effect of soy supplementation on thyroid function in women given slightly less (90 mg of total isoflavones/day) soy protein isolate and followed for 6 months. Likewise, two other studies have investigated

the longer-term effects of soy protein isolate on thyroid function. Duncan *et al*.[28] followed 18 postmenopausal women given the same dose of soy protein isolate supplement (2 mg of isoflavones/kg/day) for 3 months and found no change in thyroid hormone concentrations. In contrast, Persky *et al*.[29] studied 46 postmenopausal women who consumed either a high (90 mg/day) isoflavone soy protein isolate (ISP 90, Protein Technologies) or moderate (56 mg/day) isoflavone (ISP56, Protein Technologies) for 6 months and reported small but statistically significant changes in thyroid function. In these women, serum TSH was significantly higher at 3 and 6 months in women ingesting ISP90, serum T4 was significantly higher in women ingesting ISP56 at both 3 and 6 months, and serum T3 was significantly higher at 6 months in the women ingesting ISP90. However, it is difficult to interpret these findings since the hormone values for the women in the three groups (control, ISP56, and ISP90) differed at baseline. All values remained the within normal ranges. Our results from two separate 1-week exposures to high soy protein isolate provide additional support for the hypothesis that soy supplementation in iodine-replete populations have minimal or no effects on thyroid function.

On average, the women in our study excreted 266 _g of I/day (13 _g/dL) during the control period and 587 _g of I/day (32 _g/dL) while ingesting seaweed. Thus, our subjects were iodine sufficient at the beginning of the study and slightly above average for the United States.[30] Exposure to dietary iodine in Asia is much higher. For example, in Japan, the mean urinary iodine excretion in apparently healthy men and women in Sapporo was 5,100 _g/day (*n* _ 4,138)[31] and 211 _g/dL in Korea (*n* _ 207),[32] compared to a median of approximately 217 _g/day in 20,369 Americans.[30] When urinary iodine is standardized for creatinine content, the women in our study excreted 291 _g/g when ingesting placebo and 587 _g/g when ingesting seaweed. This is close to 673 _g/g of creatinine reported for 278 healthy adults in Korea,[33] where seaweed intake is common, and suggests that our study used seaweed exposure that approximates that found in Asia, where a variety of different kinds of seaweeds are eaten daily in small amounts.

There have been two other studies confirming the bioavailability of iodine from seaweed. Clark *et al*.[34] conducted a similar double-blind prospective 6-week clinical trial in 36 healthy euthyroid subjects. The study utilized commercial kelp tablets with an estimated 250 _g per capsule iodine content. Alfalfa (four capsules per day) was compared with high-dose kelp (four capsules per day) and lowdose kelp (two kelp capsules per day and two alfalfa capsules per day). Similar to our study, TSH increased significantly

FIG. 3. Comparison of treatment effects on the thyroid hormones TSH (**A**), T3 (**B**), and T4 (**C**).

A

B

in both kelp-supplemented groups. Serum free T4 concentrations did not change with either kelp supplementation, but total T3 did significantly after high-dose kelp; the latter finding remains unexplained. Their low-dose kelp ingestion corresponded to that in the present study, and urine iodine excretion was similar. Unlike our study in which we observed a slight but nonsignificant increase in T3, in the study by Clark *et al*.,[34] T3 decreased significantly after high-dose kelp therapy.

In the second study of iodine bioavailability from seaweed, Aquaron *et al*.[35] reported on the 48-hour short-term effect of dietary seaweed in two different geographic locations. In 18 healthy iodine-replete volunteers in Marseille, France, 90% of the seaweed iodine was excreted, but in a mildly iodine-deficient population in Brussels, Belgium, only 62% of the seaweed iodine was excreted in 48 hours. In our study in an iodine-sufficient population, 60% of the seaweed iodine was excreted in the urine. It is unclear why this was the case. When we compared within-subject variation in total urinary creatinine content of the collection specimens, the coefficient of variation averaged 15 _ 7%, with a range between 4% and 30%. We hypothesize that some of the urine collections for some women at a few time points may have been incomplete, possibly explaining the lower percentage of excreted iodine observed in our iodinereplete population.

