bend
BEAUTY

ANTI-AGING FORMULA

Scientific Research & Evidence Summary

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1.0 Synopsis

Bend Beauty Anti-Aging Formula:

- Targets the root cause of aging by transforming skin from the inside out
- Provides six powerful nutrients shown in clinical trials to enhance skin health including the omega-3s EPA and DHA, the omega-6 GLA, and the strong anti-oxidants lutein, zeaxanthin and vitamin D
- Contains natural product formulation enhancers including natural source grapefruit and tangerine flavours within the capsules and natural source grapefruit and tangerine flavours combined with monk fruit extract in the liquid version.
- Helps to increase skin hydration, firmness and elasticity, and helps to reduce roughness and redness (associated with skin inflammation) resulting in younger looking skin
- Helps improve symptoms such as roughness and redness, associated with skin inflammation that may results from atopic dermatitis (eczema)
- Helps reduce skin sensitivity to UV induced sunburn
- A ground breaking clinical trial measured an 84% increase in skin resistance to UV radiation induced sunburn after 8 weeks supplementation with Bend Beauty Anti-Aging Formula

Bend Beauty Anti-Aging Formula is a mixture of high-EPA fish oil, borage oil, the anti-oxidants zeaxanthin and lutein and vitamin D designed to optimize the Skin Climate, leading to improved skin hydration, elasticity, firmness, roughness and redness, and reduced skin sensitivity to UV induced erythema (sunburn). The grapefruit tangerine flavoured product is available in packs of 120 soft gelatin capsules or 200 mL of liquid in amber glass bottles. A daily dose of each dosage form provides 1050 mg of eicosapentaenoic acid (EPA) and 350 mg of docosahexaenoic acid (DHA) from anchovies, sardines, mackerel and/or herring whole body oil, 120 mg of gamma-linolenic acid (GLA) from Borage (*Borago officianalis*) seeds, 2.5 mg of zeaxanthin from *Capsicum annuum* L. fruit, 5 mg of lutein from *Tagetes erecta* flower, and 25 µg (1000 IU) of vitamin D3 (cholecalciferol) from lanolin.

Bend Beauty Anti-Aging Formula is authorized for sale by the Natural and Non-Prescription Health Product Directorate (NNHPD) of Health Canada. It has four skin related health claims. The formulation has been proven in a human intervention trial to provide significant skin photo-protection that increases with continued use in 100% of users (Morse 2017). Benefits reported in clinical studies for individual ingredients within the formulation include:

EPA and DHA

- Improved severity scores (Dölle 2008), scale, itch and overall subjective severity in atopic eczema (Bjorneboe 1987).
- Improved redness, scaling, inflammation, body surface area of psoriatic lesions (Mayser 1998), redness (Maurice 1987a, & 1987b), Psoriatic Association Score and itch (Lassus 1990) in psoriasis, and reduced hyperlipidemia and nephrotoxicity side effects associated with retinoid and cyclosporine therapy typically used to treat psoriasis (Allen 2001).

- Improved inflammatory and non-inflammatory acne lesions (Jung 2014).
- Increased UV- induced sunburn threshold (Pilkington 2014, Orengo 1992, Rhodes 1994, 1995 & 2003, Pilkington 2014).

GLA

- Improved itch, crusting, swelling and redness between 4 and 8 weeks after treatment was initiated in atopic eczema (Morse 1989 & 2006).
- Improved skin barrier function, water loss, dryness and itch in healthy elderly people (Brosche 2000) and

  skin moisture, water loss, elasticity, firmness, fatigue resistance, smoothness (Muggli 2005 & 2007), and scaling (De Spirt 2009) in healthy middle-aged adults.
- Reduced inflammatory lesions and sebum secretions without hormonal changes, in people with mild-to moderate facial acne (Lee 2014).

Lutein and zeaxanthin

- Improved skin lipid content, skin hydration, elasticity, sunburn threshold (Palombo 2007) in people with normal skin, and skin tone and luminance (brightness) in people with mild to moderately dry skin (Juturu 2016)

Vitamin D

- Reduced rash caused by sun exposure, when topically applied, in people who with sunlight sensitivity (Gruber-Wackernage 2011)
- Reduced Psoriasis Area Severity Index score in people with psoriasis (Perez 1996)
- Improved the SCORing Atopic Dermatitis score in people with atopic eczema (Javanbakht 2011)

All of these benefits enhance the structure and function of skin resulting in less wrinkles and more youthful appearance.

2.0 Recommended use

Bend Beauty Anti-Aging Formula (liquid and soft gelatin capsules) is licenced by the NNHPD of Health Canada. The licence numbers are NPN 80056197 and 80059934, respectively, and is associated with the following approved health claims.

Claim 1: *Helps to improve symptoms such as roughness and redness, associated with atopic dermatitis (skin inflammation).*

Claim 2: *Helps to improve skin hydration, elasticity, and firmness.*

Claim 3: *Helps in reducing skin sensitivity to UV induced erythema (sunburn).*

Claim 4: *Provides antioxidants for skin health.*

3.0 Formulation details

Bend Beauty Anti-Aging Formula is available in packs of 120 soft gelatin capsule or 200 mL of liquid in amber glass bottles. The recommended intake is one teaspoon (5 ml) of the liquid form, or 4 softgels daily. The medicinal ingredients, per serving, are listed in Table 1.

Table 1: Medicinal Ingredients per serving (1 tsp. (5 ml) or 4 softgels)

PROPER NAME	SOURCE MATERIAL(S)	DOSAGE
Eicosapentaenoic acid (EPA)	Anchovies, sardines, mackerel and/or herring	1050 mg
Docosahexaenoic acid (DHA)	Anchovies, sardines, mackerel and/or herring	350 mg
Gamma-linolenic acid (GLA)	*Borago officianalis* – seed	120 mg
Zeaxanthin	*Capsicum annuum L.* – fruit	2.5 mg
Lutein	*Tagetes erecta* - flower	5 mg
Vitamin D_3	Cholecalciferol from lanolin	25 µg (1000 IU)

Non-medicinal ingredients include grapefruit and tangerine flavours (natural source), olive oil, phospholipids, tocopherols (derived from non-GMO soy), medium chain triglycerides, canola oil, ascorbyl palmitate, and monk fruit extract in the liquid version and capsule shell components (gelatin, glycerin, purified water), beeswax, olive oil, lecithin (from sunflower oil), mint flavour (natural source), safflower oil, tocopherols (from non-GMO soy), ascorbyl palmitate, medium chain triglycerides in the capsule version.

3.1 Dosage Rationale

Numerous human intervention trials (Pilkington 2014, Rhodes 1994 & 1995, 2003, Orengo 1992) have reported significantly increased UV- induced sunburn threshold following 1800 mg – 5 g/day EPA alone (Pilkington 2014, Rhodes 2003) or with 1200 mg/day DHA (Orengo 1992, Rhodes 1994 & 1995) for as little as 4 weeks (Orengo 1992), while as little as 1800 mg of EPA daily improved symptom severity in atopic eczema (Bjorneboe 1987). Bend Beauty Anti-Aging Formula, provides slightly less (1400 mg of EPA+DHA) than the amount of omega-3 utilized in the atopic eczema trial mentioned previously. This lower dose would be expected to provide significant benefits in healthy skin in combination with the other ingredients included in the Bend Beauty Anti-Aging Formula.

Bend Beauty Anti-Aging Formula provides 120 mg of GLA daily which falls within the 80-640 mg dose of GLA reported in a meta-analysis to relieve itch, crusting, swelling and redness in atopic eczema (Morse 2006). Therefore, one would expect this dose to provide significant benefits in healthy skin in combination with the other ingredients included in the Bend Beauty Anti-Aging Formula.

Although EPA+DHA and GLA on their own produce significant benefits in skin, combining these inputs would be expected to enhance the outcome. Omega-3 supplementation increases the skin EPA: arachidonic acid (AA) ratio thereby reducing pro-inflammatory prostaglandin production (Pilkington 2014) that mediates blood vessel dilation (Rhodes 1995), under basal as well as inflammatory insult conditions (Pilkington 2014). Although AA is necessary to enhance immune function during infant development (Laitinen 2006), it is believed to contribute to inflammatory symptoms. Since combining EPA and GLA can

prevent an unwanted increase in AA (Barham 2000) that may occur with chronic borage oil supplementation (Surette 2003), formulations containing both of these nutrients may be more effective to treat inflammatory skin conditions than either alone (Boon 2004). This is the reason why Bend Beauty Anti-Aging Formula provides both EPA and GLA.

Clinical trials providing 6 mg of lutein combined with various other anti-oxidants (Morganti 2004) and with 0.18 mg of zeaxanthin (Morganti 2002) have reported UV-protective effects and enhanced skin moisture, respectively. Therefore, the 5 mg of lutein provided in Bend Beauty-Anti-aging Formula, that contains additional anti-oxidants, would be expected to provide similar effects. As well, 0.18 mg (Morganti 2002) and 0.6 mg (Pallombo 2007) of zeaxanthin daily have increased skin moistures and provided protection from UV-induced damage, improved elasticity and hydration, respectively. Therefore, a significantly higher dose of 2.5 mg of zeaxanthin as provided in the Bend Beauty Anti-Aging Formula would be expected to provide similar benefits.

Higher vitamin D status is associated with reduced systemic inflammation (Calton 2017). Therefore, one would expect any amount of vitamin D included within a product formulation to contribute to its anti-inflammatory effects. In addition, an intervention study providing 1600 IU of vitamin D significantly decreased symptom severity in people with atopic eczema (Javanbakht 2011). Therefore, the 1000 IU of vitamin D within the Bend Beauty Anti-Aging Formula would be expected to enhance the anti-inflammatory properties of the formulation.

The definitive efficacy measure of the combined ingredients within the Bend Beauty Anti-Aging Formula was a clinical trial in healthy subjects where 4 capsules per day for 8 weeks significantly increased skin resistance to UV radiation induced sunburn by 84% in 100% of the subjects (Morse 2017).

4.0 Scientific Background

Nutritional habits play an important role in skin health, contributing to skin structure, texture, and overall appearance and also affecting the risk for skin diseases and premature skin aging (Boelsma 2003). Bend Beauty Anti-Aging Formula is a unique combination of EPA, DHA, GLA, lutein, zeaxanthin, and vitamin D (cholecalciferol) that was formulated to promote skin health. Although it was developed primarily to prevent skin aging through UV protection, its nutrient composition is also ideally suited to maintain healthy skin.

Sunburn erythema and other health risks associated with excess sun exposure including premature skin aging, damage to the immune system, cataracts, and non-melanoma and melanoma skin cancers (Health Effects of UV Radiation) place huge economic burdens on societies. In Canada, in 2004, the cost for treatment of melanoma alone was $444 million and for non-melanoma was $88 million, and is expected to rise by 2031 to $696 million and $226 million, respectively (Canadian Partnership Against Cancer). 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 (Warthan 2003).

In recent years, interest in using natural products including nutrients as photo-protectors has grown (Saewan 2015, Serafini 2015, Fernández-García 2014). Orally administered nutrients showing benefit include omega-3 fatty acids derived from fish (Pilkington 2014, Rhodes 1994, 1995 & 2003, Orengo 1992) and a variety of anti-oxidants (Fernández-García 2014) including zeaxanthin and lutein, while vitamin D exhibits anti-inflammatory effects (Krishnan 2011) in vitro (Tongkao-On 2015, Song 2013, Gordon-Thomson 2012) and in vivo (Calton 2017), and reduces polymorphic light eruption when topically applied to human skin (Gruber-Wackernagel 2011). In addition, oral omega-6 fatty acids, frequently sourced from borage oil, are

now recognized contributors to inflammation resolution (Chiang 2017), and so may be valuable additions to products aimed at preventing/relieving inflammatory skin conditions like sunburn.

Similarly, supplementation has become an area of interest to help slow skin aging. Free radicals, reactive oxygen species (ROS) and lipid peroxides are involved in the pathogenesis and progression of accelerated skin aging when prolonged oxidative stress occurs. The use of antioxidant-related therapies in combination with fatty acids is of particular interest in combatting this assault and restoring skin homeostasis that can reduce the appearance of wrinkles, age spots and thickened skin. These nutrients can enhance wound healing (Hong 2014, Bohr 2013) as well as the structure, function and appearance of healthy skin (Muggli 2005 & 2007). They can also provide symptomatic relief of many skin disorders including atopic eczema (dermatitis) (Morse 2006), psoriasis and acne vulgaris (McCusker 2010), and may reduce side-effects associated with other skin treatments (Fabbrocini 2014, Allen 2001).

