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Oral Sea Buckthorn Oil Attenuates Tear Film Osmolarity and Symptoms in Individuals with Dry Eye[1–4]

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Abstract

Dry eye is a common condition that can severely impair the quality of life. We aimed to find out whether oral sea buckthorn (SB) oil, containing (n-3) and (n-6) fatty acids and antioxidants, affects dry eye. In this double-blind, randomized, parallel trial, 20- to 75-y-old women and men experiencing dry eye symptoms consumed 2 g of SB or placebo oil daily for 3 mo from fall to winter. One hundred participants were recruited and 86 completed the study. Clinical dry eye tests and symptom follow-ups were performed. Tear film hyperosmolarity is a focal factor in dry eye. There was a general increase in the osmolarity from baseline to the end of the intervention. Compared with the placebo group, the increase was significantly less in the SB group when all participants were included [intention to treat (ITT), $P = 0.04$] and when only participants consuming the study products for at least 80% of the intervention days were included [per protocol (PP), $P = 0.02$]. The maximum intensities of redness and burning tended to be lower in the SB group. In the ITT participants, the group difference was significant for redness ($P = 0.04$) but not for burning ($P = 0.05$). In the PP participants, the group difference was significant for burning ($P = 0.04$) but not for redness ($P = 0.11$). In conclusion, SB oil attenuated the increase in tear film osmolarity during the cold season and positively affected the dry eye symptoms. J. Nutr. 140: 1462–1468, 2010.

Introduction

Dry eye can cause severe discomfort and greatly compromise the quality of life (1). Depending on the population and definition, prevalence from <1% to over 30% in people aged 50 y and older has been reported (1,2). Dry eye can be caused by several interlinked factors, which makes defining, diagnosing, and treating the condition challenging. The dry eye definition by the 2007 Dry Eye Workshop emphasizes the aspects of inflammation and increased osmolarity of the tear film as features common to different forms of dry eye (3).

The 2 main types of dry eye, although sometimes difficult to clearly separate, are aqueous-deficient and evaporative dry eye. In the aqueous-deficient form, the lacrimal secretion is reduced, leading to tear hyperosmolarity and activation of inflammatory pathways. In evaporative dry eye, the lacrimal secretion is normal, but water evaporation from the ocular surface is increased. The most common cause of evaporative dry eye is meibomian gland dysfunction associated with a deficient tear film lipid layer that is not stable enough to prevent water loss (3). Hyperosmolarity resulting from either of the 2 mechanisms can damage the ocular surface epithelium by activating inflammation via the mitogen-activated protein kinase and nuclear factor-κB signaling pathways (3,4). Epithelial damage and cell death further interrupt normal functions of the eye, leading to a vicious cycle (3). Dry eye risk factors include, among others, older age, female gender, and contact lens wear (2).

Artificial tears are the common treatment for dry eye. Although they relieve symptoms, they likely do not affect the causative factors or the inflammation accompanying dry eye. Antiinflammatory drugs relieve the inflammation, but not all of them are suitable for long-term use and side effects may occur (5). Earlier studies suggest positive effects of (n-3) fatty acids, a combination of (n-6) linoleic [18:2(n-6)] and γ-linolenic [18:3 (n-6)] acids, and antioxidants on dry eye (6–11). Mechanisms suggested for these effects include inflammation attenuation, effects on tear secretion, and prevention of oxidative damage. Dietary supplementation with (n-6) fatty acids may increase the

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[3] This trial was registered at clinicaltrials.gov as NCT00739713.

[4] Supplemental Fig. 1, Supplemental information about participants' baseline characteristics, Supplemental Table 1, and Supplemental information about alternative and explorative statistical analyses are available with the online posting of this paper at jn.nutrition.org.

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level of dihomo γ-linolenic acid, which is a precursor for antiinflammatory eicosanoids (8).

Sea buckthorn (*Hippophaë rhamnoides*) (SB)[10] has been used in Asian traditional medicine for centuries, and SB berries are included in the Chinese Pharmacopeia (12,13). SB oil is rich in lipophilic antioxidants and SB seed oil especially contains high proportions of (n-3) and (n-6) fatty acids (14). Positive effects of SB oil on skin and mucous membrane have been reported (13,15,16), as well as antiatherogenic (17) and platelet aggregation-inhibiting effects (18). Our objective was to investigate whether consuming SB oil can affect dry eye. Effects on both objective clinical responses and subjective symptoms experienced by the participants were studied.

Participants and Methods

Study design and participants. The study was carried out at the University of Turku and the Turku University Hospital, Finland. Participants gave their written, informed consent to the study procedures, which were approved by the Ethics Committee of the Hospital District of Southwest Finland. Participants were 20–75 y of age and were recruited for the study by announcements on Internet pages, local newspapers, and bulletin boards around the city of Turku. A total of 100 volunteers (SB group, $n = 52$; placebo group, $n = 48$) were randomized to this double-blind, parallel, placebo-controlled trial. Eighty-six participants (SB group, $n = 45$; placebo group, $n = 41$) completed the study (**Supplemental Fig. 1**). The stratification factors in randomization were age, sex, and contact lens wear. The inclusion criterion was the participant's own experience of dry eye symptoms. The exclusion criteria were severe illness, pregnancy or breastfeeding, smoking, and regular use of strongly anticholinergic drugs.

