Author's response to reviews

Title: A pilot study into the effect of intake of Fucus vesiculosus, an edible brown seaweed, upon menstrual cycle length and hormonal status of pre-menopausal women

Authors:

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Response to Comments by Reviewer 1

Major Compulsory Revisions:

1. Comment: Sample Size: The issue of sample size needs to be addressed in this study. A sample size of 3 is insufficient to draw statistically valid conclusions. This report is likely more appropriate as a case-study report rather than a full study.

Response: These and other points mentioned by both reviewers regarding lack of a well-controlled study are well taken. Therefore, this paper has been redrafted as a case study report. In addition, as addressed below, statements regarding the weaknesses of this study are included in the discussion.

2. Comment: Subject Characteristics: There needs to be more information provided about the subjects (age, body weight, use of medications, etc.). In addition, it is of concern that these subjects have such irregular menstrual cycles as such variation does not contribute to a well-controlled research study.

Response: Subject characteristics are now included in a new table, Table 1. As mentioned in response to Comment 1, the paper now is being submitted as a case report since it is not a well-controlled research study.

3. Comment: Description of Data Collection: There needs to be more detail regarding when the samples were collected. It is described that blood was collected on day 21 (baseline) and then bladderwrack was consumed for 2 cycles of which blood was collected on day 21 of the second cycle. It is then unclear when samples were collected in the following cycles during the higher dose.

Response: A new Table 2 now reflects the study protocol and timeline to clarify when dosing and hormone measurements were ascertained.

4. Comment: Ovulation: There is no mention of ovulation testing. How can circulating hormones be adequately assessed if there is no confirmation of ovulation? For example, if the subject did not ovulate in cycle 1 and did ovulate in cycle 2, it would appear that progesterone, collected on day 21, had increased. But this would be due to ovulation, not the intervention.

Response: In the case of Subject 1 where multiple hormone measurements were taken, she suffered from luteal phase deficiency and experienced anovulatory cycles for several years prior to the study. Prior to and during the study period, she had been monitoring ovulation through basal body temperature measurements. Since the average length of her cycle was 16 days prior to treatment, baseline hormone levels were ascertained on a set day (day 12) for two consecutive cycles prior to bladderwrack treatment. Following initiation of the bladderwrack treatment, hormone levels were measured on Days 12 and 21 (which was another anovulatory cycle). All subsequent
measurements were taken on Day 21. This is now reflected in "Experimental Protocols" section. Since ovulation was not assessed for the other study subjects, all data related to their serum hormone measurements has been removed from the text.

5. Comment: Lack of washout period: The fact that the 1400 mg was consumed directly after the 700 mg means that an effect of time, rather than dose, cannot be ruled out. The author should address this in the discussion.

Response: The following statement has now been inserted in the first paragraph of the discussion: "However, since there was not a sufficient washout time between the 700 and 1400 mg/d doses, an effect of length of time of dosing rather than a dose response effect cannot be ruled out."

6. Comment: Lack of control group: This methodological limitation should be stated more clearly in the discussion section (it is mentioned in the conclusions but only briefly).

Response: This and other weaknesses of the study have now been more clearly stated in a section entitled, "Study Limitations."

Minor Essential Revisions

1. Comment: Background, page 4, line 2: is endometriosis lower in Asian women? Do statements 1 and 2 support this statement.

Response: No. The statement said that estrogen-dependent cancers were lower among Asians, although the wording may have been misleading. Since there are no references that compare rates of endometriosis in the U.S. and Japan, "endometriosis" has been omitted from this sentence for clarity.

2. Background, page 4, line 6: use of reference 3 does not seem appropriate.

Response: A more appropriate reference has now been added.

3. Background, page 4, line 12: need to provide a reference for this statement (after "hyperplasia").

Response: A reference has now been added.

4. Methods, page 6, source of kelp paragraph: It would be good to have a reference to support the statement that the doses used in this study are similar to Japanese intakes.

Response: References and an explanation of how the dose was calculated has now been added.

5. Study subjects, page 6: more detail needed. See item #2 in Compulsory Revisions

Response: This has been addressed as stated in Compulsory Revisions #2 by adding Table 1.