4.1 Bend Beauty Anti-Aging Formula to Reduce Sunburn

The efficacy and safety of Bend Beauty Anti-Aging Formula has been tested in an open label clinical trial including 20 subjects with Fitzpatrick skin phototypes I, II or III, who took four capsules daily for 8 weeks (Morse 2017). 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 weeks treatment to determine their minimal erythema dose (MED). Results were compared before and after treatment using three paired t-tests and subsequently three linear mixed models.

Treatment significantly improved tolerance to UV exposure as evidenced by increased MED at 4 and 8 weeks compared to baseline ($p<0.001$). This protection increased with prolonged use of Bend Beauty Anti-Aging Formula as demonstrated by progressively increased MED between baseline and 4 weeks, and again between 4 and 8 weeks ($p<0.001$). 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 to baseline. Treatment was well tolerated with no product associated adverse events (AE) and only a few mild and expected side effects. Results of this study showed that Bend Beauty Anti-Aging Formula safely and effectively provides significant skin photo-protection that increases with continued use.

4.2 Omega-3 Fatty Acids

Fatty acids, including the omega-3s, EPA and DHA, are found in dietary fat and are components of every cell membrane in the body. The types of fatty acids in the diet influence body composition, and are crucial to its function and health. They are structural components of phospholipids that comprise cell membranes and can affect their fluidity and flexibility thereby modulating the behaviour of membrane bound proteins including receptors, enzymes and ion channels that dictate cell function. They are involved in the transport and disposal of cholesterol, and are responsible for the impermeability of the skin to water and for regulation of permeability in the gut and other tissues. In addition, they are precursors for hormone-like substances that regulate a broad spectrum of functions including blood pressure control, inflammation and immunity (Griffiths 2006).

EPA and DHA are derived primarily from fish and fish oils. Although, our bodies naturally produce some EPA and DHA, the amount is too small and irregular to ensure proper biochemical functioning, with DHA synthesis being the most affected and males being particularly disadvantaged in this respect (Gerster 1998,

Simopoulos 1999). In addition, limited storage of the n-3 fatty acids in adipose tissue suggests that a continued dietary supply is needed (Arterburn 2006). Currently, the dietary intake of these two nutrients is grossly inadequate according to recommendations for general populations, by dozens of government departments, international bodies, and formal and informal scientific societies and groups (Global Recommendations). The current US daily dietary intake of EPA+DHA is only 40 mg in children and teens, and 90 mg in adults (Omega-3 Fatty Acids Fact Sheet). Canadian children only eat 92.5 mg EPA + DHA daily (Madden 2009), while Canadian adults eat only 47-160 mg of DHA per day (Innis 2003, Denomme 2005, Fratesi 2009, Xie 2008). This intake data, combined with the significant efficacy of omega-3s against UV skin damage (see Section 4.2.5 Human Intervention Trials), leads one to speculate that nearly the entire North American population could benefit from photo-protection through omega-3 supplementation.

4.2.1 Omega-3 Pharmacokinetics

The pharmacokinetics of fatty acid is relatively more complex than standard drug treatments where a pure, single ingredient substance is ingested, absorbed, distributed, metabolized and excreted. Since fatty acids are structural components within the body, they can become incorporated into membranes, they are precursors for a wide variety of regulatory molecules and can be processed through beta-oxidation as a source of energy. As well, this process occurs gradually, over a long period of time following repeat ingestion. Consequently, any standard models for elucidation of their pharmacokinetics are of limited use and provide little relevant or significant information. The following is an example of the inappropriateness of such studies.

A study on the pharmacokinetics of an omega-3 oil in humans compared the relative absorption and effect on platelet function, of concentrated fish oil and tuna given to 10 subjects in a randomized crossover study. Although plasma enrichment of EPA from either preparation was similar, relative absorption of EPA from tuna was significantly greater than that from fish oil (46.6 +/- 3.0 mg/L/g EPA from tuna compared with 16 +/- 1.0 mg/L/g EPA from fish oil, P less than 0.001). Relative absorption of DHA was equivalent (54.0 +/- 9.0 mg/L/g DHA from tuna, 56 +/- 9.0 mg/L/g DHA from fish oil, NS). Platelet aggregation was significantly diminished after either preparation but bleeding time, and membrane omega-3 fatty acid content were not changed. Thus, omega-3 fatty acids are well absorbed after one dose of either tuna or fish oil but EPA absorption appears to be more efficient from tuna.

Additionally, a single dose of omega-3 fatty acids decreases platelet aggregation by a mechanism not requiring incorporation into platelet membranes. (Silverman et al. 1991)

This design is completely inappropriate for assessment of a fatty acid supplement since it only included a single dose of product. Numerous studies in various disease states have shown that weeks of daily treatment is typically necessary before an accurate assessment can be made of the product effectiveness that corresponds to significant changes in membrane/blood fatty acid profiles (Horrobin. 1990).

In contrast to the above study, three dosages (3 g, 6 g, 12 g) of two different fish oil preparations for 28 days achieved a rapid increases in EPA and DHA plasma concentrations. There was a dose dependent increase in EPA concentrations whereas DHA plasma concentrations remained relatively constant in all dosages investigated. There was no differences in bioavailability EPA or DHA from the different types of fish oils (Marsen 1992).

A summary review of biodistribution, interconversion, and dose response data reported that EPA, but not DHA concentrations in plasma, increase in response to dietary EPA. Dietary DHA results in a dose-dependent, saturable increase in plasma DHA concentrations and modest increases in EPA concentrations.

Plasma DHA concentrations equilibrate in approximately 1 month and then remain at steady state throughout supplementation. DHA doses of approximately 2 g/d result in a near maximal plasma response. Both dietary DHA and EPA reduce plasma AA concentrations. Tissue contents of DHA and EPA also increase in response to supplementation with these fatty acids (Arterburn 2006).

4.2.2 Omega-3 Pharmacodynamics/Mechanism of Action

The balance of omega-6 and omega-3 polyunsaturated fatty acids determine the development and severity of the inflammatory responses. A high intake of omega-6 fatty acids, particularly AA, potentiates inflammatory processes while a higher omega-3 fatty acid intake has anti-inflammatory effects (Calder 2006). Therefore, correcting the omega-6 and omega-3 balance may benefit patients with skin inflammation. The omega-3 polyunsaturated fatty acids work through a plethora of anti-inflammatory mechanisms (Calder 2009).

Within skin, the long-chain polyunsaturated fatty acid (LC-PUFA) derivatives of the parent omega-3, alpha-linolenic acid (ALA), are immune modulators (McMusker 2010). These, along with omega-6 derived LC-PUFAs act through a variety of independent mechanisms as well as those involving peroxisome proliferator-activated receptors (PPARs) and Toll-like receptors (TLRs) to (McCusker 2010):

- maintain the startum corneum permeability barrier and skin appearance
- control maturation and differentiation of the stratum corneum and formation and secretion of lamellar bodies
- inhibit pro-inflammatory eicosanoids, cytokines [tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and interleukin (IL)-12] and lipoxygenase
- elevate the sunburn threshold
- promote tissue repair and apoptosis in malignant cells.

The outcome of these actions is clinical benefit for many skin disorders including atopic eczema (dermatitis), psoriasis, acne vulgaris, systemic lupus erythematosus, nonmelanoma skin cancer and melanoma (McCusker 2010). In addition, they reduce ultraviolet (UV) damage/photoaging (Pilkington 2014, Rhodes 2003, Orengo 1992, Rhodes 1995, Rhodes 1994), enhance wound healing (Hong 2014, Bohr 2013) and the structure, function and appearance of healthy skin (Muggli 2005, Muggli 2007), and may reduce side-effects associated with other treatments including less degree of erythema caused by isotretinoin (Fabbrocini 2014) and hyperlipidemia and nephrotoxicity side effects associated with retinoid and cyclosporine therapy typically used to treat psoriasis (Allen 2001).

4.2.3 Omega-3 Preclinical Studies

Excess inflammation and altered inflammatory mediator metabolism has been suspected to contribute to skin aging and the pathogenesis of a number of skin conditions, such as UV sensitivity (Miller 1994), acne vulgaris (reviewed in (Taylor 2011), acne rosacea (Millikan 2004), eczema (Ruzicka 1986, Fogh 1989, Ikai 1993, Bieber 2008, Nicolaou 2012), hives (Sabroe 1997), and psoriasis (Brain. 1984, Grabbe 1984, Ruzicka 1986, Stone 1990, Sabroe 1997, Nicolaou 2012).

Preclinical studies in relation to atopic eczema, show that increased EPA, DHA (Park 2011) and their metabolites (Kiss 2015) within skin, reduce inflammation partly through suppressing production of leukotriene B4 (LTB4) (Yoshida 2016) and the pro-inflammatory protein poly(ADP-ribose) polymerase

(PARP). Resolvin E1, derived from EPA, inhibits dendritic cell migration in the skin (Sawada 2015) and suppresses development of eczema-like lesions by reducing IL-4 and IFN-γ of activated CD4(+) T cells, serum IgE levels and skin infiltration by immune cells including eosinophils, mast cells, and CD4(+) and CD8(+) T cells (Kim 2012). DHA suppresses atopic dermatitis symptoms partly through transforming growth factor-β and IL-10 conversion of CD4 T cells to CD4 FoxP3 T regulatory cells by M2 macrophages (Haitz 2015).
Preclinical studies indicate that DHA supplementation reduces allergic sensitization to whey and allergic skin response symptoms to both whey and peanut (van den Elsen 2014). When combined with AA, it reduces the severity of allergen-induced dermatitis through a variety of mechanism (Weise 2011 & 2013).

Within keratinocytes, apoptosis is typically induced as a protective mechanism after acute exposure to UV radiation (Serini 2011). However, apoptosis resistance and carcinogenesis can follow chronic UV exposure (Serini 2011). Preclinical studies report that EPA and DHA can induce apoptosis and inhibit growth of pre-malignant keratinocytes (Nikolakopoulou 2013), inhibit cell proliferation in malignant melanoma cells (Zajdel 2013), delay the development of UVB-induced skin tumours and decrease tumour formation (Lou 2011).

EPA and DHA inhibit IL-8 production that may partly account for their anti- inflammatory effects yielding UV-protection in skin (Storey 2005). An in vitro study showing this included cells supplemented with ≥90% purified EPA, DHA, oleic acid, or control for 4.5 days. Prior to supplementation, UVB-induced IL-8 release increased dose-dependently in various cell lines (ie. keratinocytes, which are the most common cell type in the epidermis; and fibroblasts, which synthesize collagen). In keratinocytes, EPA and DHA reduced IL-8 secretion by 66% and 63%, respectively ($p<0.05$), and UVB-induced levels by 66% and 65% at 48 h, respectively ($p<0.01$); a similar pattern occurred in fibroblasts. Oleic acid had no influence on IL-8 release in either cell line. In addition, tumour necrosis factor-α (TNF-α)-induced IL-8 secretion by keratinocytes was reduced by 54% and 42%, respectively, by EPA and DHA ($p<0.001$). These results suggest that EPA and DHA both inhibit production of induced IL-8 in skin cells, providing a possible mechanism for their UV-protective and anti- inflammatory effects.

As well, in a 39 week study, hairless SKH-1 mice were irradiated with 30 mJ/cm^2 of UVB once daily, two times per week and consumed either a high-fat fish oil (HFFO) diet rich in omega-3 fatty acids or a high-fat mixed-lipids (HFML) diet rich in omega-6 fatty acids. Compared with the HFML diet, the HFFO diet delayed the development of UVB-induced skin tumours and decreased the formation of tumours. HFFO also significantly decreased UVB-induced increases in several pro-inflammatory mediators and decreased UVB-induced programmed cell death in the epidermis (Lou 2011).

