Maturitas xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Maturitas

journal homepage: www.elsevier.com/locate/maturitas

# Effects of sea buckthorn oil intake on vaginal atrophy in postmenopausal women: A randomized, double-blind, placebo-controlled study☆

Petra S. Larmo[a,*], Baoru Yang[b], Juha Hyssälä[c], Heikki P. Kallio[b], Risto Erkkola[d]

[a] Aromtech Ltd, Tornio, Finland
[b] Food Chemistry and Food Development, Department of Biochemistry, University of Turku, Finland
[c] Statfinn Ltd, Turku, Finland
[d] Department of Obstetrics and Gynecology, University Central Hospital of Turku, Finland

ARTICLE INFO

*Article history:*
Received 15 April 2014
Received in revised form 7 July 2014
Accepted 11 July 2014
Available online xxx

*Keywords:*
Sea buckthorn
Vaginal atrophy
Menopause
Mucous membrane

ABSTRACT

*Background:* Vaginal atrophy, the thinning and drying of vaginal mucosa, is associated with menopause. The standard estrogen treatment is not suitable for all women.
*Objective:* To investigate the effects of oral sea buckthorn (SB) oil supplementation on vaginal atrophy.
*Method:* A total of 116 postmenopausal women experiencing symptoms of vaginal dryness, itching or burning were randomized to this placebo-controlled, double-blind study. Ninety-eight participants completed the intervention of three months, during which they consumed 3 g of SB or placebo oil daily. At the beginning and end, factors of vaginal health were scored by a gynecologist, vaginal pH and moisture were measured and vaginal health index was calculated. Symptoms of atrophy and menopause were evaluated at study visits and by daily logbooks. Serum samples were collected for the analysis of circulating lipids, liver enzymes and C-reactive protein.
*Results:* Compared to placebo, there was a significantly better rate of improvement in the integrity of vaginal epithelium in the SB group when both compliant and noncompliant participants were included (odds ratio (OR) = 3.1, 95% CI 1.11–8.95). A beneficial trend was observed when only the compliant participants were included (OR = 2.9; 95% CI 0.99–8.35). There was a tendency ($P = 0.08$) toward better improvement of vaginal health index from baseline to the end in the SB group [(0.8 (SD 2.8)] compared to placebo [−0.1 (SD 2.0)].
*Conclusions:* SB oil showed beneficial effects on vaginal health, indicating it is a potential alternative for mucosal integrity for those women not able to use estrogen treatment for vaginal atrophy.


## 1. Introduction

Vaginal atrophy, the thinning and drying of vaginal mucosa, is associated with lowered levels of estrogen at menopause [1]. Atrophic tissue is prone to inflammation, petechial hemorrhages and ulceration [2]. Typical symptoms include vaginal discomfort, feelings of dryness, burning, itching and dyspareunia [1]. A prevalence of 43% for the symptoms of vaginal atrophy among postmenopausal women was recently reported in Finland and United States [3]. The average age of natural menopause in Europe is between 47 and 50 years. As the life expectancy increases, most women will spend even about one third of their life in postmenopausal state [1].

Estrogen is important for the structure of urogenital area, where it maintains the levels of collagen and elastic fibers and affects acidic mucopolysaccharides and hyaluronic acid, necessary for tissue moisture and epithelial barrier [1,2]. Estrogen promotes vaginal secretions, epithelial proliferation, vascularization and glycogen deposition in cells [2]. Exfoliating glycogen loaded superficial cells enhance the growth of lactobacilli producing lactic acid, important for the normal acidic vaginal pH [1]. The standard treatment for

*Abbreviations:* ALA, α-linolenic acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BL, baseline; CI, confidence interval; CRP, C-reactive protein; EPA, eicosapentaenoic acid; HDL, high density lipoprotein; ITT, intention to treat; LA, linoleic acid; LDL, low density lipoprotein; MI, maturation index; OR, odds ratio; PP, per protocol; $Q_1$, lower quartile; $Q_3$, upper quartile; SD, standard deviation; SB, sea buckthorn; VagHI, vaginal health index.

☆ The study was registered at clinicaltrials.gov (NCT01697085).

