Comparison of Oral Carotene Supplementation and Oral Polypodium Leucotomas in Preventing Photodamage

Jazmin Martinez

Yale Physician Associate Program, jazmin.martinez@yale.edu

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Comparison of Oral Carotene Supplementation and Oral *Polypodium Leucotomas* in Preventing Photodamage

A Thesis Presented to
The Faculty of the School of Medicine
Yale University

In Candidacy for the degree of Master of Medical Science

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Jazmin Martinez, PA-SII
Class of 2023
Yale Physician Associate Program

Suguru Imaeda, MD
Associate Professor of Dermatology
Yale University School of Medicine
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Abstract:

Skin exposure to ultraviolet (UV) irradiance is the major cause of skin disorders, such as sunburn and nonmelanoma cancer. The current standard of care for photoprotection is the daily use of sunscreen, although patient adherence and adequate application continues to be an issue. Current data show systemic photoprotection can be added to sunscreen use to reduce the risk of cutaneous diseases. This randomized controlled study will investigate whether supplementation with Polypodium leucotomas versus carotenoid supplementation versus the standard of care, provide increased photoprotection in adults with Fitzpatrick skin types II-IV. Outcomes will be measured by comparing the minimal erythema dose before and after treatment. We hypothesize the treatment group receiving oral Polypodium leucotomas will have a higher minimal erythema dose compared to the other groups. The results of this study will help inform photodamage prevention with the goal of reducing the risk of developing UV-carcinogenesis and erythema in the skin.
Chapter I: Introduction

1.1 Background

1.1.1 UV Damage and Reactive Oxygen Species

Cumulative ultraviolet radiation exposure to human skin, both UV-A and UV-B, plays a role in the destruction of healthy skin conditions, increasing the risk for several deleterious effects to develop, including skin cancer, photoaging, and immunosuppression. UVR is an established cause of nonmelanoma cancer and squamous cell cancer. Similarly, it is the primary and most preventable environmental risk factor for melanoma and non-melanoma cancers, affecting millions of people worldwide. Keratinocyte-derived skin cancers (KDSC) account for about five million of cancers in the United States, and rates continue to increase each year. The average annual cost in the United States is estimated to be $3.3 billion for treating melanoma and $4.8 billion for treating nonmelanoma skin cancers (NMSC), including squamous cell carcinoma (SCC) and basal cell carcinoma (BCC).

As a result, photoprotection plays an important role in the prevention of the damaging effects of daily exposure to UV rays. UV radiation wavelengths range from 100 nm to 400 nm and are categorized into three wavebands: UVA ranging from 315-400 nm, UVB (280-315 nm), and UVC (100-280 nm). Minimal UVC reaches the earth’s surface, so photoprotection is aimed to protect against UVA and UVB, to which the majority of DNA damage is attributed to. However, many of the marketed sunscreens in the United States do not provide balanced protection against UVA and UVB radiation, and adoption of photoprotective measures are not undertaken regularly by a large proportion of patients. Due to the detrimental effects caused by solar radiation, it is crucial to find more ways to protect against UV damage, including systemic photoprotection through oral supplementation.
UVR is a major source of oxidative stress, causing inflammation and DNA damage; and ultimately leading to photoaging and the development of several skin disorders and cancers. Overexposure of the skin to UVA and UVB damages DNA through multiple mechanisms. UVB is almost completely absorbed by the epidermis and UVA penetrates deep into the dermis, leading to oxidative stress that increases the production of reactive oxygen species (ROS). ROS are important signaling molecules involved in the progression of inflammatory disorders. They are partially reduced metabolites of oxygen that are generated as byproducts of cellular metabolism. They possess strong oxidizing properties; known to be deleterious at high concentrations in contrast to physiological conditions. They are released by polymorphonuclear neutrophils (PMNs) present at the site of inflammation and play a role in the opening of interendothelial junctions and promote the migration of inflammatory cells across the endothelial barrier. Although important in the setting of an acute injury, a chronic low-grade inflammation induced by over-exposure to UVR is harmful to the skin. ROS promotes the up-regulation of several inflammatory markers, including cyclooxygenase-2 (COX-2), which has shown to contribute to carcinogenesis.

Furthermore, the accumulation of pro-inflammatory cells and chronic low-grade inflammation increases the chronological aging process of the skin. ROS trigger lipid peroxidation of cell membranes, DNA and protein damage to tissue, inflammation, and keratinocyte apoptosis. Similarly, the expression of matrix metalloproteinases (MMPs) are activated, causing the degradation of the extracellular matrix, including collagen maintaining cells and thus compromising skin integrity. UVB-induced skin damage leads to massive infiltration by leukocytes, including neutrophils, which play a role in releasing chemokines that further activate MMPs and cause increased damage to the skin. Another consequence of UVR is
the rapid degranulation of cutaneous mast cells that release a different inflammatory mediator, known as tumor necrosis factor alpha (TNF-a). TNF-a plays a role in activating the sunburn response that leads to the activation of dermal blood vessels, manifesting as erythema. Reducing the generation of ROS is crucial in preventing and counteracting the detrimental effects of chronic UVR exposure and limiting UV-induced inflammation and erythema.

1.1.2 Sunscreens and Other Photoprotective Agents

A multi-pronged approach is recommended by dermatologists for the protection against the damages of UVR. Several behavioral and lifestyle modifications are recommended, including wearing wide-brimmed hats, avoid tanning-beds, wearing UVR protective clothing when outdoors, seeking shade, and the use of sunglasses. However, sunscreens continue to be an integral component of preventing and counteracting the damaging effects of UVR on human skin.

Although sunscreens are essential for protection, they are not enough and they are accompanied by inherent limitations. A large proportion of patients do not undertake in photoprotective practices regularly, whether due to lifestyle preferences or common misconceptions regarding photoprotection. According to a recent study, several personal barriers to sunscreen use included: dislike of feel or appearance of topically applied sunscreen, time constraints, and burden of cost. Currently, the World Health Organization (WHO), recommends sunscreen be re-applied every 2-3 hours, supported by experts who have pointed out that increased frequency of re-application can significantly enhance UVR protection in real-world testing.
Sunscreen was originally developed as a means of protection against sunburn, primarily thought to be caused by UVB radiation. It was not until years later that radiation in the UVA spectrum was also found to contribute to carcinogenesis and photoaging. This led to the introduction of UVA-absorbing compounds to the sunscreen industry, notably avobenzone. Marketed sunscreens must show coverage of both UVB and UVA radiation and testing through spectrophotometry is required by the Food and Drug Administration (FDA). The active agents of sunscreens typically fall into two major classes: organic molecules, including PABA, cinnamates, and benzophenones among others, and inorganic molecules, such as titanium dioxide and zinc oxide. Both classes of sunscreen formulation differ in their mechanism of action, as well as their inherent limitations. Organic molecules contain aromatic rings that absorb and distribute UVR, whereas inorganic molecules function as a physical filter that absorbs and scatters incident UVR.

Although modern sunscreen formulations continue to be an integral part of photoprotection and clinical trials have shown their effectiveness in preventing both invasive melanoma and SCC, they are not immune to negative side effects. Organic molecules penetrate deeper into the epidermis, leading to a higher risk of inducing irritant or allergic contact dermatitis. Studies have shown measurable levels of sunscreen chemicals in serum, human milk samples, and urine, confirming substantial absorption and distribution of organic molecules. Inorganic sunscreens penetrate human skin in a minimal degree, but tend to leave a whitish hue after application to human skin due to its property of scattering light, making it less cosmetically appealing. The short half-life, need for frequent reapplication, and potential side effects continue to limit the usefulness of sunscreens.
1.1.3 Carotene Properties and Absorption

Carotenoids are fat-soluble yellow, orange, red, and green leafy pigments synthesized exclusively by plants, and are found in vegetables and fruits, especially in tomatoes. About 850 naturally occurring carotenoids have been reported, and about 50 variants of carotenes are present in human diet. Carotenes are divided into provitamin A molecules: carotenes and xanthophylls, and their general structures consist of a 40-carbon skeleton, a polyene chain with nine conjugated double chain bonds and an end group at both ends of the polyene chain.