4.2.4 Omega-3 Human Epidemiological Studies

Epidemiological studies report that omega-3 supplementation is associated with reduced UVB-erythemal sensitivity and photo-aging (Latreille 2013). A 20 year observation study of Inuit who typically have a fish-rich diet, found low rates of melanoma and nonmelanoma skin cancer (Rhodes 2003). In a cohort of 20,785 women followed for 4.5 years, higher EPA/DHA intake was associated with an 80% lower risk of malignant melanoma (Donat-Vargas 2017). Other epidemiological studies report a direct dose-response relationship between serum concentrations of EPA and DHA and the tumour suppressor, TP53, in the whole epidermis and basal layer (van der Pols 2011), and lower incidence of squamous cell carcinoma in people with higher plasma EPA and omega-3/6 ratio (Wallingford 2013).

High dietary intake of omega-3 LC-PUFAs is associated with low acne rates, and primary signs of acne including oily skin, papules, pustules and acne cysts are significantly lower among teenagers eating diets

high in saltwater fish and seafood (Katzman 2007). Omega-3s inhibit LTB4 synthesis which reduces oil production (Katzman 2007) and inflammation through TLRs, making them likely candidates for acne treatment (McCusker 2010).

4.2.5 Omega-3 Human Intervention Trials

In randomized, double-blind, placebo-controlled (R, DB, PC) trials in people with atopic eczema, 12 weeks treatment with 5.4 g DHA daily significantly improved symptom severity scores (Dölle 2008), while 12 weeks of supplementation with 1.8 g EPA/day significantly improved scale, itch and overall subjective severity (Bjorneboe 1987).

In people with psoriasis, intravenously administered EPA+DHA improves erythema, scaling, inflammatory infiltrate, and body surface area of psoriatic lesions (Mayser 1998). High oral dosing is necessary to increase EPA+DHA skin content (Mayser 1998) that reduces inflammatory LTB4 (Ziboh 1986, Maurice 1987a, & 1987b), and reduces skin redness and scaling (Maurice 1987a, & 1987b). However, 8 weeks oral supplementation with as little as 1122 mg EPA + 756 mg DHA as ethyl ester reduced the Psoriatic

Association Score, with itch responding most rapidly (Lassus 1990). Of the 80 patients treated, 7 were completely healed, 13 were >75% healed and only 14 were non-responders. Omega-3s also reduce hyperlipidemia and nephrotoxicity side effects associated with retinoid and cyclosporine therapy respectively (Allen 2001), and so are an attractive adjunct therapy for psoriasis.

A 10 week R, PC trial treating 45 participants with mild to moderate acne taking either 2000 mg/day EPA + DHA, or 400 mg/day GLA from borage or placebo reported significant improvements in both treatment groups for inflammatory and non-inflammatory acne lesions accompanied by reduced inflammation and IL-8 (Jung 2014).

Numerous R, DB, PC (Pilkington 2014, Rhodes 2003, Orengo 1992) and open trials (Rhodes 1994 & 1995) have reported significantly increased UV- induced sunburn threshold following 1800 mg – 5 g/day EPA alone (Pilkington 2014, Rhodes 2003) or with 1200 mg/day DHA (Orengo 1992, Rhodes 1994 & 1995) for as little as 4 weeks (Orengo 1992). In addition, sunburn threshold rose progressively with increasing time throughout treatment, but fell 10 weeks after treatment cessation, indicating that routine continuous supplementation provides better protection (Rhodes 1994).

Details of the studies mentioned above follow:

4.2.5.1 Omega-3 Open Label Trials

PSORIASIS

- **Ziboh 1986** – Thirteen psoriasis patients consumed 60 to 75 grams per day of fish oil; each gram contained 180 mg EPA and 120 mg DHA, for 8 weeks. The fish oil supplementation resulted in mild to moderate improvement in psoriatic lesions in 8 patients ($p < 0.05$) and improved clinical response correlated with high EPA/DHA ratios attained in skin tissue specimens. It was suggested that a possible increase in the ratio of leukotriene B5 (derived from EPA) to leukotriene B4 [derived from AA], was responsible for the reduction in inflammation.
- **Maurice 1987a&b)** – Ten patients with severe chronic psoriasis that was resistant to conventional topical treatment were supplemented with fish oil containing approximately 12 g EPA daily for a

minimum of 6 weeks. A reduction in skin redness and scaling was observed in 8 of 10 patients after treatment. The fish oil treatment also resulted in a substantial inhibition of inflammatory leukotriene B4 production.

- Lassus 1990 – In this study, 80 patients with chronic, stable psoriasis were treated daily with 1122 mg EPA and 756 mg DHA in ethyl ester form; 34 subjects also had psoriatic arthritis. At baseline, the mean Psoriatic Association scoring index (PASI) score was 3.56. After 4 and 8 weeks of treatment, the scores dropped to 1.98 and 1.24, respectively ($P < 0.001$). The degree of itching decreased most rapidly, followed by scaling and hardening of the plaques, and skin redness was most persistent. At the end of the trial, 7 patients were completely healed and >75% healing was observed in 13 patients. Although the result was poor in 14 patients, the authors concluded that polyunsaturated fatty acids may be useful for the treatment of psoriasis and may provide an important adjuvant to standard therapy.

UV PROTECTION

- Rhodes 1994 – This study involving 15 subjects who consumed 10 g fish oil daily (1800 mg EPA and 1200 mg DHA) for either 3 or 6 months was conducted to examine the effect of fish oil on susceptibility to UVB-induced skin redness and oxidation in the skin's epidermal layer. Paired skin shave biopsies were taken from 6 subjects at baseline and 3 months, from both irradiated and control skin. Epidermal total omega-3 fatty acids increased significantly throughout the 3 months of treatment ($p < 0.01$). In addition, sunburn threshold rose progressively with increasing time throughout the fish oil treatment ($p < 0.01$) but fell again 10 weeks after stopping the fish oil treatment ($p < 0.05$). These results suggest that omega-3 fatty acids produce a pronounced reduction in UVB-induced skin reddening.

- Rhodes 1995 – This study involving 13 Caucasian subjects with moderate to severe sun allergy (11 female, two male; median age 45 years) was conducted to examine the effect of fish oil (1800 mg EPA daily and 1200 mg DHA daily) on UVB-induced PG metabolism. Fish oil was taken as 5 capsules twice daily of MaxEPA with each capsule containing 1 g of oil (18% EPA and 12% DHA). A forearm graded UVA challenge was used to assess the threshold dose for initiation of blisters. Suction blisters were also induced on the other forearm, on control skin of 11 healthy volunteers, and on skin irradiated with four times the sunburn threshold of UVB 24 h previously. Following 3 months of fish oil supplementation, the mean sunburn threshold increased ($p < 0.01$) and while the forearm graded UVA challenge was positive in 10 patients at baseline, 9 of these subjects showed reduced sensitivity to blister initiation after 3 months ($p < 0.001$). In addition, compared to control skin, the UVB- induced increase in inflammatory PGE2 production was significantly attenuated by 3 months of fish oil consumption ($p < 0.05$). These results suggest that UV-induced inflammation may be partially lowered by fish oil's PGE2-lowering effects.

4.2.5.2 Omega-3 Randomized, Double-Blind, Placebo-Controlled Trials

PSORIASIS

- Mayser 1998 – This study investigated the treatment effects of intravenous omega-3 fatty acids on chronic psoriasis. Subjects included 83 patients hospitalized for chronic plaque-type psoriasis with a severity score of at least 15 according to the Psoriasis Area and Severity Index (PASI). Subjects were randomly allocated to receive daily infusions with either an omega-3 fatty acid-based lipid emulsion

(Omegavenous;200 ml/day with 4.2 g of both EPA and DHA) or a conventional omega-6- lipid emulsion (Lipovenous; EPA+DHA < 0.1 g/100 ml) for 14 days. Results revealed decreases in total PASI scores by 11.2 and 7.5 in the omega-3 and omega-6 groups, respectively (p = 0.048). The omega-3 group was superior to the omega-6 group with respect to change in severity of psoriasis per body area, change in overall skin redness, and overall scaling, as well as change in overall assessment by the investigator and self-assessment by the patient. A decrease in total PASI of ≥50% between admission and last value was seen in 37% of the omega-3 treatment group versus 23% of the omega-6 group. The authors concluded that intravenous omega-3-fatty acid administration is effective in the treatment of chronic plaque-type psoriasis and that the effect may be related to changes in inflammatory mediator generation.

ATOPIC ECZEMA

- **Dölle 2008 –** This 8-week study investigated the efficacy of DHA in the treatment of eczema in 53 subjects (aged 18-40 years). Subjects received either 5.4 g DHA daily or a control product and at weeks 0, 4, 8, and 20, clinical outcome was assessed by the Severity Scoring of Atopic Dermatitis (SCORAD) index. DHA resulted in a significant clinical improvement of eczema whereas the control treatment did not lead to significant improvements. DHA also decreased SCORAD by week 8. The authors concluded that dietary DHA may have a beneficial impact on the outcome of eczema.

- **Bjorneboe 1987 –** This 12-week study investigated the effects of an omega-3 fatty acid supplement containing 1.8 g EPA on eczema. Results revealed that compared to controls, the treatment group experienced favorable results with regard to scale ($P < 0.05$), itch ($P < 0.05$), and overall subjective severity ($P < 0.02$).

UV PROTECTION

- **Pilkington 2014 –** This study examined the impact of EPA intake on levels of AA, EPA and their resulting eicosanoids in human skin with or without ultraviolet radiation (UVR) challenge. Seventy-nine females took 5 g EPA-rich or control lipid for 12 wk. EPA supplementation increased red blood cell and dermal EPA versus control and lowering relative AA:EPA content. Pre-supplementation, UVR increased various pro-inflammatory metabolites including prostaglandin (PGE)2, 12-hydroxyeicosatetraenoic acids, 12-HEPE and PGE3. Post-EPA, PGE2 was reduced in unchallenged skin while EPA-derived PGE3 and 12-HEPE were elevated post-UVR. Thus, post-EPA, PGE2 :PGE3 was lower in unchallenged and UVR exposed skin; and 12-hydroxyeicosatetraenoic acids:12-HEPE was lower in UVR-exposed skin. The results of this study showed that supplemental EPA augments skin EPA:AA content, shifting eicosanoid synthesis towards less pro-inflammatory species, and promoting a regulatory milieu under basal conditions and in response to inflammatory insult.

- **Rhodes 2003 –** This 3 months study in 42 healthy subjects consuming 4 g daily of purified EPA or monounsaturated (oleic acid), examined the effect of omega-3 supplementation on a range of indicators of UV-induced DNA damage in humans. Sunburn sensitivity was reduced with EPA supplementation; compared to baseline, the UVR-induced sunburn threshold increased significantly after supplementation ($p < 0.01$).

- **Orengo 1992 –** This 4-week study found that fish oil supplementation [2800 mg EPA and 1200 mg DHA] increased the sunburn threshold in 20 subjects ($p<0.02$).