The participants in both groups were similar (**Table 1**; Supplemental Information: Participants and Methods). Sixty-three percent of the women in the SB group and 64% in the placebo group were under 51 y of age, which is the median age of natural menopause among Finnish women (19). This suggests that effects of menopause and menopausal hormonal therapy on dry eye (2) were similar in both groups. The proportions of participants feeling that dry eye had a negative (severe or to some extent) effect on their quality of life were 84 and 83% in the SB and placebo groups, respectively.

Study products. During the 3-mo intervention period, participants consumed 2 g of SB or placebo oil daily (in the form of 2 capsules twice/d with a meal). Both capsules had identical opaque gelatin shells. During the trial, the participants, study personnel, and researchers did not know who was getting the SB capsules. To study the success of blinding, the participants were asked to guess whether they were receiving the SB or placebo capsules at each study visit. The participants were advised not to use other oil supplements during the trial.

The SB oil with a standardized composition and containing both seed and pulp oil was manufactured by Aromtech Ltd. (Tornio, Finland) using supercritical carbon dioxide extraction. The placebo capsules contained triacylglycerols of medium-chain fatty acids isolated from coconut and palm kernel. Absorbed medium-chain fatty acids are preferred for oxidation for energy, and their esterification and distribution from the liver to other tissues is limited (20). The compositions of the study oils are described in **Table 2**. Fatty acids were analyzed as methyl esters (21) using a gas chromatographic method (16). Tocopherols and carotenoids were analyzed using HPLC-UV/Visible methods.

Dry eye monitoring. Participants' dry eye symptoms were monitored at the beginning of the intervention (October 2008: SB, $n = 43$, placebo, $n = 38$; November 2008: SB, $n = 6$, placebo, $n = 9$), at 1 mo, and at 3 mo (January 2009: SB, $n = 34$, placebo, $n = 32$; February 2009: SB, $n = 11$, placebo, $n = 11$), when the intervention ended. To find out whether the presumed changes in the dry eye symptoms would last when the participants no longer consumed the oil capsules, a postintervention check at 1 to 2 mo after the end of the intervention period was scheduled (March 2009 for most participants).

Because of the multifactorial nature of dry eye, a combination of tests is recommended for diagnosis (2,22). At each visit, tear film osmolarity (mOsm/L) was measured using an electrochemical osmolarity meter (TearLab, OcuSense) utilizing lab-on-chip technology enabling fast measurement directly from the eye. Tear film stability was measured as tear film break-up time (TBUT; seconds until breakup of fluorescein tear film) and tear secretion was analyzed using the Schirmer test without anesthesia (length of wetting the Schirmer paper after 5 min). The participants were asked to answer a modified version of the validated dry eye symptom questionnaire the Ocular Surface Disease Index [mOSDI (23,24)]. The following modification to the OSDI questionnaire was made: question number 11 in the OSDI pad concerning the dry eye symptoms in places with low humidity was difficult for the participants to understand and was therefore excluded.

During the 3 mo that the participants consumed the study capsules, they kept logbooks concerning their dry eye symptoms. The participants were asked to record each day whether they had any eye symptoms (yes/no). In the logbook, typical dry eye symptoms (25) were listed: soreness, foreign body sensation, dryness, grittiness, burning, redness, watery eyes, or blurry vision. Participants were asked to record daily the severity of each symptom using a 4-point scale, with the severity of symptoms ranging from 0 = none to 3 = severe. In the logbook, the participants were also asked to report whether they had taken the daily dose of oil capsules, worn contact lenses, or used eye drops or other treatment for dry eye symptoms.

The main outcome measures of the study were changes in tear film osmolarity, TBUT, Schirmer, and mOSDI test scores. As secondary outcomes, the symptoms reported in the logbook were compared between groups.

Statistical analysis. Prestudy sample size estimation was based on the assumption that at the end of the observation period the mean mOSDI scores of the groups would differ by 4 points or more (assumed SD = 6

TABLE 1 Characteristics of the randomized participants at BL[1]

	SB	PL[1]
n	52	48
Women, *n (%)*	44 (85)	41 (85)
Age, *y*	45 ± 18	46 ± 17
Contact lens wearers, *n (%)*	26 (50)	23 (48)
Dry eye symptoms,[2] *n (%)*		
Soreness	23 (46)	14 (29)
Foreign body sensation	41 (82)	37 (77)
Dryness	38 (76)	39 (81)
Grittiness	29 (58)	28 (58)
Burning	14 (28)	16 (33)
Redness	39 (78)	34 (71)
Watery eyes	30 (60)	33 (69)
Use of medication associated with dry eye symptoms[3], *n (%)*		
Antihistamines[4]	12 (23)	11 (23)
Oral contraceptives/other hormonal medication	22 (42)	23 (48)
Medication for high blood pressure	7 (13)	5 (10)

[1] Values are means ± SD or *n* (%).
[2] Information missing from 2 participants in the SB group who dropped out of the study before the first clinic visit. Symptoms according the McMonnies dry eye questionnaire completed with additions (25, 48–49).
[3] Participants' medications were classified according to the McMonnies dry eye questionnaire (48). None of the participants reported regular use of diuretics, sleeping tablets, tranquilizers, or medication for duodenal ulcer, digestive problems, or depression.
[4] Use of antihistamines mostly during spring and summer, not during the intervention period.

