Original Contribution

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Protective effects of a novel nutritional and phytonutrient blend on ultraviolet radiation-induced skin damage and inflammatory response through aging defense mechanisms

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Summary

Background The human body relies on several aging defense mechanisms (ADMs) to limit damage induced from pro-aging stressors (aging aggressors). However, such protective mechanisms can be compromised, leading to accelerated aging. The skin provides a model to probe the effects of an oral nutritional intervention on ADMs in response to ultraviolet radiation (UVR)-induced damage.

Objective To determine whether supplementation with a novel nutritional and phytonutrient blend could protect against UVR-induced skin damage and positively influence facial skin attributes and characteristics by bolstering ADMs.

Methods Thirty-six healthy, nonsmoking women (40–75 years) with Fitzpatrick skin types I and II were recruited. UVR-induced erythema and the number of apoptotic cells were determined before (pre-) and after 8-week (post-) supplementation. Other clinical variables included skin carotenoid concentrations, facial skin attributes and characteristics.

Results Eight-week supplementation led to protection against UVR-induced skin damage as evidenced by reductions in erythema at all three minimal erythema doses (MEDs) (9.1 to 7.4 [$P = 0.10$]; 15.8 to 13.6 [$P = 0.02$]; and 19.6 to 17.3 [$P = 0.01$] for one, two, and three MEDs and a reduction in the average number of apoptotic cells [11.3 to 5.3, $P < 0.0001$] pre- and post-supplementation, respectively). Skin carotenoid concentrations increased from 28 111 Raman intensity units to 38 472 ($P < 0.0001$) along with noticeable improvements in facial skin attributes and characteristics: elasticity, transepidermal water loss, radiance, texture, and overall appearance (all $P < 0.05$) following supplementation.

Conclusion Eight weeks of oral supplementation positively impacted ADMs resulting in protection against UVR-induced skin damage and improvements in facial skin attributes and characteristics.

Keywords: aging defense mechanisms, erythema, apoptosis, aging aggressors, photoaging, inflammation

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Introduction

The human body is exposed to a variety of damaging insults that contribute to the aging process. Such insults can be generated either internally or externally and include stress, oxidative damage, DNA damage, pollution, metabolic by-product accumulation, advanced glycation end-products, toxins, and a variety of other factors which collectively can be described as "aging aggressors." Fortunately, humans have several mechanisms to mitigate the damage caused by aging aggressors. Examples of these protective "aging defense mechanisms" (ADMs) include DNA protection and repair mechanisms, mechanisms that regulate inflammatory responses, and antioxidant protection mechanisms. These ADMs, however, may be dysregulated over time, resulting in an inability to repair or reverse damage that occurs in response to an insult. One tissue that is particularly susceptible to environmental insults is the skin and one of the primary aggressors that cause human skin aging is ultraviolet radiation (UVR), also known as "photoaging." Photoaging, unlike chronological aging, occurs rapidly and is directly correlated with the degree of sun exposure. In light of this close correlation between an exogenous insult and tissue damage, a dermatological model affords an optimal approach to investigate the clinical impact of a nutritional supplement on ADMs in humans. Moreover, skin has been suggested to be a good surrogate of overall health and aging in humans.[1]

Skin is made up a number of cell types and has a network of mechanisms to protect against cellular damage, repair tissue, and maintain inflammatory balance. Exposure of the skin to UVR induces acute inflammation, and this reaction can be visibly observed by the appearance of erythema. Antioxidant protection is one of the most commonly studied ADMs involved in the protection of skin against UVR-induced cellular damage and erythema. Both extrinsic antioxidants (vitamin C, carotenoids, and vitamin E) derived from the diet and endogenous antioxidant enzymes (glutathione peroxidase or superoxide dismutase) have been demonstrated to play a critical role in cellular protection in various tissues, including the skin. UVR-induced skin damage at the cellular level can be quantified histologically by counting the number of apoptotic cells.[2] ADMs involved in DNA protection and repair therefore play a critical role in maintaining skin health in the presence of UVR. Skin UVR model, therefore, provides a convenient model to examine whether dietary interventions can protect the skin from UVR-induced damage by optimizing ADMs.