Carotenoids have been shown to possess various mechanisms against chronic diseases including, eye disorders, metabolic syndrome, and different types of cancer. However, this summary will focus on carotenoids in reference to dermal health. Carotenoids possess the ability to absorb excessive energy and transfer the energy through two different mechanisms: light energy state and high energy state. A singlet-singlet excitation transfer is considered a light energy state transfer and is relevant for photosynthesis. However, the higher energy state transfer, through triplet-triplet transfer that releases excessive thermal energy by polyene vibration is important in explaining the photoprotection characteristics carotenoids possess. In vitro and in vivo studies have shown that carotenoids possess the ability to suppress UVA and UVB-mediated ROS formation through chemical reactions. They can prevent photoinactivation of anti-oxidant enzymes, lipid peroxidation, and DNA damage caused by oxidative stress. Carotenoids also possess the ability to quench and neutralize reactive species, such as singlet oxygen (\(^{1}\text{O}_2\)), and to scavenge free radicals generated during photooxidative stress. They are among the most effective naturally occurring quenchers of \(^{1}\text{O}_2\), with a bimolecular rate constant in the range of \(1 \times 10^9\) to \(1 \times 10^{10}\) mol/L\(^{-1}\) s\(^{-1}\). In addition to taking up singlet oxygen, carotenoids have been shown to quench superoxide anion radicals and hydroxyl radicals by the formation of endoperoxide or
Furthermore, studies have found that a high dietary intake of beta-carotene can decrease UVR induced erythema and lead to a slight increase in the threshold of MED.

Beta-carotene has been used for the treatment of various photosensitivity disorders for decades and its role as an oral sun protectant has gained momentum. It is currently the treatment of choice to improve the photosensitivity in patients with erythropoietic protoporphyria. Doses between 60-180 mg/day for adults are recommended for positive effects and allows a maximum plasma level of 600-800 μg/dl to be reached. 80% of patients notice a decrease in photosensitivity. Partial protection against UVA and UVB radiation has been observed in studies where carotene is administered orally and its effect on UVR-induced erythema has been shown to be beneficial, especially in individuals with Fitzpatrick’s skin phototype II, who are more susceptible to the development of erythema induced by natural sunlight.

1.1.4 PLE and Systemic Absorption

Polypodium leucotomos (PL) is a tropical fern native to Central and South America, and is traditionally used in these geographical areas to treat various skin conditions. The leaves from this fern are rich in polyphenols, from which an aqueous extract can be formulated to exploit its photoprotective effects. The classes of polyphenols are divided into: phenolic acids, flavonoids, stilbenes, and ligands. The chemical nature of these phenolic rings is a hydroxyl (-OH) group attached to a carbon atom in a benzene ring. Dietary polyphenols have played important roles in human health for decades due to its antioxidant activities. Foods rich in polyphenols have been linked to lowered risks of disease states that develop due to reactive oxygen and nitrogen species, including cancer, chronic inflammation, and other degenerative diseases. Polyphenols suppress the generation of free radicals through inhibition, scavenging, and deactivating active species or
precursors of free radicals. UVR activates enzymes, such as lipoxygenase and cyclooxygenase, inducing lipid oxidation of its major surface unsaturated lipids. Lipid oxidation products impair the skin’s protective barrier and induce various skin pathologies. However, polyphenols have been shown to act as direct radical scavengers of the lipid peroxidation chain reactions, thus reducing the rate of oxidation and stopping the chain reactions from cascading. Additionally, PL enhances DNA repair and modulates the inflammatory and immune responses, showing promising effects in the prevention of photodamage and development of photo-carcinogenesis.

The most potent anti-oxidants present in PLE are ferulic and caffeic acids, which have been shown to possess a strong post-oral administration absorption. Studies have revealed that orally administered PLE significantly prevented erythema in UVR treated human skin, measured through the increase of MED. Furthermore, its photo-immunosuppression properties have been shown to modulate the expression of molecules, transcription factors, and gene expression involved in photo-carcinogenesis, correlating with changes in the levels of several markers of oxidative stress in the skin and blood. The safety of oral treatment of PLE has been supported through various studies administering carefully controlled capsules of PLE that have not produced long term severe adverse effects.

1.1.5 Systemic Photoprotection and its Role in Treatment

In recent years, there has been substantial data in support of the beneficial effects of oral photoprotection. Systemic photoprotection has been studied as a potential complementary to topical photoprotection, offering anti-photocarcinogenic, photoprotective, anti-inflammatory, and anti-oxidant properties. Although they do not directly protect the skin from UVR, they possess several advantages, including ease of use, long half-life, and resistance to external conditions.
Oral photoprotective products typically contain active molecules that activate photoprotective mechanisms, mainly antioxidant actions. The addition of natural oral antioxidants to topical sunscreen formulations, such as PLE or carotenoids, could protect against oxidative stress that could not be prevented by UVR absorption or reflectance. In addition to its antioxidant properties that have been shown to decrease the number of sunburn cells, DNA damage, and lipid peroxidation; antioxidants combined with sunscreen have produced a statistically significant increase in the measured SPF.  

The results of this study could add to the wealth of knowledge regarding the beneficial effects of naturally occurring oral antioxidants. Although sunscreen based photoprotection continues to be a major first line of prevention against UVR, future advances in photoprotection could include the addition of an oral antioxidant to decrease the risks associated with UVR exposure. Despite the widespread use of sunscreens, sunburn remains common and thus systemic photoprotection can bridge the gap between the limitations of topical sunscreens and deleterious effects inflicted by chronic UVR. Oral agents possess several advantages compared to the latter, such as their ease of use, ability to not be altered by external conditions, stable half-lives, and absorption properties. The ideal systemic photoprotective agent would possess strong antioxidant properties, making PLE or carotenoids viable options.

1.2 Statement of the Problem

Exposure to UV radiation is unavoidable and continues to be a major cause of skin disorders. The predominant exposure to UV light occurs under everyday circumstances when no topical sunscreen is applied, estimated to be about two thirds of the cumulative erythemal UV dose/year. Patient adherence to the current standard of care continues to be an issue, leading to
the vast majority of skin protection to depend on endogenous defense mechanisms. The use of systemic photoprotection is gaining momentum, and the use of micronutrients, including carotenoids and PLE has been shown to have antioxidant, anti-inflammatory, immunoregulatory, and anti-tumorigenic effects. Adjuvant photoprotection with oral PLE is expected to reduce the risk of UV-related cutaneous manifestations, contributing to lifelong skin health.15

1.3 Goals and Objectives

The primary goal of the study is to determine whether supplementation with PLE vs beta carotene increases the MED and reduces the risk of photodamage in patients with Fitzpatrick skin types II through IV. The proposed study will be a randomized placebo-controlled trial to establish a potential superior adjuvant systemic photoprotection agent that could decrease the risk of UV-related cutaneous manifestations, such as erythema of the skin. Additionally, a secondary aim of the study is to determine whether the effectiveness of the systemic agents vary by Fitzpatrick skin type

1.4 Hypothesis

There will be a statistically significant difference in the mean of MED in patients with Fitzpatrick skin types II through IV following oral PL extract supplementation with concurrent sunscreen use compared to the oral carotenoid supplementation group and the standard care group after 12 weeks.
References

Chapter II: Review of the Literature

2.1 Introduction

A thorough literature review that pertains to carotenoids, PLE, UVR, and photoprotection was conducted between August 2022 and May 2023, using several databases, including PubMed, Cochrane, and Ovid (Medline). Several search terms were used within these databases either separately or in conjunction, such as the following: Antioxidants, oral PLE, carotenoids, beta-carotene, UVR, photodamage, photoprotection, MED, photosensitivity, photocarcinogenesis, sun exposure, sunscreen agents, side effects, prevention, and dietary supplements. The search was limited to studies published since 2000 in order to focus on recent and relevant findings.

