Accepted: 31 August 2017

DOI: 10.1111/phpp.12350

ORIGINAL ARTICLE

WILEY Photodermatology, Photoimmunology & Photomedicine

An open-label clinical trial assessing the efficacy and safety of Bend Skincare Anti-Aging Formula on minimal erythema dose in skin

Nancy L. Morse[1] | Anna-Jean Reid[2] | Marc St-Onge[2]

[1]Independent Scientific Consultant, Berwick, NS, Canada

[2]Bend Beauty Inc., Halifax, NS, Canada

Correspondence
Nancy L. Morse, Independent Scientific Consultant, Berwick, NS, Canada.
E-mail: nancy.morse@eastlink.ca

Funding information
The Canadian National Research Council, Industrial Research Assistance Program Project, Grant/Award Number: 789075; Bend Beauty Inc

Summary

Background/purpose: Sunburn and other health risks associated with excess sun exposure place huge economic burdens on societies, and create discomfort and disease within susceptible individuals. Oral supplements that reduce sunburn may be advantageous. This study evaluated the safety and efficacy of Bend Skincare Anti-Aging Formula to ameliorate sunburn induced with a solar simulator.

Methods: Subjects (n = 28) with Fitzpatrick skin phototypes I, II, or III took 4 capsules daily of the supplement providing 1400 mg of eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA), 120 mg of gamma-linolenic acid (GLA), 5 mg of lutein, 2.5 mg of zeaxanthin, and 1000 IU of vitamin D3 for 8 weeks. Skin on each subject's back was exposed to a progressive sequence of timed ultraviolet (UV) radiation exposure doses at baseline, and after 4- and 8-week treatment to determine their minimal erythema dose (MED). Results were compared before and after treatment using 3 paired t tests and subsequently 3 linear mixed models.

Results: Treatment significantly improved tolerance to UV exposure as evidenced by increased MED at 4 and 8 weeks compared with baseline (P < .001). This protection increased with prolonged use of Bend Skincare Anti-Aging Formula as demonstrated by progressively increased MED between baseline and 4 weeks, and again between 4 and 8 weeks (P < .001). Nearly 86% of patients responded to treatment within 4 weeks and 100% of patients responded by the end of the study, resulting in a 39% mean increase in MED at 4 weeks, and an 84% mean increase in MED at 8 weeks compared with baseline. Treatment was well tolerated with no product associated adverse events (AE) and only a few mild and expected side effects.

Conclusion: Bend Skincare Anti-Aging Formula safely and effectively provides significant skin photoprotection that increases with continued use.

KEYWORDS
carotenoids, fish oil, skin, sunburn, UV radiation

1 | INTRODUCTION

Sunburn erythema and other health risks associated with excess sun exposure including premature skin aging, damage to the immune system, cataracts, and nonmelanoma and melanoma skin cancers[1] place huge economic burdens on societies. In Canada, in 2004, the cost for treatment of melanoma alone was $444 million, and for nonmelanoma was $88 million, and is expected to rise by 2031 to $696 million and $226 million, respectively.[2] A study including beach goers in the United States (US) found that sunburn accounts for up to 92 720 lost workdays annually, making the economic burden for lost work plus treatment in excess of $10 million annually.[3]

Erythema (skin redness) is an acute inflammatory skin response resulting from UV radiation overexposure. Both UVA (320-400 nm) and UVB (280-320 nm) contribute to this response known as sunburn, although UVB (280-320 nm) is more potent in this regard.[4] Sunburn response varies widely among individuals depending on skin type (ie people with lighter skin tone have a greater sunburn risk) and within the same person depending on factors such as age[5] and diet.[6,7]

In experimental models, sunburn sensitivity is measured using the minimal erythema dose (MED), defined as the smallest UV dose producing perceptible skin redness within distinct borders in a given period of time after exposure.[8-10] The higher the MED, the more resistant the skin is to sunburn. Therefore, increased MED following an experimental treatment signifies photoprotection.

Experimental sunburn is achieved using solar stimulators providing a continuous spectral output in the UVB (290-320 nm), UVAII (320-340 nm) and UVAI (340-400 nm) ranges that are similar to sunlight. Instrument performance, calibration, and operation requirements are defined within the US Food and Drug Administration (FDA) Federal Register[8] and are necessary to achieve reliable test results.

In recent years, interest in using natural products including nutrients as photoprotectors has grown.[11-13] Orally administered nutrients showing benefit include omega-3 fatty acids derived from fish[14-18] and a variety of antioxidants,[13] while vitamin D exhibits anti-inflammatory effects[19] in vitro[20-22] and in vivo,[23] and reduces polymorphic light eruption when topically applied to human skin.[24] In addition, oral omega-6 fatty acids, frequently sourced from borage oil, are now recognized contributors to inflammation resolution,[25] and so may be valuable additions to products aimed at preventing/relieving inflammatory skin conditions like sunburn. To date, no clinical studies have reported the combined effects of these nutrients on lowering sunburn risk.

The primary objective of this pilot trial was to evaluate and compare changes in the MED following use of an oral supplement combining benefits of omega-6 and omega-3 fatty acids with the antioxidants zeaxanthin and lutein, and vitamin D. The secondary trial purpose was to assess the safety of the supplement under the conditions of use.

2 | METHODS

This open-label clinical trial, completed within a contract testing laboratory, included recruitment of at least 30 subjects from the general population in New Jersey, United States, aged 19-65 years, who met all of the following inclusion criteria and none of the exclusion criteria.

TABLE 1 Characteristics of Fitzpatrick skin phototypes I, II, and III

Skin type	Sunburn and tanning history
I	Always burns easily; never tans
II	Always burns easily; tans minimally
III	Burns moderately; tans gradually

2.1 | Inclusion criteria

Subjects with a Fitzpatrick skin phototype I, II, or III (Table 1), aged 19-65 years, inclusive, who were considered suitable by a nurse or physician prior to their trial initiation; those who had protected the test site from sun for 4 weeks prior to trial initiation, had completed a medical history form, had understood and executed an informed consent form, and who were considered dependable and capable of understanding and following directions.