We developed a novel nutritional and phytonutrient blend designed to deliver anti-aging benefits by bolstering ADMs. The unique blend was developed based on a combination of the anti-aging nutrition science literature and by screening ingredients for the ability to elicit gene expression patterns similar to caloric restriction (S.M. Wood, A.F. Mastaloudis, S.N. Hester; unpublished data). Ingredients in the novel nutritional and phytonutrient blend have been shown to positively modulate ADMs including antioxidant/cellular protection/repair,[3–5] cellular stress response,[6–9] metabolism,[10,11] and inflammatory balance.[12–15] The objective of this study was to determine whether supplementation with a novel nutritional and phytonutrient blend could protect against UVR-induced skin damage and positively influence facial skin attributes and characteristics of healthy women by bolstering ADMs.

Materials and methods

Participants

Healthy, nonsmoking women between the ages of 40 and 75 years and body mass index (BMI) between 19 and 30 kg/m^2 with Fitzpatrick skin types I and II were recruited to participate. Participants had to present with moderate signs of skin aging based on a dermatologist assessment. Exclusion criteria included the following: (1) history of chronic diseases, skin diseases, or skin abnormalities; (2) regular consumption of dietary supplements containing carotenoids, vitamin D, fish oil, eicosapentaenoic acid (EPA), docosahexaenoic (DHA), or resveratrol; (3) consumption of more than one serving of fatty fish per week; or (4) having used an anti-aging topical skin care treatment within 30 days of study enrollment. Participants who were pregnant, nursing, or planning to become pregnant were also excluded. The study was approved by an Institutional Review Board (Concordia Clinical Research, Cedar Knolls, NJ, USA) and conducted according to the Helsinki Declaration as revised in 1983. All participants signed an informed consent document prior to enrollment, and the study was registered on ClinicalTrials.gov (#NCT02525224).

Study design

The study protocol is outlined in Fig. 1. This was an open label study designed to investigate effects before and after 8-week oral supplementation with a novel nutritional and phytonutrient blend against UVR-

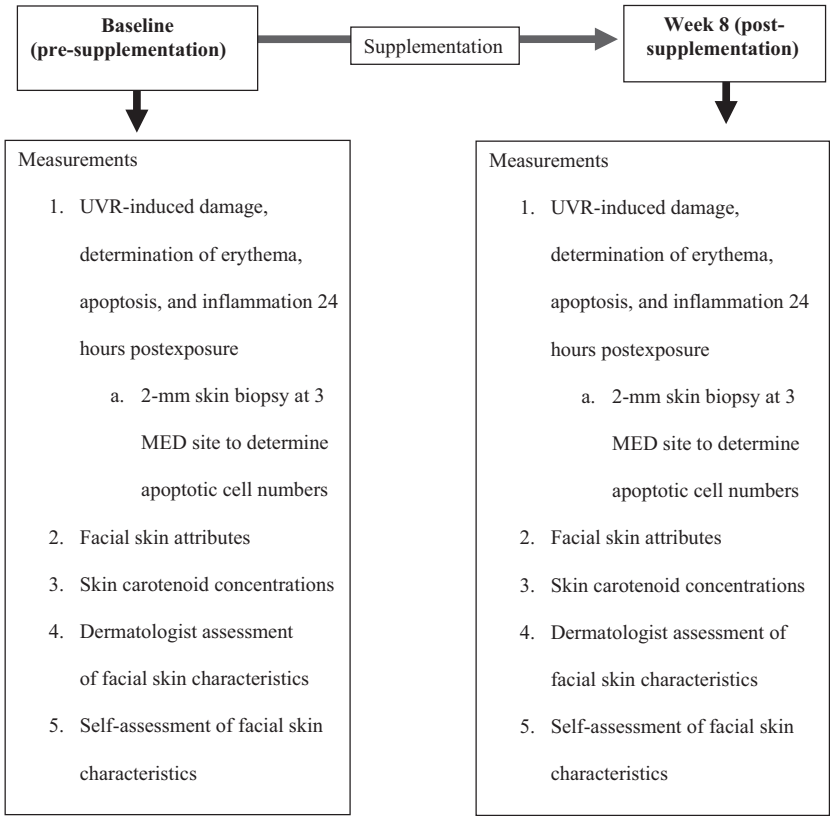

Figure 1 Study design.

