Intake of *Fucus vesiculosus*, an Edible Brown Seaweed, Affects the Menstrual Cycle and Hormonal Status of Pre-menopausal Women

Christine F. Skibola

School of Public Health, Molecular Epidemiology and Toxicology Laboratory,
University of California, Berkeley, CA 94720

Running Title: Seaweed consumption affects human menstrual cycling and hormone levels

Address for Correspondence:

Christine F. Skibola, Ph.D.

Division of Environmental Health Sciences

School of Public Health

217 Earl Warren Hall

University of California

Berkeley, California 94720-7360

(510)643-5041 tel/(510)642-0427 fax

email: chrisfs@uclink.berkeley.edu

Abstract

Background: Epidemiological studies show that incidence rates of estrogen-dependent diseases are among the highest in Western countries and lower in the East. These variations may be attributable to differences in dietary and environmental exposures associated with modern lifestyles that may promote estrogenic stimulation resulting in higher circulating estrogen and shorter menstrual cycle lengths. Seaweed consumption accounts for 10-25% of the Japanese diet, though few studies have explored the impact of seaweed intake on the menstrual cycle.

Methods: To determine whether seaweed consumption reduces circulating 17β-estradiol levels and attenuates menstrual cycle irregularities, the brown seaweed, bladderwrack, was administered to three pre-menopausal women with abnormal menstrual cycling patterns and/or menstrual-related disease histories.

Results: Following treatment, all women reported significant increases in menstrual cycle lengths, ranging from an increase of 5.5 to 14 days. In addition, a series of hormone measurements taken in one woman revealed significant anti-estrogenic and pro-progestagenic effects following kelp administration. Mean baseline 17β-estradiol levels were reduced from 626 ± 91 to 164 ± 30 pg/ml ($p=0.04$) following 700 mg/d, which decreased further to 93.0 ± 3.5pg/ml ($p=0.03$) with the higher dose (1.4 g/d). Mean baseline progesterone levels rose from 0.58 ± 0.14 to 8.4 ± 2.6 ng/ml with the 700 mg/d dose ($p=0.1$), which increased further to 17.0 ± 0.7 ng/ml with the 1.4 g/d dose ($p=0.002$).

Conclusion: These data suggest that dietary bladderwrack can prolong the length of the menstrual cycle and exert anti-estrogenic effects in premenopausal women. Furthermore, seaweed may be another important dietary component apart from soy that is responsible for the reduced risk of estrogen-related cancers observed in Japanese populations.

Background

Epidemiological studies show that incidence rates of estrogen-dependent diseases such as endometriosis, and cancers of the breast, endometrium and ovary are among the highest in Western, industrialized countries, while rates for estrogen-related cancers are much lower in China and Japan (1, 2). These disparities may be attributable, in part, to differences in dietary and environmental exposures associated with affluent and modern lifestyles that promote estrogenic stimulation and hormone imbalances (3). Although the mechanisms are not fully understood, epidemiological and experimental data suggest that exposure to estrogens, through endogenous production and exogenous exposures resulting in an imbalance in the estrogen/progesterone ratio, may be the most critical determinants in disease risk (4-6). In estrogen-sensitive tissues, estrogen triggers cell proliferation, and through prolonged stimulation, unopposed by adequate progesterone production, hyperplasia and possibly neoplasia can occur. Reproductive factors associated with increased exposure to menstruation resulting in persistent and sustained estrogenic stimulation, such as shorter menstrual cycles, reduced parity, early menarche, and late menopause, are known to increase risk of endometriosis and estrogen-dependent cancers (7, 8), while post-menopausal obesity, hormone replacement therapy and alcohol consumption are associated with increased breast cancer risk (9-11). Therefore, limiting exposure to estrogens and reducing the overall number of menstrual cycles in one's lifetime through dietary and lifestyle changes may be the simplest means to reduce disease risk. In particular, the identification of dietary compounds that have estrogen reducing and/or progesterone enhancing effects holds great promise in developing chemopreventive strategies to abrogate risk of these diseases.