2.2 | Exclusion criteria

Subjects who had a history of abnormal response to sunlight, such as lupus erythematosus or skin cancer; subjects who had a sunburn, suntan, uneven skin color or visible disease that would interfere with test result evaluations; subjects with known allergy or intolerance to the test material ingredients (ie fish oil, soy); subjects who had nevi, blemishes, or moles, which, in the opinion of the principal investigator (PI), would interfere with the trial results, but including subjects with excessive hair who clipped their hair; subjects who were in ill health or taking medication other than birth control, which could influence the purpose, integrity or outcome of the trial; subjects who used a tanning bed or overexposed themselves to sunlight on the skin test site; females who were pregnant, planning to become pregnant, or nursing during the course of the trial; subjects who had participated in testing procedures that precluded a sufficient area being clear of all previous skin tanning and subjects who had experimented on the skin test site within the previous 2 months.

2.3 | Intervention

Participants were instructed to consume 4 Bend Skincare Anti-Aging Formula soft gelatin capsules supplied by Bend Beauty Inc., Halifax, N.S. Canada, once daily for 8 weeks. The total daily dose contained 1400 mg of eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) derived from fish oil, 120 mg of GLA derived from borage oil, 5 mg of lutein derived from marigold flower oleoresin, 2.5 mg of zeaxanthin derived from Capsicum annuum (paprika) fruit, and 1000 IU of vitamin D3. Subjects were required to record the time of supplement use in a daily diary to monitor compliance, as well as comments on the product's attributes. Capsule counts were maintained before and after treatment to determine the percentage of

capsules consumed relative to the expected quantity based on the dosing instructions.

2.4 | Outcomes

The primary outcome was a statistically significant increase ($P < .05$) in MED following 4- and 8-week treatment relative to baseline, and between 4-week treatment and 8-week treatment. There were no changes to the trial's primary outcome after the trial commenced. The secondary outcome was a progressive positive percentage change in MED from baseline to after 4- and 8-week treatment. Unsolicited patient-reported outcomes were recorded in the daily diaries.

2.5 | Determination of MED

The MED of each subject was determined within the first 7 days of the initial time point (when supplementation began), and again at 4 and 8 weeks following supplementation, using instrumentation specifications, qualifications, and calibrations as well as MED assessment scoring requirements defined by the US FDA.[8] Subjects were seated upright during the UV irradiation and MED assessments. During irradiation, unprotected skin on each subject's back was exposed to full-spectrum UV radiation with a continuous spectral output similar to sunlight (UVB range 290 nm-320 nm, UVAII range 320 nm-340 nm, and UVAI range 340-400 nm) using a solar simulator (Solar Light Company, Philadelphia, PA, USA).

At baseline, different sections of each subject's back were exposed to a progressive sequence of timed UV radiation exposures, each of which was graduated incrementally by 25% over the previous exposure. The initial dose of radiation varied according to their Fitzpatrick skin phototype (eg those with Type I received a lower dose than Type III) such that different subjects were exposed to different dosage sequences.

A trained grader evaluated the skin test sites for erythema between 16 and 24 hours after irradiation using the MED Scoring Scale described in Table 2. The MED was defined as the quantity of erythema-effective energy (mJ/cm^2) that produced mild but definite erythema within clearly defined borders (ie "1" on the MED Scoring Scale). If a baseline MED could not be determined by the grader during the first irradiation sequence, as second irradiation occurred. Subjects were removed from the study if the second irradiation sequence did not yield a baseline MED.

TABLE 2 Minimal erythema dose scoring scale

Score	Description
0	No reaction
0.5	Equivocal reaction, barely perceptible erythema with no clearly defined border
1	Mild but definite erythema with clearly defined borders
2	Moderate clearly defined erythema
3	Strong erythema, edema
4	Bulla or vesiculation

After 4 weeks of supplementation, the MED was determined again as previously described on a skin test site in close proximity to the baseline MED skin test site. However, this time the starting dose of UV radiation was adjusted such that the baseline MED became the midpoint irradiation dose within the sequence of 25% incremental doses applied. If the MED could not be determined after the first irradiation sequence, a second sequence was conducted. If the MED could not be determined with the second irradiation sequence, the subject was allowed to remain in the study, but they were not included in the statistical analysis. Similarly, subjects returned to the test facility after 8 weeks of supplementation. This time the MED was determined using an irradiation sequence in which the week 4 MED was the midpoint. Evaluation of the irradiated test site was conducted as described for the baseline and week 4 time points.

Throughout the study, subjects were required to maintain a daily diary of test material use and to record comments pertaining to their experiences with the test material.

2.6 | Sample size

Subjects could freely withdraw from the trial at any time and for any reason, in accordance with the World Medical Association Declaration of Helsinki (as amended). The PI could also withdraw subjects from the trial for safety, lack of efficacy, or administrative reasons. Possible reasons may have included experiencing a serious or intolerable adverse events (AE); development during the course of the trial, of any symptoms or conditions listed in the exclusion criteria, including pregnancy; consumption of contraindicated medications; a protocol violation such as failure to comply with the specified treatment regimen or failure to comply with the visit schedule; a request by the subject to discontinue due to a clinical event for which the PI did not consider removal from the trial to be necessary or any other nonspecific, subject-initiated reason.

2.7 | Statistical methods

Per-protocol analysis was performed using 3 paired *t* tests to determine if any statistically significant differences in MED occurred between baseline and week 4, between baseline and week 8 and between weeks 4 and 8. Results were deemed statistically significantly different if $P < .05$. However, the paired *t* test does not take into account any random effects, and the *P*-values are not adjusted for repeated testing. Therefore, to confirm the validity of conclusions based on the paired *t* test analysis, 3 subsequent linear mixed models were built and analyzed in R using ANOVA to generate *P*-values for comparing those models. A linear mixed model explicitly considers both random-effect and fixed-effect covariates and therefore is advantageous when both effects are present, as in our data. The random variation in baseline MED was modeled where *Subjects* were considered as random-effect covariates, while the *Weeks* and *Skin Types* were considered as fixed effects and successively added in the following 3 models: Group 0—MED ~ (1|Subject); Group 1—MED ~ Week + (1|Subject); Group 2—MED ~ Week + Skin + (1|Subject).

2.8 | Adverse Events

Each subject was monitored for the signs and symptoms of AEs during each examination by the trial personnel and through spontaneous reports from the subjects. These included AEs resulting from concurrent illnesses, reactions to concomitant medications or progressive disease states. All AEs whether volunteered, elicited or noted during each visit were recorded using Common Terminology Criteria for Adverse Events v.4. All AEs were evaluated by the PI for their relationship to, or association with the test material (or other causes) and for their intensity. Any actions taken (eg discontinuation of test material, administration of treatment, etc.) and the resulting outcome of the AE were recorded. Subjects who withdrew from the trial due to an AE were followed until the outcome of the event was determined, and a written summary of the event and follow-up was maintained. The Allendale Institutional Review Board (an independent group of professionals utilized by the contract testing laboratory, as required by FDA federal regulations) was informed of any serious AEs as instructed in the study protocol.