2.2 Review of Relevant Studies

This section summarizes the existing evidence pertaining to the use of carotenoids and PLE in the prevention of UVR-associated skin damage. There has been great interest in the use of these agents as an adjuvant prevention due to their antioxidant, anti-inflammatory, immunoregulatory, and anti-tumorigenic properties. However, a direct comparison between carotenoids and PLE is limited and relatively new, and more data is warranted to detect a difference between agents. Therefore, the focus of review is on previous literature that has investigated the relationship between photoprotection and oral carotenoids, as well as oral PLE.

2.2.1 Studies Analyzing the Relationship Between Carotenoids and UVR

Many studies have investigated the relationship and efficacy of carotenoids and its protective effect against UVR-induced skin damage. A 2017 single-blinded randomized controlled trial conducted by Rizwan et al assessed whether lycopene, a powerful carotenoid
with potent antioxidant properties, can protect the skin against UVR-induced effects. They found no statistically significant difference in visual assessment of the MED between the groups after 3 months of supplementation. However, an objective assessment of erythema, using an erythema meter, did show a significant erythema dose-response curve shift to the right (p<0.05) post supplementation in the active group. Some limitations of the study include that it was not sufficiently powered to detect a difference between the group receiving daily lycopene supplementation compared to placebo and its population consisted of solely Caucasian women, limiting generalizability. Similarly, another limitation was that visual assessment of erythema, as used in this study, was demonstrated to not be as sensitive as objective erythemal measurements. This may have contributed to the lack of a statistically significant difference in MED between groups since MED is derived using an interval scale of UVR change, meaning that small alterations in erythema may have gone undetected.

Grether-Beck et al performed a randomized, double-blinded, placebo-controlled crossover study assessing the capacity of carotenoid rich tomato nutrient complex (TNC) and lutein to protect against UVR at a molecular level. This trial used lycopene-rich TNC softgel capsules, lutein softgel capsules, and placebo softgel capsules and included a sample size of 65 healthy participants. They found a significant reduction of UVR-induced gene expression of heme-oxygenase 1, intercellular adhesion molecule 1 (ICAM-1) and matrix metallopeptidase 1 mRNA (P<0.05) in those treated with lutein or TNC, in contrast to those treated with placebo capsules. However, differences were observed in the lutein arm, in which significantly lower photoprotection was detected against UVA-induced ICAM-1 expression when lutein was given in the second phase of treatment. These findings may indicate that oral TNC and lutein not only protect human skin against UVB/A, but also longwave UVA1 radiation, and oral photoprotection
can be demonstrated at a molecular level. Strengths of this study include its design as a crossover study with a reasonable sample size. The crossover design increases the power and decreases confounding covariates as each volunteer was their own control. Some limitations of this study include inappropriate washout phases, as observed in the lutein arm where lower photoprotection was observed if lutein intake was preceded by 16 weeks of restricted diet due to washout and placebo treatment.

In 2019, Groten et al conducted a double-blinded, randomized placebo-controlled, multicenter study to assess whether a similar carotenoid-rich tomato nutrient complex (TNC) could protect against UVB-induced erythema, measured as an increase in MED. A sample size of 149 healthy participants were randomized to receive either carotenoid-rich TNC softgel capsules or placebo, which appeared identical and consisted of medium-chain triglycerides, for a period of 12 weeks. They found no significant change in the MED between groups post supplementation, given as a median 0.080 (0.035, 0.147), but in the second primary outcome, measured as the change of erythema (Δa), did observe a significant difference between the two treatment groups (n= 71 for carotenoid-rich TNC, n=74 for placebo, p=0.019). Additionally, intake of the active supplement significantly protected against UVB-induced upregulation of markers, IL6 and TNFα, associated with immunosuppression or inflammation, as noted in biopsies taken at visits 4 and 8 (p=0.012, p=0.033). These findings corroborate with previous studies that have found protection against UV-induced erythema formation, measured as Δa, and UVB-induced upregulation of IL6 and TNFα. Limitations of this study that may explain the failure to determine a difference in MED between groups is that a higher UV dose was applied (1.25 MED instead of 1 MED), as well as a higher sensitivity due to an objective detection device.
Baswan et al performed a similar randomized controlled trial in 2020 to further evaluate the photoprotective effects of daily supplementation with carotenoids against UVA radiation-induced pigmentation. The trial included 60 healthy participants that were randomized to receive either the multi-carotenoid supplement, containing β-carotene 4.25 mg, α-carotene 1.10 mg, lutein 1.12 mg, and zeaxanthin 0.053 mg per softgel capsule or placebo for a duration of 12 weeks. They measured skin sensitivity to UVA irradiation as the minimal persistent pigmentation (MPPD), in which an increase in MPPD indicates increased skin protection. Although they found no significant changes in the mean values of MPPD between groups, they did find a significant increase in the individual typology angles (ITA°), after 8 weeks (P=.000) and at 12 weeks (P=.000) relative to placebo. The ITA° characterizes the melanic level of the skin and a decrease in this value indicates increased pigmentation. Quantitative assessment of skin pigmentation by chromametry supported the finding that oral intake of carotenoids provided significant skin protection against UVA radiation. A strength of this study is its generalizability, since subjects who participated were not asked to follow extensive dietary restrictions, but rather were asked to keep their normal dietary habits. However, a limitation of this study is its fairly small sample size that may have not been powered enough to detect a difference in MPPD between groups.

In 2021, Baswan et al performed a literature review of RCTs investigating the ingestion of carotenoids in relation to skin-related clinical outcomes.¹ They included 25 human clinical intervention studies that spanned the last 5 decades. Studies that were not RCTs placebo-controlled were only included if more than 10 subjects participated and the duration lasted 4 weeks or longer. Their primary outcomes were changes in MED, skin carotenoid levels, colorimetry, gene expression markers, trans epidermal water loss (TEWL), skin texture and
appearance, and carotenoid distribution in skin. Of the 25 articles they evaluated, 19 were RCTs, 1 was a prospective study, and 4 were intervention studies, for a total of 966 patients.

Overall, their comprehensive review demonstrated that carotenoids possess significant photoprotective effects against sunlight-induced erythema, are effective against suppressing various biomarkers induced by oxidative stress, and prevent skin lipid peroxidation. Most of the reported clinical studies strongly support the meta-analysis conducted by Kopke et al in 2008, which found a significant effect of β-carotene supplementation on the development of a sunburn reaction (P=0.0089) in the size of 0.8 standard deviations (95% CI 0.2, 1.4). Similarly, regression plot analysis revealed that photoprotection required a minimum of 10 weeks of treatment with a mean increase of the protective effect of 0.5 standard deviations with every additional month of treatment, confirming the time-dependent manner β-carotene supplementation provides.

It is important to note that recent studies that found little to no photoprotection with carotenoid supplementation could be explained by differences in dosing and duration of the treatment before the irradiation occurred. As described previously, clinical data gathered thus far indicates significant protection requires at least 10 weeks of supplementation and the recommended dose for total carotenoids falls within the range of 17.7-39.9 mg/d. The studies that have reported no photoprotective effects were conducted for a period of 3-8 weeks. Recent research has also shifted to more reliable and reproducible clinical and molecular endpoints than erythema as an indicator of photoprotection. Other endpoints such as MPPD and gene expression should be considered, given the studies that have utilized subjective assessments of UVB-induced erythema and have found no significant differences. A strength of this literature review
was that it was successful at analyzing the current trends between carotenoids and photoprotection and the pitfalls of studies using MED as an endpoint.