Studies show that Japanese women have longer menstrual cycle lengths (greater than the 28 day average) and lower circulating estrogen levels compared to Western populations (12-14), which until now has been at least partly attributed to the increased intake of soy protein among Asian populations (15-17). Another less explored component but main staple of the Japanese diet is seaweed, which accounts for 10-25% of their food intake (18, 19). A major source of dietary seaweed among Japanese populations is the edible brown kelp, wakame (*Undaria pinnatifida*) and kombu (*Laminaria japonica*). These species and the Atlantic brown kelp, bladderwrack *(Fucus vesiculosus)*, have been shown to exert powerful anti-hypertensive activity related to angiotensin-I-converting enzyme inhibition (20), to possess antibacterial and antioxidant properties related to their high polyphenolic content (21), and to prevent dioxin absorption and accelerate dioxin excretion in rats (22). Other chemopreventive properties such as antiviral activity (23, 24), immunostimulatory effects (25), anti-proliferative effects on 7,12-dimethylbenz(a)-anthracene-induced rat mammary tumors (26, 27), and anti–tumor and anti-metastatic activities in xenograft mouse models (28), have been associated with the high level of sulfated polysaccharides, also known as fucoidans, found in brown seaweed.

Intake of bladderwrack, as well as other brown kelp species, also has been shown to alter cholesterol metabolism and to significantly lower plasma cholesterol levels (29, 30). A possible mechanism of action involves competitive inhibition by fucosterols found in kelp. Since cholesterol is the precursor involved in steroid hormone biosynthesis, a reduction in cholesterol bioavailability could lower circulating plasma 17β-estradiol

levels that may lead to alterations in menstrual cycling patterns in pre-menopausal women. Until now, no studies have been performed in humans to determine the effects of brown kelp on menstrual cycling patterns and sex hormone status in pre-menopausal women, particularly in women with or at risk for estrogen-dependent diseases. To explore the hypothesis that kelp consumption could reduce circulating17β-estradiol levels and attenuate menstrual cycle irregularities, bladderwrack was administered to three pre-menopausal women with abnormal menstrual cycling patterns and/or menstrual-related disease histories.

Methods

Source and dose of bladderwrack (Fucus vesiculosus)

Dried, powdered bladderwrack (Fucus vesiculosus) was obtained from Maine Coast Sea Vegetables (Franklin, ME) and encapsulated in 350 mg capsules. Two capsules were administered daily for the low dose treatment (700 mg) and four capsules were administered daily for the high dose treatment (1.4 g). Bladderwrack dosage levels were chosen to fall within the range of normal dietary seaweed intakes typical in Japanese populations.

Study subjects

Three pre-menopausal women with abnormal menstrual cycling histories volunteered for the present study. Subject 1 had a history of hypermenorrhea (excessive menstrual bleeding), polymenorrhea (shorter than average menstrual cycle length of 28 days), and was diagnosed with luteal phase deficiency and endometriosis (through laparoscopy).

Subject 2 suffered from hypermenorrhea and polymenorrhea. Subject 3 suffered from hypermenorrhea and was diagnosed with endometriosis. All three women reported a history of dysmenorrhea (painful menses). No hormones were taken for >3 months prior to the inception of the study. No soy protein products were consumed during the study period.

The protocol was approved by the Committee for the Protection of Human Subjects of the University of California at Berkeley. The nature of the study was explained, and written informed consent was obtained from all study subjects.