* Corresponding author at: Aromtech Ltd, Veturitallintie 1, FI-95410 Tornio, Finland. Tel.: +358 20 789 020; fax: +358 207 789 028.
*E-mail address:* petra.larmo@aromtech.com (P.S. Larmo).

http://dx.doi.org/10.1016/j.maturitas.2014.07.010

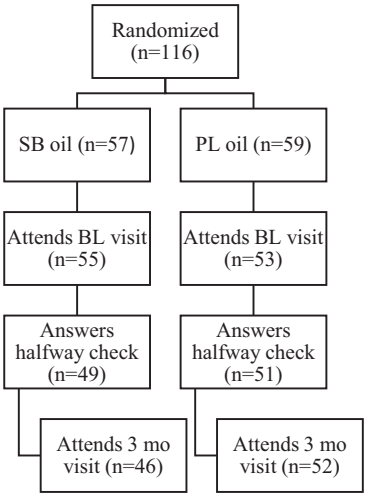


**Fig. 1.** Flow of participants during the study.

vaginal atrophy is estrogen, either topically or *via* systemic route. Though effective, this is not suitable for breast cancer patients. Also many healthy women are not willing to use hormone replacement therapy due to its association with higher risk of breast and endometrial cancer and venous thromboembolism [1,4,5].

Sea buckthorn (*Hippophaë rhamnoides*) oil has in the Central Asia traditionally been used for the treatment of inflammations in the genital organs and uterus [6]. Intake of sea buckthorn (SB) oil produced with a supercritical carbon dioxide extraction process has in clinical studies shown beneficial effects on serum lipids and lipoproteins [7], dry eye [8], markers of endothelial inflammation [9], and platelet aggregation [10]. In a double-blind, randomized, cross-over study in women suffering from Sjögren's syndrome, SB oil relieved the dryness-associated symptoms of genital mucous membranes [11]. The objective of this study was to investigate the effects of oral SB oil on vaginal atrophy among post-menopausal women.

## 2. Participants and methods

### 2.1. Study design and participants

This randomized, double-blind, placebo-controlled study was carried out at the Gynecological Center of Turku, a private clinic in Turku, Finland during a period from October 2012 to March 2013. The study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland and registered at clinicaltrials.gov (NCT01697085). A total of 116 postmenopausal women, recruited by announcements in local newspapers, were randomized to SB and placebo groups. Inclusion criteria were: age of 55–75 years and experience of moderate or severe dryness/burning/itching of vaginal mucous membranes. Exclusion criterion was the use of systemic or local estrogen treatment. The participants were advised not to use other oil supplements during the study and the wash-out period of one month before the trial. They attended two study visits: at baseline, and at three months when the intervention ended. In addition, they were interviewed by the study gynecologist at halfway of the intervention.

The flow of participants is presented in Fig. 1. The characteristics of participants at baseline visit are presented in Table 1. The main outcomes were the effects of SB oil on the vaginal pH, moisture and health, and the symptoms of vaginal atrophy. As secondary measures, also the effects on vasomotor and psychological symptoms associated with menopause, on skin and other mucous membranes and on serum markers associated with cardiovascular disease and metabolic syndrome were investigated.

**Table 1**
Characteristics of the ITT participants at baseline visit.[a]

| | SB[b] | PL |
|---|---|---|
| Age, y | 64(5) | 62(5) |
| BMI, kg/m$^2$ | 27(4) | 27(5) |
| Time from last periods, mo | 166(94) | 167(107) |
| Experience of vaginal symptoms, *n* (%) | | |
| Dryness | | |
| None | 0(0) | 0(0) |
| Mild | 24(45) | 14(28) |
| Moderate | 23(43) | 27(53) |
| Severe | 6(11) | 10(20) |
| Burning | | |
| None | 11(22) | 13(26) |
| Mild | 21(41) | 17(34) |
| Moderate | 18(35) | 20(40) |
| Severe | 1(2) | 0(0) |
| Itching | | |
| None | 11(22) | 12(25) |
| Mild | 24(48) | 20(41) |
| Moderate | 14(28) | 15(31) |
| Severe | 1(2) | 2(4) |
| Smoking, *n* (%) | 5(9) | 4(8) |
| Previous use of systemic estrogen medication, *n* (%) | 32(64) | 20(42) |
| Previous use of topical estrogen medication, *n* (%) | 26(50) | 27(55) |
| Diagnosed chronic illness or condition requiring medication, *n* (%)[c] | 31(61) | 25(52) |

[a] Values are means (SD) or *n* (%) of participants.
[b] SB, *n* = 47–55; PL, *n* = 48–53.
[c] The most common chronic illnesses or conditions requiring medication in both study groups were hypertension or elevated serum lipid levels (SB *n* = 14; PL *n* = 20), and hypo/hyperactivity of the thyroid gland (SB *n* = 12, PL *n* = 9). Four participants in the SB group and two in the placebo group had breast cancer that was being treated or had been treated before.