A summary table of the Human Intervention Trials follows:

REFERENCE	TRIAL DESIGN	POPULATION	TREATMENT	RESULTS
Ziboh 1986	Open label	13 **psoriasis** patients	60 – 75 g per day of fish oil (each gram contained 180 mg EPA and 120 mg DHA), for 8 weeks.	• Mild to moderate improvement in psoriatic lesions in 8 patients ($p < 0.05$) and improved clinical response correlated with high EPA/DHA ratios attained in skin tissue specimens. • A possible increase in the ratio LTB5 (derived from EPA) to LTB4 [derived from AA], was responsible for inflammation reduction.
Maurice 1987a&b	Open label	10 patients with severe chronic **psoriasis** that was resistant to conventional topical treatment	Fish oil containing approximately 12 g EPA daily for a minimum of 6 weeks.	• A reduction in skin redness and scaling was observed in 8 of 10 patients • Substantial inhibition of inflammatory leukotriene B4 production.
Lassus 1990	Open label	80 patients with chronic, stable **psoriasis**; 34 subjects also had psoriatic arthritis	1122 mg EPA and 756 mg DHA in ethyl ester daily for 8 weeks	• Mean Psoriatic Association scoring index (PASI) dropped to 1.98 and 1.24, four and eight weeks post treatment respectively ($P < 0.001$). • Itching decreased most rapidly, followed by scaling and hardening of the plaques • Skin redness was most persistent. • At trial end, 7 patients were completely healed and >75% healing was observed in 13 patients.
Rhodes 1994	Open label	15 **healthy** subjects	10 g fish oil daily (1800 mg EPA and 1200 mg DHA) for either 3 or 6 months	• Total omega-3 fatty acids increased significantly ($p < 0.01$). • Sunburn threshold rose progressively with increasing time throughout the fish oil treatment ($p < 0.01$) but fell 10 weeks after stopping treatment ($p < 0.05$).
Rhodes 1995.	Open label, case-control	13 caucasian subjects with **moderate to severe sun allergy**	MaxEPA Fish oil (1800 mg EPA and 1200 mg DHA daily) for 3	• Mean sunburn threshold increased ($p < 0.01$) • Forearm graded UVA challenge was positive in 10 patients at baseline, 9 of

		(11 female, two male; median age 45 years) and 11 healthy volunteers	months	these subjects showed reduced sensitivity to blister initiation after 3 months ($p < 0.001$). • Compared to control skin, the UVB-induced increase in inflammatory PGE2 production was significantly attenuated ($p < 0.05$). • These results suggest that UV-induced inflammation may be partially lowered by fish oil's PGE2-lowering effects.
Mayser 1998	R, DB, PC	83 patients hospitalized for **chronic plaque-type psoriasis** with a severity score of at least 15 according to the Psoriasis Area and Severity Index (PASI).	Daily infusions with either an omega-3 fatty acid-based lipid emulsion (Omegavenous; 200 ml/day with 4.2 g of both EPA and DHA) or a conventional omega-6 lipid emulsion (Lipovenous; EPA+DHA < 0.1 g/100 ml) for 14 days	• Total PASI scores decreased by 11.2 and 7.5 in the omega-3 and omega-6 groups, respectively ($p = 0.048$). • Symptom severity per body area, skin redness, scaling, and overall assessment by the investigator and the patient improved more in the omega-3 group than the omega-6 group. • A decrease in total PASI of ≥50% between admission and last value was seen in 37% of the omega-3 treatment group versus 23% of the omega-6 group.
Dölle 2008	R, DB, PC	53 subjects with **atopic eczema** (aged 18 – 40 years).	5.4 g DHA or placebo daily at weeks 0, 4, 8, and 20	• DHA resulted in a significant clinical improvement of eczema whereas the control treatment did not lead to significant improvements. • DHA decreased SCORAD scores by week 8.
Bjorneboe 1987	R, DB, PC	Subjects with **atopic eczema**	1.8 g EPA or placebo for 12 weeks	• Compared to controls, the treatment group had improved scale ($P < 0.05$), itch ($P < 0.05$), and overall subjective severity ($P < 0.02$).
Pilkington 2014	R, DB, PC	79 **healthy** females	5 g EPA-rich or placebo for 12 weeks	• EPA increased RBC and dermal EPA versus control and lowering relative AA : EPA content. • Pre-EPA, UVR increased various pro-inflammatory metabolites including

				prostaglandin (PGE)2, 12-hydroxyeicosatetraenoic acids, 12-HEPE and PGE3. • Post-EPA, PGE2 was reduced in unchallenged skin while EPA-derived PGE3 and 12-HEPE were elevated post-UVR. • Thus, post-EPA, PGE2 :PGE3 was lower in unchallenged and UVR exposed skin; and 12-hydroxyeicosatetraenoic acids:12-HEPE was lower in UVR-exposed skin. • EPA supplemental augments skin EPA:AA content, shifting eicosanoid synthesis towards less pro-inflammatory species, and promoting a regulatory milieu under basal conditions and in response to inflammatory insult.
Rhodes 2003	R, DB, PC	42 **healthy** subjects	4 g daily of purified EPA or monounsaturated (oleic acid) placebo for 3 months	• Sunburn sensitivity was reduced with EPA supplementation; compared to baseline. • UVR-induced sunburn threshold increased significantly after supplementation ($p < 0.01$).
Orengo 1992	R, DB, PC	20 **healthy** subjects	Fish oil supplementation [2800 mg EPA and 1200 mg DHA daily} for 4 weeks	• Sunburn threshold significantly increased following supplementation ($p<0.02$).

4.3 Gamma-linolenic Acid

Gamm-linolenic acid (GLA) is an omega-6 fatty acid derived from LA. Dietary GLA is relatively scarce, but it is a significant component of human milk and is abundant in evening primrose oil (EPO) and borage oil (Horrobin 1990). No LC-PUFA has been researched more thoroughly in human skin related intervention trials than GLA.

4.3.1 GLA Pharmacokinetics

Similar to omega-3s, elucidation of the pharmacokinetics of omega-6 fatty acids using standard techniques are of limited use. One study reporting the pharmacokinetics of EPO in humans, was a serum level-time course of 8 fatty acids after the administration of Epogam (a brand of EPO). From 6 volunteers, serum concentration time curves of GLA and 7 other fatty acids were profiled 24 h with and without the administration of Epogam. Six capsules of Epogam were administered to each subject in the morning at 7:00 and further 6 capsules in the evening at 19:00. On the days of investigation the volunteers had a diet of low

fat meals. The serum concentrations of the fatty acids were determined as their methyl esters by means of gas chromatography mass spectrometry. GLA shows an absorption-elimination pattern after the administration of Epogam and its AUC24h and Cmax were significantly increased over the baseline values. After the evening administration, t(max) was shorter (2.7 +/- 1.2 h) than after the morning administration (4.4 +/- 1.9 h). The other fatty acids showed no significant increase in their concentrations, especially DGLA and AA, which are metabolic products of GLA. The authors concluded that an effect of the administration of GLA on the serum concentrations of DGLA and AA could not clearly be established in healthy volunteers (Martens-Lobenhoffer and Meyer 1998).

4.3.2 GLA Pharmacodynamics/Mechanism of Action

The parent omega-6 fatty acid, linoleic acid (LA) and its products including GLA are structural precursors of the stratum corneum ceramides and can impact inflammation (McMusker 2010). See section 4.2.2 Omega-3 Pharmacodynamics/Mechanism of Action for further details on the mechanism of action.
GLA exhibits anti-inflammatory properties through its intermediate product, dihommogamma-linolenic acid (DGLA), where an increase in DGLA relative to AA within inflammatory cells may attenuate the biosynthesis of inflammatory AA metabolites (Kapoor 2006). This dependence on conversion of various dietary fatty acids including LA and GLA to further metabolites to maintain homeostasis, can be particularly significant in certain segments of the population that carry underperforming alleles for some of the enzymes in the fatty acid metabolic pathway. For example, in people with atopic eczema, there is defective enzyme conversion of LA through to its metabolites including GLA, based on a study showing strong associations between blood fatty acid composition and variants in the following two human genes (Schaeffer 2006):

- FADS2 that codes for delta-6-desaturase (D6D), the enzyme that converts
    - LA to GLA
    - and is required along with various elongases to convert omega-3 LC-PUFAs between ALA to EPA as well as EPA to DHA
- FADS1 that codes for delta-5-desaturase (D5D), the enzymes that converts
    - DGLA to AA
    - and is required along with an elongase to convert to omega-3 LC-PUFAs between ALA to EPA

These abnormalities in atopics raised the possibility that supplementation with particular LC-PUFAs including GLA could bypass these defects and provide clinical benefit.

Dependence on conversion of dietary fatty acids to further metabolites is further complicated because LA and ALA are converted to their corresponding metabolites through a shared set of enzymes that alternately desaturate and elongate the molecules. This results in competition between the omega-6s and omega-3s. For example, higher intake of EPA can block the conversion of DGLA to AA and thereby increase the concentration of DGLA and subsequently, the production of anti-inflammatory prostaglandin E1 from DGLA. As a consequence, combinations of omega-3 and omega-6 fatty acids may be more beneficial in treating inflammatory skin conditions than omega-3 or omega-6 fatty polyunsaturated fatty acids alone (Boon 2004). Further, combining EPA with GLA has been shown to prevent the unwanted increase in AA (Barham 2000) that may occur with chronic borage oil supplementation (Surette 2003). This lends credibility to the formulations of Bend Beauty Anti-Aging Formula that combines EPA, DHA, and GLA for their combined anti- inflammatory activities.

4.3.3 GLA Human Epidemiological Studies

The notion that LC-PUFA deficiency might contribute to atopic eczema was first proposed by Hansen in the 1930s, who noted that EFA deficient animals had skin lesions similar to people with the condition, and atopic eczema patients being treated with a substantial quantity of LA had near normal blood LA levels, whereas their AA levels were far below normal (Hansen 1937). These results were substantiated in a case-control study in the 1980s when LA was reported to be slightly above normal, but its metabolites including GLA, DGLA and AA were all significantly below normal (Manku 1984), indicating a defect in D6D activity.

4.3.4 GLA Human Intervention Trials

4.3.4.1 GLA Meta-analyses

ATOPIC ECZEMA

To date, at least 5 meta-analyses and/or systematic reviews have evaluated the impact of GLA on atopic eczema with varying outcomes owing to study designs and selection criteria (Horrobin 2000, Morse 1989, Morse 2006, Bamford 2013, Foster 2010).

- Morse 1989 – A meta-analysis of over 300 patients ranging from 1 to 60 years of age taking from 80-480 mg GLA/day from EPO with most taking 320 mg daily for 12 weeks, found those with the greatest increases in erythrocyte membrane GLA, DGLA and AA following treatment, also had the greatest skin improvements. These improvements exceeded those achieved using their conventional long-term maintenance therapy, for overall global assessment and in particular for itch (Morse 1989). Another review found GLA combined with emollients was more efficacious than when combined with mild potent steroids (Morse 2006). Such masking effects attributed to steroids are best overcome, without causing symptom flare-ups, by weaning patients off of steroids starting 8-12 weeks into their GLA treatment to allow time for GLA induced beneficial membrane fatty acid changes to occur.
- Morse 2006 – A meta-analysis of 26 R, DB, PC spanning over two decades of clinical testing included 1207 patients aged 1 to 28.5 years, who took Efamol® EPO providing 80-640 mg of GLA daily for 3 to 16 weeks. Results of this study agreed with those of the Morse 1989 meta-analysis, showing that GLA is a safe and effective remedy for symptomatic relief of atopic eczema with simultaneous benefits on itch, crusting, oedema and redness that become apparent between 4 and 8 weeks after treatment is initiated. However, the magnitude of this effect is reduced in association with increasing frequency of potent steroid use. (Further discussion pertaining to steroid interference with GLA efficacy can be found in Horrobin 2000.) As well, GLA was deemed to be most effective in a subset of patients with elevated immunoglobulin (Ig) E.
- Foster 2010 – A 2010 critical review of 11 R, DB, PC clinical studies including 103 to 1000 mg/day of GLA from borage oil, reported that studies were small or had methodological limitations, and results were highly variable. But most showed at least some benefit to patients with less severe eczema. The 4 negative outcome trials also had large placebo effects, which could have confounded the results.
- Bamford 2013 – Contrasting the positive results mentioned above, a 2013 Cochrane Review of R, PC trials including 1596 participants from 19 EPO and 8 borage oil studies, reported no significant improvement in global eczema symptoms compared to placebo according to both doctors and patients. However, this analysis did not include many of the studies reported in the previous meta-analyses and that could have impacted the conclusion.

4.3.4.2 GLA Open Label Trials

ATOPIC ECZEMA

- Stewart 1991 – A study investigated the effects of 4000 to 6000 mg EPO (a source of GLA) in 179 patients with moderate to severe eczema. The majority (62%) of patients saw improvements in at least some of the symptoms assessed (skin redness, dryness, scaling, itch, and edema) with EPO treatment. Many of the patients also reduced or stopped taking their conventional medication for the treatment of eczema, which is a testament to the significant improvements noted from EPO supplementation. The authors concluded that treatment with EPO provided clinically important improvements in patients with eczema and provides a useful addition to the range of treatments available for this condition.
- Kawamura 2011 – In a 12-week study, 112 subjects with dry skin and mild eczema consumed GLA-containing foods (200 mg GLA daily). GLA was found to have beneficial effects on skin water loss. The efficacy of GLA was demonstrated to be statistically significant in subjects with pro-inflammatory features, suggesting that skin barrier improvement may be associated with the generation of
anti-inflammatory metabolites from GLA. The clinical physician also confirmed that none of the study subjects showed any noteworthy side effects. The authors concluded that the consumption of GLA-enriched food appears to be safe and seems to improve skin barrier function in subjects with dry skin conditions and mild eczema.