Experimental Protocols

All women provided self-reported menstrual cycling histories for the three months prior to the treatment period. Following a blood draw that was taken on day 21 of the menstrual cycle to obtain baseline plasma 17β-estradiol and progesterone measurements, Subjects 2 and 3 were administered 700 mg/day of encapsulated powdered bladderwrack and followed for two consecutive menstrual cycles. On day 21 of the second cycle, 17β-estradiol and progesterone measurements were retaken. Subjects 1 and 3 agreed to continue the experiment for two additional cycles at which time they received a daily dose of 1.4 g/day kelp. In the case of Subject 1, sex hormone levels were ascertained for two consecutive cycles prior to treatment and for two cycles during the low-dose (700 mg/d) and for two cycles during the high-dose (1.4 g/d) treatment period. Since the average length of her cycle was only 16 days prior to treatment, plasma 17β-estradiol and progesterone levels were measured on day 12 during the pre-treatment period, and on

days 12 and 21 for the first cycle and on day 21 thereafter during the treatment period. Menstrual cycling logs were maintained on all subjects during the entire course of the experiment.

Hormone Assays

All hormone assays were performed blind and in duplicate using radioimmunoassays by an outside-certified clinical laboratory.

Statistical Methods

Statistical analyses were performed by unpaired t-tests (2-sided) with a commercially available statistical software package (Stata, College Station, Texas). All statistical tests were considered significant for $p \leq 0.05$. Results are referred to as borderline significant for $0.05 < p \leq 0.10$.

Results

There were no adverse side effects reported and bladderwrack was well tolerated by all three women.

Effects of treatment on length of menstrual cycle and total days of menstruation

Following treatment, all women exhibited a significant increase in menstrual cycle lengths (Figure 1A). Subjects 1 and 3 both experienced a normalization of cycling patterns. Specifically, in Subject 1, who had a 30-year history of irregular menses, the menstrual cycle length increased from an average of 16.3 ± 0.6 days to 26.0 ± 1.4 days with

the low dose treatment (p<0.002), which increased by approximately 5 additional days to 31.2±1.1 days following administration of the higher dose (p<0.001) Figure 1B. In Subject 2, the average cycle length increased 5.5 days, from 23.0±1.7 to 28.5±0.7 days (p=0.03). Subject 3 exhibited a 4-day increase in menstrual cycle length from 27.3±0.6 to 31.5±0.7 days with the 700 mg dose (p=0.005) that increased by approximately 6 more days to 36.0±2.8 days with the 1.4g dose (p=0.01).

Along with increased menstrual cycle lengths, all women reported marked reductions in blood flow and average number of days of menstruation following bladderwrack treatment. Subject 1 reported the most significant reduction in total days of menstruation, changing from an average 9.3±0.6 to 6.3±1.8 days (p=0.06) with the low dose and to 4.5+0.7 average days with the high dose (p<0.003). Subject 2, who only took the low dose, also experienced a marked reduction in number of days of menstruation, from 8.0±1.0 to 5.3±2.5 days (p=0.06). Subject 3 exhibited a decrease in total menstruating days averaging from 6.3±1.5 to 5.8±0.4 days (p=0.65) with the low dose, and to 3.5±0.7 days (p=0.10) with the 1.4 g/d dose. Subjects 1 and 3, both who suffered from endometriosis, reported substantial alleviation from pain during menstruation and throughout the menstrual cycle following bladderwrack treatment.

Effects of treatment on serum estradiol and progesterone levels

A significant anti-estrogenic and pro-progestagenic dose response was observed in plasma estradiol and progesterone levels in Subject 1 (Table 2). Specifically, the mean baseline 17β-estradiol levels were reduced from 626±91 to 164±30 pg/ml (p=0.04) with

the low dose (700 mg/d), which decreased further to 92.5±3.5 pg/ml (p=0.03) with the higher dose (1.4 g/d). A more unexpected finding in Subject 1 was that the mean baseline progesterone level rose from 0.58±0.14 to 8.4±2.6 ng/ml with the lower 700 mg/d dose (p=0.1), which increased further to 16.8±0.7 ng/ml with the 1.4 g/d dose (p=0.002).