[10] Abbreviations used: BL, baseline; ITT, intention to treat; mOSDI, modified Ocular Surface Disease Index; PL, placebo; PP, per protocol; OSDI, Ocular Surface Disease Index; TBUT, tear film break-up time; SB, sea buckthorn.

TABLE 2 Fatty acids, carotenoids, and tocopherols in the daily dose of SB and placebo oil[1]

	SB	PL
Fatty acid	*mg/ 2 g oil*	
8:0		884 ± 11
10:0		733 ± 4
12:0		1 ± 0
14:0	2 ± 0	2 ± 0
16:1(n-7)	346 ± 48	
16:0	338 ± 47	
18:2(n-6)	245 ± 34	
18:3(n-3)	149 ± 21	
18:1(n-9)	316 ± 45	
18:1(n-7)	108 ± 15	
18:0	31 ± 4	
20:0	6 ± 1	
Carotenoids	1.8 ± 0.0	
α-Tocopherol	6.0 ± 0.4	0.2 ± 0.0
γ-Tocopherol	0.8 ± 0.1	

[1] Values are means ± SD, n = 8 (SB) or 3 (PL).

points). With a sample size of 37 participants/group, the study would have a power of ~80% to detect the difference between treatments (2-sided tests, 0.05 significance level). To tolerate a drop-out rate of ~25%, a total of 100 participants were recruited.

For the clinical tests and mOSDI, changes from baseline (BL) values were used as dependent variables in the statistical analyses. Variables of change were analyzed with 2-way ANOVA with a repeated-measure term included in the model (SAS MIXED procedure). BL values of clinical tests and mOSDI, age, contact lens wear, and sex were considered as potential covariates in the model. Only significant covariates ($P < 0.05$) and those with significant interactions with other variables in the model ($P < 0.05$) were included in the final model [for osmolarity: BL osmolarity, BL Schirmer, age; for mOSDI: BL mOSDI; for TBUT left eye: BL TBUT left eye, BL osmolarity; for TBUT right eye: BL TBUT right eye, and contact lens wear (significant interaction terms); for Schirmer: BL Schirmer, age]. The group-change interaction was included to calculate the estimates of changes. The results were adjusted for multiplicity using Bonferroni correction. Alternative analyses without covariates were also done. In addition, explorative analyses were carried out separately for the contact lens wearers and those not wearing contact lenses using only the model without covariates. For additional alternative methods and explorative analyses concerning the clinical tests and mOSDI, see Supplemental Information: Participants and Methods.

For each individual symptom in the logbook, the ratio of symptom days:days with no symptoms was calculated. The symptom:day ratio was also calculated for overall eye symptoms (the logbook question was "do you have eye symptoms: yes/no"). The symptom sum was calculated by summing the daily intervention period intensity scores. The proportions of participants having symptom score 0, 1, 2, or 3 as their maximum symptom in each group was calculated. The differences between groups of maximum symptoms were tested using the Cochran-Mantel-Hanzel test. The differences between groups of ratio and sum of symptom days were estimated from an ANOVA model and from an analysis of covariance model using SAS MIXED procedure. Age and use of contact lenses were introduced as covariates in the analysis of covariance model. In addition, the symptom:day ratio and symptom sum analyses were carried out separately for subgroups of participants above and below 45 y of age, and for contact lens wearers and those not wearing contact lenses. The logbook analyses were carried out by Statfinn Ltd (Turku, Finland). The logbook background information data were not compared statistically between groups.

The primary data analyses were done including all randomized participants [intention to treat (ITT) participants]. Unless otherwise noted, the presented results concern the ITT participants. In addition, analyses including only participants who consumed the study capsules for at least 80% of the observation period days [per protocol (PP) participants] were conducted. Two-sided tests, significance levels of 0.05 and SAS software version 9.2 (SAS Institute) were used throughout. Values in the text are means ± SD.

Results

Participant compliance and blinding. In the SB group, 87%, and in the placebo group, 85% of the participants completed the whole study including the postcheck after the intervention period (Supplemental Fig. 1). The number of PP participants was 43 in the SB group and 38 in the placebo group. At the beginning of the intervention period when the participants had not yet consumed any capsules, 69% of the participants in the SB group guessed that they were receiving SB oil capsules. The proportion of SB guesses was 78% in the placebo group. At the 1-mo visit, 45% in the SB and 44% in the placebo group guessed they were receiving SB oil. After consuming the capsules for 3 mo and at a postcheck visit, the proportion of those guessing they were receiving SB oil was greater in the SB group (3 mo: 51 vs. 33%; postcheck: 58 vs. 30%).