PEOPLE WITH HEALTHY SKIN

- Brosche 2000 – In a 2-month study, 29 healthy elderly people (mean age 68.6 years) received a daily dose of 360 or 720 mg GLA from borage oil (Quintesal 180, manufacturer Galderma Laboratorium GmbH, Freiburg, Germany). The consumption of borage oil induced a statistically significant improvement of skin barrier function, which was reflected in a mean decrease of 10.8% in skin water loss ($p<0.05$). Dry skin was claimed to be reduced from 42% to 14% with no significant alterations in skin hydration measured and while 34% of the people noted itch at baseline, 0% noted itch after borage oil supplementation. The fatty acid composition of erythrocyte membrane phospholipids demonstrated an increase of GLA and DGLA and an increase in the DGLA/AA ratio. The authors concluded that borage oil consumption may lead to alterations in fatty acid metabolism and improved skin function in elderly people.

ACNE

- Lee 2014 – Acne is reportedly improved through 5-alpha-reductase inhibition that can be achieved via GLA supplementation. Twelve weeks treatment with 320 mg/day GLA significantly reduced inflammatory lesions and sebum secretions without hormonal changes, in 10 patients with mild-to moderate facial acne vulgaris.

4.3.4.3 GLA Randomized, Double-Blind, Placebo-Controlled Trials

Independent R, DB, PC trials in atopic eczema patients, published subsequent to the meat-analyses described in *Section 4.3.4.1 GLA Meta-analyses*, including similar dosages and treatment durations to the meta-analyses described previously, showed GLA improves symptoms within 8 weeks while increasing blood levels of GLA and AA in a dose dependent manner (Chung 2013), and increases plasma DGLA while increased GLA levels at 4 weeks correlate with symptom improvement (Simon 2014). Thus, patients without increased plasma GLA could be non-responders and their therapy ceased while those with increased GLA levels could proceed and expect to achieve successful outcomes. In addition, GLA significantly improved barrier function through reduced transepidermal water loss (TEWL), especially in those with high levels of pro-inflammatory eicosanoids and/or low levels of anti-inflammatory eicosanoids suggesting that GLA acts partly through its anti-inflammatory effects (Kawamura 2011).

Details of some additional studies follow:

ATOPIC ECZEMA

- **Bahmer 1992** – A trial of 12 patients (aged 20 to 48 years) investigated the efficacy of 3000 mg [720 mg of GLA] oral borage supplementation to change the severity of eczema. Using the Atopic Dermatitis Area and Severity Index (ADASI) score, analysis revealed that 5 of 7 patients receiving borage oil experienced significant improvements in eczema severity. On the other hand, in the placebo group, which consisted of 5 adults, only 1 person experienced improvement while the score worsened for 3 people.

- **Buslau 1996** – This study investigated the efficacy of 2000 mg borage oil daily in the treatment of mild to moderate eczema in 50 adults (aged 18–61 years). Subjects were allowed to use topical ointments and creams but no other drugs throughout the study duration. Over the 12-week study period, 78% of the borage oil group had significant improvements in the ADASI score compared to 43% in the placebo group ($p < 0.05$). The borage oil group also experienced a significant rise in serum dihomo-α-linolenic acid (DGLA) levels ($p < 0.05$), which is a product of GLA metabolism. The borage oil group also experienced some increases in GLA as well as arachidonic acid (AA), a key inflammatory intermediate. The authors concluded that these results demonstrate the beneficial role of borage oil in the treatment of mild to moderate eczema.

- **Landi 1993** – This 8 week trial investigated the efficacy of 4000 mg borage oil daily in the treatment of eczema in 24 young adults (aged 12–27 years). The extent and severity of the eczema was assessed by the same dermatologist at baseline and every 4 weeks thereafter. Borage oil was found to significantly improve overall severity, response, area of eczema involvement, and individual symptoms.

- **Melnik 1995** – In this 12-week single-center study involving 20 adults (aged 18-53 years), the efficacy of borage oil (Glandol) in the treatment of eczema was compared with an EPO-containing product (Epoleum; is a source of GLA). A trend analysis was carried out in 10 patients of each group and significant improvements were noted in the Atopic Dermatitis and Severity Index in 9 of 10 patients in the EPO group.

PEOPLE WITH HEALTHY SKIN

The structure and function of healthy skin can be improved with GLA as indicated in a number of PC trials. Twelve weeks treatment with 345 mg/day significantly improved skin moisture, water loss, elasticity, firmness, fatigue resistance and smoothness by 13, 8, 5, 17, 14 and 22% respectively (Muggli 2005), while 475 mg/day significantly decreased water loss and improved skin moisture, smoothness and scaling (De Spirt 2009). These benefits reduce the appearance of wrinkles and provide a more youthful looking skin. Similar benefits were achieved when skin was irritated with a strong detergent prior to GLA treatment, indicating that GLA can both repair damaged skin as well as improve healthy skin (Muggli 2007). Such effects reduce inflammation, and perhaps even reduce sensitization that contributes to developing conditions such as house wife's eczema and contact dermatitis. Details of a few of these studies follows.

- De Spirt 2009 – In a 12week trial, women ingested flaxseed oil, borage oil, or a placebo containing medium-chain fatty acids on a daily basis. The dose was 2.2 g total fatty acids per day with ALA and LA as major constituents in the flaxseed oil group whereas LA and GLA predominated in the borage oil group (i.e., 475 mg per day GLA in the borage oil group). After 6 weeks of supplementation, skin water loss decreased in both treatment groups by about 10 %. After 12 weeks of treatment with flaxseed and borage oil, skin hydration significantly increased and roughness and scaling of the skin significantly decreased compared to baseline (both $p < 0.05$). Except for hydration, none of the parameters was affected in the placebo group. These results suggest that dietary fats have the ability to modulate skin properties.
- Muggli 2005 – This 12 week study in healthy adults tested the effect of Efamol EPO (source of GLA) on skin moisture, skin water loss, redness, firmness, elasticity, fatigue resistance, and roughness. The dosage of Efamol EPO was 3000 mg daily (345 mg GLA). After 12 weeks of treatment, all measured variables, with the exception of skin redness, were significantly different in the EPO group compared with placebo. Skin moisture, skin water loss, elasticity, firmness, fatigue resistance, and roughness significantly improved by 12.9, 7.7, 4.7, 16.7, 14.2, and 21.7%, respectively. The authors concluded that these findings lend further support to the notion that GLA is a conditionally essential fatty acid for the skin.

A summary table of the Human Intervention Trials follows:

REFERENCE	TRIAL DESIGN	POPULATION	TREATMENT	RESULTS
Morse 1989	Meta-analysis	300 patients ranging from 1 to 60 years of age with **atopic eczema**	80 – 480 mg GLA/day from EPO with most taking 320 mg daily for 12 weeks	• Those with the greatest increases in erythrocyte membrane GLA, DGLA and AA following treatment, also had the greatest skin improvements. • Improvements exceeded those achieved using their conventional long-term maintenance therapy, for overall global assessment and in particular for itch
Morse 2006	Meta-analysis	26 R, DB, PC trials spanning over two decades including 1207 patients aged 1 to 28.5 years	Efamol® EPO providing 80-640 mg of GLA or placebo daily for 3 to 16 weeks	• GLA is a safe and effective remedy for symptomatic relief of atopic eczema with simultaneous benefits on itch, crusting, oedema and redness that become apparent between 4 and 8 weeks after treatment is initiated.

		with **atopic eczema**		• The magnitude of the treatment effect is reduced in association with increasing frequency of potent steroid use.
Foster 2010	Meta-analysis	11 R, DB, PC clinical studies with **atopic eczema**	103 to 1000 mg/day of GLA from borage oil or placebo	• Studies were small or had methodological limitations • Results were highly variable • Most studies showed at least some benefit to patients with less severe eczema. The 4 negative outcome trials also had large placebo effects, which could have confounded the results.
Bamford 2013	Meta-analysis	1596 participants with **atopic eczema** from 19 EPO and 8 borage oil studies	EPO, borage or placebo	• No significant improvement in global eczema symptoms compared to placebo according to both doctors and patients. • This analysis did not include many of the studies reported in the previous meta-analyses and that could have impacted the conclusion.
Stewart 1991 –	Open Label	179 patients with **moderate to severe eczema.**	4000 to 6000 mg EPO (a source of GLA)	• 62% of patients saw improvements in at least some of the symptoms assessed (skin redness, dryness, scaling, itch, and edema) with EPO treatment. • Many patients reduced or stopped taking their conventional medication, a testament to the significant improvements noted from EPO supplementation.
Kawamura 2011	Open Label	112 subjects with **dry skin and mild eczema**	GLA-containing foods (200 mg GLA daily) for 12 weeks	• GLA had beneficial effects on skin water loss. • Those with pro-inflammatory features had significant improvements, suggesting that skin barrier improvement may be associated with the generation of anti-inflammatory metabolites from GLA. • There were no side effects.

Brosche 2000	Open Label	29 **healthy** elderly people (mean age 68.6 years)	360 or 720 mg GLA daily from borage oil (Quintesal 180, manufacturer Galderma Laboratorium GmbH, Freiburg, Germany) for 2 months.	• Borage oil produced a statistically significant improvement in skin barrier function as evidenced by a mean decrease of 10.8% in skin water loss ($p<0.05$). • Dry skin reduced from 42% to 14% with no significant alterations in skin hydration • 34% of the people noted itch at baseline, 0% noted itch after borage oil supplementation. • RBC GLA, DGLA and DGLA/AA ratio increased following treatment.
Lee 2014	Open Label	10 patients with **mild-to moderate facial acne vulgari.**	Twelve weeks treatment with 320 mg/day GLA	• Significantly reduced inflammatory lesions and sebum secretions without hormonal changes
Bahmer 1992	R, DB, PC	12 **atopic eczema** patients (aged 20 to 48 years)	3000 mg (720 mg of GLA) oral borage	• 5 of 7 patients receiving borage oil had significantly improved eczema severity using the Atopic Dermatitis Area and Severity Index (ADASI) score. • Only 1 of 5 in the placebo group experienced improvement while the score worsened for 3 people.
Buslau 1996	R, DB, PC	50 adults (aged 18–61 years) with **mild to moderate eczema**	2000 mg borage oil daily for 12 weeks. Subjects were allowed to use topical ointments and creams but no other drugs throughout the study duration.	• 78% of the borage oil group members improved with the ADASI score compared to 43% in the placebo group ($p < 0.05$). • Borage oil significantly increased serum dihomo-α-linolenic acid (DGLA) ($p < 0.05$) (a product of GLA metabolism), and substantially increased GLA and arachidonic acid (AA).- a key inflammatory intermediate.
Landi 1993-	R, DB, PC	24 young adults (aged 12 – 27 years) with **atopic eczema**	4000 mg borage oil daily for 8 weeks	• Borage oil significantly improved overall severity, response, area of eczema involvement, and individual symptoms.

Melnik 1995-	R, DB, PC, single -center	20 adults (aged 18-53 years) with **atopic eczema**	Borage oil (Glandol) and EPO-containing product (Epoleum; as a source of GLA) for 12 weeks	• The Atopic Dermatitis and Severity Index in 9 of 10 patients in the EPO group improved.
De Spirt 2009	R, DB, PC	**Healthy women**	2.2 g Flax oil per day providing ALA and LA, or borage oil providing 475 mg GLA per day or placebo for 12 weeks	• After 6 weeks, skin water loss decreased in both treatment groups by about 10 %. • After 12 weeks skin hydration significantly increased and roughness and scaling of the skin significantly decreased compared to baseline (both $p < 0.05$) in both groups. • Except for hydration, none of the parameters was affected in the placebo group. These results suggest that dietary fats have the ability to modulate skin properties.
Muggli 2005 -	R, DB, PC	40 **healthy** males and females between the ages of 32 and 56 years of age	3000 mg daily Efamol EPO providing 345 mg GLA or placebo for 12 weeks	• Skin moisture, water loss, elasticity, firmness, fatigue resistance, and roughness significantly improved by 12.9, 7.7, 4.7, 16.7, 14.2, and 21.7%, respectively.
Muggli 2007	R, DB, PC	40 **healthy** males and females between 32 and 60 years of age that had dry and rough skin.	Experimental skin irritation was induced by a strong detergent prior to treatment with 3000 mg daily Efamol EPO providing 345 mg GLA or placebo for 12 weeks	• Skin moisture, water loss, elasticity, firmness, fatigue resistance, and roughness significantly improved by 13.9, 10.2, 3.5, 19.2, 22.2, and 13.0%, respectively.