### 2.2. Study products

Participants consumed 3 g of SB or placebo oil daily in the form of 3 capsules twice a day. The dose was chosen based on our previous study with Sjögren's syndrome patients [11]. The capsules shells were opaque vegetable capsules with identical outlook. The SB oil used in the study was a standardized mixture of SB berry and seed oils produced using supercritical carbon dioxide extraction by Aromtech Ltd (Tornio, Finland). The main fatty acids in SB oil were palmitoleic [16:1$n-7$, 24% of fatty acids (w/w)], palmitic (16:0, 22%), linoleic (18:2$n-6$, 18%), oleic (18:1$n-9$, 16%), α-linolenic (18:3$n-3$, 13%) and *cis*-vaccenic (18:1$n-7$, 6%) acids. The oil contained 0.11% carotenoids (mainly β-carotene, 0.09% of the oil) and 0.44% vitamin E. Phytosterols composed 1.0% of the SB oil, analyzed as free sterols. The placebo oil was triacylglycerols of medium chain fatty acids fractionated from palm and coconut oils. The main fatty acids were caprylic (8:0, 60%) and capric acid (10:0, 40%). No vitamin E compounds were detected and the carotenoids were below the limit of quantification. Methods for the analysis of oils are presented in the Supplementary material.

### 2.3. Effects on vaginal mucous membranes

Vaginal mucous membranes were evaluated by the same gynecologist at the beginning and end of the intervention. Vaginal elasticity, epithelial integrity, moisture and fluid volume were scored from 1 to 5 according to Bachmann et al. [12]. pH of the vaginal wall was measured using a pH meter (Flexilog, Model No 2000, Oakfield Instruments Ltd., Oxon, UK). For the analysis of vaginal health index (VagHI), the pH was scored from 1 to 5 [12]. A higher

Please cite this article in press as: Larmo PS, et al. Effects of sea buckthorn oil intake on vaginal atrophy in postmenopausal women: A randomized, double-blind, placebo-controlled study. Maturitas (2014), http://dx.doi.org/10.1016/j.maturitas.2014.07.010

value in VagHI, calculated as a sum of scores for elasticity, epithelial integrity, moisture, fluid volume and pH-scores, indicates less atrophy [12]. The vaginal moisture was assessed by measuring the length of wetting a Schirmer paper applied on the vaginal wall for 1 min (mm/min) [13]. The vaginal maturation index (MI) was evaluated from Pap smear from a random sample of 30 participants (SB, $n = 15$; placebo, $n = 15$). In MI, the presence of superficial cells indicates the estrogenic influence while the presence of parabasal cells only is the sign of the absence of that.

### 2.4. Effects on experience of symptoms

The participants were asked to evaluate the severity of their symptoms of vaginal dryness, burning and itching on a scale from 0 (none) to 3 (severe) at the beginning, halfway and end of the intervention. In addition, they kept a daily logbook, reporting symptoms associated with vaginal atrophy (dryness, burning, itching, pain, secretion), urinary tract (inconvenience, excessive need for urinating, incontinence), dryness of other mucous membranes (nose, mouth, eyes), dryness of skin (scalp, skin in general), intercourse (pain, hemorrhage) and general symptoms associated with menopause (ten symptoms listed, including vasomotor and psychological symptoms). Each symptom was assessed on a scale from 0 (none) to 3 (severe). In the logbooks, the participants reported whether they had taken the study capsules and if there were any changes in their medical diagnosis. At halfway and at the end of the study the participants were asked whether they felt the intervention had in general beneficial (yes/no) or adverse (yes/no) effects to them.

### 2.5. Effects on serum markers

Blood samples were taken after a 12-h fast at the beginning and end of the intervention. Serum triacylglycerols, and total and HDL cholesterol concentrations were measured by standard enzymatic methods using Roche Diagnostics reagents (Roche Diagnostics GmbH, Mannheim, Germany) with a fully automated analyzer Cobas 8000 (Roche Diagnostics GmbH, Mannheim, Germany). LDL cholesterol was calculated using the Friedewald formula. Alanine and aspartate aminotransferases, ALT and AST respectively, in lithium heparin plasma were analyzed with photometric methods on a Cobas 8000 automatic analyzer (Roche Diagnostics GmbH, Mannheim, Germany). C-reactive protein (CRP) in serum was analyzed with an immunonephelometric assay on a Siemens Dade Behring BN II Nephelometer analyzer (Siemens Healthcare Diagnostics Inc., Siemens AG, Munich, Germany). Analyses of the blood samples were carried out by Tykslab (Hospital District of Southwest Finland, Turku, Finland).

### 2.6. Statistical analyses

The sample size was estimated based on the assumption that at the end of the study there would be a group difference of 2 (SD 0.7) units in the vaginal pH, 2.5 (SD 5) mm/min in the moisture test with a paper strip, or a difference of 0.55 (SD 1) units in the severity of vaginal symptoms. A difference of 26 percentage points in the proportions of participants experiencing beneficial effects on symptoms in the treatment groups was assumed. These effects were considered to be clinically relevant. With a sample size of approximately 50 participants/group the study would have a power of ≈80% to observe these differences (two-sided tests, 0.05 significance level). A drop-out rate of ≈15% was assumed. The participants were randomized using age, intensity of vaginal dryness/burning/itching and smoking status as stratification factors. For details of randomization and allocation concealment, see Supplementary material.