For Subjects 2 and 3, only one pre-treatment and post-treatment 17β-estradiol and progesterone measurement was ascertained. However, a decrease in baseline 17β-estradiol levels for both Subject 2 and 3 was observed. For Subject 2, levels went from 92 pg/ml to 64 pg/ml, and for Subject 3, levels went from 49 pg/ml to 20 pg/ml following treatment. However, there were no marked increases in progesterone levels following treatment. Progesterone levels for Subject 2 went from 6.0 to 8.7 ng/ml, and in Subject 3 from 2.2 to 0.62 ng/ml following 700 mg/d kelp treatment.

Discussion

The results of this preliminary pilot study suggest that bladderwrack consumption can effectively increase the length of the menstrual cycle and reduce the total number of days of menstruation in pre-menopausal women. These effects were most marked in the two women that had shorter than average cycles (16 and 23 days) versus the normal range of 26 to 29 days seen in women in Western populations. Menstrual cycles were further lengthened with increasing dose suggesting a linear dose-response effect. These marked increases in menstrual cycle length may have beneficial health effects in lowering risk of estrogen-dependent diseases such as endometriosis and ovarian, endometrial, and breast

cancers as reported in a number of studies (13, 31-34). Menstrual characteristics are surrogate markers that may reflect a woman's overall exposure to and production of endogenous hormones. Shorter menstrual cycle lengths and prolonged menstruation confer longer follicular and luteal phases where estrogen and progesterone levels and endometrial and breast cell proliferation rates are at their highest. A nearly fourfold increase in mitotic activity in the breast lobules occurs during the luteal phase of the menstrual cycle (35), while the highest proliferation rates (nearly 100-fold) in the endometrium occur during the follicular phase (36). Therefore, fewer menstrual cycles over a woman's lifetime would decrease the amount of time during which the breast and endometrial epithelia would be exposed to high levels of proliferation, which may decrease overall disease risk.

Bladderwrack consumption was shown to markedly reduce circulating 17β-estradiol levels and increase progesterone levels, which led to the normalization of an imbalance in the estrogen/progesterone ratio in Subject 1 who suffered from progesterone deficiency and endometriosis. Progesterone antagonizes the proliferative effects of 17β-estradiol on the endometrium and in the breast, suggesting that this unique bioactivity could abrogate a situation favorable to the development of estrogen-dependent diseases. Further, the alleviation of hypermenorrhea and dysmenorrhea associated with endometriosis reported by Subject 1 following bladderwrack intervention suggests that correcting an imbalance in the estrogen/progesterone ratio may be a potential strategy in treating endometriosis.

Conclusions

The observed responses to bladderwrack consumption in this study suggest that dietary modification may lead to significant changes in the regulation of the menstrual cycle. Such changes may be beneficial particularly with regard to endometriosis and other estrogen-dependent diseases. While this study needs to be repeated in a larger population with placebo controls, results from these preliminary experiments suggest that bladderwrack can exert anti-estrogenic and pro-progestagenic effects in humans and that it may provide some relief in the treatment of endometriosis. Although these reported effects are generally in a beneficial direction, their clinical significance is yet to be determined. Animal and *in vitro* studies are currently underway to elucidate the potential mechanisms and clinical relevance of bladderwrack bioactivity, and to identify and isolate the active components involved.

Competing Interests

Christine Skibola has declared no interests.