In Japan and Korea, exposure to high iodine-containing foods usually begins *in utero* and via breast milk.[36] A study of 50 lactating Korean mothers reported the average maternal iodine intake immediately postpartum was 2,744 _g/day, decreasing to 1,295 _g/day at 4 weeks. The iodine concentration of the colostrum was 2,170 _g/L, and breast milk at 4 weeks contained 892 _g/L. This supports the possibility that early exposure to high iodine may be important in the habituation of people living in Asia to high ambient levels of dietary iodine.

Even though we report a small rise in serum TSH with moderate seaweed iodine exposure, thyroid disease in Japan is less common than in the United States. The prevalence of hyperthyroidism (TSH _0.15 _U/L) among 4,110 people living on Hokkaido in Japan was 0.6% among people living in the capitol of Sapporo and 1.1% among people living along the coast, with the latter presumably having more seaweed in their diets.[31] By comparison, the prevalence of hyperthyroidism (TSH _0.1 _U/L) among 13,344 adults with no underlying thyroid problems in the United States was 1.3%.[37] A slightly more stringent definition of hyperthyroidism in the United States (_0.1 _U/L) than in Japan (_0.15 _U/L) may partially explain the minor differences in rates between the two countries. Hypothyroidism (TSH _5.0 _U/L) prevalence in Japan was 1.3% in Sapporo and 3.8% along the coast.[31] The U.S. prevalence of hypothyroidism (TSH _4.5 _U/L) was 4.6%.[37] Again, the slightly different cutoff points may explain the greater prevalence in

the United States. Age-standardized incidence of thyroid cancer rates are higher in the United States (6.2/100,000) compared to Japan (4.8/100,000), but the death rates from thyroid cancer are twice as high in Japan (0.6) as in the United States (0.3).[19] Using autopsy studies to estimate the rate of undetected thyroid cancer at death, the prevalence in Japan was 35%,[38] compared to 3.6% in the United States.[39] This high prevalence of occult thyroid tumors (35%) but uncommon clinical diagnosis (4.8 age-standardized rate) and mortality (0.6 age-standardized rate) in Japan suggests that factors such as diet or environment play a role in thyroid cancer initiation and progression. People who immigrate from Japan to the United States have higher rates of many cancers, including thyroid cancer.[40]

The safety of high seaweed iodine supplements in the United States, where people have not been exposed since infancy, may be different than it is in Japan. Based on reviews of thyroid status of people in the United States, between 9.5% to 24% of women older than 60 years of age had evidence of thyroid dysfunction (TSH _5 mIU/L).[37,41] Even among people known to have hypothyroidism, a recent study found that 40% of people who were taking thyroid medications had elevated or suppressed serum TSH levels,[41] suggesting poor patient compliance, inadequate supervision, or variability in T4 content of the prescribed tablets. These baseline characteristics of U.S. populations suggest that low iodine-containing seaweeds would be safe, but the more typical high iodine seaweeds (kelp) deserve further study. Since older women have a high prevalence of Hashimoto's thyroiditis, which predisposes them to iodineinduced hypothyroidism, or nodular goiter, predisposing them to iodine-induced hyperthyroidism, careful monitoring of thyroid function is advisable in iodine-exposed women with positive thyroid antibodies or nodular goiter.[42]

With any dietary change there is the question of possible harm. In addition to thyroid function, long-term exposure to seaweed and thyroid cancer risk need to be considered. Reports of an association between thyroid disease and breast cancer have been found in some, but not all, epidemiologic studies.[43] In the largest case control study including 9,257 American women (4,575 cases and 4,682 controls), the only significant association between thyroid disease and breast cancer was that parous women who had been treated for thyroid cancer had an increased risk of breast cancer.[44] Likewise, a large retrospective study of 41,686 breast cancer patients and 3,662 thyroid cancer patients seen at MD Anderson Hospital reported a significantly increased risk of developing breast cancer after thyroid cancer in young women.[45] This finding was explored further using the National Cancer Institute's Surveillance, Epidemiology and End Results database with 1,333,115 person-years of data. Women who were diagnosed with thyroid cancer had a significantly increased risk of developing breast cancer. Again, those who had been treated for thyroid cancer had an increased risk of breast cancer (relative risk 1.18, *P* _ .007).

The effect was most pronounced in premenopausal women (relative risk 1.42, *P* _ .001).[46] It is possible that therapy of thyroid cancer with large doses of ^{121}I may play a role in this association since the nonlactating breast weakly concentrates iodine.