**Table 2**
Vaginal health index, pH and moisture of vaginal mucosa among ITT participants at baseline and the change from baseline to end of the intervention at three months.[a,b]

| | SB[c] | | PL | | *P*-value |
|---|---|---|---|---|---|
| | 0 mo | 3 mo–0 mo | 0 mo | 3 mo–0 mo | |
| Vaginal health index | 11.9 (2.9) | 0.8 (2.8) | 12.2 (2.5) | −0.1 (2.0) | 0.08 |
| pH | 6.6 (1.1) | −0.2 (1.0) | 6.6 (1.1) | −0.2 (1.1) | 1.00 |
| Moisture test, mm/min | 2.4 (1.0) | −0.9 (1.1) | 2.6 (1.4) | −1.0 (1.5) | 0.62 |

[a] Values are means (SD).
[b] Two-way ANCOVA with baseline measure as covariate was used for the analysis of group differences.
[c] SB, $n = 42–54$; PL, $n = 49–55$.

The group differences in the changes of vaginal pH, vaginal moisture with Schirmer paper and serum total and LDL cholesterol were analyzed by ANOVA. Because of the nonparametric distribution, other markers from the serum/plasma samples were analyzed using Wilcoxon–Mann–Whitney *U*-test. VagHI was analyzed using ANCOVA with the baseline measures as a covariate to compensate for the group difference at baseline. Vaginal elasticity, fluid volume, pH-score, epithelial integrity and moisture-score were analyzed by comparing the proportions of participants with improvement and deterioration/no change in the groups by logistic regression with baseline as a covariate. The same method was used for the analysis of vaginal symptoms reported at study visits and halfway.

For the symptoms in logbooks, a mean was calculated for each month. The groups were compared by Wilcoxon–Mann–Whitney *U*-test. Group changes in the proportions of cell types in Pap smear samples, and in the scores for the factors of VagHI were compared by Wilcoxon–Mann–Whitney *U*-test. The proportions of participants reporting general beneficial or adverse effects were compared using chi-squared or Fisher's exact test. Two-sided tests and significance levels of 0.05 were used throughout. The analyses were performed by SAS software (SAS Institute Inc, Cary, NC, USA). In reporting and comparisons, missing data was excluded. No adjustments for multiple testing were used [14].

Participants who in the daily logbooks reported consuming the study capsules according to the protocol for ≥80% of the days, were considered compliant. The primary statistical analysis was carried out including all participants (compliant and non-compliant: the intention to treat (ITT) participants). Additionally, a second set of analyses was carried out including only the compliant participants (the per protocol (PP) participants). For the PP data 15 participants were excluded based on the intake of study capsules. For the analysis of serum/plasma markers, participants using medications affecting serum lipids, and those having CRP concentrations >10 mg/L (indicating acute infection or inflammation), were excluded from the PP participants (excluded $n = 25$). In the text, results are those of ITT participants, unless otherwise indicated. When conclusions from the ITT and PP participants differ, both are presented. For other results of PP participants, see Supplementary material.

## 3. Results

### 3.1. Effects on vaginal mucous membranes

SB oil induced a beneficial increasing trend on VagHI, whereas a decrease was observed in the placebo group (Table 2). In the SB group, there was a significantly better rate of improvement in the score for vaginal epithelial integrity compared to placebo (Table 3). Among the PP participants, a non-significant trend for enhanced epithelial integrity in the SB group was observed [(Supplementary Table S2: Improvement in 36 and 17% of participants in

Please cite this article in press as: Larmo PS, et al. Effects of sea buckthorn oil intake on vaginal atrophy in postmenopausal women: A randomized, double-blind, placebo-controlled study. Maturitas (2014), http://dx.doi.org/10.1016/j.maturitas.2014.07.010

**Table 3**
Changes in the vaginal elasticity, fluid volume, pH-score, epithelial integrity and moisture among the ITT participants during the three months intervention.[a,b]

| | No change or deterioration, *n* (%) | | Improvement, *n* (%) | | Odds ratio | 95% CI |
|---|---|---|---|---|---|---|
| | SB[c] | PL | SB | PL | | |
| Elasticity | 28 (67) | 37 (74) | 14 (33) | 13 (26) | 1.48 | 0.52–4.19 |
| Fluid volume | 37 (88) | 45 (90) | 5 (12) | 5 (10) | 1.50 | 0.32–7.14 |
| pH-score | 33 (79) | 39 (77) | 9 (21) | 12 (24) | 0.92 | 0.34–2.51 |
| Epithelial integrity | 27 (64) | 42 (84) | 15 (36) | 8 (16) | 3.14 | 1.11–8.95 |
| Moisture | 29 (69) | 40 (80) | 13 (31) | 10 (20) | 2.11 | 0.74–6.02 |

[a] Values are *n* (%) of participants.
[b] Logistic regression with baseline value as covariate was used for the analysis of group differences.
[c] SB, *n* = 42; PL, *n* = 50.