4.4 Zeaxanthin and Lutein

Zeaxanthin and lutein are in the xanthophylls category of dietary carotenoids and are best known for their importance to maturing eye health and other age related conditions. However, their importance to skin care is becoming apparent. The chemical structure of lutein and zeaxanthin are very similar, differing only in the placement of one double bond. Lutein absorbs blue light and therefore appears yellow at low concentrations and orange-red at high concentrations. These yellow to red pigments are found in green leafy vegetable like spinach, and colorful fruits and vegetable like oranges and sweet corn. Lutein is one of the most common carotenoids in nature and in the human diet (Erdman 2015). Although there are currently no Recommended Daily Intakes (RDI) for lutein or zeaxanthin, they are an essential part of our diet because they cannot be made internally (Böhm 2002). The combined daily dietary intake of lutein plus zeaxanthin ranges between 2 and 26 mg, for which a lutein-to-zeaxanthin ratio of roughly 5:1 is generally assumed (Thürmann 2005).

4.4.1 Zeaxanthin and Lutein Pharmacokinetics

Lutein and zeaxanthin differ from other carotenoids in that they each have two hydroxyl groups, one on each side of the molecule. Zeaxanthin is a stereoisomer of lutein, differing only in the location of a double bond in one of the hydroxyl groups. The hydroxyl groups appear to control the biological function of these two xanthophylls (Johnson 2002). Dietary lutein can be converted to meso-zeaxanthin within the body. Because xanthophylls are fat-soluble nutrients, bioavailability to tissues is dependent on a number of factors, including nutrient source (whole food or supplement), state of the food (raw, cooked, or processed), extent of disruption of the cellular matrix via mastication and digestive enzymes, and absorption by the enterocytes of the intestinal mucosa (primarily the duodenum). Cooking of lutein/zeaxanthin-containing foods may increase bioavailability by disrupting the cellular matrix and the carotenoid-protein complexes (Castenmiller 1999).

After lutein and zeaxanthin are absorbed by the enterocytes they are transported across the intestinal lumen and incorporated into the chylomicrons. They reach the circulating blood and are subsequently taken up by hepatocytes, entering the hepatic circulation where they are incorporated into lipoproteins. In humans, low- and high-density
lipoproteins transport lutein and zeaxanthin via the systemic circulation to various tissues (Yeum 2002)

Data on xanthophyll absorption is limited, but studies involving single dietary doses indicate lutein reaches peak concentrations in chylomicrons at approximately two hours post-ingestion (O'Neill 1998) and peaks in serum at about 16 hours post-ingestion (Kostic 1995) Lutein absorption from lutein supplements is almost twice that from vegetable sources (Castenmiller 1999) Non-dietary factors affecting absorption and bioavailability of lutein and zeaxanthin include age, body composition, gender, malabsorption of fats, alcohol consumption, smoking, and liver or kidney disease (Williams 1998, Brady 1996, Alberg 2002, Albanes 1997). Carotenoids are best absorbed in the presence of fat, but as little as 3-5 g in a meal appear to ensure carotenoid absorption (van Het Hof 2002).

In a study of two volunteers treated with a 30 mg of lutein daily for 140 day, plasma concentrations plateaued after 20–40 d, with a 10-fold increase from baseline, and returned to baseline concentrations 40–50 d after supplementation was discontinued (Landrum 1997). Depletion studies estimate the terminal half-life of lutein to be roughly 76 days in healthy subjects (Burri 2001). Long-term supplementation with 4.1 and 20.5 mg lutein increased plasma lutein concentrations approximately 3.5- and 10-fold, respectively (Thürmann 2005).

4.4.2 Zeaxanthin and Lutein Pharmacodynamics/Mechanism of Action

Zeaxanthin and lutein are powerful antioxidants. In the skin, they help protect light-exposed tissue (Stahl 2005, Darvin 2011) and prevent oxidative tissue damage by scavenging singlet oxygen molecules and quenching free radicals (Lee 1990). This physical quenching mechanism leaves the carotenoid intact so that a regeneration mechanism is unnecessary for the carotenoid to continue functioning as an antioxidant (Sies 1995, Stahl 2002). They also prevent lipid peroxidation. Such oxidative tissue damage, along with immunosuppression and mutations caused by UV radiation, contribute to the appearance of wrinkles, dark spots and thickened skin characteristic of aging skin, and promote skin cancer development (Lee 2004).

Carotenoids, including lutein and zeaxanthin, appear to be depleted in the skin under conditions of prolonged UV light exposure (Ribaya-Mercado 1995, Sorg 2002). Skin exposure to UV rays generates reactive oxygen species, and causes inflammation in skin cells, and erythema. Intake of lutein and zeaxanthin, reduces this inflammatory response (Stahl 2002).

Zeaxanthin is not only an effective anti-oxidant in vivo, it also reduces hydroperoxide formation in dietary oils, making it an attractive ingredient to reduce oxidative degradation within fatty acid supplements (Lee 1990).

4.4.3 Zeaxanthin and Lutein Preclinical Studies

The antioxidative effectiveness of four carotenoids, including zeaxanthin, was analyzed by monitoring the formation of hydroperoxides in methyl linoleate solution. All carotenoids tested were found to inhibit hydroperoxide formation when compared to a control solution with no added carotenoids. These results suggest that zeaxanthin can prevent oxidation in lipid systems (Terao 1989).

The anti-oxidant capability of zeaxanthin has been compared to that of the highly potent synthetic anti-oxidant, Trolox. In one study, testing three different zeaxanthin isomers, two had greater free radical scavenging capacity and prevented oxidation better than Trolox, while one isomer was less effective (Böhm 2002). In a second study, zeaxanthin was reportedly more effective than Trolox (Miller 1996).

The antioxidant activity of lutein was examined by using the photochemiluminescence assay and the beta-carotene-linoleic acid model system. Lutein showed a greater antioxidant activity than two other common carotenoids, beta-carotene and lycopene. (Wang 2006).

Preclinical studies have shown that lutein supplementation causes 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 is significantly reduced. Authors of a study showing these effects suggested that lutein modulates the skin's response to UV radiation and may contribute to the defence against some of the deleterious effects of sunlight (Lee 2004).

In dermal fibroblasts, melanoma cells, and ultraviolet radiation exposed fibroblasts, lutein is required to help regulate the extracellular matrix remodeling to maintain cell membrane integrity (Philips 2007).

4.4.4 Zeaxanthin and Lutein Human Epidemiological Studies

Researchers have demonstrated the presence of lutein and its oxidative metabolites in human skin (Wingerath 1998, Khachik 1995). Although a direct association between skin levels of lutein and lutein consumption has not been established, higher skin lutein levels are observed in humans who take a regular lutein-containing, multivitamin supplement (Peng 1995).

4.4.5 Zeaxanthin and Lutein Human Intervention Trials

Lutein and zeaxanthin studies have revealed positive effects on skin hydration, elasticity and sunburn threshold (Palombo 2007). A summary of relevant clinical trials follows.

4.4.5.1 Zeaxanthin and Lutein Open Label Trials

- Nakagawa 2009 – A study investigating whether orally administered lutein inhibits oxidation within the blood cells included 6 healthy subjects taking one capsule containing 9.67 mg lutein once daily for 4 weeks. Results revealed that lutein administration caused lutein incorporation into human blood cells. In addition, blood cell oxidative damage decreased after the ingestion for 2 and 4 weeks. The antioxidative effect of lutein was confirmed on blood cell membranes, suggesting that lutein has the potential to act as an important antioxidant molecule in blood cells.

4.4.5.2 Zeaxanthin and Lutein Randomized, Double-Blind, Placebo-Controlled Trials

- Palombo 2007 – This 12-week randomized, placebo-controlled, multi-center study was performed to evaluate the effect of lutein and zeaxanthin administered orally and applied topically upon human skin. Forty healthy women (aged 25–50 years) exhibiting signs and symptoms of premature skin aging ingested one active capsule (containing 5 mg lutein and 0.3 mg zeaxanthin) or a placebo twice daily with meals. Oral supplementation with the carotenoids led to a significant increase in skin lipids ($p < 0.05$), skin hydration, ($p < 0.05$), skin elasticity ($p < 0.05$), and sunburn threshold ($p < 0.05$). These results suggest that a dose of 10 mg lutein and 0.6 mg zeaxanthin daily provided protection from UV-induced damage.

- Juturu 2016 – This study included 46 healthy subjects aged 18-45 years with mild-to-moderate dry skin, who took a supplement containing 10 mg lutein and 2 mg zeaxanthin isomers or a placebo daily for 12 weeks. Overall skin tone was significantly improved in the active group compared to placebo ($P<0.0237$), and luminance values were significantly increased in the active group. Mean minimal erythemal dose was increased with active supplementation, as was individual typological angle which signifies lighter skin tone. Overall, results of this study showed that lutein and zeaxanthin supplementation improved skin tone, luminance (brightness) and sunburn sensitivity.

- Morganti 2002 – In an 8 week trial that included 30 females (aged 48 to 59 years) with moderate xerosis (abnormal dryness of the skin or mucus membranes) and photo-aging (damage from prolonged exposure to UV radiation), subjects consumed an oral antioxidant complex that contained 6 mg of lutein and 0.18 mg zeaxanthin daily, among other antioxidants. The amount of oxidation present in the skin significantly decreased within the first 2-week period of consuming the antioxidant complex and continued to decrease throughout the entire study period. In addition, skin moisture increased as early as 2 weeks and continued to increase throughout the study. These results suggest that the antioxidant treatment may be a promising therapeutic approach to reduce the oxidative stress of people affected by photo-aging.

- Morganti 2004 – This 8-week was conducted on 50 healthy smokers. Subjects in the treatment groups applied sunscreen (SPF 20) and were given capsules twice a day, containing either 3 mg lutein, 45 mg ascorbic acid, 5 mg alpha tocopherol, and 2.5 mg alpha-lipoic acid (lutein treatment group) or capsules with 13 mg carotenoids, 2 mg lycopene, 30 mg alpha-tocopherol, 60 mg ascorbic acid, and 10 mg polyphenol (carotenoid treatment group). The control group only applied sunscreen. Throughout the study period, the treatment groups had significant reductions in free radicals in the blood compared to the control group ($p<0.005$) indicating the UV-protective effect

of lutein as well as other carotenoids. All groups had a highly significant ($p < 0.005$) increase in skin hydration throughout the duration of the treatment compared to baseline, however, the difference between groups was not significant. These results indicate that a dose of 6 mg of lutein per day combined with other anti-oxidants can have UV-protective effects for the skin.

- Schwartz 2016 – Twelve weeks supplementation with a supplement containing with zeaxanthin, algae extracts, peptides, and hyaluronatein, improved hydration within 2 weeks and total wrinkle count by 4 weeks in 31 subjects. Statistically significant differences from baseline scores for average wrinkles severity were seen for week 12 visit for the active group compared to placebo.
- Heinrich 2003 – This study investigated the effect of mixed carotenoid including lutein and zeaxanthin and straight beta-carotene supplementation on skin erythema after exposure to UV light. Subjects were divided into Group 1 receiving 24 mg beta-carotene and Group 2 receiving 24 mg mixed carotenoids (8 mg each of beta-carotene, lutein, and lycopene) daily for 12 weeks. Following treatment, Group 2 patients had increased serum levels of all three nutrients and a significant decrease in erythema was observed in both groups after UV exposure compared to pre-supplementation. This study suggests carotenoids deposited in the skin may protect against erythema and inflammation resulting from UV rays.