induced tissue damage of non-sun-exposed skin (buttock), skin carotenoid concentrations (palm of hand), and facial skin characteristics. The primary end point was UVR-induced cellular damage assessed by erythema and the number of apoptotic cells at the highest damaging dose of UVR. Secondary variables included facial skin attributes (i.e., elasticity and water loss), dermatologist- and self-assessed facial skin characteristics (lines, firmness, radiance, texture, and overall appearance), and skin carotenoid concentrations.

Novel nutritional and phytonutrient blend

Each daily dose (four capsules) contained the following: 1000 mg EPA + DHA from ultra-pure fish oil concentrate (2110 mg), 30 mg resveratrol (from *Polygonum cuspidatum* root), 75 mg quercetin (from *Dimorphandra mollis* fruit extract), 140 mg purple corn (*Zea mays* L.) cob extract (delivering 10 mg anthocyanins), 37.5 mg rosemary (*Rosmarinus officinalis* L.) leaf extract (delivering 1.5 mg carnosic acid), 200 mg citrus bioflavonoids (delivering 100 mg naringin and 100 mg hesperidin), 30 mg coenzyme Q_{10}, 100 mg alpha lipoic acid, 1 mg astaxanthin (a carotenoid from *Haematococcus pluvialis* algae), 5 mg lycopene (a carotenoid), 4 mg lutein (a carotenoid from Marigold flower [*Targetes erecta*]), 1000 IU vitamin D_3 (as cholecalciferol), 40 μg vitamin K_2 (as menaquinone-7), and 50 mg d-limonene (from *Citrus sinensis* peel oil). The novel nutritional and phytonutrient blend was manufactured by NSE Products (Provo, UT, USA).

Participants were instructed to consume four capsules per day, two capsules with breakfast and two capsules with dinner daily for 8 weeks. Participants were encouraged to consume a minimum of 5 g of fat with meals to facilitate absorption of fat-soluble nutrients. Compliance was determined by the number of capsules distributed and the number returned by participants at 8 weeks. Adverse reactions as defined as any untoward medical occurrence (sign, symptom, or laboratory finding), regardless of severity and whether or not attributed to the supplements, were reported to the physician/researcher.

Ultraviolet radiation-induced cell damage

At baseline (pre-) and at 8 weeks (post-)supplementation, previously non-sun-exposed buttock skin was exposed to three solar minimal erythema doses (one

MED, two MED, and three MED) on 1-cm-diameter areas by simulated radiation (model 16S-150v.3 powered by a xenon lamp power supply model XPS 200; Solar Light Co., Glenside, PA, USA). One MED was defined as the lowest dose of UVR (mJ/cm^2) causing a visually perceptible erythema at 24 h post-UVR exposure. The same procedure was repeated on each subject at the end of the study post-supplementation, but on the opposite buttock.

Erythema

Erythema was determined by dermospectrophotometer measurement of the three irradiated sites 24 h postexposure and compared to nonexposed skin in the same area.

Apoptosis

Twenty-four hours post-UVR exposure, a skin punch biopsy (2 mm) was taken at the three MED site after measurement of erythema. The tissue collected from the punch biopsy was immediately placed in formalin. Samples were sectioned (thinly sliced) and mounted on slides. Six sections were then stained with hematoxylin and eosin (Cockerell Laboratories, Dallas, TX, USA). Each section was examined under light microscopy, and the sum of apoptotic cells for all six sections was recorded. The slides were read in a blinded fashion by a board-certified dermatologist, and the average number of apoptotic cells was recorded at baseline and after 8-week supplementation.

Skin carotenoid concentrations

Skin carotenoids concentrations were measured noninvasively in the palm of the hand using Raman spectroscopy (BioPhotonic Scanner; NSE Products, Provo, UT) as described in previous clinical studies.[16–18] Each subject was measured twice at each visit, and measurements were recorded in Raman intensity units (RIUs). In the event, the two measurements had a difference greater than 3000 RIUs, a third measurement was taken, and the values were averaged.