Clinical tests and mOSDI. During the intervention, the tear film osmolarity increased in both the SB and placebo groups. When the changes were adjusted for significant covariates, the increase was significantly less in the SB group (**Table 3**, ITT participants). The conclusion was the same for the PP participants (P = 0.02 for the change from BL to 3 mo; other comparisons to BL did not differ between the groups; Supplemental Table 1). In the case of no covariate adjustments in the statistical model, the differences between groups were not significant (data not shown). Changes in TBUT, Schirmer, and mOSDI results did not differ between groups in the ITT (Table 3) or PP participants (Supplemental Table 1). The subgroup analyses based on contact lens wear produced nonsignificant results (data not shown).

The number of participants having BL tear film osmolarity above or TBUT/Schirmer flow below the suggested diagnostic cutoff values (22,26–28) was small in both groups despite the fact that all participants experienced dry eye symptoms (BL osmolarity > 316 mOsm/L: SB, n = 11, placebo, n = 19; TBUT mean < 10 s: SB, n = 9, placebo, n = 21; Schirmer flow < 5 mm/5 min: SB, n = 6, placebo, n = 8). For the results of additional alternative method and explorative analyses concerning the clinical tests and mOSDI, see Supplemental Information: Results.

Logbook symptoms. The logbook records of dry eye symptoms showed significantly lower maximum scores for redness in the SB group in the ITT participants. The proportion of participants reporting 3 as their maximum redness score was 6% in the SB group and 36% in the PL group (P = 0.04; **Table 4**). The difference between groups was not significant in the PP participants (P = 0.11), although the trend was the same. The number of PP participants recording 3 as the maximum of redness was 2 (5%) in the SB group and 14 (37%) in the placebo group (Supplemental Table 2). In ITT participants (Table 4), the difference between groups in burning sensation was not significant. In PP participants, the maximum burning scores were significantly lower in the SB group. The number of participants recording 3 as the maximum of burning was 5 (12%) in the SB group and 12 (32%) in the placebo group (P = 0.04) (**Supplemental Table 2**). The age- and contact lens wear-adjusted analyses confirmed the conclusion that the maximum redness

TABLE 3 Clinical dry eye tests and mOSDI scores in the ITT participants at BL, during, and at the end of the 3-mo intervention and at postcheck[1]

						Change[2]		
	Group	BL	1 mo	3 mo	Postcheck	1 mo − BL	3 mo − BL	Postcheck − BL
						P-value		
Osmolarity[3] (right eye), *mOsm/L*	SB	308 ± 10	308 ± 11	316 ± 15	311 ± 13	0.28	0.04	1.00
	PL	312 ± 16	314 ± 9	324 ± 17	314 ± 13			
TBUT[4] (left eye), *s*	SB	13 ± 5	13 ± 6	13 ± 7	13 ± 5	0.17	1.00	1.00
	PL	11 ± 5	11 ± 5	12 ± 6	12 ± 5			
TBUT[4] (right eye), *s*	SB	14 ± 5	15 ± 6	13 ± 6	14 ± 5	0.70	1.00	1.00
	PL	12 ± 6	13 ± 5	13 ± 6	13 ± 4			
Schirmer[5] (right eye), *mm/5 min*	SB	13 ± 10	11 ± 8	13 ± 10	11 ± 8	1.00	1.00	1.00
	PL	11 ± 9	10 ± 10	11 ± 8	10 ± 8			
mOSDI,[6] *questionnaire score*	SB	21 ± 12	18 ± 12	16 ± 13	18 ± 13	1.00	0.91	0.58
	PL	24 ± 11	17 ± 9	20 ± 14	17 ± 11			

[1] Values are means ± SD. Postcheck 1 to 2 mo after the intervention end.
[2] Two-way ANOVA with repeated-measures, change-group-interaction term, and significant covariates included (see ''Statistics'' for details). Significant covariates included in the statistical model: osmolarity: BL osmolarity, BL Schirmer, age; mOSDI: BL mOSDI; TBUT left eye: BL TBUT left eye, BL osmolarity; TBUT right eye: BL TBUT right eye and contact lens wear (significant interaction terms); Schirmer: BL Schirmer, age. Bonferroni adjusted for multiple comparisons.
[3] *n* = 44–49 in the SB group, 39–47 in the PL group.
[4] *n* = 44–47 in the SB group, 39–46 in the PL group.
[5] *n* = 42–49 in the SB group, 37–45 in the PL group.
[6] *n* = 45–49 in the SB group, 39–47 in the PL group.

and burning scores were lower in the SB group in the ITT and PP participants, respectively (Supplemental Tables 3 and 4).

Other individual symptoms in the ITT or PP participants did not differ significantly between the groups (Table 4: ITT participants; Supplemental Table 2: PP participants, **Supplementary Tables 3** and **4**: adjusted analyses). However, there was a significant difference in the proportion of days recorded as eye symptom days (question: "Do you have eye symptoms": Yes/no," without further specification of the symptom or evaluation of intensity; see Participants and Methods) in contact lens wearers in the ITT participants. Percentage of symptom days was 65 ± 29% and 81 ± 26% in contact lens wearers in the SB and placebo groups, respectively ($P = 0.049$). In PP participants, the percent of days with symptoms in the SB group (65 ± 30%) did not differ from the placebo group (77 ± 27%; $P = 0.19$).