2.2.2 Studies Analyzing the Relationship Between PLE and UVR

Middelkamp-Hup et al performed a randomized controlled trial to assess the efficacy of oral PLE in decreasing psoralen + UVA (PUVA) induced phototoxicity of human skin and its protective effects on various histologic parameters. They enrolled 10 healthy volunteers between the ages of 24 to 47 with skin phototype II or III. Participants with a personal or family history of photosensitivity or taking any drug that might alter the skin response to UVR were excluded from the study. As primary outcome they measured the minimal phototoxic dose (MPD) for each individual and obtained skin biopsy specimens. They found that oral PLE supplementation significantly decreased the acute PUVA-induced phototoxic reaction and also diminished the cutaneous pigmentary response, including decreased erythema and edema (P<0.005). Moreover, histologic comparison between the group receiving oral PLE with PUVA and PUVA alone showed noticeably more maturation disarray, micro-vesiculation, and vacuolization of keratinocytes in the PUVA alone group. In the PLE treated group, the number of sunburn cells/mm epidermis was significantly lower when compared with PUVA alone (P=0.05), there was less depletion of Langerhans cells/mm² epidermis in response to UVR (P≤0.01), and these cells preserved their size and dendritic appearance. However, limitations of this study include its small sample size, and its observations of PLE as an effective chemoprotector were made in the acute phase of UV-induced damage. Therefore, follow-up studies with a larger, more diverse sample size and longer duration of study are warranted to generalize the findings and assess the photoprotective efficacy of PLE in chronic UVR exposure.
Villa et al conducted a similar randomized controlled trial in 2010 to investigate the efficacy of PL against UVA radiation. They aimed to detect and quantify PLs’ UVA-induced photoaging marker, common deletion (CD), after UVA irradiation. 10 healthy participants were enrolled and exclusion criteria included subjects with a history of current or planned pregnancy, skin cancer, photosensitivity, radiation other than sunlight, asbestos exposure, smoking during the previous 6 months, applying/consuming any drug that might alter skin responses to UV radiation, and subjects unable to undergo skin biopsies. They found at two times the MED, average CD values in the placebo group increased by 217% over baseline, while values in the PL-treated group decreased by 84% (P=0.06). Similarly, at three times the MED, those values increased 760% and 61% respectively (P=0.07). Chronic UVA exposure is associated with elevation in CD expression and in this study, increments in CD expression are noted after acute UVA exposure. After an interaction analysis, PL was found to exhibit a trend towards preventing the increase of CD expression levels as the UVA dose increased. Limitations of this study include a small sample size that prevents the findings from being extrapolated and leads to higher variability. A larger study is warranted to further support and characterize PL’s role in preventing UVA-induced skin photodamage.

In 2015, El Haj et al conducted a literature review on the photoprotective properties of PLE in the context of sunburn, photo dermatoses, chronic skin damage, and photoaging, and skin cancer. They included 39 articles of dermatological relevance that commented on the antioxidant, immunoregulatory, anti-inflammatory, and antitumorigenic effects of PLE and excluded studies in patients with psoriasis, atopic dermatitis, vitiligo, and melasma. They found that PLE reduced UV-induced inflammatory responses, accelerates the removal of UV-induced photoproducts, decreases UV mediated oxidative DNA mutations, and has some protective
effects against photoaging and PUVA-induced phototoxicity. Its antioxidant properties were supported by several studies that used PUVA therapy, which produces ROS and free radicals, inducing erythema, edema, and pain. PL-treated skin showed a statistically lower grade of erythema and edema than sites exposed to PUVA alone (P<0.005). These studies highlight the potential of PL to quench oxidative stress and its ability to enhance endogenous antioxidant systems.

Moreover, studies have demonstrated the anti-inflammatory properties of PL, such as its ability to inhibit the expression of UV-induced inflammatory markers, including COX-2, and account for decreased leukocyte extravasation and mast cell infiltration in irradiated areas by 60% (P<0.001) and 50% decrease in macrophages (P<0.02). They also found studies that supported the immunoregulatory effects of PL, established PL inhibits trans-uroconic acid (t-UCA) photoisomerization in a dose-dependent manner, and prevents the oxidative breakdown of t-UCA in the presence of free radicals. t-UCA is a natural epidermic constituent, located in the upper layers of the stratum corneum, that provides some endogenous UV protection. However, it is converted to cis-UCA by UVB, which is known to be involved in immunosuppression induced by UV-irradiation.

Strengths of this literature review are that it focused on the photoprotective effects of PL and excluded patients with certain dermatological conditions that could have confounded the results. A limitation of this literature review is the use of non-current research studies. Although this ensures completeness and comprehensiveness of the review, some of the studies discussed were performed on mice. This limits the generalizability of certain studies to a human population.
In 2017, Emanuele et al conducted a randomized controlled trial comparing the effects of PLE versus topical PL on skin biophysical skin parameters. They enrolled 40 healthy adult volunteers in a 1:1 fashion to receive either a fixed PL/pomegranate combination (PPmix) 480 mg/day or topical PL (Fernblock) for 3 months. The biophysical parameters assessed at baseline and after 3 months of treatment included skin sebum content, hydration, TEWL, erythema index, melanin index, and elasticity. All participants enrolled were free of any known dermatological conditions and the exclusion criteria included a history of significant neurologic, psychiatric, hematological, endocrine, cardiovascular, respiratory, renal, hepatic, or gastrointestinal disease, or coagulation deficits. Women who were pregnant or breastfeeding were also excluded and participants were not allowed to use any topical skin intervention throughout the entire study duration.

They found hydration and elasticity significantly improved and TEWL reduced in both groups (P<0.001) without significant intergroup differences. Similarly, the erythema index decreased after both treatments, although the fixed PL/pomegranate combination was significantly more effective (P<0.001) compared with topical PL. Moreover, the melanin index and skin sebum content were significantly reduced by the fixed PL/pomegranate combination, whereas the topical PL alone did not change these biophysical parameters (P<0.001). A limitation of this study is the composition of the fixed PL/pomegranate combination, since a more marked decrease in the skin erythema and pigmentation could be explained by the additive effects exerted by the active compounds of pomegranate. Furthermore, only participants with II and III Fitzpatrick skin types were enrolled and was limited to Caucasian individuals, limiting the study’s generalizability to different skin types and populations. A strength of this study
includes well-matched characteristics in both groups, with no statistically significant differences in baseline skin biophysical parameters, minimizing their confounding impact on the results.

2.2.3 Review of Safety Profile for Carotenoids and PLE

Although the use of topical sunscreens continues to be the most widely used method of photoprotection, the use of systemic photoprotection has demonstrated various advantages, including a more uniform coverage and increased compliance. PLE and carotenoids have shown a strong potential as adjuncts to sunscreen through their antioxidant properties. Carotenoids have been studied extensively and studies have demonstrated an association between higher levels of total plasma carotenoid and improved health outcomes. Epidemiological evidence suggests a plasma level of 0.4 micromole/liter β-carotene supports preventative health benefits, which can be achieved with a daily intake within 2-4 mg. Commercially available β-carotene supplements usually contain between 1.5 to 15 mg per capsule. However, reports on β-carotene consumption in the United States demonstrates most individuals fall in the range of 1-2 mg/d.