Facial skin attributes

Skin elasticity
Skin elasticity was measured on facial skin 3 cm below the outer corner of the eye using an elasticity cutometer (Dermalab, Cortex Technologies, Hadslund, Denmark).

Epidermal water loss
Transepidermal water loss (TEWL) was measured on the face by Evaporimeter (Dermalab, Cortex Technologies).

Facial skin characteristics

Facial skin characteristics were evaluated by a board-certified dermatologist at each visit. In order to minimize bias, the dermatologist was blinded as to participants' visit when making the assessments. Skin characteristics included: lines/wrinkles, firmness, radiance, texture/smoothness, and overall appearance using a five-point ordinal scale (1 = youthful and 5 = aged). Participants also completed a self-assessment survey of facial skin characteristics (lines/wrinkles, firmness, radiance, texture/smoothness, and overall appearance) at baseline and 8-week postsupplementation using a similar five-point ordinal scale (1 = youthful and 5 = aged).

Statistical methods

Erythema and apoptotic cell counts were analyzed by repeated-measures ANOVA. The linear model fit to the skin carotenoid concentration, elasticity, and TEWL metrics included a random participants' effect (making the analysis equivalent to a multifactor paired *t*-test), main effects for time. The analysis performed on the dermatologist's and participants' assessments on skin characteristics was a nonparametric Wilcoxon sign-rank test. *P*-values less than or equal to 0.05 were considered significant.

Results

Thirty-six healthy female participants were enrolled and completed the study. Average age of participants was 58 (range of 43–75 years). Average BMI was 27 kg/m^2 (range of 19–30). No participants withdrew from the study, and average supplement compliance was 97.3% (range of 87–100%).

Ultraviolet radiation-induced cell damage

Skin erythema
There were no differences between baseline and 8 weeks of supplementation in the normal skin readings at the nonexposed, 0 MED site ($P = 0.75$). However, there was a trend toward less erythema between baseline and 8 weeks at the one MED site ($P = 0.10$) and significantly less erythema at 8 weeks compared

to baseline at the two MED ($P = 0.02$) and three MED exposure sites ($P = 0.01$, Table 1).

Apoptosis

There were significantly fewer apoptotic cells at the 3MED UVR exposure site following supplementation compared to baseline, 11.3 ± 0.9 cells/mm^2 SE (range of 3–31) at baseline; 5.3 ± 1.0 cells/mm^2 SE (range of 0–13) at 8 weeks ($P < 0.0001$), representing a 50% reduction in the number of apoptotic cells following 8-week supplementation (Table 1).

Skin carotenoid concentrations and skin attributes

Skin carotenoid concentrations increased from baseline 28 111 RIUs mean ± 1787 SE to 38 472 ± 1787 ($P < 0.0001$) following 8-week supplementation (Table 2). Significant increases in skin elasticity ($P < 0.005$) and TEWL ($P < 0.005$) following supplementation were also observed (Table 2).

Facial skin characteristics

Evaluation of skin characteristics by a dermatologist noted significant improvements in skin radiance ($P < 0.0001$), texture ($P < 0.0001$), and overall appearance ($P < 0.0001$) following 8-week supplementation; no differences in firmness or fine lines were detected (Table 3). Based on participants' self-assessments, significant improvements in skin firmness ($P < 0.05$) and radiance ($P < 0.05$) were reported following supplementation, yet no changes in texture, lines, or overall appearance were noted (Table 3).

Tolerance to the novel nutritional and phytonutrient blend

Six participants reported adverse events that were mild and transitory: mild stomach upset ($n = 2$), flatulence ($n = 2$), and polyphagia ($n = 2$). All of them were considered possibly related to the supplement, but no subject was withdrawn or stopped consumption of the supplement as a result of an adverse event.