Logbook background information. The participants kept the logbook record for a few days longer in the SB group (86 ± 18 d)

TABLE 4 Dry eye symptoms reported in symptom logbooks in the SB and PL groups during the 3-mo intervention in the ITT participants[1]

		Symptom:day ratio[2]		Symptom sum[3]		Max symptom score, *n (%)*				
Symptom	Group	%	*P*-value	Score	*P*-value	0	1	2	3	*P*-value
Soreness	SB	35 ± 33	0.92	27 ± 36	0.83	10 (20)	17 (35)	18 (37)	4 (8)	0.34
	PL[4]	34 ± 33		28 ± 37		11 (24)	7 (16)	18 (40)	8 (18)	
Foreign body sensation	SB	44 ± 36	0.81	35 ± 43	0.52	4 (8)	12 (25)	24 (49)	9 (18)	0.60
	PL	46 ± 34		41 ± 44		3 (7)	10 (22)	22 (49)	10 (22)	
Dryness	SB	69 ± 33	0.79	58 ± 48	0.40	4 (8)	11 (22)	23 (47)	11 (22)	0.16
	PL	71 ± 34		67 ± 51		4 (9)	2 (4)	25 (56)	14 (31)	
Grittiness	SB	37 ± 36	0.23	33 ± 53	0.47	8 (16)	16 (33)	17 (35)	8 (16)	0.21
	PL	45 ± 34		40 ± 46		4 (9)	13 (29)	18 (40)	10 (22)	
Burning	SB[5]	19 ± 25	0.08	17 ± 35	0.20	15 (31)	12 (25)	16 (33)	5 (10)	0.05
	PL	29 ± 32		27 ± 41		10 (22)	8 (18)	14 (31)	13 (29)	
Redness	SB[5]	40 ± 37	0.95	33 ± 41	0.63	10 (20)	8 (16)	27 (55)	3 (6)	0.04
	PL	41 ± 35		37 ± 48		6 (13)	8 (18)	15 (33)	16 (36)	
Watery eyes	SB	44 ± 36	0.64	33 ± 43	0.94	5 (10)	16 (33)	20 (41)	8 (16)	0.86
	PL	40 ± 38		34 ± 46		7 (16)	13 (29)	16 (36)	9 (20)	
Blurry vision	SB	40 ± 36	0.42	34 ± 48	0.74	8 (16)	20 (41)	16 (33)	5 (10)	0.46
	PL	34 ± 37		31 ± 51		15 (33)	9 (20)	17 (38)	4 (9)	

[1] Values are means ± SD, *n* = 49 (SB) or 45 (PL) unless otherwise noted.
[2] Proportion of intervention days on which participants reported having the particular symptom.
[3] Sum of symptom intensity scores during the intervention.
[4] *n* = 44.
[5] *n* = 48.

compared with the placebo group (82 ± 20 d). During the intervention period, the contact lens wearers in the SB group wore lenses more often and for longer times compared with the placebo group (mean percentage of intervention days: 45 ± 33 vs. 27 ± 30; maximum h/d: 14 ± 4 h vs. 11 ± 6 h). Use of eye drops or other treatment for dry eye symptoms was slightly more frequent in the placebo group (mean percentage of days: 17 ± 30% vs. 19 ± 32%).

Discussion

Previous dry eye oil intervention studies have focused on the combination of (n-6) linoleic acid and γ-linolenic acid (7–10,29) or γ-linolenic acid alone (30). Most (7–10,29), but not all (30), of the (n-6) fatty acid studies have shown positive effects. The mechanism of the positive effect is likely to be related to modulation of inflammation (8,10). In humans, linoleic acid can be converted to γ-linolenic acid and further to dihomo-γ-linolenic acid [20:3(n-6)], a precursor for antiinflammatory and tear production-stimulating eicosanoid prostaglandin E_1 (8,22, 29,31).

Meibomian gland secretion, meibum, is an important source of lipids in the outermost lipid layer of the tear film, preventing water loss. Differences in the meibum lipids between healthy and dry eye patients have been detected (22,29,32). Linoleic acid and γ-linolenic acid may change the properties of meibum in patients with meibomian gland dysfunction, indicating effects on meibum composition (29). The possible pathways of lipid composition modification may be manifold, however, because the lid bacteria also affect the meibomian secretion composition and tear film stability (3).