Carotenoids have been shown to be well tolerated across different populations and provide great health benefits. In 2018, Ito et al conducted a randomized controlled trial that obtained serum from participants at baseline and after supplementation with carotenoids to perform general biochemical evaluations for safety evaluation. Participants received either carotenoid-rich, astaxanthin 4 mg, or placebo for 9 weeks. They observed no adverse effects or severe changes in participants’ biochemical evaluations of blood tests. Similarly, Groten et al performed a randomized controlled trial in 2019, in which 149 participants received carotenoid-rich TNC or placebo were subjected to a 5-week washout phase followed by a 12-week treatment phase. The carotenoid-rich TNC capsule contained 15 mg lycopene, 5.8 mg phytoene and
phytofluene, 0.8 mg β-carotene, 5.6 mg tocopherols from tomato extract, and 4 mg carnosic acid from rosemary extract. They found the active supplement was well tolerated with no reported adverse effects.\textsuperscript{13}

A literature review performed in 2020 by Mitra et al describes in-depth the health benefits of lutein, a carotenoid compound. Most notable of lutein’s health benefits is its ability to protect skin against light-induced injury through its potent antioxidant properties. Studies have revealed that dietary supplementation with lutein has potential to reduce the development of hyperplasia and edema triggered by UV exposure.\textsuperscript{14} The beneficial effects of lutein and other carotenoids are related to the manner in which they are absorbed, transported, stored, and metabolized. Interactions between carotenoids have been reported in various human and animal studies, including an inhibitory effect of lutein on β-carotene absorption but not on β-carotene cleavage.\textsuperscript{15} However, studies have shown that lutein supplementation combined with vitamin C or E may increase effectiveness, making it more bioavailable. Similarly, smaller doses of lutein in more bioavailable forms have been proposed to increase carotenoid bioavailability. Molldrem et al aimed to evaluate and compare lutein uptake and clearance in humans through a randomized controlled trial. They found that 20-mg crystalline lutein supplement compared with 1.7 mg lutein dissolved in 1.3 mL canola oil showed an increase in mean serum concentration of 0.31 μmol/L after 7 days, suggesting higher bioavailability (P<0.0001).\textsuperscript{16} Moreover, a randomized 2-way crossover study conducted in 2014 by Kopec et al aimed to determine the effects of dietary lipids on the bioavailability of carotenoids. Two separate sets of 12 healthy participants were subjected to receive either a meal with avocado or without avocado in addition to their source of carotenoids, a high β-carotene variety of tomatoes in study 1 and raw carrots in study 2. They found that consumption of lipid-rich avocado enhanced the absorption of β-carotene by 2.4 fold
in study 1 (P<0.0001). Similarly, in study 2 the absorption of β-carotene increased by 6.6 and 4.8 fold (P<0.0001). These results further support previous findings that increasing amounts of lipids increases carotenoid absorption in healthy humans, therefore allowing for maximum delivery of active vitamin A.\textsuperscript{17} In 2010, Cho et al conducted a study to examine the effects of a low dose (30mg/day) versus a high dose (90mg/day) amount of β-carotene for 90 days. They found in the low dose group, procollagen type I levels significantly increased by 4.4 fold compared to the baseline values (P<0.05). Moreover, the high dose of β-carotene supplement had deleterious effects where it increased cutaneous reactivity to UV and UV-induced cutaneous damage, suggesting this high dose of β-carotene was not beneficial nor recommended.\textsuperscript{18}

Overall, the oral administration of carotenoids is safe with minimal to no adverse effects reported, and effective in providing photoprotection against photodamage via UV-induced DNA damage. However, its supplementation is not recommended in individuals that currently smoke or have a history of smoking or asbestos exposure. A systematic review and meta-analysis conducted by Druesne-Pecollo et al in 2010 reviewed the effect of β-carotene supplementation on cancer incidence. They included 9 randomized controlled trials that were reviewed by cancer site, β-carotene supplementation characteristics, and study population. Overall, they found no effect of β-carotene supplementation on the incidence of all cancers combined (RR, 1.01; 95% CI, 0.98-1.04). However, the incidence of lung cancers were significantly increased in individuals supplemented with β-carotene at 20-30 mg/day (RR< 1.16; 95% CI, 1.06-1.27), as well as in smokers and asbestos workers (RR 1.20; 95% CI, 1.07-1.34 and RR, 1.54; 95% CI, 1.08-2.19) compared to the placebo group.\textsuperscript{19} A limitation of this systematic review and meta-analysis is possible confounding, as it found that daily β-carotene supplementation as well as exposure to tobacco or asbestos do influence the risk of lung cancer in individuals receiving oral
supplementation with β-carotene compared to controls. Therefore, it is not possible to accurately conclude which of the 2 criteria is the most important risk factor. Furthermore, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) and Carotene and Retinol Efficacy Trial (CARET) were characterized by a higher proportion of lung cancers compared to other RCTs, partially explaining the effect observed.

PLE has also been shown to have several beneficial properties and has been historically used to treat inflammatory disorders for over 4 decades. Middelkamp-Hup et al studied 10 healthy participants to assess PLE’s efficacy in decreasing oral PUVA-induced phototoxicity in human skin. Those receiving 7.5 mg/kg oral PLE demonstrated reduced phototoxicity clinically and histologically with no adverse events reported during the study. In a slightly different study, Middelkamp-Hup et al conducted a double-blind randomized controlled trial to examine whether PLE improves narrow band (NB)-UVB-induced repigmentation in patients with vitiligo. The treatment group received 250 mg oral PLE three times daily and were exposed to NB-UVB twice weekly. They found repigmentation was higher in the PLE group compared to placebo in irradiated skin (44% vs. 27%, P=0.06). Four patients in the PLE group and 5 patients in the placebo group reported mild gastrointestinal (GI) complaints. Considering patients in the placebo group also reported similar complaints, it is unclear whether PLE supplementation was the main cause of these symptoms. Caccialanza et al conducted a study to assess whether oral PLE could be an effective photoprotectant in patients with idiopathic photodermatoses (PMLE) exposed to sunlight. They enrolled 26 patients with a diagnosis of PMLE and 2 patients with solar urticaria and participants received 480 mg/day of oral PLE. Their skin response was compared with previous evaluations in the absence of PLE use. They found a statistically significant reduction of the cutaneous eruption and subjective symptoms (P<0.05). Although one patient withdrew
from the study due to an exacerbation of irritable bowel syndrome (IBS), authors did not relate the exacerbation to consumption of PLE. They emphasized that data regarding acute and chronic PLE toxicity has been favorable and essentially devoid of adverse effects, and found the tolerance of PLE to be excellent.\textsuperscript{21}

A literature review was conducted by Winkelmann et al in 2015 to evaluate the safety of PLE. A PUBMED search for any RCTs related to PLE or anapsos, a synonym, was performed and the primary safety endpoint was any mention of adverse event, side effect, or toxicity. They included 19 human and 6 basic science studies in their review that spanned over 40 years of research, and studies that were not randomized or placebo controlled were only included if performed on a large sample size. Overall, oral PLE was administered at daily doses ranging from 120 mg to 1080 mg and no adverse effects were reported.\textsuperscript{22} Common side effects included mild-moderate GI complaints and pruritus and were only found in very small numbers of participants overall (2\%). Furthermore, these symptoms resolved with drug cessation and there were no long-term sequelae. This review strongly supported years of anecdotal use and multiple studies that have found oral PLE to be safe and can be prescribed confidently for long-term use. A strength of this review includes its large sample size, as it included over 40 years of research on PLE and its use as a versatile photoprotectant and in treating several photo-aggravated dermatologic disorders.

A similar literature review was conducted by Berman et al in 2016 to understand the basis for the protection and effectiveness of PLE in cutaneous diseases.\textsuperscript{23} They included in vivo animal, in vitro human, and human clinical studies. A relevant study they looked at was a randomized, double-blind, placebo-controlled study undertaken to determine the safety of capsules containing PLE. The capsules contained 240 mg of carefully controlled extract of PL
and subjects were instructed to take it orally twice daily at 8 AM and 2 PM for 2 months. Safety was assessed on Day 0, 14, 28, and 56 and safety assessments included vital signs, complete blood count (CBC), a comprehensive metabolic panel (CMP), prothrombin time and partial thromboplastin time (PT-PTT), and any adverse events. All 40 subjects completed the study and no treatment-related adverse events were reported throughout the duration of the study. No significant changes were noted in physical examinations, clinical laboratory parameters, or vital signs. These findings further support the excellent safety profile of PLE and its potential as an adjunct to sunscreens and other methods of photoprotection.²⁴

2.3 Review of Studies to Identify Possible Confounding Variables

A review of prior studies investigating the relationship of antioxidants, such as carotenoids and PLE, between UVR and photoprotection exposes a number of confounding variables. Baseline characteristics including photosensitivity disorders, use of prescriptive or over the counter drugs known to interact with PLE or carotenoids, dry skin or rash, and history of skin cancer were used as exclusion criteria for a number of studies.