2.9 | Ethics Approval

The study was conducted in accordance with the current Declaration of Helsinki, with the ICH Guideline E6 Good Clinical Practices, the requirements of the FDA 21 CFR Parts 50 and 56, and with standard operating procedures of the contract testing laboratory. Written informed consent was obtained from each subject enrolled in the study. The protocol, informed consent form, and relevant procedures were approved by the Allendale Investigational Review Board on 6 February 2014. Trial registration was not applicable under FDAAA801 requirements.

3 | RESULTS

This study, conducted between 14 April 2014 and 18 June 2014, ended after the last follow-up visit by the last patient was completed for the week 8 assessment. There were no amendments to the protocol after trial commencement. However, there were a number of deviations, none of which had any significant impact on the trial results, as follows: The baseline MEDs for 3 subjects were not evaluated within 16-24 hours after irradiation because the subjects failed to return to the testing facility in a timely fashion such that their evaluations took place 1, 24, and 110 minutes beyond the allowed 24 hour upper time limit; however, a MED was obtained for each subject; 2 subjects did not record taking the test material within the daily diary on 1 day; one subject took 2 capsules on Day 0 instead of 4 capsules; One subject, on 4 occasions, took 2 capsules per day instead of 4 capsules; at week 4, one subject said they recorded when the test material was taken, even though they forgot to take it on a few of those days; and the week 8 MEDs for 2 subjects were not evaluated 16-24 hours after irradiation.

Thirty-three subjects were enrolled and 28 completed the study. The reasons for subject drop out/withdrawal were as follows: Two did not meet the inclusion criteria, one withdrew consent, one was disqualified due to excessive sun exposure in the test area and one took exclusionary medication. The demographics for the subjects included in the study at baseline are presented in Table 3. The subject's ages ranged from 21 to 64 years (mean 47.4 ± 11.5); 5 subjects had Skin Type I (17.9%), 15 subjects had Skin Type II (53.6%), and 8 subjects had Skin Type III (28.6%); and their baseline MEDs ranged from 12.0 to 49.3 mJ/cm^2 (mean 23.5 ± 9.7).

Supplement intake compliance was approximately 96% throughout the study.

3.1 | Outcomes

Results for the paired *t* tests are presented in Table 3. The amount of energy needed to produce the MED significantly increased ($P < .001$) compared with baseline after 4 and 8 weeks of supplementation. The mean MED at baseline was 23.5 mJ/cm^2, and increased to 33.9 and 43.8 mJ/cm^2 after 4 and 8 weeks of supplementation, respectively. There was also a significant increase in energy required to achieve a MED between 4 and 8 weeks ($P < .001$). These results corresponded to a mean increase of 39% in MED at 4 weeks and an 84% mean increase in MED at 8 weeks, compared with baseline (Table 3, Figure 1). In addition, these increases were observed throughout the test population with 85.7% and 100% of the subjects requiring an increase in energy to produce a MED at week 4 and week 8, respectively (Figure 2).

Results of the linear mixed models analyzes confirmed our conclusion based on the paired *t* test and showed that the MED significantly increased after treatment (Table 3). Adding the *Week* covariate had a significant effect on the MED ($P < .001$), adding both the *Week* and the *Skin Type* covariates had a significant effect on the MED ($P < .001$), and adding the *Skin Type* covariate had a weaker but still significant effect on the MED ($P < .001$). Combined, these results confirm that the MED does increase significantly due to treatment effects.

A few patients reported outcomes within their daily diaries (Table 4) including 5 mild side effects and 5 perceived enhanced skin, hair, or overall health effects.

No serious AEs related to the supplement were reported by the subjects or noted by the trial monitors. There were 2 AEs unrelated to supplement use as follow: One subject developed a cough and took a cough medicine on 1 day and another subject rolled her ankle. The latter subsequently consumed anti-inflammatory medication that prevented continuation within the trial.

4 | DISCUSSION

The need for effective UV skin protection is important from both an economic and personal perspective. Human skin exposed to UV radiation produces reactive oxygen species leading to DNA, cell, and tissue damage that can alter immune function and generate health issues ranging in severity from skin pigmentation and photoaging [4]

TABLE 3 Patient demographics and clinical characteristics at baseline of patients who completed the study and their minimal erythema dose (MED) results and statistical analysis following treatment

			MED (mJ/cm^2)			Change in MED from baseline (%)	
Subject number	Age	Skin type	Baseline	Week 4	Week 8	Week 4	Week 8
2	36	II	16.1	20.4	25.2	26.7	56.5
3	60	II	20.2	20.3	25.2	0.5	24.8
4	31	I	15.8	19.7	19.6	24.7	24.1
6	58	II	20.2	25.3	31.7	25.2	56.9
7	43	II	20.2	31.7	31.8	56.9	57.4
8	46	III	25.2	31.7	62.1	25.8	146.4
9	51	I	15.8	19.7	31.0	24.7	96.2
10	45	II	12.9	16.2	31.5	25.6	144.2
11	21	II	31.7	49.3	39.5	55.5	24.6
12	41	III	49.3	62.0	61.8	25.8	25.4
13	21	III	31.0	60.5	75.5	95.2	143.5
14	55	II	25.2	49.5	61.6	96.4	144.4
15	51	II	25.2	39.6	49.5	57.1	96.4
16	44	II	25.2	39.6	61.9	57.1	145.6
17	59	I	12.7	16.2	20.2	27.6	59.1
18	64	III	20.2	31.7	49.7	56.9	146.0
19	60	II	20.2	25.3	31.7	25.2	56.9
21	31	III	38.7	75.9	95.0	96.1	145.5
22	54	II	25.2	39.6	61.9	57.1	145.6
23	60	II	25.2	20.3	39.6	−19.4	57.1
24	49	III	45.0	56.3	70.3	25.1	56.2
26	58	II	12.0	18.5	23.0	54.2	91.7
27	48	I	19.8	24.6	24.4	24.2	23.2
28	52	II	17.6	17.5	27.4	−0.6	55.7
29	41	III	20.2	31.7	39.6	56.9	96.0
30	60	II	12.9	12.9	20.2	0.0	56.6
31	38	III	38.9	75.9	95.0	95.1	144.2
32	50	I	16.1	16.1	20.1	0.0	24.8
Mean			23.5*	33.9*	43.8*	39.1	83.8
Standard deviation			9.7	18.5	22.3	31.5	47.9