In a more general sense, the role of iodine, as opposed to thyroid function, may be important, as there is some suggestion that iodine deficiency is a risk factor for breast cancer.[47–49] Iodine is critical for the health of newborn infants, and during lactation and in rapidly dividing breast cancer cells. These observations led to the identification of the mammary gland iodide transporter (sodium iodide symporter) protein,[50] important in breast cell differentiation during breast development. Additionally, therapeutic success using oral iodine for breast fibrocystic disease has been reported.[51,52] Venuri[47] hypothesized that iodine is a primitive antioxidant that has been evolutionarily conserved, and provides protection to cell membranes. The form of iodine may be particularly important for breast cancer. Aceves *et al*.[53] have reviewed the literature of known effects of iodine on breast cancer and concluded that in its oxidative form, it is a potent antioxidant, inducing antiproliferative and apoptotic actions via iodinated lipids called iodolactones. Potassium iodine, as used in iodine supplementation programs, does not have these effects. The *in vivo* data are also suggestive of a role for seaweed iodine in mammary tissue protection. Three studies by Funahashi and coworkers explored the relationship of iodine and iodine in seaweed as factors in inhibition of 7,12-dimethylbenz[*a*]anthracene-induced mammary tumors in rats.[54–56] As a possible mechanism, they reported a high correlation between serum iodine and apoptosis of mammary cancer cells. These results, along with those we[57] and others[58–60] have reported for dietary seaweed as inhibitory of 7,12-dimethylbenz[*a*]anthracene-induced mammary tumors, are supportive of the idea that seaweed, possibly via iodine, could be involved in breast cancer prevention.

CONCLUSION

In an iodine-replete population of healthy postmenopausal women with normal thyroid function, the ingestion of an additional 475 _g of I/day was associated with a small but significant increase in serum TSH. Soy protein isolate had no effect on thyroid function or iodine excretion. A history of treatment for early breast cancer (Stage I or II) did not alter the changes observed in the seaweed-associated increase in TSH or iodine excretion. However, our sample size was small, and all women in the present study were screened to rule out underlying thyroid disease. It is unknown how seaweed ingestion might affect women with underlying thyroid disease. Given appropriate medical supervision and prior screening for allergies to iodine and thyroid function, consumption of low iodine seaweed and soy protein isolate is unlikely to cause serious side effects.

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of breast cancer. *Gan To Kagaku Ryoho* 2000;27:251–255.

TABLE IODINE CONTENT OF COMMERCIALLY AVAILABLE EDIBLE SEAWEEDS

American						
Alaria esculenta wakame	Maine	Whole	7			
	Maine	Whole	5			
	Maine	Whole	3			
	Namibia	Whole	6			
	Japan	Whole	3			
	Maine	Whole	3			
	Japan	Whole	6			
Ascophyllum nodosum			110			
Ecklonia maxima			431			
Eisenia bicyclis			646			
Fucus vesiculosis			2123			
Hizikia fusiforme			586			
Laminaria			276			
		Whole	629			
		Capsule	30			
		Whole	104			
		Capsule	392			
		Whole	352			
		Whole	56			
			82			
			153			
			3			
			5			
			7756			
			746			
			1259			
			1350			
			1356			
			1513			
			1862			
			26			
			200			
			362			
			665			
			117			
			520			
	Maine					
	British Columbia Canada					
	Washington					
	Maine					
	British Columbia Canada					
	Maine					
	Maine	Whole	6	1997	563	
	Japan					

Maine
Iceland
Maine
Japan
California
Washington
Tasmania
Powdered
Whole
Granules
Whole
Sheet
Whole
Whole
Tablets
4
6
6
3
3
7
5
4
2353
2984
8165
72
16
871
30
22
65
910
373
23
2
231
1
1
Knotted wrack
Paddle weed
Arame
Bladderwrack
Hijiki
Kelp
Oarweed
(L. longicruis)
Kelpb
Kombub
Wild kelpb
Kelpb
Oarweed
Fingered tangle
(I. digitata)
Mitsuishi-kombu
(I. angustata)
Fingered tangle
Fingered tangle
Dulse
Nori, purple laver
Sea palm
Horsetail tangle,
Mekabu (Undaria
spore)
Wakame
Wakame
Wakame
Mekabu
Wakame
Palmaria palmata
Porphyra tenera
Postelsia palmaeformis
Sargassum
Undaria pinnatifida
Powder

Whole
Whole
Powder
Whole
5
4
6
5
6
32
41
42
53
115
4
14
17
3
42
Tasmania
Tasmania
Japan
Tasmania
New Zealand
"Common names according to Madlener (42) and Arasaki and Arasaki (11).
bNo species indicated.

have been reported as food, medicine, and as part of religious celebrations in precolonial times (23,24), and seaweeds are still part of the diets of many indigenous people living in Asia, Polynesia and the Pacific Islands.