A summary table of the Human Intervention Trials follows:

REFERENCE	TRIAL DESIGN	POPULATION	TREATMENT	RESULTS
Nakagawa 2009	Open label	6 **healthy** subjects	One capsule containing 9.67 mg lutein once daily for 4 weeks.	• Lutein incorporated into blood cells. • Blood cell oxidative damage decreased after the ingestion • Antioxidative effect was confirmed on blood cell membranes
Palombo 2007 –	R, DB, PC	Forty **healthy** women (aged 25 – 50 years) exhibiting signs and symptoms of premature skin aging	One capsule (containing 5 mg lutein and 0.3 mg zeaxanthin) or a placebo twice daily with meals for 12 weeks	• Significant increases in skin lipids ($p < 0.05$), skin hydration, ($p < 0.05$), skin elasticity ($p < 0.05$), and sunburn threshold ($p < 0.05$) following treatment.
Juturu 2016	R, DB, PC	46 **healthy** subjects aged 18-45 years with mild-to-moderate dry skin	10 mg lutein and 2 mg zeaxanthin isomers or a placebo daily for 12 weeks.	• Overall skin tone ($P<0.0237$) and luminance values were significantly improved in the active group compared to placebo • Mean minimal erythemal dose and individual typological angle, which signifies lighter skin tone, was increased with active.

				• Overall, showed that lutein and zeaxanthin improved skin tone, luminance (brightness) and sunburn sensitivity.
Morganti 2002	R, DB, PC	30 females (aged 48 to 59 years) with **moderate xerosis (abnormal dryness** of the skin or mucus membranes) and photo-aging (damage from prolonged exposure to UV radiation)	Oral antioxidant complex that contained 6 mg of lutein and 0.18 mg zeaxanthin daily, among other antioxidants for 8 weeks	• The amount of oxidation present in the skin significantly decreased within the first 2-weeks and continued to decrease throughout the study period. • Skin moisture within 2 weeks and continued to increase throughout the study.
Morganti 2004,	R, DB, PC	50 **healthy** smokers	Treatment groups applied sunscreen (SPF 20) and were given capsules twice a day, containing either 3 mg lutein, 45 mg ascorbic acid, 5 mg alpha tocopherol, and 2.5 mg alpha-lipoic acid (lutein treatment group) or capsules with 13 mg carotenoids, 2 mg lycopene, 30 mg alpha-tocopherol, 60 mg ascorbic acid, and 10 mg polyphenol (carotenoid treatment group) for 8 weeks. The control group only applied sunscreen.	• The treatment groups had significantly reduced free radicals in blood compared to control ($p<0.005$) • All groups had a highly significant ($p <0.005$) increase in skin hydration compared to baseline, however the difference between groups was not significant.
Schwartz 2016	R, DB, PC	31 **healthy** subjects	a supplement containing with zeaxanthin, algae extracts, peptides, and hyaluronatein for 12 weeks	• Improved hydration within 2 weeks and total wrinkle count by 4 weeks

				• Statistically significant differences from baseline scores for average wrinkles severity at week 12 visit in the active group compared to placebo
Heinrich 2003	R, DB, PC	24 volunteers with **skin type II**	Group 1 receiving 24 mg beta-carotene and Group 2 receiving 24 mg mixed carotenoids (8 mg each of beta-carotene, lutein, and lycopene) daily for 12 weeks.	• Group 2 patients had increased serum levels of all three nutrients • Significant decrease in erythema was observed in both groups after UV exposure compared to pre-supplementation.

4.5 Vitamin D

Vitamin D is a fat soluble vitamin found in some foods including fish and eggs, and can also be manufactured in skin upon exposure to UVB rays from sunlight. Vitamin D is required to absorb calcium and promote bone growth, but its requirement for skin health is becoming increasingly obvious. There is evidence of widespread sub-clinical vitamin D deficiency (Hyppönen 2007) that is aggravated by long hours of work indoors and avoidance of sunshine aimed at reducing skin cancer risk (Hyppönen 2010).

Vitamin D exists in several different forms including D1 - D5 that differ primarily in their side chains. The two major forms are vitamin D2 or ergocalciferol, and vitamin D3 or cholecalciferol. These are known collectively as calciferol. The majority of circulating vitamin D, known as serum 25-hydroxyvitamin D [25(OH)D] and that is necessary to maintain health and function of the immune, reproductive, muscular, skeletal and integumentary system, originates from vitamin D3 (cholecalciferol) and reflects endogenous synthesis from exposure to sunlight as well as intake from the diet (Calvo 2005).

There are very few dietary sources of vitamin D. Oily fish such as herring, mackerel, pilchards, sardines and tuna are rich sources but their consumption in some countries is low. The only other useful sources are eggs, fortified margarines (required in some countries by law to contain vitamin D) and some fortified yoghurts and breakfast cereals. However, a recent global review of vitamin D status has shown that its intake is often too low to sustain healthy circulating 25(OH)D in countries without mandatory staple food fortification and is even too low in countries that do fortify due to low milk consumption, vegetarianism, non-supplement use and low fish intake (Calvo 2005). Supplement use contributed 6-47% of the average vitamin D intake in some countries. In 2005, the average dietary intake of vitamin D was in the range of 3 ug = 120 IU per day in most countries and did not exceed 9 ug = 361 IU per day in any of the countries surveyed including the United States, Canada, the United Kingdom, Ireland, Scotland, Australia, Europe, Japan and various other countries. The recommended daily intake of vitamin D for adults 19–70 years old is 600 IU and for those aged 71 years and older it is 800 IU (NIH).

Vitamin D deficiency is defined as serum 25(OH)D of less than 25-50 nmol/L. Approximately one billion people worldwide are estimated to be vitamin D deficient with people living in Europe, the Middle East, China and Japan at particular risk (Vieth 2007, Lips 2007). Deficiency is more common in women than men (9.2% verses 6.6%) (Hyppönen 2010).

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 (Hoel 2016). For these reasons alone, vitamin D is considered an important nutrient for inclusion in dietary supplements aimed at photo-protection. However, vitamin D also regulates numerous functions within the skin (Bikle 2012), exhibits anti-inflammatory effects (Krishnan 2011) in vitro (Tongkao-On 2015, Song 2013, Gordon-Thomson 2012) and in vivo (Calton 2017), and reduces polymorphic light eruption when topically applied to human skin (Gruber-Wackernage 2011).

4.5.1 Vitamin D Pharmacokinetics

Vitamin D within the body is derived from two sources:

- D3 (cholecalciferol), the more bioactive form is produced in the skin on exposure to UVB radiation, and is also present is some animal foods (e.g. fish and liver) and supplements.
- D2 (ergocalciferol) is found in vegetable food sources.

For most people, the major supply is from the action of sunlight on skin with food sources contributing only a small amount. Solar ultraviolet-B radiation (UVB; wavelengths of 290 to 315 nanometers) stimulates the production of vitamin D3 from 7-dehydrocholesterol (7-DHC) in the epidermis of the skin. The efficiency of vitamin D3 synthesis depends on the number of UVB photons that penetrate the epidermis. During the winter months in some countries however, there is no UVB radiation of the appropriate wavelength needed for vitamin D production. Thus, winter levels are dependent on the amount of vitamin D formed during the previous summer and there is a marked seasonal variation in plasma levels. Vitamin D status is also very much dependent on the time spent out of doors during the day, which itself depends on mobility, institutionalisation, weather and cultural influences upon skin exposure (COMA 1991).

The vitamin D from food or supplements is absorbed in the part of the small intestine immediately downstream from the stomach. Stomach juices, pancreatic secretions, bile from the liver and the integrity of the wall of the intestine all have some influence on how much of it is absorbed. Since vitamin D is a fat soluble vitamin, its absorption can be maximized by consuming sufficient healthy fats. In addition, maintaining healthy gut flora enhances absorption within the small intestine, provided sufficient quantities of bile salts are also available (Vitamin D Council).

Vitamin D3 is a lipophilic molecule similar to cholesterol and so requires a protein carrier for solubility in plasma. When absorbed from the gut, vitamin D enters the circulation on chylomicrons first, and it is only slowly transferred to vitamin D binding protein (DBP). Vitamin D3 made during skin synthesis is bound rapidly to DBP and so is introduced into metabolism quicker than diet derived vitamin D. Peripheral tissues, such as adipose tissue and muscle, uptake vitamin D via the action of lipoprotein lipase. The liver absorbs what is left in the chylomicron remnant and quickly removes it from the bloodstream. Vitamin D has a short plasma half-life of 4 – 6 h while the whole-body half-life is 2 months (Jone 2008).

Vitamin D is metabolized first to 25OHD, then to the hormonal form 1,25-dihydroxyvitamin D (1,25(OH)2D). The three main steps in vitamin D metabolism, 25-hydroxylation, 1α-hydroxylation, and 24-hydroxylation are all performed by cytochrome P450 mixed-function oxidases (CYPs). These enzymes are located either in the endoplasmic reticulum (e.g., CYP2R1) or in the mitochondria (e.g., CYP27A1, CYP27B1, and CYP24A1). 1,25(OH)2D is the ligand for the vitamin D receptor (VDR), a transcription factor, binding to sites in the DNA called vitamin D response elements (VDREs). There are thousands of these binding sites regulating hundreds of genes in a cell-specific fashion in nearly every tissue within the body, including skin (Bikle 2014).

4.5.2 Vitamin D Pharmacodynamics/Mechanism of Action

The keratinocytes of the skin are unique because they possess both the enzymatic machinery to product vitamin D, to metabolize it to active metabolites, in particular 1,25(OH)(2)D, and the VDR that enables the keratinocytes to respond to the 1,25(OH)(2)D thus generated. Numerous functions of the skin are regulated by vitamin D and/or its receptor. These include inhibition of proliferation, stimulation of differentiation including formation of the permeability barrier, promotion of innate immunity, regulation of the hair follicle cycle, and suppression of tumor formation (Bikle 2012).

4.5.3 Vitamin D Preclinical Studies

In vitro studies have shown that vitamin D reduces UV-induced damage, including inflammation, sunburn, and photo-associated immunosuppression, and carcinogenesis (Tongkao-On 2015, Song 2013, Gordon-Thomson 2012).

Nonmelanoma skin cancer is a condition of increased proliferation and decreased differentiation of keratinocytes (Bikle 2014). Mice lacking the VDR in their keratinocytes are predisposed to UVB and chemically induced skin cancer (Teichert et al., 2011), and topical application of 1,25(OH)2D appears to be photoprotective (Mason and Reich-rath, 2013).

4.5.4 Vitamin D Human Epidemiological Studies

A human epidemiology study has associated higher vitamin D status with reduced systemic inflammation (Calton 2017). The aim of the study was to track seasonal variations in 25(OH)D and investigate the influence on whole body energy metabolism, circulating peripheral blood mononuclear cell (PBMC) bioenergetic profiles, and markers of systemic inflammation. The study included 30 adult Australians of European origin, aged between 20 and 70 years who supplied blood samples for testing in the summer and winter. 25(OH)D increased from winter to summer and was accompanied by significant improvements in indices of insulin sensitivity and mitochondrial parameters. Markers of systemic inflammation including MCP-1, IL-6, IL-8, IL-10, and IL-12p70 decreased significantly in summer compared to winter. Participants who entered winter with a low 25(OH)D (<50 nmol/L), had the greatest alteration in bioenergetic parameters in summer, relative to those with winter 25(OH)D concentrations of 50–75 nmol/L or >75 nmol/L.