Linear mixed model analysis

Group	*df*	AIC	BIC	logLik	Deviance	Chisq	Chi *df*	Pr (>Chisq)
0 compared to 1								
0	3	718.62	725.91	−356.31	712.62			
1	4	670.04	679.77	−331.02	662.04	50.574	1	1.147e-12, $P < .001$
0 compared to 2								
0	3	718.62	725.91	−356.31	712.62			
2	5	651.64	663.79	−320.82	641.64	70.98	2	3.863e-16, $P < .001$
1 compared to 2								
	4	670.04	679.77	−331.02	662.04			
	5	651.64	663.79	−320.82	641.64	20.405	1	6.265e-06, $P < .001$

*Results of the paired t tests where $df = 27$ for all: Baseline vs Week 4, $t = 5.104$, two-tailed $P < .001$, $r = .893$; Baseline vs Week 8, $t = 6.885$, two-tailed $P < .001$, $r = .909$; Week 4 vs Week 8, $t = 5.953$, two-tailed $P < .001$, $r = .924$.

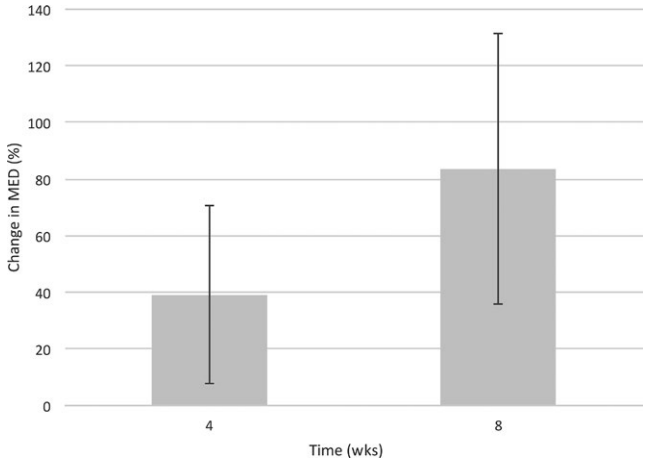

FIGURE 1 Mean percent change in minimal erythema dose (MED) compared with baseline after supplementation for 4 and 8 wk

to cancer.[1] Strategies to mitigate such risks, including dietary supplementation, would be advantageous. This study showed that oral supplementation with Bend Skincare Anti-Aging Formula safely and effectively reduced the likelihood and/or severity of the inflammatory response associated with sunburn within a given UV exposure time and dose. It is the first study to report the combined efficacy and safety of oral supplementation with omega-6 and omega-3 fatty acids, zeaxanthin, lutein, and vitamin D to provide such photoprotection.

To the author's knowledge, no human intervention studies have investigated the protective effects of GLA against UV damage in skin. However, GLA has proven anti-inflammatory effects in skin as evidenced in atopic eczema trials,[26-28] possibly due to its metabolic conversion to anti-inflammatory PGE1[29] and lipoxin,[30] a specialized proresolving mediator derived from arachidonic acid (AA) that activates inflammation resolution.[25] Hence, GLA may lessen sunburn associated inflammation.

The fish oil derived omega-3s, EPA, and DHA, inhibit IL-8 production that may partly account for their anti-inflammatory effects yielding UV-protection in skin.[31] In human epidemiological studies, omega-3 intake is associated with reduced UVB-erythemal sensitivity and photoaging.[32] Omega-3s also alter the skin EPA:AA ratio, which during the acute stage of inflammation, reduces pro-inflammatory prostaglandin and leukotriene production[14] thereby reducing blood vessel dilation,[16] under basal as well as inflammatory insult conditions.[14]

Numerous randomized, double-blind, placebo-controlled,[14,17,18] and open trials[15,16] have reported significantly increased UV-induced sunburn threshold (a defined dose causing perceptible erythema similar to a MED) following 1800 mg—5 g/d EPA alone [14,17] or with 1200 mg/d DHA [15,16,18] for as little as 4 weeks.[18] In addition, sunburn threshold rose progressively throughout treatment, but fell 10 weeks after treatment cessation, indicating that constant supplementation is needed for continuous protection.[15] This enhanced efficacy with prolonged use is consistent with the results obtained in our study, even though our dosage of total omega-3s was slightly less (ie 1400 mg EPA + DHA daily).

The current United States daily dietary intake of EPA + DHA is only 40 mg in children and teens, and 90 mg in adults.[33] And even though 7.8% of adults and 1.1% of children use EPA/DHA supplements,[34,35] they only contribute additionally, about 10 mg of DHA and 20 mg of EPA to adult intakes.[36] Canadian children only eat 92.5 mg EPA + DHA daily,[37] while Canadian adults eat only 47-160 mg of DHA per day.[38-41] These deficient intakes are despite recommendations beyond that for general populations, by dozens of government departments, international bodies, and formal and informal scientific societies and groups, for both general and specific populations.[42] This intake data, combined with the significant efficacy of omega-3s against UV skin damage, lead one to speculate that nearly the entire North American population could benefit from photoprotection through omega-3 supplementation.

UV radiation promotes skin cancer development through mutations, immunosuppression, and inducing oxidative stress.[6] Carotenoids are important components of the antioxidant network and help protect light-exposed tissue.[43,44] Zeaxanthin and lutein are 2 such carotenoids that work by scavenging singlet oxygen molecules and quenching free radicals responsible for oxidative tissue damage.[45] They cannot be de novo synthesized and therefore must be diet derived.[46] Zeaxanthin is not only an effective antioxidant in vivo, it also reduces hydroperoxide formation in dietary oils, making it an attractive ingredient to reduce oxidative degradation within fatty acid supplements.[45]

The antioxidant capability of zeaxanthin has been compared to that of the highly potent synthetic antioxidant, Trolox. In one study, testing 3 different zeaxanthin isomers, 2 had greater free radical scavenging capacity, and prevented oxidation better than Trolox, while one isomer was less effective.[47] In a second study, zeaxanthin was reportedly more effective than Trolox.[48] A 12 week multicentered, randomized, placebo-controlled trial supplementing, forty women with signs and symptoms of premature skin aging, with 0.6 mg of zeaxanthin and 10 mg of lutein daily, reported a significant increase in skin lipids, skin hydration, skin elasticity, and sunburn threshold (all $P < .05$).[46]