Dietary factors such as use of dietary supplements (vitamin/herbal), diet composition, and eating habits (3 meals/day) were controlled or kept consistent between the arms of studies to limit their effects on carotenoid or PLE levels.¹ Participants were also asked not to change their regular diet or follow an extensive dietary restriction to increase generalizability and relevance.¹,²⁵

Environmental factors such as geographical location and season will also be controlled, recruiting participants from northern regions of the United States to reduce the amount of skin damage and sun exposure participants may have at baseline. Furthermore, the study will be
conducted during fall/winter months and participants will be asked to refrain from sunbathing and tanning for the entire duration of the study.\textsuperscript{1,13}

Carryover effects are also a potential confounder in clinical trials. To minimize this effect, subjects who were involved in another trial within 4 weeks prior to our trial will be excluded.\textsuperscript{1,20,25} A washout phase will also be carried out prior to initiating oral supplementation with carotenoids or PLE.\textsuperscript{13}

2.4 Review of Relevant Methodology

2.4.1 Study Design

This study will be a multi-center, randomized, double-blind, placebo-controlled trial which is a study design that has been used by multiple previous trials evaluating the photoprotective effects of PLE or carotenoids against UV-induced damage. The use of placebo capsules will ensure both patients and researchers are blinded in the study. Furthermore, block randomization will be used to prevent imbalances in sample allocation and eliminate differences between groups, due to the use of multiple centers. Informed consent of the subjects will be obtained prior to inclusion into the clinical investigation in accordance with the institutional ethics and guidelines. All patient information will be maintained strictly in accordance with Health Insurance Portability and Accountability Act (HIPAA). The implementation of a 3-week washout period prior to receiving treatment will minimize the number of variables and allow for more reassurance that the effects measured are due to the experimental therapy. Furthermore, our washout period will protect participants from potential drug interactions.

2.4.2 Study Population and Selection Criteria
Inclusion criteria requires all participants to be healthy adult individuals with Fitzpatrick skin types II, III, or IV. Participants will be eligible for inclusion if they are between the ages of 20-40 at the time informed consent was provided. Subjects must be willing to accept the test for UV-induced erythema on their buttock skin and have punch biopsy specimens taken for immunohistochemistry analysis. Patients must be willing and able to visit the administrative facility on all follow-up visits for the duration of the study and provide informed written consent. Unlike previous studies, this study will include Fitzpatrick skin types III and IV which are often underrepresented groups in previous studies. Participants who are pregnant or lactating, have a photosensitivity disorder, personal or family history of skin cancer, pre-existing intense skin tanning response, abnormal response to sunlight, dry skin or rash, special diet (e.g. vegan, vegetarian, high protein), or taking any prescriptive or over the counter drugs known to interact with PLE or carotene will not be included in the study for safety reasons. Patients who exhibit regular sun exposure behavior or use of indoor tanning will also be excluded due to possible confounding.

2.4.3 Intervention and Method of Administration

Several studies have reviewed the efficacy and safety of PLE and carotenoid oral supplementation. A comprehensive toxicology safety assessment on PLE showed no treatment-related adverse effects after 90 days of doses administered at 300, 600, 1200, and 5000 mg/kg bw/day, making the dose used in this study safe to use over an extended period of time. They found low occurrence of adverse events in clinical trials involving PLE and its long history of traditional use without safety concerns supports their conclusion that PLE is safe to consume. According to the FDA, administration of 1080 mg PLE in divided doses to healthy individuals
with skin type III and IV provided total protection against sunburn reaction following acute summer midday UV exposure, equivalent to exposure at doses up to three MED. Middelkamp-Hup et al used two consecutive doses of PLE, 180 mg each, administered orally in a dose of 7.5 mg/kg body weight, approximately 12 hours apart prior to UV exposure. Participants received the first dose of oral PLE the evening before the second exposure, and the second and final dose of oral PLE was given the next day. They found PLE provided photoprotective effects, including a significant decrease in erythema and sunburn cells, with intake of only two doses (P<0.05), and no adverse effects reported. Ahmed et al used a dose of 240 mg oral PLE three times daily for 12 weeks and similarly, reported no adverse events. In hopes to demonstrate photoprotective effects and provide healthy and safe doses of PLE, we propose using 180 mg of PLE administered orally twice daily in a dose of 7.5 mg/kg body weight.

The safety of oral carotenoids has also been studied, using carotenoid-rich astaxanthin, and it appears that no human studies have identified any significant toxicity at any doses over any length of time. At least 87 clinical trials involving 2,000+ participants were included in this review, using short-term daily doses (up to 100 mg), and long-term daily doses, (8-12 mg). Other than reddening of stool; considered a minor event, no indicators of liver toxicity or severe events were recorded. Baswan et al used a multi-carotenoid supplement that was taken three times daily, containing β-carotene 4.24 mg, α-carotene 1.10 mg, lutein 1.12 mg, and zeaxanthin 0.053 mg, for a duration of 12 weeks. This dose was well tolerated by subjects and no adverse effects were reported. Stahl et al used a carotenoid supplement, 25 mg total carotenoids/day, for a total of 12 weeks. The capsule was comprised of 13.0 mg all-trans-β-carotene, 10.5 mg 9-cis β-carotene, 0.3 mg other cis isomers of β-carotene, 0.75 mg α-carotene, 0.18 mg cryptoxanthin, 0.15 mg zeaxanthin, and 0.12 mg lutein. Compliance was monitored by questionnaires and analyses of
carotenoid serum concentration. This dose provided subjects with protective effects against erythema formation after UVR irradiance and was well tolerated with no adverse effects reported. The recommended dose for total carotenoids falls in the range of 17.7-39.8 mg/d. We propose using 30 mg of oral carotenoid supplement for a duration of 12 weeks to investigate the photoprotective effects of carotenoids using a safe and recommended dose.

2.4.4 Outcome Measures

The primary outcome measure of this study will be the efficacy of the intervention groups to provide photoprotection against UV irradiation measured by the mean change in MED. MED will be determined using objective visual grading to increase sensitivity, reliability and reproducibility. Other variables will include result based on Fitzpatrick skin type and geographical location. Results will be analyzed using the analysis of variance test (ANOVA). Data will be collected at baseline and after 4, 8, and 12 weeks of treatment.

2.4.5 Safety Concerns

Baseline laboratory tests will be taken after the 3-week washout phase and subsequent labs will be taken at randomization, after 4 weeks, 8 weeks, and 12 weeks of treatment. General biochemical examination of blood will be undertaken, including aspartate aminotransferase, alanine transaminase, alkaline phosphatase, lactate dehydrogenase, creatinine, uric acid, total cholesterol, low density-lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, glucose, sodium, chloride, potassium, and hematologic tests including white blood cell, red blood cell, hemoglobin, hematocrit, and platelet for safety evaluation. Other studies have similarly checked liver and kidney function tests, calcium/creatinine ratios, and complete
blood counts. For safety reasons, participants will cease treatment if any of these previous lab findings are >20% above normal range, if the participant becomes pregnant, or if they develop a severe adverse event.