In a rat feeding study, a combination of long-chain (n-3) fatty acids [eicosapentaenoic acid, 20:5(n-3) and docosahexaenoic acid, 22:6(n-3)] and γ-linolenic acid was found to be superior for the alleviation of dry eye compared with (n-3) or (n-6) fatty acids alone (33). High dietary intake of (n-3) fatty acids is associated with a decreased risk of dry eye in women, whereas a high (n-6): (n-3) ratio in fatty acid consumption is associated with increased risk (6). In humans, (n-3) and (n-6) fatty acids can be converted to fatty acids of the same n-family in metabolic pathways sharing common enzymes. Long-chain fatty acids from both families are substrates for eicosanoids and other messenger molecules, competing for the same enzymes in these conversions too. Thus, the amount of (n-6) fatty acids affects the metabolism of (n-3) fatty acids and vice versa. Messengers from both fatty acid families have anti- and proinflammatory properties, but in general the (n-3) effects are more antiinflammatory (34). The (n-3) fatty acids may also affect the expression of inflammatory genes (35).

The only (n-6) fatty acid in the SB study oil was linoleic acid. In previous studies with positive results, linoleic acid doses many times higher (7), lower (9,10,29), and of the same magnitude (8) have been used, but always in combination with γ-linolenic acid. The α-linolenic acid content in the daily dose of SB oil corresponds to 6–9% of the mean daily intake of Finns (36). About one-half of the fatty acids in the SB oil were monounsaturated fatty acids with 16 or 18 carbons. A small percentage of ≤C18 monounsaturated fatty acids in meibum wax and sterol esters and FFA is associated with a paste-like meibum texture, whereas a high proportion of these fatty acids is found in meibomian seborrhoea patients with more fluid-like meibum (37). Accordingly, it is possible that not only the PUFA in SB oil have effects on factors related to dry eye.

The daily SB oil contained α-tocopherol at 58–82% of the Finnish mean intake (36). In contrast to the placebo, SB also contained γ-tocopherol. Both forms of vitamin E have antioxidant activity, but in macrophages and epithelial cells, the antiinflammatory potential of γ-tocopherol is stronger (38). The carotenoid content and type in SB vary. According to Raffo et al. (39), the main carotenoids in SB are β-carotene and zeaxanthin, which both have antioxidant, and most likely also antiinflammatory, activity (40). Based on published information about the proportion of each carotenoid type (39,41), we estimated that the daily β-carotene in the study SB oil equates to ~10% or less of the daily vitamin A intake of Finns (36). Supplements containing carotenoids and/or vitamin E in combination with other antioxidants have shown positive effects on dry eye and ocular surface (11,42). Antioxidants may protect the eye from oxidative damage leading to activation of inflammatory cascades (42). A tear film stabilizing effect also has been reported (11,42).

During the intervention, there was a trend toward higher values of tear film osmolarity in both groups. The increase was significantly less in the SB group in both ITT and PP participants. From the start to the end of the intervention period, the climate temperature in Turku dropped considerably from +8.1°C in October 2008 to −4.5°C in February 2009 (Finnish Meteorological Institute, Helsinki, Finland). During the cold months, the air humidity is low indoors and outdoors. Low relative humidity increases tear evaporation rates (43), and dry eye symptoms are more common during periods when indoor heating systems are used (44). The core mechanisms of dry eye are tear hyperosmolarity, which is common to different dry eye types, and tear film instability. Both mechanisms can initiate dry eye, lead to activation of inflammation cascades, and fortify each other (3). It is possible that most of the above-mentioned SB components have played a role in the detected positive SB oil effect through different mechanisms.

Judging by the BL values of clinical dry eye tests and mOSDI scores, the participants' dry eye was not very severe. Still, a vast majority of participants felt that dry eye negatively affected their quality of life. Because the inclusion criterion was widely the experience of dry eye symptoms, different dry eye types were represented in our study. The dry eye definition includes the aspect of symptoms of discomfort (3), and it is known that the association between symptoms and clinical signs is poor (45). Earlier fatty acid intervention trials have shown symptom improvement also when the effects on clinical signs have been less inconsistent (7,8,10). In our study, the logbook results indicated milder maximum symptoms of burning (significant in PP participants) and redness (significant in ITT participants) in the SB group. Although for these symptoms the results were not consistently significant in both PP and ITT participants, the trend was always the same. Liu et al. (46) suggest that the burning sensation especially is related to tear film instability and to transient hyperosmolarity spikes resulting from it.

The proportion of participants guessing they were in the SB group was ~45% in both groups after consuming the capsules for 1 mo. According to Desbiens (47), a balanced guessing pattern (even if it is not equal to 50-50) after a few doses indicates that blinding was achieved and there were no treatment or side effects. Change toward an unbalanced guessing pattern indicates a beneficial or side effect. In our trial, the shift to unbalanced guesses took place at 3 mo, when the difference in osmolarity change was also significant between groups, indicating positive effects of SB oil treatment.

The results of this study suggest that SB oil consumption can attenuate the increase in tear film osmolarity occurring during the cold season. It may also influence the maximum intensity of redness and burning symptoms in participants with dry eye. Contact lens wearers reported fewer overall eye symptom days in the SB group. Participants in this trial represented different dry eye types derived from various causative factors. Further studies should investigate the effects of SB oil on more defined populations and aim to determine the mechanisms of a positive SB oil effect.