Discussion

In the present study, 8 weeks supplementation with the novel nutritional and phytonutrient blend provided cellular protection against UVR-induced cellular damage by bolstering ADMs including DNA damage response, inflammatory balance, and antioxidant protection. Furthermore, participants experienced significant and noticeable facial skin benefits including increases in skin elasticity and TEWL and improvements in skin radiance, texture, and overall appearance based on dermatologist assessment.

The novel nutritional and phytonutrient blend was designed to target and bolster ADMs in order to protect against aging aggressors, delivering anti-aging benefits. The present study used skin as a model to test the effects of the novel nutritional and phytonutrient blend on multiple ADMs related to cellular stress and DNA damage in response to UVR-induced tissue damage. We found that the novel nutritional and phytonutrient blend protected against UVR-induced cellular damage

Table 2 Skin carotenoid concentration and skin attributes (elasticity and transepidermal water loss) at baseline and following 8-week supplementation; data presented as least square means ± SE with ranges in parentheses

	Skin carotenoid concentration RIU	Elasticity MPa	Transepidermal water loss g/h/m^2
Baseline	28 111 ± 1787 (12 000–60 000)	32.8 ± 2.7 (10–64)	10.2 ± 0.5 (6–17)
8 weeks	38 472 ± 1787* (20 000–63 000)	41.4 ± 2.7† (17–73)	11.7 ± 0.5† (8–18)

*$P < 0.0001$; $^\dagger P < 0.005$ compared to baseline and after 8 weeks of supplementation.

Table 1 Skin erythema score and number of apoptotic cells from skin biopsy at the 3 MED site; skin erythema was determined by dermospectrophotometric measurement of the three irradiated sites 24 h post-UVR-exposure at baseline and 8 weeks; data presented as least square means ± SE with ranges in parentheses

	0 MED	1 MED	2 MED	3 MED	Apoptotic cell count cells/mm^2
Baseline	6.2 ± 0.6	9.1 ± 0.6	15.8 ± 0.6	19.6 ± 0.6	11.3 ± 0.9 (3–31)
8 weeks	6.1 ± 0.6	7.4 ± 0.6*	13.6 ± 0.6†	17.3 ± 0.6‡	5.3 ± 0.9§ (0–13)

*$P = 0.10$; $^\dagger P = 0.02$; $^\ddagger P = 0.01$; $^\S P < 0.0001$ compared to baseline and after 8 weeks of supplementation.

Table 3 Facial skin characteristics as determined by dermatologist and self-assessment (1–5 scale with 1 = youthful and 5 = aged)

	Radiance	Texture	Firmness	Lines	Overall appearance
Dermatologist assessment					
Baseline	3.5 ± 0.09	3.4 ± 0.10	3.6 ± 0.08	3.4 ± 0.04	3.5 ± 0.10
8 weeks	2.8 ± 0.09*	2.7 ± 0.10*	3.5 ± 0.08	3.4 ± 0.04	3.0 ± 0.10*
Self-assessment					
Baseline	2.6 ± 0.14	2.4 ± 0.21	2.7 ± 0.12	2.6 ± 0.12	2.6 ± 0.12
8 weeks	2.3 ± 0.14†	2.3 ± 0.21	2.3 ± 0.12†	2.5 ± 0.12	2.5 ± 0.12

*$P < 0.0001$; †$P < 0.05$ compared to baseline and after 8 weeks of supplementation.

as evidenced by an attenuation of erythema at all doses of UVR exposure (one MED −19%; $P = 0.10$, two MED −14%; $P = 0.02$, and three MED −12%; $P = 0.01$) and a reduction in the number of apoptotic cells at the 3 MED exposure site by ~50% ($P < 0.0001$). Nutrients included in the novel nutritional and phytonutrient blend, for example, fish oil, lycopene, and vitamin D have been shown to offer protection against UVR-induced cellular damage, but at higher levels and for longer periods of supplementation.[19,20] The dramatic protective effects of the blend in such a short period of time and at the lower doses in the present study suggest synergistic effects of the novel nutritional and phytonutrient blend. This synergy is supported by others that have found that carotenoid supplementation (25 mg total carotenoids) plus vitamin E (335 mg RRR-α-tocopherol) was more effective than the same level of carotenoids alone in reducing skin erythema following 8 weeks of supplementation,[21] and as compared to only 10 mg total carotenoids in the present study. The fact that we observed such dramatic effects with a formulation containing lower levels and supplemented for shorter periods of time indicates that it is the total blend of ingredients, not just the contribution of one or two individual ingredients, conferring the protective effects of the novel nutrient and phytonutrient blend.