2.4.6 Sample Size Calculation

The proposed study will examine the efficacy of carotenoid vs. PLE supplementation as an adjunct photoprotectant in individuals with Fitzpatrick skin types II, III, and IV. There are no prior completed clinical trials that compare these two types of antioxidants. Therefore, the sample size calculation was performed using data from previous clinical studies. A 2018 randomized, double-blind, placebo-controlled trial evaluated the effects of dietary supplementation with carotenoid-rich, astaxanthin on UV induced skin deterioration in healthy participants. They found the astaxanthin group showed a significant increase in MED from baseline compared with the placebo group after supplementation (7 SD, P<0.05). This expected change in standard deviation between the MED in the placebo group compared to the astaxanthin group was used for the sample size calculation. It covers subjects aged from 30 to 59 years old and set the evaluation of MED as their primary outcome. Dropout and nonadherence rate will be accounted for at a higher rate than most previous studies, as our study design will require longer duration of supplementation and several follow-up visits to the administrative facility throughout the study. Therefore, the sample size of this proposed study will account for a 15% drop out rate. Ninety-five percent confidence intervals will be used as proportions of successes.

2.5 Conclusion
In conclusion, the deliberate search of the medical literature presented demonstrates the effectiveness of systemic photoprotective agents, such as carotenoids and PLE, in reducing photodamage.\textsuperscript{1, 4, 5, 12, 13, 14} Human studies have also shown an increase in the UV dose required for immediate pigment darkening, MED, and minimal phototoxic dose.\textsuperscript{24,28} These effects are likely due to their antioxidant properties seen both in-vitro and in-vivo, in which they act by increasing the antioxidant efficacy of endogenous pathways and decreasing ROS that induce DNA damage.\textsuperscript{29} These findings would suggest that oral systemic photoprotection through supplementation of carotenoids or PLE could provide patients with increased photoprotection against UVR-damage and counter the long-term effects of sun exposure.

This study will be the first study to compare the efficacy of oral supplementation with carotenoids vs PLE in patients with Fitzpatrick skin types II, III, and IV. By conducting this study, we will gain insight on whether carotenoids or PLE supplementation are superior to one another and could provide a relatively safe and uniform coverage adjuvant option to topical sunscreens.
References


Chapter III: Methods

3.1 Study Design

We will conduct a multi-centered, double blind, randomized placebo-controlled trial to evaluate the efficacy of supplemental PL extract vs multi-carotene supplement for the prevention of photodamage after UV radiance exposure in Fitzpatrick skin type II, III, and IV healthy patients. This study will evaluate and compare the photoprotective effects of daily supplementation with PL extract against carotenoids and a placebo capsule, assessing for clinical and histologic response after the treatment period. Enrolled subjects will undergo a 3-week washout period, followed by an intervention of daily oral supplementation for 12 weeks. Two 6 mm punch biopsies will be obtained from the participants buttocks 24 h after irradiation. A biopsy will be taken from nonirradiated skin and UVB/A irradiated skin at the end of the washout and treatment phase. Irradiation will be done using a solar simulator, Sellamed 2000 Device, with an emission spectrum of 340-400 nm, and the participants MED will be determined as the lowest dose causing visible erythemal changes to the skin.¹ The biopsies will be analyzed for UV-induced biomarkers and sunburn cells.

3.2 Study Population and Sampling

The study population will consist of healthy patients aged 20-40 with Fitzpatrick skin types II, III, or IV. These patients will be recruited from 7 dermatology centers across the USA who meet the inclusion and exclusion criteria. Blocked randomization will be used to form the study population. Treatment groups will be stratified based on gender and age category to ensure equal distribution of populations within the three groups.
3.2.1 Inclusion Criteria

Adults between the age of 20-40 with Fitzpatrick skin types II, III, or IV will be enrolled in the study. Participants must be willing to refrain from the use of sunbathing or tanning and the use of dietary supplements (herbal, vitamin) for the entire duration of the study. Additionally, participants are urged to keep a balanced diet and be willing to visit the clinical site multiple times for follow up throughout the study.

3.2.2 Exclusion Criteria

Patients with a personal or family history of skin cancer, photosensitivity disorders, pre-existing intense skin tanning response, abnormal response to sunlight, dry skin or rash, pregnant or breastfeeding women, special diet (e.g. vegan, vegetarian, high protein) will be excluded from the study. Additionally, patients taking any prescriptive or over the counter drugs known to interact with PL or carotene will also be excluded.

3.3 Recruitment

Recruitment will take place at various dermatology centers within the Yale Health system and across the USA including: Yale Dermatology clinics located in New Haven, Middlebury, and Branford, North Metro Dermatology (North Oaks, MN), Weill Cornell Medical Center (New York, NY), Ohio Dermatology Center (New Albany, OH) and Illinois Skin Center (Chicago, IL). These sites are located in northern geographical locations to eliminate the potential problem of recruiting participants with extensive sun damage and sun exposure. Block randomization will be performed at each individual site to ensure balance in sample allocation. The dermatology clinics at these sites will be asked to participate in this study and help with the recruitment for eligible
patients. Those patients meeting inclusion criteria and expressing interest in participating will be enrolled in the study. Prior to randomization, these patients will be assessed against the exclusion criteria, then will be asked to sign written informed consent forms (Appendix A).

3.4 Subject Protection and Confidentiality

This study will require approval by Yale’s Institutional Review Board (IRB) and Human Investigation Committee (HIC), along with the IRB from each respective medical center and hospital participating in the study. All subjects will provide informed consent prior to induction into the study. Consent forms will be available in English and Spanish, as well as other languages as necessary. Confidentiality of all patients will be maintained throughout the study in accordance to HIPAA. This will be reinforced through the use of a randomly generated identification number assigned to each participant. All clinicians and personnel involved in the study will be required to undergo HIPAA training, if not already compliant.

3.5 Study Variables and Measures

Participants who have met the inclusion criteria and assessed against the exclusion criteria will be randomized into three different groups: the control group, which will receive the standard of care and placebo capsule, the oral PLE supplement in conjunction with sunscreen, or the oral multi-carotene supplement and sunscreen group. At enrollment of each subject, a baseline minimal erythema dose and dermal biopsy will be obtained at the end of the washout phase. These same measurements will be repeated again at the end of the treatment phase. Within the 12 week period, blood samples and daily logs will be obtained to monitor patient adherence.
The carotene treatment group will receive 30 mg of β-carotenoid supplement per day, divided into two consecutive doses of 15 mg. The PLE treatment group will receive two capsules containing 180 mg of PLE administered orally in a dose of 7.5 mg/kg body weight. The control group will receive a capsule identical in appearance to both intervention groups but with a filling agent and no active ingredients. All groups will be instructed to take the capsules with the same frequency and return for the same regularity of monitoring and outcome measurement to evaluate for UV photoprotection.

The primary outcome of the study will be the efficacy of the intervention groups to provide photoprotection against UV irradiation measured by the mean change in MED. Other variables will include Fitzpatrick skin type and geographical location.

3.6 Assignment of Intervention and Blinding

Throughout the 7 dermatology clinics, rolling recruitment and block randomization will take place. Eligible participants will be allocated to three different groups: PLE group vs beta-carotene group vs control, in a 3:3:2 fashion with a block size of eight. Once a sample of 406 participants is met, recruitment will end and participants will be assigned their unique identification number to ensure confidentiality. The study will be double-blinded, with participants and study personnel unaware of the treatment allocation.

3.7 Data Collection

Participants will undergo a full body skin examination and blood samples will be drawn and collected for hematological analysis. A complete metabolic panel, complete blood count, and serum antioxidant concentration will be checked for baseline levels on the day of enrollment.
Participants that meet the inclusion and exclusion criteria will be randomized and assigned to treatment arms in a 3:3:2 fashion. The MED of each participant will be assessed at baseline to determine their sensitivity to UVB irradiation. Subjects will be irradiated in an area of the lower back, using the Sellamed 2000 Device. MED will be determined by objective grading 20-24 hours after irradiation. Two 6 mm punch biopsies will be obtained from the participants buttocks 24 h after irradiation with the Sellamed 2000 Device. A biopsy will be taken from nonirradiated skin and UVB/A irradiated skin at the end of the washout and treatment phase for histological analysis. Participants will receive a 12-week supply of either the placebo, multi-carotenoids, or PLE and will complete a demographic survey. They will be given explicit instructions on the dosage, frequency, and adherence necessary to remain in the study. After 12 weeks of supplementation, data will be collected by the patient’s dermatologist via full body skin check and general biochemical examination of blood. Side effects will be monitored throughout the duration of the study.