Japanese culture. In a recent article on improving public health nutrition in Japan, a recommendation for increasing seaweed consumption was included (17).

The actual amount of seaweed consumed is difficult to quantify because it is often as flavoring to noodles, soups, garnishes, and added as part of mixed vegetable dishes, as well as being a food that is served as a distinct entity as a snack, salad or side dish. In addition, seaweed is a part of military and religious ceremonial celebration foods in Japan. Soups containing seaweed (miso or wakame) have traditionally been included as part of most meals in Japan (8), although this is changing toward more Western foods (18).

Estimated iodine intake of people in Japan, mostly from seaweed, ranges from 200 to 20,000 .u.g/ d, with the average estimate of SOO-1000 .u.g/d (19). The average seaweed intake in Japan is approximately 4-7 g/d (11,20,21). Commercial data on seaweed sales in Japan estimate that the national average seaweed consumption per person is 4 kg/yr, or closer to 10 g per person per day (22).

In Japan, 21 species of seaweed are routinely included in the diet and in Korea more than 40 kinds of seaweed are commonly used as food (11). Elsewhere in the Pacific basin, in Hawaii and other Polynesian islands, 29 kinds of seaweed

FIG.I.
species.
Y-'
Seaweeds (Common Names)
Iodine content of dietary seaweeds compared by

7500l seaweed. Samples cut from growing juvenile (<50 cm), had approximately twice the amount of iodine per gram as found in samples from adult seaweed.

Figure 3 presents the iodine content in different parts of two kinds of seaweed (Ecklonia maxima and Laminaria pa//ida). The iodine content in the inedible stipes was approximately 50% lower than the blades. Meristematic tissue, the growing area at the base of the blade of the seaweed, was available for Laminaria from Namibia. The higher concentration of iodine in this area of the seaweed suggests that iodine is important to rapidly dividing seaweed cells.

On beach Floating
(n = 9) (n = 6)
Sunbleached Blades
Adult Juvenile
(n = 12) (n = 3)
Growing Blades

FIG.2. Iodine content of Laminaria pallida compared by harvesting

condition and age of seaweed sample.

Seaweeds are increasingly common foods and food supplements in the United States. As international foods have become commonplace throughout the world, seaweed consumption has increased. One study of marketing trends reported that 15% of Americans enjoy Japanese cooking (25). Popular claims for seaweed are that they provide an all-natural source of minerals. Such claims of efficacy have placed seaweeds in the role of nutriceuticals as well as regular food.

Discussion

Dietary seaweeds have great variability in iodine content. The problem is further compounded by the use of some high-iodine seaweeds as flavoring in some traditional Asian soup stocks and other dishes, in which the seaweed is removed before serving. A range of post harvest factors affects iodine content of seaweed, including preparation and storage conditions.

The iodine in common edible seaweeds is mostly water soluble, with highest levels reported in kelp (Laminaria), of which 99.2% is water soluble (27), although another edible seaweed, Sargassum, had only 40% water soluble iodine. The bioavailability of the seaweed iodine to humans has been reported (28-30). Hou et al. (27) reported that the chemical species of iodine in common seaweeds were primarily I-, 66% (Sargassum) to 88% in kelp (Laminaria). Organic iodine ranged from 10% in kelp to 29% in Sargassum, and iodate (103-) from 1.4% in kelp to 4.5% in Sargassum (27). Geographic variation in iodine content is also a factor, and iodine values for seaweeds from our study and other published work is presented in Table 2. For example, iodine in wakame (Undaria) was 23 times higher in the sample from China, and 2.5 times higher in the samples analyzed by Lee et al. (31) than in the samples we analyzed from New Zealand, Australia, and Japan. Kelp varied from an average of 1542 JLg/ g in the 10 species of Laminaria in our study to 5307 JLg/ g in the values reported for French Laminaria (30). The red seaweeds (Rhodophyta) analyzed (dulse and nori) were consistently lower in iodine, less than 100 JLg/ g.