SB and placebo groups, respectively, OR = 2.9 (95% CI 0.99–8.35)]. The median ($Q_1$, $Q_3$) changes in the scores for vaginal epithelial integrity from baseline to end were 0.00 (0.00, 1.00) and 0.00 (0.00, 0.00) in the SB and placebo groups, respectively ($P = 0.02$), indicating improvement in SB group. The mean (SD) changes in integrity scores were 0.52 (1.02) and 0.04 (0.78) in the SB and placebo groups, respectively. The effect on integrity change was significant among the PP participants as well ($P = 0.03$). SB oil did not have a significant effect on vaginal elasticity, fluid volume, or moisture, though there was a non-significant tendency for better improvement rate compared to placebo (Table 3). Vaginal pH and moisture measured with Schirmer paper were unaffected (Table 2). SB oil did not affect the proportions of cell types determined for vaginal MI, indicating a lack of estrogen effect (Supplementary Table S3). Results concerning the PP participants are presented in Supplementary Tables S1, S2 and S4.

### 3.2. Effects on experience of symptoms

Based on the evaluations by the participants at study visits, SB oil did not affect the experience of vaginal dryness, burning or itching (Table 4, Results for PP participants: Supplementary Table S5). In the daily logbooks, participants in the SB group reported more intense joint pain during the last month of the study. The median ($Q_1$, $Q_3$) severities were 0.85 (0.25, 1.21) and 0.16 (0.00, 0.89) in the SB and placebo groups, respectively ($P = 0.01$). There was a non-significant tendency toward more joint pain in the SB group already at baseline: the median ($Q_1$, $Q_3$) severity score during the first week was 0.86 (0.14, 1.71) in the SB, and 0.57 (0.00, 1.29) in the placebo group ($P = 0.13$). The other logbook symptoms did not differ between the groups among the ITT participants (data not shown). Among PP participants, conclusions from the logbooks were the same, except for the experience of night sweating. In PP participants, significantly less night sweating in SB group compared to placebo was experienced during month three. The median ($Q_1$, $Q_3$) severities were 0.00 (0.00, 0.39) and 0.20 (0.00, 1.00) in the SB and placebo groups, respectively ($P = 0.03$). During the first week, the reported night sweating did not clearly differ between the groups [SB median ($Q_1$, $Q_3$) of 0.36 (0.00, 1.00); placebo 0.29 (0.00, 1.00), $P = 0.72$].

At the end of the study 59% ($n = 26$) of participants in SB and 49% ($n = 25$) in the placebo group reported that they felt beneficial effects due to intake of the study product ($P = 0.37$). Experience of adverse effects by the study capsules was reported by 16% ($n = 7$) of participants in the SB and 14% ($n = 7$) in the placebo group ($P = 0.74$). The most common side effects were gastrointestinal symptoms, experienced by 11 ($n = 5$) and 4% ($n = 2$) of participants, in the SB and placebo groups, respectively ($P = 0.24$). For details, see Supplementary material.

### 3.3. Effects on serum markers

The changes in circulating cholesterol, triaclyglycerols, CRP or liver enzymes from the beginning to the end of the intervention did not differ between SB and placebo groups (Table 5; PP participants: Supplementary Table S6).

## 4. Discussion

Our study suggests that intake of SB oil improves integrity of the vaginal epithelium among postmenopausal women. A trend toward improved VagHI was observed, but this was not statistically significant. Previously, plant estrogens [5], vitamin D [15], and botanical preparations based on black cohosh and St. John's wort [16] have been associated with effects on vaginal atrophy and/or the vasomotor and psychological symptoms of menopause.

The daily dose of phytosterols, associated with estrogenic effects [17] from SB oil was ≈30 mg. Depending on the estimate [17,18], this is between 8–38% of the average daily intake in Western diets. SB did not affect vaginal pH or maturation of epithelial cells, indicating a lack of effect on the activity of vaginal estrogen receptors.

The cleavage of β-carotene to retinol in humans is feedback-regulated, and only the required amount is produced. β-Carotene in the daily dosage of SB oil corresponds to approximately 1.4 mg vitamin A [19], essential for normal mucosal epithelia [19,20]. Beneficial effect of vitamin A and β-carotene supplementation on bacterial vaginosis among pregnant women, possibly due to enhanced vaginal epithelial barrier, has been observed in a randomized controlled study [20].