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Figure 1

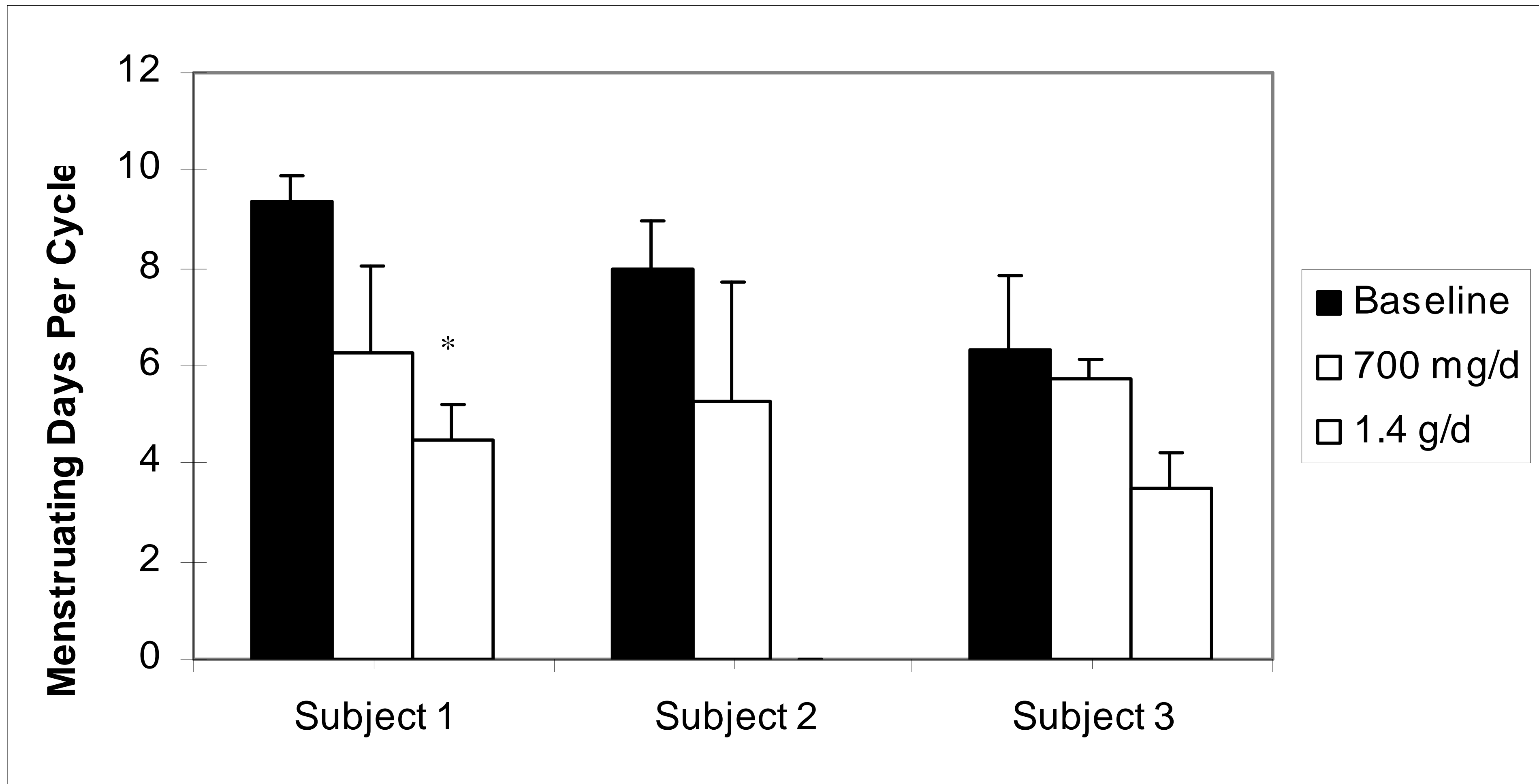

Figure 1a. Average menstrual cycle length in days for Subjects 1-3 at baseline (black bars) and following bladderwrack administration of 700 mg/d (diagonal striped bars) and 1.4 g/d (white bars).

Whiskers indicate standard deviations.

*P value <0.05.