This is the first study to examine the effects of an oral nutritional supplement including any of the ingredients in the novel nutrient and phytonutrient blend on UVR-induced apoptosis in a human clinical trial *in vivo*. We identified only one previous study investigating the effects of a vitamin and antioxidant formula on UV-induced apoptosis. In that study, Ma *et al.* reported that supplementation with moderate amounts of retinol, β-carotene, α-tocopherol, ascorbic acid, and selenium for 8 weeks was protective; however, the level of protection in the present study was much more dramatic.[22] The present study definitively demonstrates that the novel nutrient and phytonutrient blend dramatically impacted the DNA damage response ADM, protecting against UVR-induced cell death as evidenced by a > 50% reduction in apoptosis after 8-week supplementation.

Inflammation is an important ADM which contributes to cellular response and repair. However, if the inflammatory response is unregulated and is allowed to persist, it becomes a negative event in the aging process. In this study, the cellular injury was induced by UVR exposure and erythema was quantified as a marker of inflammation. Following supplementation for 8 weeks, there was a marked reduction in erythema at the sites of UVR exposure (Table 1) with a corresponding reduction in erythema outside of the UVR exposed area at all three doses of UVR. Figure 2 shows photographs of two representative participants' skin erythema 24 h post-UVR exposure with 0 MED, one MED, two MED, and three MED. The marked reduction in erythema outside the injury site (inflammation) after 8-week supplementation is positive evidence that the novel nutritional and phytonutrient blend provided benefits beyond the UVR exposure site by attenuating damage and modulating the inflammatory response.

It is assumed that inflammatory balance was modulated by several of the ingredients included in the novel nutritional and phytonutrient blend. For example, EPA and DHA give rise to anti-inflammatory prostaglandins, eicosanoids, and resolvins. Changing the fatty acid composition of cells involved in the inflammatory response also effects production of peptide mediators of inflammation (adhesion molecules, cytokines, etc.).[23] Other ingredients such as resveratrol and astaxanthin have also been shown to be effective in positively modulating inflammatory balance, even in healthy populations. For example, normal, healthy weight participants were randomized to placebo or 40 mg resveratrol daily for 6 weeks and experienced a reduction in plasma concentrations of inflammatory biomarkers TNF-α and IL-6.[14] Other researchers have found that resveratrol can decrease inflammatory cytokine signaling in mononuclear cells following supplementation.[24] *In vitro* work conducted by Lee *et al.*[25] demonstrated that