The essential fatty acids in SB oil may have contributed as structural components. LA, in particular, is important for the water permeability barrier of skin as a component of ceramides [21]. Atrophic vaginal epithelium is associated with inflammation [2]. Of the compounds in SB oil, anti-inflammatory activity has been attributed to carotenoids [22], tocopherols, tocotrienols [23] and β-sitosterol [24]. The low LA to ALA ratio favors production of eicosanoids of lower pro-inflammatory activity [25]. Sjögren's syndrome is a chronic autoimmune disease associated with inflammation and dry mucous membranes. Previously, intake of 3 g of combined SB seed and pulp oil daily for three months was found to relief symptoms of itching, burning, pain, secretion and dryness of genital mucosa among women with Sjögren's syndrome [11].

While more than half of participants in the SB group experienced improvement in vaginal symptoms from baseline to the end, the difference between groups was not significant, indicating a placebo effect. Results from PP participants suggest effects on night sweating, but this was not significant among the primary ITT population. Previously, long-chain n-3 fatty acids have shown alleviation of symptoms of depression and/or hot flashes among peri- and postmenopausal women, possibly *via* modulation of neurotransmitters involved in thermoregulation [26,27]. Among breast cancer survivors, vitamin E slightly decreased frequency of hot flashes [28].

Joint pain reported in the logbooks during the last month was more severe in the SB group compared to placebo. A biological rationale for this is difficult to explain. There was a statistically non-significant tendency for more symptoms in the SB group at baseline already. Previously, opposite, anti-inflammatory effects by SB oil

Please cite this article in press as: Larmo PS, et al. Effects of sea buckthorn oil intake on vaginal atrophy in postmenopausal women: A randomized, double-blind, placebo-controlled study. Maturitas (2014), http://dx.doi.org/10.1016/j.maturitas.2014.07.010

**Table 4**
Changes in the experience of vaginal dryness, burning and itching among the ITT participants during three months invention.[a,b]

| | No change or deterioration, *n* (%) | | Improvement, *n* (%) | | Odds ratio | 95% CI |
|---|---|---|---|---|---|---|
| | SB[c] | PL | SB | PL | | |
| Dryness | 21 (48) | 12 (25) | 23 (52) | 37 (76) | 0.44 | 0.16–1.19 |
| Burning | 14 (35) | 17 (37) | 26 (65) | 29 (63) | 0.83 | 0.25–2.76 |
| Itching | 18 (45) | 19 (40) | 22 (55) | 28 (60) | 0.78 | 0.25–2.47 |

[a] Values are *n* (%) of participants.
[b] Logistic regression with baseline value as covariate was used for the analysis of group differences.
[c] SB, *n* = 40–44; PL, *n* = 46–49.

**Table 5**
Serum triacylglycerols, cholesterol, CRP, ALT and AST among ITT participants at baseline and the change from baseline to end of intervention at three months.[a,b]

| | SB[c] | | PL | | *P*-value |
|---|---|---|---|---|---|
| | 0 mo | 3 mo–0 mo | 0 mo | 3 mo–0 mo | |
| Total chol, mmol/L | 5.7 (1.0) | −0.3 (0.6) | 5.8 (1.0) | −0.2 (0.6) | 0.42 |
| LDL chol, mmol/L | 3.3 (0.9) | −0.2 (0.6) | 3.3 (1.0) | −0.1 (0.5) | 0.83 |
| HDL chol, mmol/L | 1.9 (0.5) | −0.1 (0.2) | 1.9 (0.5) | −0.1 (0.2) | 0.72 |
| Triacylglycerols, mmol/L | 1.1 (0.5) | 0.0 (0.4) | 1.2 (0.6) | 0.1 (0.4) | 0.36 |
| HDL chol/total chol, % | 33.0 (9.0) | 0.2 (4.1) | 35.0 (10.0) | −0.5 (4.9) | 0.89 |
| uCRP, mg/L | 2.8 (5.7) | 0.2 (2.6) | 1.7 (2.4) | −0.3 (2.6) | 0.24 |
| ALT, U/L | 23.3 (10.8) | 1.3 (17.2) | 24.4 (11.0) | 0.5 (10.6) | 0.51 |
| AST, U/L | 24.5 (5.8) | 0.8 (9.6) | 24.2 (6.9) | 0.7 (6.6) | 0.52 |

[a] Values are means (SD).
[b] ANOVA was used for the analysis of group differences of total cholesterol and LDL cholesterol. Wilcoxon–Mann–Whitney *U*-test was used for the analysis of the other parameters.
[c] SB, *n* = 44–54; PL, *n* = 51–55.

and its components have been observed [11,29]. The most reported adverse effects in both groups were gastro-intestinal symptoms, which are commonly associated with oil supplements [27]. SB oil did not affect the circulating triacylglycerols or cholesterol. In our earlier study, SB oil lowered serum total, IDL, and LDL cholesterol and subfractions of IDL and LDL among women with a metabolic profile with a higher cardio-metabolic risk, whereas the effects among women with a lower risk profile at baseline were modest [7].