In studies of iodine loss caused by storage conditions, Marchal et al. (29) reported that iodine content remained more or less constant when stored in watertight bags or boxes, but lost almost half of its iodine content in the first 40 days when stored in open containers or in paper bags, especially under

Methods and Materials

We obtained seaweed samples from health food stores in central Massachusetts, by contacting seaweed harvesters in Tasmania, Maine, and British Columbia, and collecting samples of kelp (Laminaria and Ecklonia) of known age and condition from Namibia. All samples were in the form that an average consumer might buy.

Samples were analyzed according to the standard determination of total iodine as outlined by Benotti et al. (26). This uses the reduction-oxidation reaction between ceric and arsenite catalyzed by iodide. The iodine concentration is proportional to its catalytic activity. Twenty-five 50-mg samples were digested with chloric acid and diluted with iodine free deionized water. They were then measured spectrometrically at 420 nm with a Technicon Autoanalyzer (Technicon Instrument, Inc, Tarry town, NY). Values in the seaweed were based on an iodine standard curve. At least three aliquots from each seaweed were analyzed for iodine content. The mean value is presented:!: standard deviation (SD).

5000
4000
Ql
C
~ 3000
C)
-a, 2000
~
1000

Results

Iodine content of seaweeds varied from 16 .ug/ 9 to 8165 .ug/g {Table 1). The highest iodine-containing sample came from kelp granules that had been made from Laminaria digitata harvested off the coast of Iceland. The kelp granules

were made of dried and pulverized seaweed.
In Figure 1 we compare the common edible seaweeds by iodine content. It is interesting to note that American wakame {Alaria) and kelp {Laminaria), both remarkably similar in appearance, have a marked difference in iodine content, even when both specimens were harvested on the same day by the same harvester from the same bay.
Iodine has been reported to vary with age and condition of the plant, with iodine loss thought to occur rapidly once a seaweed is no longer growing. In Figure 2, sun-bleached seaweed collected from the beach had the lowest iodine content, followed by samples collected from floating drifts of
Stipe Blade
(n = 9) (n = 9)
Ecklonia
Stipe Blade Meristem
(n = 3) (n = 3) (n = 6)
laminaria
FIG.3. Iodine content compared by part of adult fresh sea.
weed.

TABLE 2. COMPARISON OF SEAWEED IODINE BY GENUS, GEOGRAPHIC LOCATION, AND STUDY
Lee et al.
(31)
JLg/g
Hou and Yan
(43)
.ug/g
Aquaron et al.
(30)
JLg/g
Van Net ten et al.
(44)
,ug/g
This study
JLg/g
Seaweed origin u.s., Canada,
Namibia,
Tasmania,
Japan
586
72
629
8165
UK China France British Columbia
714a
44
391
67
600
436
815
Arame
Dulse
Hijiki
Kelp granules.
tablets (salt
substitute)
Kelp/kombu
Nori
1542b
16
2650
43
3040
36
5307 2110
17
185
60
102
151
Wakame 66c 161a 1571
Alaria
a A verage of two reported values.
b Average of 10 kinds of kelp analyzed
c A verage of three sample sites.
humid conditions, in which moisture might condense to concentrate