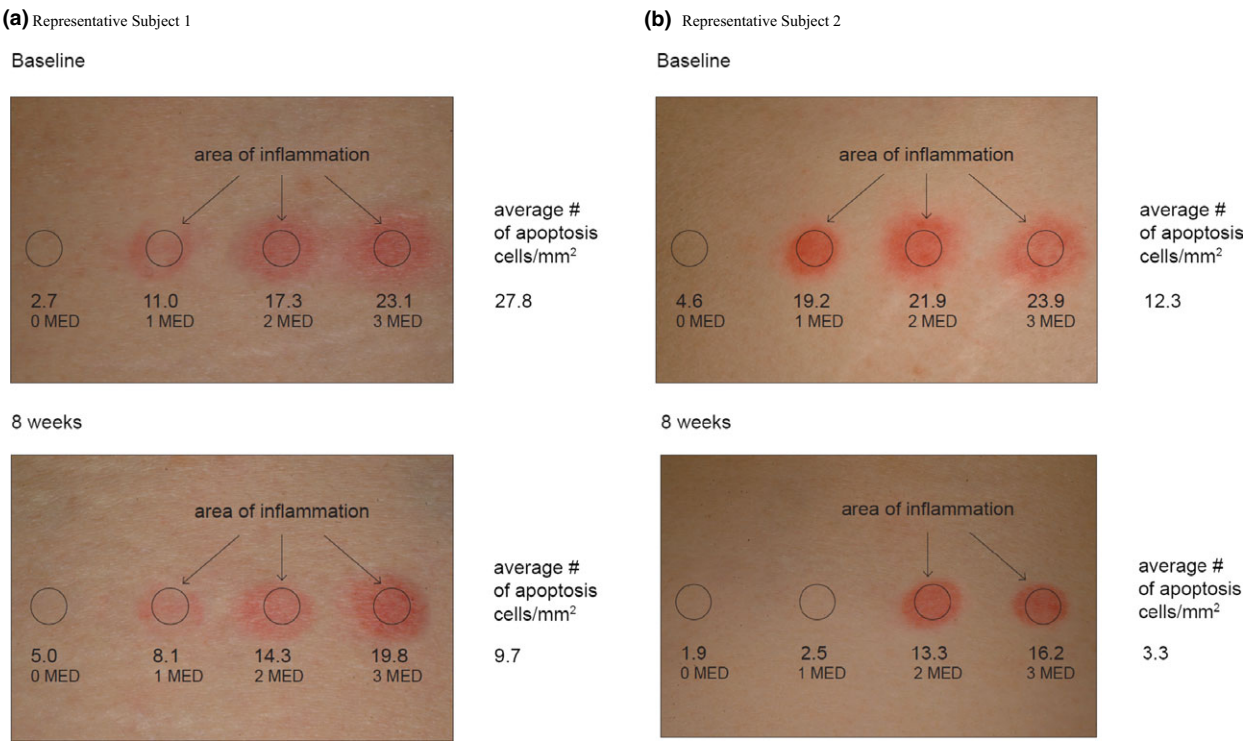

Figure 2 Photographs of skin erythema, skin erythema score, and number of apoptotic cells induced by UVR exposure in two representative subjects at baseline and after 8 weeks of supplementation (a. subject 1 b. subject 2). Photographs illustrate erythema and inflammation at all UVR doses (0 MED, one MED, two MED, and three MED). Black circles indicate UVR exposure area. MED, minimal erythema dose.

astaxanthin prevents the inflammatory process by blocking the expression of pro-inflammatory genes by suppressing the nuclear factor kappaB activation. Astaxanthin has also been found to inhibit the production of nitric oxide, prostaglandin E2, and the pro-inflammatory cytokines, TNF-α and IL-1β.[25] Researchers have noted that single ingredients, for example, EPA, can influence mediators of inflammation, but have no effect on skin erythema.[26] Therefore, it is assumed that the combination of ingredients in the blend affected several ADMs contributing to decreases in skin erythema and inflammation. Although there is clear evidence of a qualitative reduction in inflammation that could be attributed to several ingredients, a limitation of the present study is that measurement of circulating inflammatory biomarkers was beyond the scope of the study, and were not measured but should be explored in future studies.

Human skin has an inherent antioxidant capacity, an ADM, to attenuate the potential damage caused by reactive species generated by UVR exposure. This inherent capacity can be significantly depleted by moderate UV light exposure. Several nutrients have been shown to be protective; for example, intakes of dietary antioxidants, including lutein, lycopene, vitamin E, and flavonoids, have all been demonstrated to reduce UVR-induced oxidative stress.[27] Healthy women who exhibited signs of premature skin aging were given a dietary supplement containing 5 mg lutein and 0.3 mg zeaxanthin for 12 weeks and skin damage was induced by UVR exposure. Supplementation led to improvements in lipid peroxidation (as measured by malondialdehyde) and photoprotective activity in skin.[28] In the current study, there was a dramatic increase in skin carotenoids (Table 2) indicating increased antioxidant protection, an ADM, in the skin. However, not all of the antioxidant protection observed in the present study can be attributed to the carotenoid content of the blend (lutein, lycopene, and astaxanthin) as skin carotenoid concentrations were not correlated with erythema or apoptosis (data not shown), further suggesting that it was the novel blend of ingredients, rather than individual ingredients, responsible for the protective effects observed. It should be emphasized that the novel nutritional and phytonutrient blend is not intended to be a substitute for sunscreen although it did attenuate UVR-

induced erythema and cellular damage, but rather a compliment to topical skin care products to prevent and/or delay skin aging.