4.5.5 Vitamin D Human Intervention Trials

4.5.5.1 Vitamin D Randomized, Double-Blind, Placebo-Controlled Trials

- **Gruber-Wackernage 2011** – When topically applied to human skin, vitamin D reduces polymorphic light eruption. An intra-individual half-body trial including 13 patients with polymorphic light eruption, investigated the preventive effect of a topically applied calcipotriol-containing cream. They pretreated their skin on two symmetrically located test fields with calcipotriol or placebo cream twice daily for 7 days before the start of photoprovocation testing with solar-simulated UV radiation. Compared with placebo calcipotrial pretreatment significantly reduced symptoms on average by 32% throughout the observation period starting at 48 h until 144 h after the first photoprovocation exposure. Calcipotriol diminished the polymorphic light eruption test score in all 12 photoprovocable patients (P = 0·0005).
- **Perez 1996 –** Psoriasis is a disorder with hyperproliferation and decreased or abnormal differentiation driven by an abnormal immunologic component (Bikle, 2012 & 2014). A single centre study investigated the efficacy and safety of oral calcitriol (1,25-dihydroxyvitamin D3) in the treatment of psoriasis. Of the 85 patients who received calcitriol, 88.0% had some improvement in their disease while 26.5, 36.2, and 25.3%, experienced complete, moderate and slight improvement. The mean Psoriasis Area Severity Index score (PASI) decreased from 18.4 at baseline to 9.7 and 7.8 after 6 and 24 months of calcitriol treatment, respectively. The authors concluded that oral calcitriol is effective and safe for the treatment of psoriasis.
- **Javanbakht 2011 –** This trial involving 45 subjects was conducted to assess the effects of vitamins D and E supplementation on the clinical manifestation of eczema. Subjects were randomly divided into four groups and treated for 60 days: placebo; group D [1600 IU vitamin D(3)]; group E (600 IU synthetic all-rac-alpha-tocopherol); and group DE [1600 IU vitamin D(3) plus 600 IU synthetic all-rac-alpha-tocopherol]. The clinical improvement was evaluated with SCORing Atopic Dermatitis (SCORAD). Results revealed that SCORAD was reduced after 60 days in groups D, E and DE by 34.8%, 35.7% and 64.3%, respectively ($p = 0.004$). Objective SCORAD also showed significant improvement. There was a positive correlation between SCORAD and intensity (objective, subjective, and extent) ($p < 0.001$). This study supports the contributing and beneficial effects of vitamins D in the treatment of eczema.

A summary table of the Human Intervention Trials follows:

REFERENCE	TRIAL DESIGN	POPULATION	TREATMENT	RESULTS
Gruber-Wackernage 2011	R, DB, PC	13 patients with **polymorphic light eruption**,	Subjects pretreated their skin on two symmetrically located test fields with calcipotriol or placebo cream twice daily for 7 days before the start of photoprovocation testing with solar-simulated UV radiation.	• Compared with placebo calcipotrial pretreatment significantly reduced symptoms on average by 32% throughout the observation period starting at 48 h until 144 h after the first photoprovocation exposure.

				• Calcipotriol diminished the polymorphic light eruption test score in all 12 photoprovocable patients (P = 0·0005).
Perez 1996	R, DB, PC	85 **psoriasis** patients	Oral calcitriol (1,25-dihydroxyvitamin D3) or placebo for 24 months	• 88.0% had some improvement in their disease while 26.5, 36.2, and 25.3%, experienced complete, moderate and slight improvement. • The mean Psoriasis Area Severity Index score (PASI) decreased from 18.4 at baseline to 9.7 and 7.8 after 6 and 24 months of calcitriol treatment, respectively.
Javanbakht 2011	R, DB, PC	45 subjects with **atopic eczema**	Four groups treated for 60 days with placebo or group D [1600 IU vitamin D(3)]; group E (600 IU synthetic all-rac-alpha-tocopherol); and group DE [1600 IU vitamin D(3) plus 600 IU synthetic all-rac-alpha-tocopherol].	• SCORAD was reduced after 60 days in groups D, E and DE by 34.8%, 35.7% and 64.3%, respectively (p = 0.004). • Objective SCORAD also showed significant improvement. There was a positive correlation between SCORAD and intensity (objective, subjective, and extent) (p < 0.001).

4.6 Human Intervention Trials including Combinations of Ingredients within Bend

Skincare Anti-Aging Formula

4.6.1 Open Label Trials

ACNE

- **Rubin 2008** – Five subjects with mild to moderate acne, self-administered the supplement, Perfect Skin, Genuine Health Inc., Toronto, Canada, that contains 250 mg EPA, 3.75 mg zinc gluconate, 50 mcg selenium, 50 mcg chromium, and 50 mg epigallocatechin gallate per capsule. The 5 subjects took 4 capsules per day for at least 2 months. Four subjects had a reduction in total lesion count, with a range of 11 to 41 less lesions after 2 months. The average total lesion count among inflammatory lesions. No subject experienced a worsening of inflammatory acne lesions during the 2 months and all had at least some reduction in inflammatory papules (small rounded bumps rising from the skin), with the average inflammatory lesion count dropping from 20.8 at baseline to 6.8 after 2 months.

4.6.2 Randomized, double-blind, placebo-controlled trial

PEOPLE WITH HEALTHY SKIN

- **Segger 2008 –** A single-blind study included 24 healthy women (aged 40 to 60 years) taking an oil formulation that contained 1168 mg EPA, 745 mg DHA, and 193 mg GLA to verify its ability to alter skin elasticity, skin water loss, and skin roughness. After 3 months of treatment with the oil formulation, skin elasticity increased by 10%, which was considered statistically significant compared to the control group (P = 0.0298). There was also a trend, albeit statistically insignificant, towards a positive influence on the skin's barrier function. The authors concluded that this oral preparation rich in natural stable fish oil can improve skin elasticity.

ACNE

- **Jung 2014** – A 10 week R, PC trial treating 45 participants with mild to moderate acne taking either 2000 mg/day EPA + DHA, or 400 mg/day GLA from borage or placebo reported significant improvements in both treatment groups for inflammatory and non-inflammatory acne lesions accompanied by reduced inflammation and IL-8 . Patient subjective assessment of improvement showed a similar result. Heamatoxylin & eosin staining of acne lesions demonstrated reductions in inflammation and immunohistochemical staining intensity for interleukin-8. No severe adverse effect was reported. This study shows for the first time that omega-3 fatty acid and γ-linoleic acid could be used as adjuvant treatments for acne patients.
- Fabbrocini 2014 - GLA in combination with anti-oxidants prevented dry skin associated with the use of oral isotretinoin. The study included 48 patients with nodular acne (32 females and 16 males) treated with either isotretinoin therapy (20-30 mg/day) + a dietary supplement containing GLA, LA vitamin E, vitamin C, beta-carotene, coenzyme Q10 and *Vitis vitifera* twice daily, or only isotretinoin (20-30 mg/day) for 6 months. Patients treated with dietary supplement had lower side effects, with a less degree of erythema and dryness, and greater degree of hydration; a greater adherence to therapy was also reported.

A summary table of the Human Intervention Trials follows:

REFERENCE	TRIAL DESIGN	POPULATION	TREATMENT	RESULTS
Rubin 2008	Open label	5 subjects with **mild to moderate acne**	4 capsules per day of the supplement *Perfect Skin*, Genuine Health Inc, Toronto, Canada, that contains 250 mg EPA, 3.75 mg zinc gluconate, 50 mcg selenium, 50 mcg chromium, and 50 mg epigallocatechin gallate per capsule for at least 2 months.	• 4 subjects had a reduced total lesion count, with a range of 11 to 41 less lesions after 2 months. • The average total lesion count among the group dropped from 62.8 to 40.4 with the most significant difference noted in the area of inflammatory lesions. • No subject experienced a worsening of inflammatory acne lesions during the 2 months and all had at least some reduction in inflammatory papules (small rounded bumps rising from the skin), with the average inflammatory lesion count dropping from 20.8 at baseline to 6.8 after 2 months.
Segger 2008	R, DB, PC	24 **healthy** women (aged 40 to 60 years)	An oil formulation containing 1168 mg EPA, 745 mg DHA, and 193 mg GLA for 3 months	• Skin elasticity increased by 10 compared to the control group (P = 0.0298). • There was also a trend towards a positive influence on the skin's barrier function.
Jung 2014	R, DB, PC	45 participants with **mild to moderate acne**	2000 mg/day EPA + DHA, or 400 mg/day GLA from borage or placebo	• Significant improvements in both treatment groups for inflammatory and non-inflammatory acne lesions accompanied by reduced inflammation and IL-8. • Patient subjective assessment of improvement showed a similar result. • Heamatoxylin & eosin staining of acne lesions showed reductions in inflammation and immunohistochemical staining intensity for interleukin-8. • No severe adverse effect was reported.

Fabbrocini 2014	R, DB, PC	48 patients with **nodular acne** (32 females and 16 males)	Either isotretinoin therapy (20-30 mg/day) + a dietary supplement containing GLA, LA vitamin E, vitamin C, beta-carotene, coenzyme Q10 and *Vitis vitifera* twice daily, or only isotretinoin (20-30 mg/day) for 6 months.	• Patients treated with dietary supplement had lower side effects, with a less degree of erythema and dryness, and greater degree of hydration. • A greater adherence to therapy was also reported.

5.0 Safety

5.1 Fatty Acids

The Canadian Natural and Non-Prescription Health Products Directorate, Heath Canada, has approved extended use of up to 5 g of EPA+DHA daily in adults with lower amounts based on body weight in children, without need for cautions, warning, contraindication or known adverse reactions displayed on product labels (Fish Oil Product Monograph). A similar safety profile has been approved for up to 1.35 g/day of GLA from borage oil in adult populations (Borage Oil Product Monograph). Bend Beauty Anti-Aging Formula provides substantially less than either of these amounts and so would not be expected to produce any known adverse reactions attributable to its fatty acid content.

Although increasing dietary LC- PUFA intake is beneficial to skin health, the same dietary changes may adversely affect some indices of lipid peroxidation. Therefore, adequate intake of antioxidants is necessary during LC- PUFA supplementation to prevent harmful oxidative stress (Jenkinson 1999). Inclusion of zeaxanthin and lutein in the Bend Beauty Anti-Aging Formula ensures powerful anti-oxidant capacity.

5.2 Zeaxanthin and Lutein

No toxicities or adverse reactions have been reported in the scientific literature for lutein/zeaxanthin at doses of up to 40 mg daily for two months (Dagnelie 2000, Hendler 2008). Fijians consume an average of (25 mg lutein daily throughout their lifetime without any toxic effects (Le Marchand 1995). Ames testing has demonstrated an absence of any mutagenic effect for purified lutein (Gonzalez 1997). Lutein was not only found to be non-mutagenic at 334, 668 and 1335 μg/plate doses, but it showed an anti-mutagenic effect in a dose-dependent manner. Similar results were found in a chromosome aberration test using Chinese hamster ovary cells for the evaluation of clastogenicity and anti-clastogenicity of lutein at 66.8, 133.5 and 267.0 mg/L (Wang 2006).

The NNHPD of Health Canada has issued a monograph for lutein and zeaxanthin that includes safety information (Marigold Monograph). Neither the U.S. Food and Drug Administration nor the European Food Safety Authority consider lutein an essential nutrient or have acted to set a tolerable upper intake level for either lutein or zeaxanthin (Carotenoids). However, the observed safe level (OSL) for lutein, based on a non-government organization evaluation, is 20 mg/day (Shao Shao2006). An acceptable daily intake level for zeaxanthin has been proposed as 0.75 mg/kg of body weight/day, which equals 53 mg/day for a 70 kg adult (Edwards 2016). In humans, an intake of 20 mg of zeaxanthin per day for up to six months had no adverse effects (Edwards 2016). Based on these observations, there is no concern that the quantities of either lutein or zeaxanthin provided in a daily dose of Bend Beauty Anti-Aging Formula would produce any toxic effects.

5.3 Vitamin D

There has been little toxicity reported in adults taking doses of Vitamin D as high as 10,000 IU/d (250 μg/d) (Hathcock 2007, Heaney 2008, Vieth 1999) although toxicity becomes generally present at 20,000 IU/d (500 μg/d). Recently, a randomized, controlled trial, including 350 women with a singleton pregnancy at 12 to 16 weeks' gestation (i.e. a high risk group compared to a non-pregnant, normal population) were supplemented with 400 (10μg), 2000 (50μg), or 4000 IU(100 μg) of vitamin D per day until delivery. There were no differences between groups on any safety measure. Not a single adverse event was attributed to vitamin D supplementation or circulating 25(OH)D levels (Hollis 2011). Bend Beauty Anti-Aging Formula provides 1000 IU (25 μg) per day of vitamin D, which is 4 times less than the maximum dose provided in this study of high risk subjects. Therefore, it is unlikely that the quantity of vitamin D provided in Bend Beauty Anti-Aging Formula would produce any toxic effects.

5.4 Risk Information

Efficacy and safety information similar to the above, has been taken into consideration during regulatory approval of Bend Beauty Anti-Aging Formula for its intended use, by the NNHPD of Health Canada. Any associated risks for use of the product, based on recommendations approved by them, are described below.

5.4.1 Cautions and Warnings – None

5.4.2 Contraindications – None

5.4.3 Known adverse reactions – None

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