Lutein modulates skin's response to UV radiation, thereby contributing to defense against some of sunlight's deleterious effects. A preclinical study found that oral lutein supplementation caused lutein accumulation in the skin while decreasing reactive oxygen species generation following UVB exposure. In addition, tissue swelling and contact hypersensitivity typically induced by UVB exposure were significantly reduced.[6]

US public health authorities recommend that men, women, and children reduce sunlight exposure to prevent skin cancer. However, increasing numbers of Americans suffer from vitamin D deficiencies and serious health problems caused by insufficient sun exposure necessary for endogenous vitamin D synthesis. In addition, sunscreens reduce vitamin D production, prompting recommendations to warn about vitamin D deficiency associated with their use.[49] For these reasons alone, vitamin D is considered an important nutrient for inclusion in dietary supplements aimed at photoprotection. However, vitamin D also exhibits anti-inflammatory effects.[19] In vitro studies have shown that vitamin D reduces UV-induced damage, including inflammation,

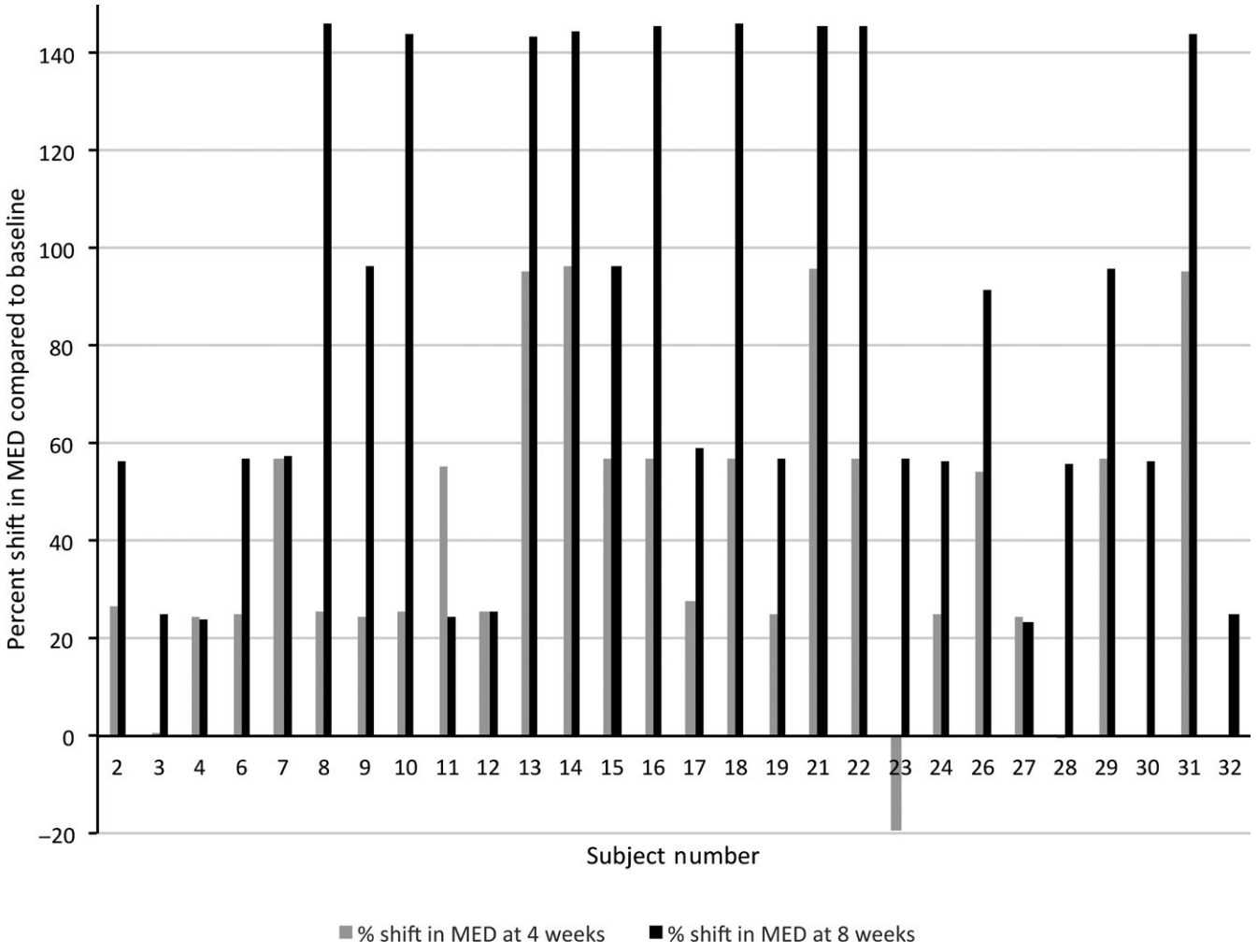

FIGURE 2 Percentage change in energy to produce a minimal erythema dose (MED) relative to baseline, within each subject, following treatment for 4 and 8 wk

sunburn, and photo-associated immunosuppression, and carcinogenesis,[20-22] while a human epidemiology study associated higher vitamin D status with reduced systemic inflammation.[23] When topically applied to human skin, Vitamin D reduces polymorphic light eruption.[24]