6. Comment: Experimental Protocols, page 7: need more detail. Item #3 in Compulsory Revisions

Response: This has been addressed as stated in Compulsory Revisions #3 response by adding Table 2.

7. Comment: Hormone assays, page 8: need more detail here. Specify the hormones measured and the assay variability.

Response: These details have now been added to the "Hormone assays" section.
8. Comment: Statistics and Results: The statistics should be done on all 3 subjects combined rather than just within each subject individually.

Response: Since all levels of treatment were not undertaken by all 3 subjects, it would be confusing to statistically analyze in combination the effects of bladderwrack treatment on menstruation in all 3 women. Since the hormone data on Subjects 2 and 3 will no longer be included in the text, statistics on hormonal analyses are only presented for Subject 1.

9. Comment: Table and Figures: There needs to be more detail provided in the footnotes of the tables and figures as to how these averages were generated (ie. From 2 menstrual cycles before the study, etc.). If this were more clearly stated in the methods, it may then not be necessary in the tables and figures.

Response: These details are now in the footnotes of Figures 1 and 2.

10. Comment: Tables and Figures: It is unclear how the 700 mg/day has more than one measurement per subject as the methods indicate that blood was collected on day 21 of the second cycle of consuming 700 mg/d. That is only one measurement, which would not have any variability associated with it if presented within one subject.

Response: The treatment protocol is now clarified in the text and in Table 2.

Response to Comments by Reviewer 2

Major Compulsory Revisions

1. Comment: At most, with the small number of subjects in this study, it should be designated a pilot study. How was the sample size calculated? Sample size too small at this point.

Response: As mentioned in an earlier response to comments by Reviewer # 1, these and other points brought up regarding the lack of a well-controlled study design are acknowledged. Therefore, this paper has been redrafted as a case study report.

2. Comment: How was dose of supplement established? - explain this further with appropriate references.

Response: Appropriate references and a description of how the dose was established has now been added in the section entitled, "Source and dose of bladderwrack (Fucus vesiculosus)".

3. Comment: Timing of blood draw in the 3 subjects - how was this adjusted for menstrual cycle points- especially when these subjects had irregular periods? How was luteal and follicular phase established, as this would be critical when hormonal measures are evaluated. Hormonal surges at points in the menstrual cycle may provide false elevated hormonal values.

Response: In the case of Subject 1 where multiple hormone measurements were taken, she suffered from luteal phase deficiency and experienced anovulatory cycles for several years prior to the study. Prior to and during the study period, she had been monitoring ovulation through basal body temperature measurements. Since the average length of her cycle was 16 days prior to treatment, baseline hormone levels were ascertained on a set day (day 12) for two consecutive cycles prior to bladderwrack treatment. Following initiation of the bladderwrack treatment, hormone levels were measured on Days 12 and 21 (which was another anovulatory cycle). All subsequent measurements were taken on Day 21. This is now reflected in "Experimental Protocols" section. Since ovulation was not assessed in the other study subjects, all data related to their serum hormone measurements has been removed from the text.
4. Comment: How were patients monitored for compliance to study agent/supplement?

Response: A sentence stating "Subjects were monitored at least weekly to insure compliance to the supplement regimen" has been added to the "Experiment Protocols" section.

5. Comment: Define abnormality of menstrual cycle.

Response: Under the heading "Case Presentation," what was considered an abnormal menstrual cycle in this study has now been inserted in the text as follows: "An abnormal menstrual cycle was defined as one of the following: a menstrual cycle of <26 or >32 days in length; menstrual cycles consisting of >8 menstruating days; or anovulatory menstrual cycling."

6. Comment: Discussion: Discuss the limitation of this study with a small sample size.

Response: A section entitled "Study Limitations" has now been added to the paper which addresses the small sample size of the study, lack of a control population, etc.

7. Comment: What about the dangers of elevated progesterone? Discuss the implication of this in the breast and endometrium.

Response: A paragraph addressing the dangers of elevated progesterone and its effect on the breast and endometrium has been added to the discussion.

8. Comment: Add future directions section at the end.

Response: A section headed "Future Directions" has now been added to the end of the paper.