The protective effects and influences of the blend on ADMs (DNA damage response, inflammatory balance, and antioxidant protection) in response to UVR-induced damage in the present study translated to improvements in facial skin appearance that were independent of the resistance to UVR-induced damage. Measurable improvements in facial skin attributes as evidenced by increased facial skin elasticity ($P < 0.005$) were observed. Somewhat surprisingly, there was an increase in the facial skin attribute TEWL ($P < 0.005$) (Table 2) that was within the normal range of this parameter. While an increase in TEWL seems counter-intuitive, it may be explained by seasonal changes in humidity and temperature over the course of the study which was conducted between the months of June and September in North Carolina. Other researchers have reported that environmental factors can influence TEWL.[29] Another explanation may be that the increase represents a more youthful skin attribute profile as researchers have noted that youthful skin has higher TEWL.[30] Remarkably, noticeable improvements in skin characteristics as determined by dermatologist's assessment (radiance, $P < 0.0001$; texture, $P < 0.0001$; and overall appearance, $P < 0.0001$) as well as by self-assessments by participants (radiance, $P < 0.05$ and firmness, $P < 0.05$) following short-term, 8-week, oral supplementation were observed (Table 3). These findings are in line with the work of Palombo *et al.*[28] who reported that lutein supplementation at 10 mg/day plus zeaxanthin at 0.6 mg/day improved skin elasticity and hydration following 12-week supplementation. The present study found similar, if not more robust improvements in these skin attributes following supplementation with only 5 mg lutein and only 8-week supplementation. These data further support the hypothesis that it was the full blend of ingredients acting synergistically that led to improvements in facial skin attributes and characteristics, rather than one or two ingredients conferring these benefits.

There are a few limitations of this study. Firstly, only women were included, and although no males were studied, we would anticipate similar effects would be found in males (e.g. protection from UVR-induced erythema and apoptosis). However, changes in dermatological and self-assessment could be different by gender and should be explored in future studies. Secondly, the effects were noted in a relatively short period of supplementation, 8 weeks, and longer term studies should be conducted to determine if the novel nutritional and phytonutrient blend could provide even greater benefits if supplemented for a longer period. Another limitation was the inability to identify the specific ingredient(s) that influenced specific changes or benefits (e.g., facial skin attributes and UVR-induced erythema). By design, the results noted can only be attributed to the blend of ingredients and not to a specific ingredient or subset of ingredients. Instead, the purpose of the present study was to investigate the synergistic effects of the novel nutritional and phytonutrient blend on multiple ADMs using the skin as an easily accessible experimental model. The premise of the current study is that it was the blend of ingredients, rather than any one individual ingredient responsible for the positive outcomes. A final limitation of the present study was the lack of a control group. While the subjects effectively acted as their own controls, a placebo group would have made it more straightforward to interpret the results of the TEWL, whether or not the increase in TEWL represented a seasonal change or a shift toward a more youthful state.

In summary, this study used skin as a model to evaluate ADMs and demonstrated that the novel nutritional and phytonutrient blend provided several skin benefits in a relatively short period of supplementation, 8 weeks. The novel nutritional and phytonutrient blend provided protection against UVR-induced cell injury (erythema), cell death (apoptosis), antioxidant protection (skin carotenoids), and influenced inflammation both within and outside the area of UVR exposure (erythema). In addition to these cellular protection benefits, improvements in several facial skin characteristics and attributes were observed. In conclusion, this novel nutritional and phytonutrient blend conferred youth preservation benefits by bolstering ADMs, protecting against UVR-induced cellular damage and death, and at the same time, restored a more youthful facial appearance. Future studies ought to be conducted to understand the impact of the novel nutritional and phytonutrient blend on ADMs and youth preservation benefits, in both genders and over a longer period of time, and in other tissues and body systems.

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