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Literature Cited

1. Pflugfelder SC. Prevalence, burden, and pharmacoeconomics of dry eye disease. Am J Manag Care. 2008;14:S102–6.
2. The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop (2007). Ocul Surf. 2007;5:93–107.
3. The definition and classification of dry eye disease: report of the definition and classification subcommittee of the international dry eye WorkShop (2007). Ocul Surf. 2007;5:75–92.
4. Luo L, Li DQ, Corrales RM, Pflugfelder SC. Hyperosmolar saline is a proinflammatory stress on the mouse ocular surface. Eye Contact Lens. 2005;31:186–93.
5. Lemp MA. Management of dry eye disease. Am J Manag Care. 2008;14:S88–101.
6. Miljanovic B, Trivedi KA, Dana MR, Gilbard JP, Buring JE, Schaumberg DA. Relation between dietary n-3 and n-6 fatty acids and clinically diagnosed dry eye syndrome in women. Am J Clin Nutr. 2005;82: 887–93.
7. Kokke KH, Morris JA, Lawrenson JG. Oral omega-6 essential fatty acid treatment in contact lens associated dry eye. Cont Lens Anterior Eye. 2008;31:141–6.
8. Aragona P, Bucolo C, Spinella R, Giuffrida S, Ferreri G. Systemic omega-6 essential fatty acid treatment and PGE1 tear content in Sjögren's syndrome patients. Invest Ophthalmol Vis Sci. 2005;46:4474–9.
9. Macri A, Giuffrida S, Amico V, Iester M, Traverso CE. Effect of linoleic acid and γ-linolenic acid on tear production, tear clearance and on the ocular surface after photorefractive keratectomy. Graefes Arch Clin Exp Ophthalmol. 2003;241:561–6.
10. Barabino S, Rolando M, Camicione P, Ravera G, Zanardi S, Giuffrida S, Calabria G. Systemic linoleic and γ-linolenic acid therapy in dry eye syndrome with an inflammatory component. Cornea. 2003;22:97–101.
11. Blades KJ, Patel S, Aidoo KE. Oral antioxidant therapy for marginal dry eye. Eur J Clin Nutr. 2001;55:589–97.
12. Pharmacopeia of the People's Republic of China. English ed, 1st vol. Beijing: Chemical Industry Press; 2000.
13. Upadhyay NK, Kumar R, Mandotra SK, Meena RN, Siddiqui MS, Sawhney RC, Gupta A. Safety and healing efficacy of sea buckthorn (Hippophaë rhamnoides L.) seed oil on burn wounds in rats. Food Chem Toxicol. 2009;47:1146–53.
14. St George SD, Cenkowski S. Influence of harvest time on the quality of oil-based compounds in sea buckthorn (Hippophae rhamnoides L. ssp. sinensis) seed and fruit. J Agric Food Chem. 2007;55:8054–61.
15. Yang B, Erkkola R. Sea buckthorn oils, mucous membranes and Sjögren's syndrome with special reference to latest studies. In: Singh V, editor. Seabuckthorn (Hippophae L.): a multipurpose wonder plant. 3rd vol. Delhi: Daya Publishing House; 2008. p. 254–67.
16. Yang B, Kalimo KO, Mattila LM, Kallio SE, Katajisto JK, Peltota OJ, Kallio HP. Effects of dietary supplementation with sea buckthorn (Hippophae rhamnoides) seed and pulp oils on atopic dermatitis. J Nutr Biochem. 1999;10:622–30.
17. Basu M, Prasad R, Jayamurthy P, Pal K, Arumughan C, Sawhney RC. Anti-atherogenic effects of seabuckthorn (Hippophaea rhamnoides) seed oil. Phytomedicine. 2007;14:770–7.
18. Johansson AK, Korte H, Yang BR, Stanley JC, Kallio HP. Sea buckthorn berry oil inhibits platelet aggregation. J Nutr Biochem. 2000;11:491–5.
19. Luoto R, Kaprio J, Uutela A. Age at natural menopause and sociodemographic status in Finland. Am J Epidemiol. 1994;139:64–76.
20. Marten B, Pfeuffer M, Schrezenmeir J. Medium-chain triglycerides. Int Dairy J. 2006;16:1374–82.
21. Ågren JJ, Julkunen A, Penttilä I. Rapid separation of serum lipids for fatty acid analysis by a single aminopropyl column. J Lipid Res. 1992;33:1871–6.
22. Methodologies to diagnose and monitor dry eye disease: report of the Diagnostic Methodology Subcommittee of the International Dry Eye WorkShop (2007). Ocul Surf. 2007;5:108–23.
23. Schiffman RM, Christianson MD, Jacobsen G, Hirsch JD, Reis BL. Reliability and validity of the ocular surface disease index. Arch Ophthalmol. 2000;118:615–21.
24. Ozcura F, Aydin S, Helvaci MR. Ocular surface disease index for the diagnosis of dry eye syndrome. Ocul Immunol Inflamm. 2007;15:389–93.
25. Nichols KK. Patient-reported symptoms in dry dye disease. Ocul Surf. 2006;4:137–45.
26. Tomlinson A, Khanal S, Ramaesh K, Diaper C, McFadyen A. Tear film osmolarity: determination of a referent for dry eye diagnosis. Invest Ophthalmol Vis Sci. 2006;47:4309–15.
27. Lucca JA, Nunez JN, Farris RL. A comparison of diagnostic tests for keratoconjunctivitis sicca: Lactoplate, Schirmer, and tear osmolarity. CLAO J. 1990;16:109–12.
28. Lemp MA, Hamill JR Jr. Factors affecting tear film breakup in normal eyes. Arch Ophthalmol. 1973;89:103–5.
29. Pinna A, Piccinini P, Carta F. Effect of oral linoleic and γ-linolenic acid on meibomian gland dysfunction. Cornea. 2007;26:260–4.
30. Theander E, Horrobin DF, Jacobsson LT, Manthorpe R. Gammalinolenic acid treatment of fatigue associated with primary Sjögren's syndrome. Scand J Rheumatol. 2002;31:72–9.
31. Pholpramool C. Secretory effect of prostaglandins on the rabbit lacrimal gland in vivo. Prostaglandins Med. 1979;3:185–92.
32. Butovich IA. On the lipid composition of human meibum and tears: comparative analysis of nonpolar lipids. Invest Ophthalmol Vis Sci. 2008;49:3779–89.
33. Viau S, Maire MA, Pasquis B, Gregoire S, Acar N, Bron AM, Bretillon L, Creuzot-Garcher CP, Joffre C. Efficacy of a 2-month dietary supplementation with polyunsaturated fatty acids in dry eye induced by scopolamine in a rat model. Graefes Arch Clin Exp Ophthalmol. 2009;247:1039–50.
34. Schmitz G, Ecker J. The opposing effects of n-3 and n-6 fatty acids. Prog Lipid Res. 2008;47:147–55.
35. Calder PC. Polyunsaturated fatty acids and inflammatory processes: new twists in an old tale. Biochimie. 2009;91:791–5.
36. Paturi M, Tapanainen H, Reinivuo H, Pietinen P. Finravinto 2007. The National FINDIET 2007 Survey. Publications of the National Public Health Institute, Department of Health Promotion and Chronic Disease Prevention, Nutrition Unit. Helsinki, Finland [cited 2009 15 Sep]. Available from: http://www.ktl.fi/portal/suomi/osastot/eteo/yksikot/ravitsemusyksikko/finravinto_-tutkimus/.
37. Shine WE, McCulley JP. Association of meibum oleic acid with meibomian seborrhea. Cornea. 2000;19:72–4.
38. Jiang Q, Elson-Schwab I, Courtemanche C, Ames BN. γ-Tocopherol and its major metabolite, in contrast to α-tocopherol, inhibit cyclooxygenase activity in macrophages and epithelial cells. Proc Natl Acad Sci USA. 2000;97:11494–9.
39. Raffo A, Paoletti F, Antonelli M. Changes in sugar, organic acid, flavonol and carotenoid composition during ripening of berries of three