Currently, it is difficult to ascertain the relative contribution of each compound within the Bend Skincare Anti-Aging Formula or any possible synergies, due to insufficient data for comparison. GLA, vitamin D, zeaxanthin, and lutein have not been tested singly in human interventions trials for their protective effects against UV damage in skin. Only one study has reported the combined effects of zeaxanthin and lutein on MED, but the data were included as a graph that did not enable calculation of an accurate numerical change in MED from baseline.[46] Methodological variations within the Omega-3–related trials[14-18] including varying dosages of EPA and/or DHA, treatment durations, and methods of assessing treatment effects, make it difficult to draw any firm conclusions. Rhodes et al[17] reported a UVR-induced erythemal threshold rising from a mean of 36 mJ/cm^2 at baseline to 49 mJ/cm^2 (1.36-fold increase) after supplementation with 4 g daily of purified EPA for 3 months, a MED of UVB irradiation increasing from 19.8 mJ/cm^2 at baseline to 33.8 mJ/cm^2 (1.7-fold increase) following 3 months treatment,[16] and from 18.9 mJ/cm^2 at baseline to 41.1 mJ/cm^2 (2.17-fold increase) following 6 months treatment [15] with 1800 mg EPA + 1200 mg DHA daily. Orengo et al[18] reported a MED of approximately 13.25 mJ/cm^2 at baseline that increased to roughly 15.5 mJ/cm^2 (1.2-fold increase) following 4 weeks treatment with 2800 mg EPA and 1200 mg DHA daily. Our study provided less active (1400 mg of EPA + DHA) than any of those studies. It also had a shorter treatment duration (8 weeks) than 3 of 4 of those studies. However, it achieved a greater (1.8-fold) increase in MED than 3 of 4 of those studies. Whether or not this enhanced efficacy measured in our study, was due to individual potency or synergistic effects of ingredients other than the omega-3s within the Bend Skincare Anti-Aging Formula, is difficult to know.

The safety of Bend Skincare Anti-Aging Formula was demonstrated by the lack of product associated AEs and the limited number of only mild side effects in the patient-reported outcomes. All of the mild gastrointestinal side effects (excluding possible dry skin) are consistent with typical side effects reported for other fish oil[50] and borage oil[51]

TABLE 4 Patient-reported outcomes

Outcomes	Number of subjects	Comment
Mild side effects	1	Stomach pains if not taken after eating
	2	Creates burping (or repeats on me)
	1	Possible dry skin
	1	After taste, always take with food
Perceived enhanced health	1	Skin looked healthier and soft
	1	Reduced acne and skin were brighter
	1	Had more energy
	1	Stools softer
	1	Had thicker hair

containing preparations and so are not unique to the test supplement. None of these side effects, or cautions, warning, contraindication or known adverse reactions, are required to be declared on product labels by the Canadian Natural and Non-Prescription Health Products Directorate (NNHPD) associated with either fish oil[52] or borage[53] oil use. Therefore, the side effects observed in this study are considered to be of no significant health consequence.

The strengths of this study included rigorous monitoring of UV radiation instrument performance, subject's product dosing compliance, and only minor deviations to the study protocol. Failure to address subject ethnicity within the inclusion/exclusion criteria may be perceived as a trial weakness. However, although there may be real differences in the skin biology of different demographic groups, sorting people effectively into categories has been problematic given the prevailing definitions of race, ethnicity, photo skin type, and pigmentation,[54] making it difficult to define those that should have been included and those that should have been excluded from the study based on their ethnic background. The main weakness of the study was the subjective nature of the MED scoring scale and that the trial design was open label. In addition, a control group of individuals receiving placebo or no intervention and graders being blinded to the intervention when reading MEDs would have made the results of this study more convincing. A randomized, double-blind, placebo-controlled trial, including a nonsubjective assessment of skin redness using a devise such as a spectrophotometer[9] rather than visual inspection, might be appropriate to confirm the beneficial results achieved in this trial. It should also include quantification of pro- vs anti-inflammatory cytokines to pin point the mechanisms underlying the apparent anti-inflammatory effects indicated by changes in MED, as well as markers for UV-induced DHA damage such as cyclobutane pyrimidine dimers [55] or oxidative DNA damage such as 8oxo-guanine,[56] to determine the extent of UV protection against DNA damage, if any. Such future studies are necessary because pure suppression of inflammation without any substantial protection against damage raises the question whether mechanisms of skin adaptation against UV radiation [57] could be adversely affected.

5 | CONCLUSION

Bend Skincare Anti-Aging Formula statistically significantly increased the amount of energy needed to produce a MED in subjects treated for as little as 4 weeks. This increase indicated a resistance to UV-induced redness characteristic of sunburn. In addition, this photoprotection increased with continued product use. Oral supplementation with omega-6 and omega-3 fatty acids combined with lutein, zeaxanthin, and vitamin D may effectively reduce sunburn risk.

ACKNOWLEDGEMENTS

Nancy Morse wrote the manuscript, Anna-Jean Reid reviewed the data, draft and final manuscript, Marc St-Onge reviewed the draft and final manuscript. Huaichun Wang under the advice of Professor Edward Susko, Statistics Consulting Service, Dalhousie University, Halifax, NS, Canada, built and compared the linear mixed models. The test product was supplied by Bend Beauty Inc. Halifax, NS, Canada.

CONFLICTS OF INTERESTS

The content of this manuscript has not been previously presented or published. Nancy Morse was hired as a consultant for Bend Beauty Inc., Halifax, NS, Canada, to write and submit the manuscript for publication. Anna-Jean Reid is an employee and Marc St-Onge is the President of Bend Beauty Inc. Huaichun Wang under the advice of Professor Edward Susko, Statistics Consulting Service, Dalhousie University, Halifax, NS, Canada, was hired as a consultant for Bend Beauty Inc., Halifax, NS, Canada, to build and compare the linear mixed models. This work was funded by Bend Beauty Inc. and supported by a grant from the Canadian National Research Council, Industrial Research Assistance Program Project 789075. There is no association between the Allendale Investigational Review Board and Bend Beauty Inc. other than through utilization of the Allendale Investigational Review Board for ethics approval of the study by the contract testing laboratory that completed the study.

ORCID

Nancy L. Morse http://orcid.org/0000-0002-0921-6225

REFERENCES

1. Health effects of UV radiation. United States: Environmental Protection Agency; 2017. http: www.epa.gov/sunsafety/health-effects-uv-radiation. Accessed March 27, 2017.
2. Canadian Partnership Against Cancer. The economic burden of skin cancer in Canada: current and projected Canada: Canadian Cancer Society; 2010 Feb 26. http://www.cancer.ca/en/get-involved/take-action/what-we-are-doing/financial-hardship-of-cancer-in-canada-mb/?region=mb. Accessed March 27, 2017.
3. Warthan MM, Sewell DS, Marlow RA, Warthan ML, Wagner RF. The economic impact of acute sunburn. *Arch Dermatol*. 2003;139:1003-1006.