Figure 2

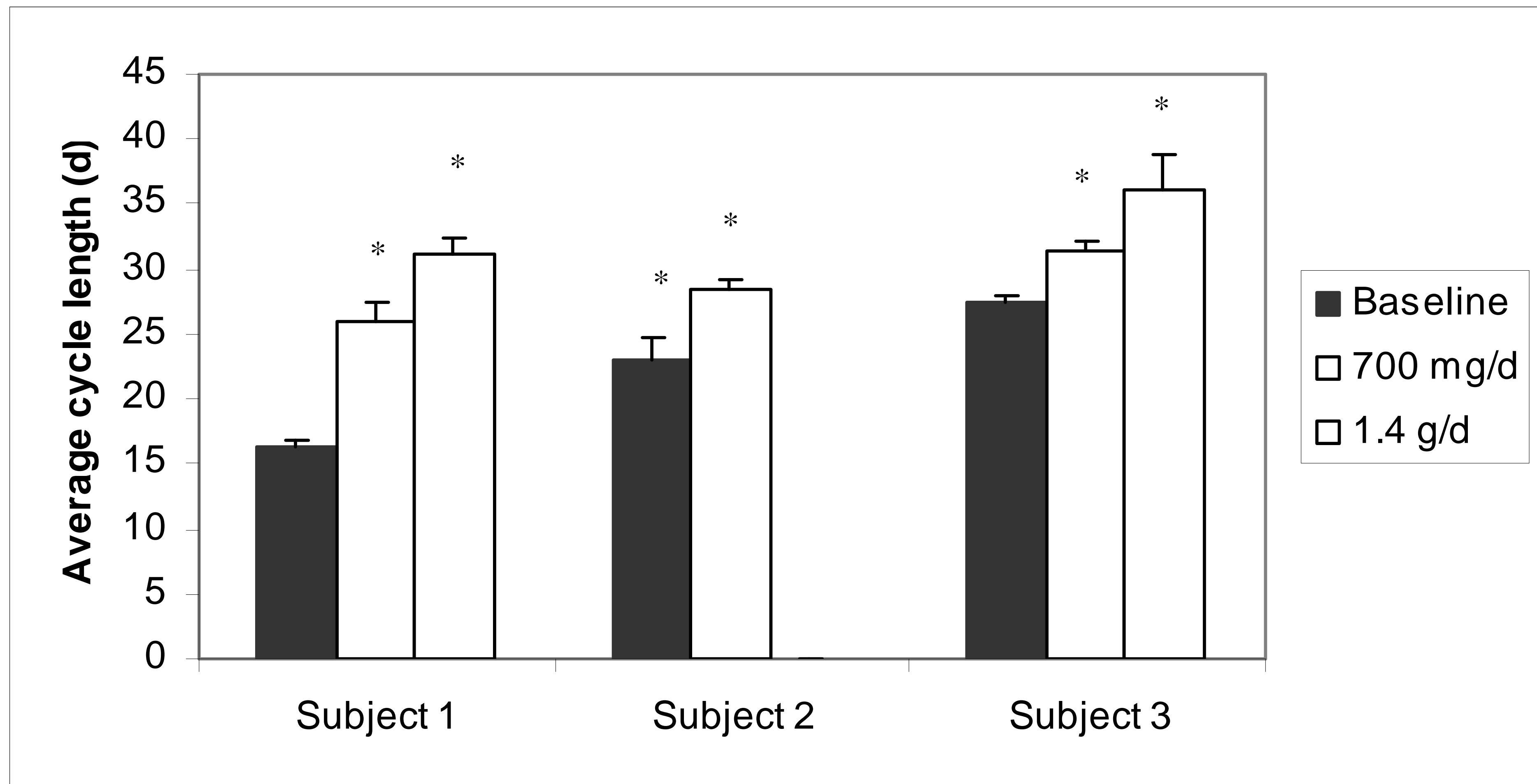

Figure 1a. Average menstrual cycle length in days for Subjects 1-3 at baseline (black bars) and following bladderwrack administration of 700 mg/d (diagonal striped bars) and 1.4 g/d (white bars).

Whiskers indicate standard deviations.

*P value <0.05.

Table 1. Average circulating plasma 17-β estradiol and and progesterone levels prior to and during kelp intervention for Subject 1.

	Pre-treatment	700 mg/d dose	P-value	1.4 g/d dose	P-value
17-β estradiol (pg/ml)	625$\pm$91	164$\pm$30	0.04	92.5$\pm$3.5	0.03
Progesterone (ng/ml)	0.58$\pm$0.14	8.4$\pm$2.6	0.1	16.8$\pm$0.7	0.002

Figure 3