seabuckthorn (Hippophae rhamnoides L.) cultivars. Eur Food Res Technol. 2004;219:360–8.
40. Salminen A, Lehtonen M, Suuronen T, Kaarniranta K, Huuskonen J. Terpenoids: natural inhibitors of NF-κB signaling with anti-inflammatory and anticancer potential. Cell Mol Life Sci. 2008;65:2979–99.
41. Yang BR, Kallio H. Composition and physiological effects of sea buckthorn (Hippophaë) lipids. Trends Food Sci Technol. 2002;13:160–7.
42. Peponis V, Papathanasiou M, Kapranou A, Magkou C, Tyligada A, Melidonis A, Drosos T, Sitaras NM. Protective role of oral antioxidant supplementation in ocular surface of diabetic patients. Br J Ophthalmol. 2002;86:1369–73.
43. Uchiyama E, Aronowicz JD, Butovich IA, McCulley JP. Increased evaporative rates in laboratory testing conditions simulating airplane cabin relative humidity: an important factor for dry eye syndrome. Eye Contact Lens. 2007;33:174–6.
44. Moss SE, Klein R, Klein BE. Prevalence of and risk factors for dry eye syndrome. Arch Ophthalmol. 2000;118:1264–8.
45. Nichols KK, Nichols JJ, Mitchell GL. The lack of association between signs and symptoms in patients with dry eye disease. Cornea. 2004;23:762–70.
46. Liu H, Begley C, Chen M, Bradley A, Bonanno J, McNamara NA, Nelson JD, Simpson T. A link between tear instability and hyperosmolarity in dry eye. Invest Ophthalmol Vis Sci. 2009;50:3671–9.
47. Desbiens NA. In randomized controlled trials, should subjects in both placebo and drug groups be expected to guess that they are taking drug 50% of the time? Med Hypotheses. 2002;59:227–32.
48. McMonnies CW. Key questions in a dry eye history. J Am Optom Assoc. 1986;57:512–7.
49. Nichols KK, Nichols JJ, Mitchell GL. The reliability and validity of McMonnies dry eye index. Cornea. 2004;23:365–71.