4. Fourtanier A, Moyal D, Seité S. Sunscreens containing the broad-spectrum UVA absorber, Mexoryl SX, prevent the cutaneous detrimental effects of UV exposure: a review of clinical study results. *Photodermatol Photoimmunol Photomed*. 2008;24:164-674.
5. Arutyunyan S, Alfonso SV, Hernandez N, Favreau T, Fernández MI. Predictors of sunburn risk among Florida residents. *J Am Osteopath Assoc*. 2017;117:150-157.
6. Lee EH, Faulhaber D, Hanson KM, et al. Dietary lutein reduces ultraviolet radiation-induced inflammation and immunosuppression. *J Invest Dermatol*. 2004;122:510-517.
7. Stahl W, Sies H. Carotenoids and flavonoids contribute to nutritional protection against skin damage from sunlight. *Mol Biotechnol*. 2007;37:26-30.
8. Labeling and effectiveness testing; sunscreen drug products for over-the-counter human use. United States: FDA 2011. https://www.federalregister.gov/documents/2011/06/17/2011-14766/labeling-and-effectiveness-testing-sunscreen-drug-products-for-over-the-counter-human-use Accessed April 29, 2017.
9. Heckman CJ, Chandler R, Kloss JD, et al. Minimal erythema dose (MED) testing. *J Vis Exp*. 2013;75:1-5.
10. Pérez-Ferriols A. The minimal erythema dose (MED) project. In search of consensus on phototesting. *Actas Dermosifiliogr*. 2013;104:541-542.
11. Saewan N, Jimtaisong A. Natural products as photoprotection. *J Cosmet Dermatol*. 2015;14:47-63.
12. Serafini MR, Guimarães AG, Quintans JS, Araújo AA, Nunes PS, Quintans-Júnior LJ. Natural compounds for solar photoprotection: a patent review. *Expert Opin Ther Pat*. 2015;25:467-478.
13. Fernández-García E. Skin protection against UV light by dietary antioxidants. *Food Funct*. 2014;5:1994-2003.
14. Pilkington SM, Rhodes LE, Al-Aasswad NM, Massey KA, Nicolaou A. Impact of EPA ingestion on COX- and LOX-mediated eicosanoid synthesis in skin with and without a pro-inflammatory UVR challenge–report of a randomised controlled study in humans. *Mol Nutr Food Res*. 2014;58:580-590.
15. Rhodes LE, O'Farrell S, Jackson MJ, Friedmann PS. Dietary fish-–oil supplementation in humans reduces UVB–erythemal sensitivity but increases epidermal lipid peroxidation. *J Invest Dermatol*. 1994;103:151-154.
16. Rhodes LE, Durham BH, Fraser WD, Friedmann PS. Dietary fish oil reduces basal and ultraviolet B-generated PGE2 levels in skin and increases the threshold to provocation of polymorphic light eruption. *J Invest Dermatol*. 1995;105:532-535.
17. Rhodes LE, Shahbakhti H, Azurdia RM, et al. Effect of eicosapentaenoic acid, an omega-3 polyunsaturated fatty acid, on UVR-–related cancer risk in humans. An assessment of early genotoxic markers. *Carcinogenesis*. 2003;24:919-925.
18. Orengo IF, Black HS, Wolf JE. Influence of fish oil supplementation on the minimal erythema dose in humans. *Arch Dermatol*. 1992;284:219-221.
19. Krishnan AV, Feldman D. Mechanisms of the anti-cancer and anti-inflammatory actions of vitamin D. *Annu Rev Pharmacol Toxicol*. 2011;51:311-336.
20. Tongkao-On W, Carter S, Reeve VE, et al. CYP11A1 in skin: an alternative route to photoprotection by vitamin D compounds. *J Steroid Biochem Mol Biol*. 2015;148:72-78.
21. Song EJ, Gordon-Thomson C, Cole L, et al. 1α,25-Dihydroxyvitamin D3 reduces several types of UV-induced DNA damage and contributes to photoprotection. *J Steroid Biochem Mol Biol*. 2013;136:131-138.
22. Gordon-Thomson C, Gupta R, Tongkao-on W, Ryan A, Halliday GM, Mason RS. 1α,25 dihydroxyvitamin D3 enhances cellular defences against UV-induced oxidative and other forms of DNA damage in skin. *Photochem Photobiol Sci*. 2012;11:1837-1847.
23. Calton EK, Keane KN, Raizel R, Rowlands J, Soares MJ, Newsholme P. Winter to summer change in vitamin D status reduces systemic inflammation and bioenergetic activity of human peripheral blood mononuclear cells. *Redox Biol*. 2017;12:814-820.
24. Gruber-Wackernagel A, Bambach I, Legat FJ, et al. Randomized double-blinded placebo-controlled intra-individual trial on topical treatment with a 1,25-dihydroxyvitamin D_3 analogue in polymorphic light eruption. *Br J Dermatol*. 2011;165:152-163.
25. Chiang N, Serhan CN. Structural elucidation and physiologic functions of specialized pro-resolving mediators and their receptors. *Mol Aspects Med* 2017;pii S0098-2997(17)30019-5. doi: 10.1016/j.mam.2017.03.005. [Epub ahead of print]
26. Morse PF, Horrobin DF, Manku MS, et al. Meta-analysis of placebo-controlled studies of the efficacy of Epogam in the treatment of atopic ezema. *Br J Dermatol*. 1989;121:75-90.
27. Morse NL, Clough PM. A meta−analysis of randomized, placebo-controlled clinical trials of Efamol evening primrose oil in atopic eczema. Where do we go from here in light of more recent discoveries? *Curr Pharm Biotechnol*. 2006;7:503-524.
28. Foster RH, Hardy G, Alany RG. Borage oil in the treatment of atopic dermatitis. *Nutrition*. 2010;26:708-718.
29. Horrobin DF. Essential fatty acid metabolism and its modification in atopic eczema. *Am J Clin Nutr*. 2000;71:367S-372S.
30. Wu SH, Chen XQ, Liu B, Wu HJ, Dong L. Efficacy and safety of 15(R/S)-methyl-lipoxin A(4) in topical treatment of infantile eczema. *Br J Dermatol*. 2013;168:172-178.
31. Storey A, McArdle F, Friedmann PS, Jackson MJ, Rhodes LE. Eicosapentaenoic acid and docosahexaenoic acid reduce UVB- and TNF-alpha induced IL-8 secretion in keratinocytes and UVB-induced IL-8 in fibroblasts. *J Invest Dermatol*. 2005;124:248-255.
32. Latreille J, Kesse-Guyot E, Malvy D, et al. Association between dietary intake of n-3 polyunsaturated fatty acids and severity of skin photoaging in a middle-aged Caucasian population. *J Dermatol Sci*. 2013;72:233-239.
33. Omega-3 fatty acids, fact sheet for health professionals. United States: National Institutes of Health, Office of Dietary Supplements; 2016. https://ods.od.nih.gov/factsheets/Omega3FattyAcids-HealthProfessional/#h4. Accessed May 1, 2017.
34. Clarke TC, Black LI, Stussman BJ, Barnes PM, Nahin RL. *Trends in the Use of Complementary Health Approaches Among Adults: United States, 2002–2012*. Natl Health Stat Report. 2015; 79:1-16.
35. Black LI, Clarke TC, Barnes PM, Stussman BJ, Nahin RL. *Use of Complementary Health Approaches Among Children aged 4–17 Years in the United States: National Health Interview Survey, 2007–2012*. Natl Health Stat Report. 2015;78:1-19.
36. Papanikolaou Y, Brooks J, Reider C, Fulgoni VL 3rd. U.S. adults are not meeting recommended levels for fish and omega-3 fatty acid intake: results of an analysis using observational data from NHANES 2003–2008. *Nutr J*. 2014;13:31.
37. Madden SMM, Garrioch CF, Holub BJ. Direct diet quantification indicates low intakes of (n-3) fatty acids in children 4 to 8 years old. *J Nutr*. 2009;139:528-532.
38. Innis SM, Elias SL. Intakes of essential n-6 and n-3 polyunsaturated fatty acids among pregnant Canadian women. *Am J Clin Nutr*. 2003;77:473-478.
39. Denomme J, Stark K, Holub B. Directly quantitated dietary n-3 fatty acid intakes of pregnant Canadian women are lower than current dietary recommendations. *J Nutr*. 2005;135:206-211.
40. Fratesi JA, Hogg RC, Young-Newton GS, et al. Direct quantitation of omega-3 fatty acid intake of Canadian residents of a long-term care facility. *Appl Physiol Nutr Metab*. 2009;34:1-9.
41. Xie L, Innis SM. Genetic variants of the FADS1 FADS2 gene cluster are associated with altered (n-6) and (n-3) essential fatty acids in plasma and erythrocyte phospholipids in women during pregnancy and in breast milk during lactation. *J Nutr*. 2008;138:2222-2228.
42. Global recommendations for EPA and DHA intake. United States: Global Organization for EPA and DHA Omega-3; 2014. http://www.issfal.