This study has limitations. The sample size for the vaginal MI was small, which hinders drawing definite conclusions. Based on the relatively low concentration of phytosterols in the oil, our hypothesis was that estrogenic effects would be unlikely. We chose to investigate the MI only in a sample of participants to observe trends and to make tentative conclusions. The lack of change in vaginal pH supports the reasoning of no estrogen effect by SB oil. The study period was limited in duration. With food supplements, compared to drugs, the effects typically take longer time to develop. It is possible that with prolonged supplementation more pronounced effects would have been observed. The relevance of intervention length was recently emphasized in a review of studies concerning the effects of isoflavones on vaginal atrophy [5]. Participants of the current study were postmenopausal women and caution should be exercised in extrapolating the results to different populations.

In conclusion, our study indicates beneficial effects by intake of SB oil on vaginal atrophy among postmenopausal women. The improving effect on the integrity of the vaginal epithelium was most likely due to combined effect of the bioactive compounds in sea buckthorn oil, *via* mechanisms other than the activation of estrogen receptors. Due to the increasing number of women in postmenopausal state, and the risks associated with the standard treatment of vaginal atrophy with estrogen, there is a need for alternative means for maintaining healthy vaginal mucosa.

## Contributors

PSL, BY, HPK and RE designed the study. PSL and RE co-ordinated the study, and collected and interpreted the data. JH carried out the statistical analyses. PSL, BY, JH, HPK and RE wrote the manuscript.

## Competing interest

PSL is an employee of Aromtech Ltd. Other authors declare no competing interest.

## Funding

The study was funded by Tekes – the Finnish Funding Agency for Innovation (grant number 301/09) and Aromtech Ltd.

## Ethical approval

The study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland (Turku, Finland).

## Acknowledgements

We thank the participants of the study. We thank the laboratory personnel of Aromtech Ltd and Raija Nurmi for technical assistance and Riku Kivimäki for the randomization of participants.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.maturitas.2014.07.010.

## References

[1] Castelo-Branco C, Cancelo MJ, Villero J, Nohales F, Julia MD. Management of post-menopausal vaginal atrophy and atrophic vaginitis. Maturitas 2005;52S:S46–52.
[2] Palacios S. Managing urogenital atrophy. Maturitas 2009;4:315–8.
[3] Nappi RE, Kokot-Kierepa M. Women's voices in the menopause: results from an international survey on vaginal atrophy. Maturitas 2010;3:233–8.
[4] Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Lancet 1997;9084:1047–59.

Please cite this article in press as: Larmo PS, et al. Effects of sea buckthorn oil intake on vaginal atrophy in postmenopausal women: A randomized, double-blind, placebo-controlled study. Maturitas (2014), http://dx.doi.org/10.1016/j.maturitas.2014.07.010