water soluble iodine from the seaweed.
Additional factors known to affect iodine content of seaweed are season, salinity of the water, and depth of the seaweed, coldness of the water, distance from the equator, postharvest storage conditions, and possibly other factors (32).
Other factors that affect the iodine content of seaweed include the part of the seaweed used. The stipe (stalk), although generally regarded as inedible, could be included in specimens harvested for health food supplements. In our study, the meristematic tissue at the base of the blade had the highest iodine content. Harvesting regulations in places such as Maine require leaving the base and at least 16 inches of seaweed, including the holdfast, stipe, and first few inches of the blade, which would include the meristematic tissue, on the rock. If harvesters did this, consumers would be protected from the high iodine containing meristematic tissue. However, harvesters do not routinely follow these guidelines either in the United States or internationally (33).
Food preparation and cooking methods are other factors in determining final iodine content of foods. Iodine in seaweed is highly water-soluble. One study of the effects of cooking on kelp reported that after 15 minutes of boiling, 99% of the seaweed iodine could be found in the cooking water (34). Although not specifically related to seaweed iodine, cooking loss of iodine from iodized salt has been studied. Goindi et al. (35) reported that the method of cooking was important in iodine loss, with losses ranging from 6% when roasted, to 20% when steamed or deep fried, to 27% when shallow fried, to 37%-82% with boiling (36). Although the previous values are for iodine in other foods, it is likely that cooked seaweed would be subject to similar rates of loss. A meal is never made from only seaweed. Seaweed, when eaten in a meal with other foods with goitrogenic potential, such as cassava, Brassica (broccoli, cabbage, cauliflower, bok choy, etc.) and soy, may mitigate the effect of the high iodine in the seaweed.
The activity level of the person eating the seaweed has also been reported to be significant in terms of assessing exposure to seaweed iodine (37). In a study of Japanese male university students, iodine losses in sweat during athletic training were high, suggesting that exercise in hot humid climates could increase iodine daily requirements.
Although exposure to high concentrations of iodine will transiently decrease thyroid hormone synthesis for approximately 24 hours (acute Wolff-Chaikoff effect [38]), continued exposure to excess iodine results in a decrease in the iodide concentrating ability of the thyroid by decreasing the thyroid sodium iodide symporter (NIS), permitting normal thyroid hormone synthesis to resume (38,39). However, some individuals do not escape or adapt to the transient decrease in iodine-induced thyroid hormone synthesis (i.e., those with autoimmune thyroid disease [Hashimoto's thyroiditis]), and continued excess iodine ingestion may induce hypothyroiditis (40). Thus, excess ingestion of seaweeds could, in susceptible subjects, induce hypothyroidism and, far less commonly in the United States, hyperthyroidism. Episodic dietary exposure to high-iodine-containing foods could pose health risks for iodine sensitive patrons. For example, the iodine content of nori, the flat sheets of seaweed used to make sushi, contain trivial amounts of iodine (16 JLg/ g), but a bowl of miso soup made with a kelp flavored stock, even without the presence of seaweed in the final soup, could contain more than 1000 JLg of iodine. These sources of natural variation in iodine content contribute to the confusion in describing a particular

species as "safe" for iodine sensitive individuals.
Seaweed iodine presents an interesting study in how people in Japan and Korea, with habitual exposure to seaweedcontaining diets could be relatively immune to any effects of high iodine intake, but a single seaweed rich Japanese meal could present health risks to iodine sensitive unhabituated diners in low-iodine consuming countries. A greater awareness of the variability of iodine content of seaweeds will help in defining high-risk foods for sensitive individuals.

The observation of Paracelsus (1493-1541), the founder of toxicology, " All substances are poisons; there is none which is not a poison. The right dose differentiates a poison and a remedy" (41), may need to be amended for iodine in seaweed. The safe dose of seaweed may depend on the kind of seaweed, but also the storage conditions, cooking methods, the climate where the person resides, the amount of physical exercise a person does, the presence of goitrogenic foods eaten with the meal, and the frequency of seaweed consumption.

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An integrative model for dietary seaweed inhibition of breast cancer
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Seaweeds were first recorded as a treatment for breast cancer by the ancient Egyptians almost 4,000 years ago. Epidemiologic data supports a role for dietary seaweed in the prevention of breast cancer. Premenopausal breast cancer rates for women in Japan are about one third that of US women and this protective effect is even greater for postmenopausal women, who have only ninth the US rate of breast cancer. Japanese women who migrate to the US have about a doubling in breast cancer risk after living in the US for 10 years, strongly supporting the idea that environmental factors, rather than genetics, are the primary risk factors. A 50% reduction in breast cancer relative risk has been reported for Japanese women who eat = 3 cups/d of seaweed-rich miso soup (compared to 1 cup/d). Other case-control studies in Japan have reported that eating seaweed significantly reduced risks of colorectal cancer, stomach cancer, and esophageal cancer.

In vitro and *in vivo* studies have shown inhibition of a range of tumor types, and were most effective in preventing tumor development and metastasis, rather than in treatment of existing tumors. This is consistent with clinical data that show Japanese women who do develop breast cancer live significantly longer.

To investigate the causal pathway for the protective properties of seaweeds, we compared the effects of several algae water extracts on proliferation of estrogen positive (MCF-7) and estrogen negative (MDA-MB231) breast cancer cells, using the MTS assay. None of the seaweeds tested inhibited the hormone dependent MCF-7 cells, but several were effective against estrogen independent MDA-MB-231 cells. *Undaria pinnatifida*, one of the most popular brown seaweeds consumed in Japan and Korea, showed the strongest antiproliferative effects. DNA synthesis was decreased, but apoptosis, as measured by caspase 3/7 stimulation, was unremarkable.

