Novel Nutritional Formula Protects Against Cellular Damage

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ABSTRACT

Human cells possess protection and repair mechanisms that may be described as "aging defense mechanisms—ADMs." Nutrients are important cofactors for cellular function and protection; similarly, natural ingredients, including phytonutrients, have been shown to support key ADMs. The purpose of this study was to determine the effects of a novel nutritional supplement—NNS (containing fish oil, vitamins K_2 and D, alpha-lipoic acid, Co-enzyme Q_{10}, quercetin, citrus bioflavonoids—naringin and hesperidin, d-limonene, resveratrol, carotenoids—lycopene, lutein, and astaxanthin, purple corn and rosemary extracts) on ADMs (i.e. cellular protection). Forty healthy, nonsmoking men and women between the ages of 40 and 75 years of age with Fitzpatrick skin types I and II were recruited to participate. Cellular injury was induced by 1 minimal erythema dose (MED), 2MED, and 3MED solar simulated ultraviolet radiation (UVR) exposure to non-sun exposed skin. Assessments at baseline and 8 weeks post supplementation included: skin erythema at all three MED doses and biopsy of the 3MED site to determine # of apoptotic cells. In addition, total skin carotenoid levels were assessed non-invasively using Raman spectroscopy (BioPhotonic Scanner, Nu Skin Enterprises, Provo, Utah). There was a dramatic effect of the NNS on ADMs following 8 weeks supplementation. Decreases in skin erythema at all three MED doses: 1MED (p=0.019), 2MED (p<0.001), and 3MED (p=0.001) were observed. Supplementation with the NNS led to a significant reduction in the mean number of apoptotic cells, 11.6 at baseline vs. 5.7 cells/mm^2 at the 3MED dose at week 8, suggesting that the NNDS protected against both UVR-induced DNA damage and apoptosis. Finally, skin carotenoid levels increased from 28,600 Raman Intensity Units (RIUs) to 38,775 RIUs (p<0.001) indicative of an increase in antioxidant protection in the skin tissue post-supplementation. In conclusion, 8 weeks supplementation with the NNS supported key ADMs related to cellular health and function including protection against UVR induced cellular damage, apoptosis, inflammation and erythema.

INTRODUCTION

The human body has many mechanisms to respond to or limit the damage induced from environmental stressors. Many times the ability to repair or internal protective mechanisms are not sufficient to combat insults and aging is manifested. These insults can be generated both internally and externally and are defined as "aging aggressors." The mechanisms by which the body, tissues, or cells deal with these aggressors are described as "aging defense mechanisms" (ADMs).

Examples of Aging Defense Mechanisms:

- Antioxidants and Protection Mechanisms
- Detoxification and Stress Response Mechanisms
- DNA Protection and Repair Mechanisms
- Tissue Renewal Mechanisms
- Mechanisms Regulating Metabolism
- Mechanisms Regulating Inflammatory Balance

One tissue that is particularly susceptible to environmental insults is the skin; furthermore it is an easily accessible tissue making it ideal to examine mechanisms of protection against aging.

Exposure of the skin to ultraviolet radiation (UVR) induces acute inflammation and characterized clinically by erythema or redness; furthermore, damaging UVR can also cause apoptosis of skin cells. Several studies have confirmed that acute exposure of human skin to UVR leads to oxidation of cellular biomolecules and to depletion of endogenous antioxidants. Skin UVR model provides a means to evaluate nutritional ingredients and protective effect of aging defense mechanisms like antioxidant protection, cellular stress response, and inflammatory balance.

We formulated a novel nutritional supplement (NNS) of natural ingredients to support several ADMs: Fish oil [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)], resveratrol, quercetin, purple corn extract, rosemary leaf extract, citrus bioflavonoids, Coenzyme Q_{10}, alpha lipoic acid, astaxanthin, lycopene, lutein, vitamin D_3, vitamin K_2 (as menaquinone-7), and d-limonene.

In this study we determined the impact of the NNS on ADMs associated with antioxidant and DNA protection, cellular stress response, and inflammatory balance mechanisms in response to damaging UVR.

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OBJECTIVE

Determine the effects of a novel nutritional supplement (NNS) on aging defense mechanisms (ADMs) of the skin of healthy adults in response to increasing doses of damaging UVR.

SUBJECTS & METHODS

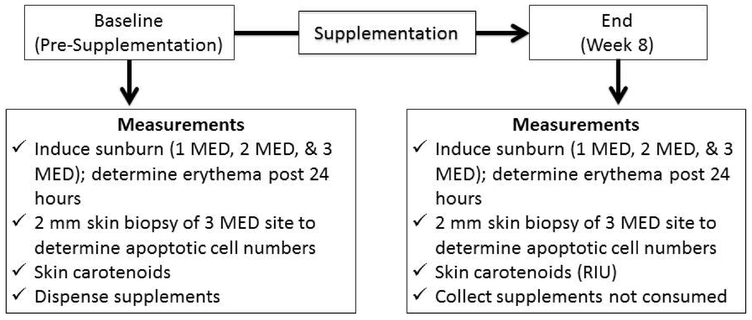
Inclusion

- Forty healthy nonsmoking subjects (n=36 females and n=4 males)
- 40 to 75 years of age
- Fitzpatrick skin types I and II
- Body mass index (BMI) between 19 and 30 (kg/m2)

Exclusion

- Participants with a history of chronic diseases, skin diseases or abnormalities currently under treatment
- Consuming dietary supplements containing carotenoids, vitamin D, EPA, DHA, & resveratrol
- Ate more than 1 fatty fish per week
- Using or having used an anti-aging treatment skin care product within 30 days of study
- Pregnant, planning to become pregnant, or nursing

The study was approved by an Institutional Review Board and conducted according to Helsinki Declaration. The study was registered on ClinicalTrials.gov (#NCT02525224).