org/GlobalRecommendationsSummary19Nov2014Landscape_-3-.pdf. Accessed May 1, 2017.
43. Stahl W1, Sies H. Bioactivity and protective effects of natural carotenoids. *Biochim Biophys Acta* 2005;1740:101-107.
44. Darvin ME, Sterry W, Lademann J, Vergou T. The role of carotenoids in human skin. *Molecules*. 2011;16:10491-10506.
45. Lee SH, Min DB. Effects, quenching mechanisms, and kinetics of carotenoids in chlorophyll-sensitized photooxidation of soybean oil. *J Agric Food Chem*. 1990;38:1630-1634.
46. Palombo P, Fabrizi G, Ruocco V, et al. Beneficial long-term effects of combined oral/topical anti-oxidant treatment with the carotenoids lutein and zeaxanthin on human skin: a double-blind, placebo-controlled study. *Skin Pharmacol Physiol*. 2007;20:199-210.
47. Böhm V, Puspitasari-Nienaber NL, Ferruzzi MG, Schwartz SJ. Trolox equivalent anti-oxidant capacity of different geometrical isomers of alpha-carotene, beta-carotene, lycopene, and zeaxanthin. *J Agric Food Chem*. 2002;50:221-226.
48. Miller NJ, Sampson J, Candeias LP, Bramley PM, Rice-Evans CA. Anti-oxidant activities of carotenes and xanthophylls. *FEBS Lett*. 1996;384:240-242.
49. Hoel DG, Berwick M, de Gruijl FR, Holick MF. The risks and benefits of sun exposure 2016. *Dermatoendocrinol*. 2016;1-17.
50. Jeansen S, Witkamp RF, Garthoff JA, van Helvoort A, Calder PC. Fish oil LC-PUFAs do not affect blood coagulation parameters and bleeding manifestations: analysis of 8 clinical studies with selected patient groups on omega-3-enriched medical nutrition. *Clin Nutr* 2017;pii: S0261-5614(17)30118-8. doi: 10.1016/j.clnu.2017.03.027. [Epub ahead of print]
51. Takwale A, Tan E, Agarwal S, et al. Efficacy and tolerability of borage oil in adults and children with atopic eczema: randomised, double blind, placebo controlled, parallel group trial. *BMJ*. 2003;327:1-4.
52. Fish oil product monograph. Canada: Natural and Non-Prescription Health Products Directorate, July 10, 2013. http://webprod.hc-sc.gc.ca/nhpid-bdipsn/atReq.do?atid=fish.oil.huile.poisson&lang=eng. Accessed April 28, 2017.
53. Borage oil product monograph. Canada: Natural and Non-Prescription Health Products Directorate, June 19, 2009. http://webprod.hc-sc.gc.ca/nhpid-bdipsn/monoReq.do?id=49&lang=eng. Accessed April 28, 2017.
54. Kimball AB. Skin differences, needs, and disorders across global populations. *J Investig Dermatol Symp Proc*. 2008;13:2-5.
55. Miller SA, Coelho SG, Yamaguchi Y, Hearing VJ, Beer JZ, de Gruijl F. The evaluation of noninvasive measurements of erythema as a potential surrogate for DNA damage in repetitively UV-exposed human skin. *Photochem Photobiol* 2017; Accessed on April 20, 2017. [Epub ahead of print]
56. Bjørås M, Seeberg E, Luna L, Pearl LH, Barrett TE. Reciprocal "flipping" underlies substrate recognition and catalytic activation by the human 8-oxo-guanine DNA glycosylase. *J Mol Biol*. 2002;317:171-177.
57. de Winter S, Vink AA, Roza L, Pavel S. Solar-simulated skin adaptation and its effect on subsequent UV-induced epidermal DNA damage. *J Invest Dermatol*. 2001;117:678-682.

How to cite this article: Morse NL, Reid A-J, St-Onge M. An open-label clinical trial assessing the efficacy and safety of Bend Skincare Anti-Aging Formula on minimal erythema dose in skin. *Photodermatol Photoimmunol Photomed*. 2017;00:1-10. https://doi.org/10.1111/phpp.12350