We then explored the effects of seaweed extracts on the extracellular matrix, staining for urokinase activity. Seaweed extract profoundly inhibited urokinase activity in MDA-MB-231 cells, but had no effect on MCF-7 cells. In a concurrent randomized double-blinded cross-over study of the effects of dietary seaweed in 15 healthy postmenopausal women, there was a highly significant ($p = 0.009$) decrease in urinary excretion of urokinase associated with ingestion of seaweed for the ten women who had been treated for breast cancer.

Additional DNA microarray analyses are ongoing and data will be presented.

Seaweed, soy, and estrogen metabolism in healthy postmenopausal American women

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Introduction: Seaweed and soy foods are common in Japan and Korea where the incidence and mortality of breast cancer are significantly lower than in the US. Most attention has focused on soy foods and their phytoestrogen content. Seaweeds are known to have an antibiotic effect in vitro and in vivo studies support the idea that dietary seaweed modifies gastrointestinal bacteria populations. We investigated the possibility that dietary seaweed could act as a probiotic when consumed with soy, and enhance the gastrointestinal metabolism of phytoestrogens, especially the increasing the production of equol. Equol production is associated with decreased breast cancer risk.

Study Design: Women were randomized to either seaweed or placebo for six weeks, followed by a week when soy supplementation (2 mg isoflavones/kg body weight) was added. A 3-week washout period separated the two arms of the study, after which women were crossed over to the alternate intervention arm. Blood samples for estradiol, estrone, and sex hormone binding globulin were obtained at each clinic visit, and 48-hour urine specimens were provided at each of the time points for phytoestrogen and estrogen metabolite determination.

Methods: In a double-blinded placebo-controlled clinical trial, 15 healthy postmenopausal women (average age = 58 years) were recruited to our 17 week study. Six of the women had been treated for early breast cancer but were disease free at the time of the study. None of the women had taken antibiotics in the preceding 3 months or during the study. All were non-smokers and drank alcohol once or fewer times per week.

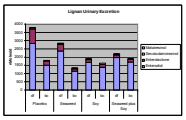
Results: Serum levels of estradiol were lower for women who had never had breast cancer (p=0.04). SHBG levels significantly decreased for both groups of women during the seaweed plus soy supplementation period. No urinary phytoestrogen excretion was reported during the placebo or seaweed interventions, but urinary phytoestrogens were detected during both the soy and seaweed plus soy intervention periods. O-DMA and equol production was significantly different among women who had had breast cancer (p=0.03; p=0.02). Equol production was only seen in women who had never been treated for breast cancer. Urinary lignan excretion was higher among disease free women (p=0.06);

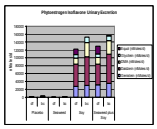
		Breast Cancer n=6	Disease free n=9
Age	(yr ± SD)	58.8 ± 7.9	59.6 ± 10.1
BMI	(± SD)	28.8 ± 3.1	25.1 ± 3.2

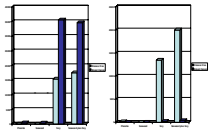
Randomized Blinded Crossover

Placebo 6 wks — Soy 1 wk — Washout 3 wks — Seaweed 6 wks — Soy 1 wk — Washout 3 wks

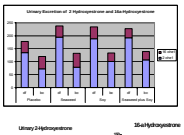
Seaweed 6 wks — Soy 1 wk — Washout 3 wks — Placebo 6 wks — Soy 1 wk — Washout 3 wks

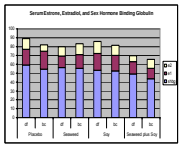




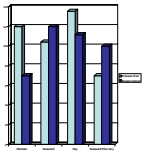


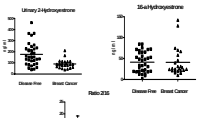
O-DMA Equol





Estradiol





This study was funded by the Susan G. Komen Foundation

Conclusions:
The presence of seaweed in the Asian diet may act as a probiotic, enhancing intestinal conversion of equol and O-DMA. Since seaweed and soy are often eaten together, some of the benefits of soy may accrue from the combination.

The differences between women who have never had breast Cancer and those who have had breast cancer suggest that there may be persistent baseline differences due to disease status.