Methods

- Open label study. Subjects consumed 2 capsules, 2 times per day with morning and evening meals.
- Measurements were taken at baseline (before supplementation) and 8 weeks post supplementation (end)
- Cellular injury induced by solar simulated radiation (model 16S-150v.3 powered by a xenon lamp power supply model XPS 200, Solar Light Co. Glenside, PA) at 3 minimal erythema doses (1 MED, 2 MED, and 3 MED) on 1 cm^2 non-sun exposed buttock skin and compared to non-exposed skin in the same area
- The MED was defined as the lowest dose of UVR (mJ/cm^2) causing a visually perceptible erythema at 24-h post-UVR exposure
- Erythema was determined 24 hour post exposure with dermospectrophotometer measurement of the irradiated site.
- Skin punch biopsies(2 mm) were taken at baseline and end of study at the 3 MED site. The specimens were processed and stained for hematoxylin and eosin by Cockerell Laboratories, Dallas, TX to determine the number of apoptotic cells.
- Six cross section slides were read in blinded fashion by board certified dermatologist and then averaged for each subject.
- Total skin carotenoid levels were assessed non-invasively using Raman spectroscopy (BioPhotonic Scanner, Nu Skin Enterprises, Provo, Utah).
- The linear model fit to the Erythema metric included a random subjects effect, main effects for time, gender, MED level, and interaction effects involving these factors. Apoptotic cell numbers and skin carotenoid levels for time effects. *P*-values ≤ to 0.05 were considered significant.

RESULTS

All subjects completed the study with 97% compliance to the NNS.

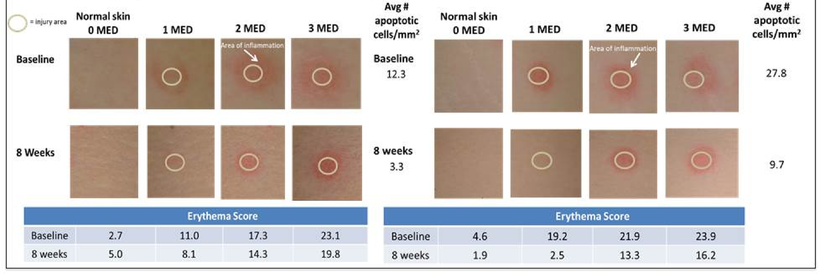
Table 1. Skin erythema induced by three solar simulated radiation minimal erythema dose (1 MED, 2 MED, and 3 MED) on non-sun exposed skin compared to non-exposed skin. Erythema was determined with dermospectrophotometer measurement of the irradiated sites 24 hours post exposure. Data presented as least square means ± standard error of the means (SEM). *P<0.05 as compared to baseline levels

	Normal Skin	1 MED	2 MED	3 MED
Baseline (n=40)	**6.0 ± 1.0**	**8.6 ± 1.0**	**16.3 ± 1.0**	**19.5 ± 1.0**
Females (n=36)	6.1 ± 0.6	9.1 ± 0.6	15.8 ± 0.6	19.6 ± 0.6
Males (n=4)	5.9 ± 1.9	8.2 ± 1.9	16.8 ± 1.9	19.5 ± 1.9
8 weeks (n=40)	**5.9 ± 1.0**	**7.6 ± 1.0***	**14.4 ± 1.0***	**17.7 ± 1.0***
Females (n=36)	6.0 ± 0.6	7.3 ± 0.6*	13.5 ± 0.6*	17.3 ± 0.6*
Males (n=4)	5.8 ± 1.9	8.0 ± 1.9	15.4 ± 1.9	18.1 ± 1.9

Table 2. Number of apoptotic cells from 3 MED dose site and skin carotenoid levels (RIUs) at baseline and 8 wks post-supplementation. Data presented as least square means ± standard error of the means and ranges in parentheses. *P<0.05 as compared to baseline levels

	Total (cells/field)	Females (cells/field)	Males (cells/field)	Skin Carotenoid Levels (RIUs)
Baseline	**11.6 ± 1.1** (2.5 to 30.8)	**11.3 ± 1.0** (2.5 to 30.8)	**14.7 ± 6.1** (6.8 to 26.7)	**28,600 ± 2,927** (12,000 to 60,000)
8 weeks	**5.7 ± 0.6*** (0.0 to 12.7)	**5.2 ± 0.6*** (0.0 to 12.5)	**10.2 ± 0.6** (8.5 to 11.3)	**38,775 ± 2,927*** (20,000 to 63,000)

Figure 1. Skin photographs and erythema scores of two representative female subjects depicting erythema and inflammation induced by 3 minimal erythema doses (normal skin, 1 MED, 2 MED, 3 MED) at baseline and after 8 weeks supplementation. Note the reduction in the number of apoptotic cell numbers from biopsies of the 3 MED site at 8 wks.



SUMMARY & CONCLUSIONS

8 weeks supplementation with the NNS led to dramatic protective effects against UVR induced exposure as evidenced by:

- Bolstered ADMs (antioxidant and DNA protection, cellular stress response, and inflammatory balance mechanisms)
- Significant decreases in skin erythema (P<0.05) at all three MED doses
- Significant reduction in the mean number of apoptotic cells, 11.6 at baseline vs. 5.7 cells/mm^2 (P<0.05) at the 3 MED dose, suggesting that the NNS protected against both UVR-induced DNA damage and apoptosis.
- Increased skin carotenoid levels indicative of an increase in antioxidant protection.
- Additional clinical studies are warranted to examine influence of NNS on other ADMs.

Conclusion: 8 weeks supplementation with the NNS supported key ADMs related to cellular health and function including protection against UVR induced cellular damage, apoptosis, inflammation and erythema.