[5] Bedell S, Nachtigall M, Naftolin F. The pros and cons of plant estrogens for menopause. J Steroid Biochem Mol Biol 2014;139:225–36.
[6] Suryakumar G, Gupta A. Medicinal and therapeutic potential of sea buckthorn (*Hippophae rhamnoides* L.). J Ethnopharmacol 2011;2:268–78.
[7] Larmo PS, Kangas AJ, Soininen P, Lehtonen HM, Suomela JP, Yang B, et al. Effects of sea buckthorn and bilberry on serum metabolites differ according to baseline metabolic profiles in overweight women: a randomized crossover trial. Am J Clin Nutr 2013;4:941–51.
[8] Larmo P, Järvinen R, Setälä N, Yang B, Viitanen M, Engblom J, et al. Oral sea buckthorn oil attenuates tear film osmolarity and symptoms in individuals with dry eye. J Nutr 2010;140:1462–8.
[9] Lehtonen H, Suomela J, Tahvonen R, Yang B, Venojärvi M, Viikari J, et al. Different berries and berry fractions have various but slightly positive effects on the associated variables of metabolic diseases on overweight and obese women. Eur J Clin Nutr 2011;3:394–401.
[10] Johansson A, Laine T, Linna M, Kallio H. Variability in oil content and fatty acid composition in wild northern currants. Eur Food Res Technol 2000;211:277–83.
[11] Yang B, Erkkola R. Sea buckthorn oils, mucous membranes and Sjögren's syndrome with special reference to latest studies. In: Singh V, Kallio H, Mörsel T, Sawhney RC, Bala M, et al., editors. Seabuckthorn (*Hippophaë* L.). A multipurpose wonder plant. Advances in research and development, vol. III. New Delhi, India: Dya Publishing House; 2008. p. 254–67.
[12] Bachmann GA, Notelovitz M, Gonzales S, Thomson C, Morecraft B. Vaginal dryness in menopausal women: clinical characteristics and nonhormonal treatment. Clin Pract Sex 1991;9:25–32.
[13] Carranza-Lira S, Fragoso-Diaz N, MacGregor-Gooch AL, Garduno-Hernandez MP, Rios-Calderon K, Aparicio H. Vaginal dryness assessment in postmenopausal women using pH test strip. Maturitas 2003;1:55–8.
[14] European Medicines Agency. IHC Topic E 9. Statistical principles for clinical trials. CPMP/ACH/363/96; September 1998.
[15] Yildirim B, Kaleli B, Duzcan E, Topuz O. The effects of postmenopausal vitamin D treatment on vaginal atrophy. Maturitas 2004;4:334–7.
[16] Laakmann E, Grajecki D, Doege K, Zu Eulenburg C, Buhling KJ. Efficacy of *Cimicifuga racemosa*, *Hypericum perforatum* and *Agnus castus* in the treatment of climacteric complaints: a systematic review. Gynecol Endocrinol 2012;9:703–9.
[17] Ju YH, Clausen LM, Allred KF, Almada AL, Helferich WG. Beta-sitosterol, beta-sitosterol glucoside, and a mixture of beta-sitosterol and beta-sitosterol glucoside modulate the growth of estrogen-responsive breast cancer cells in vitro and in ovariectomized athymic mice. J Nutr 2004;5:1145–51.
[18] Fritsche S, Steinhart H. Occurrence of hormonally active compounds in food: a review. Eur Food Res Technol 1999;209:153–79.
[19] Ross CA. Vitamin A and carotenoids. In: Shils ME, Shike M, Ross CA, Cabarello B, Cousins R, editors. Modern nutrition in health and disease. 10th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2006. p. 351–75.
[20] Christian P, Labrique AB, Ali H, Richman MJ, Wu L, Rashid M, et al. Maternal vitamin A and beta-carotene supplementation and risk of bacterial vaginosis: a randomized controlled trial in rural Bangladesh. Am J Clin Nutr 2011;6:1643–9.
[21] McCusker MM, Grant-Kels JM. Healing fats of the skin: the structural and immunologic roles of the omega-6 and omega-3 fatty acids. Clin Dermatol 2010;4:440–51.
[22] Watzl B, Kulling SE, Moseneder J, Barth SW, Bub A. A 4-wk intervention with high intake of carotenoid-rich vegetables and fruit reduces plasma C-reactive protein in healthy, nonsmoking men. Am J Clin Nutr 2005;5:1052–8.
[23] Reiter E, Jiang Q, Christen S. Anti-inflammatory properties of alpha- and gamma-tocopherol. Mol Asp Med 2007;5–6:668–91.
[24] Ku CM, Lin JY. Anti-inflammatory effects of 27 selected terpenoid compounds tested through modulating Th1/Th2 cytokine secretion profiles using murine primary splenocytes. Food Chem 2013;2:1104–13.
[25] Ziboh VA, Miller CC, Cho Y. Metabolism of polyunsaturated fatty acids by skin epidermal enzymes: generation of antiinflammatory and antiproliferative metabolites. Am J Clin Nutr 2000;1(Suppl.):361S–6S.
[26] Freeman MP, Hibbeln JR, Silver M, Hirschberg AM, Wang B, Yule AM, et al. Omega-3 fatty acids for major depressive disorder associated with the menopausal transition: a preliminary open trial. Menopause 2011;3:279–84.
[27] Lucas M, Asselin G, Merette C, Poulin MJ, Dodin S. Effects of ethyl-eicosapentaenoic acid omega-3 fatty acid supplementation on hot flashes and quality of life among middle-aged women: a double-blind, placebo-controlled, randomized clinical trial. Menopause 2009;2:357–66.
[28] Barton DL, Loprinzi CL, Quella SK, Sloan JA, Veeder MH, Egner JR, et al. Prospective evaluation of vitamin E for hot flashes in breast cancer survivors. J Clin Oncol 1998;2:495–500.
[29] Upadhyay NK, Kumar R, Mandotra SK, Meena RN, Siddiqui MS, Sawhney RC, et al. Safety and healing efficacy of sea buckthorn (*Hippophae rhamnoides* L.) seed oil on burn wounds in rats. Food Chem Toxicol 2009;6:1146–53.

Please cite this article in press as: Larmo PS, et al. Effects of sea buckthorn oil intake on vaginal atrophy in postmenopausal women: A randomized, double-blind, placebo-controlled study. Maturitas (2014), http://dx.doi.org/10.1016/j.maturitas.2014.07.010