3.8 Adherence

Adherence will be monitored through blood samples collected at baseline and at each follow-up visit. Blood samples will analyze metabolites and to further improve adherence, we will ask patients to set a daily recurring reminder for the time of day they choose to take their medication. Participants will also be encouraged to keep track of their medication through the use of a daily log.

3.9 Monitoring of adverse events and safety
Baseline laboratory tests will be checked at the beginning of the study after the 3-week washout phase. Subsequent testing for safety and monitoring will be checked at every follow-up visit, including after 4 weeks, 8 weeks, and 12 weeks of treatment. Labs such as a complete metabolic panel and complete blood count will be monitored for safety concerns. Furthermore, participants will cease treatment if any of these previous lab findings are >20% above normal range, if the participant becomes pregnant, or if they develop a severe adverse event.

3.10 Sample Size Calculation

Using the Power and Precision 4 Software, a formal sample size calculation was conducted for this study. A sample size of 406 participants using a one-tailed test and alpha of 0.05 was determined. This sample size will allow 80% power to detect whether oral supplementation with PL extract increases the mean value of MED after 12 weeks in comparison to placebo and the oral carotene treatment group. The sample size calculation was based on a previous clinical study discussed in Chapter II. The calculated sample size of 406 patients total will be randomized in a 3:3:2 fashion, with a total of 153 participants in the carotene group, 153 in the control group, and 100 in the PL extract group. Adjusting for a 15% dropout/nonadherence rate, the recruitment goal will be 467 participants to be enrolled and randomized into the study. The sample size calculation is further detailed in Appendix B.

3.11 Analysis

This study will utilize an intention-to-treat analysis of all randomized patients. Analysis will compare the MED assessments taken at each visit between the three groups. Results will be considered statistically significant if the P-value is less than 0.05. Demographic data such as age,
gender, ethnicity, race, geographical location, and Fitzpatrick skin types will be collected for all patients to compare the control and treatment groups. The categorical variables, including gender, ethnicity, race, geographical location, and Fitzpatrick skin types will be compared using the chi-square test and will be reported as frequencies (%). Age, a continuous variable, will be compared using the analysis of variance test (ANOVA) and will be reported as means (standard deviations). The primary outcome of minimal erythema dose will be analyzed by an ANOVA test and will be reported as mean change and standard deviation. The secondary outcome of intensity of erythema formation will be objectively assessed using the standardized and validated scale, Investigator Global Assessment (IGA) of erythema. Histology findings will be analyzed as percent reduction in biomarkers associated with UV damage to evaluate the effect of PLE and carotene. The biomarkers assessed will include proliferating cell nuclear antigen, sunburn cells, cyclobutene pyrimidine dimers, COX-2, and Ki67. Hypothesis testing will be done using a one-tailed hypothesis to increase the statistical power of the study at the same alpha level as a two-tailed test. A one-tailed test is appropriate for our superiority trial and allows for a reasonable sample size.

3.12 Timeline and Resources

The total duration of this study, including recruitment, randomization, data collection and analysis will be two years. Rolling recruitment and enrollment will begin in January 2024 and will continue for 10 months, given our large sample size and to allow for follow-up. However, all of the sites chosen for our study are high volume dermatology facilities, therefore reaching the recruitment goal of 467 participants is feasible. Our personnel will meet with subjects to assess their baseline MED and obtain two 6 mm punch biopsies for histological analysis. Oral
supplementation, trial instructions, and instructions on adherence will be provided by their dermatologist. Furthermore, follow up visits will be conducted by their dermatologist after 4 weeks, 8 weeks, and 12 weeks of treatment for safety monitoring, and at the research facility for UVB irradiation and MED data collection. Our personnel will objectively measure the MED after 20-24 hours of irradiation using a skin color measurement device, spectrophotometer, to ensure reliability and replication of results. Data collection will end at the beginning of January 2025 and data analysis conducted by our personnel will occur after 12 weeks of treatment.

The study will be headquartered in New Haven, CT. Each facility will require two research assistants who will be responsible for recruiting subjects and confirming they meet inclusion and exclusion criteria. They will also be responsible for obtaining written informed consent and discussing data collection with the treating dermatologist. Dermatologists at each site will be provided with the patient’s oral supplementation including either the three-month supply of daily placebo, multi-carotenoid capsules, or PLE capsules to be given during each follow-up visit.
References


Chapter IV: Conclusion

4.1 Advantages and Disadvantages

There are several advantages to our proposed research study. It will be the first clinical trial comparing the efficacy of oral carotenoids versus oral PLE as a systemic adjuvant to sunscreen in individuals with Fitzpatrick skin types II-IV. Our interventions could establish a potential superior adjuvant systemic photoprotection agent that could decrease the risk of UV-related cutaneous manifestations. As previously noted, cumulative UVR exposure plays a critical role in photodamage, leading to increased risk of photo-carcinogenesis, such as basal cell carcinoma, immunosuppression, and exacerbation of photodermatoses.¹ Although topical photoprotection is the first line of prevention to combat photoaging and skin cancer, its inadequate use, compliance, and lack of optimization continue to limit its usefulness.² Several oral photoprotective agents have been studied as potential adjunct systemic photoprotection. However, this study will provide a way to evaluate the differences in efficacy between two of the most commonly systemic agents investigated.

This study will be a double blinded, randomized placebo-controlled trial with a 3-week washout period. The washout period will decrease carryover effects that could influence the results. Block randomization and stratification by site should ensure a balanced and equal distribution sample size, as well as reduce selection bias, and minimize the effects of other confounders. The large sample size of this trial ensures that the study is powered to show a more precise estimate of the treatment effect, generalize the results, and the ability to withstand participant dropout. The use of multiple sites localized to northern regions of the U.S. will allow us to meet our target sample size within the recruitment timeline and will ensure that the results are generalizable to different populations and ethnic groups. Furthermore, our study will be
conducted during fall and winter months to prevent excessive sunlight radiation to confound our results.

There are some disadvantages to our study. The use of multiple dermatology sites across different states could lead to some differences in how the study is conducted between sites. However, this will be minimized by providing research assistants in these sites with clear and standardized instructions to follow. Another disadvantage is that diet will not be controlled, allowing subjects to continue their normal dietary habits, which could lead to differences in baseline antioxidant levels. These differences will be minimized by excluding participants in special diets, including vegan, vegetarian, and high protein. Furthermore, the duration of the study may limit the durability of the effect. While we plan to evaluate the effectiveness of oral carotenoids and PLE against photodamage for 12 weeks, exposure to UVR is chronic and lifelong. Oral supplementation may produce a temporary increase in MED, but if carotenene or PLE levels do not reach target sites, there will be minimal long-term clinical benefit for patients. Further research following the treatment groups through time and measuring time dependency of carotenoid/PLE uptake and turnover in the skin is warranted.³

4.2 Clinical and Public Health Significance

The skin is exposed daily to the detrimental effects of UV radiation, which in the absence of any topical photoprotective intervention, is left to solely depend on endogenous protection. Sunscreens continue to be the first line of prevention to combat photodamage and combined with preventative strategies, including photoprotective clothing and avoiding sun exposure, provide ideal photoprotection. However, its usefulness is limited by the lack of consumer awareness for adequate use of sunscreens, and adoption of these strategies, rendering them ineffective.
Therefore, boosting the skin’s innate defense system through consumption of dietary phytonutrients could offer complementary protection.

Our goal is that this study will lead to an adjunct therapy that clinicians can recommend to patients to improve their photoprotection. Carotenoids and PLE are known to possess antioxidant, anti-inflammatory, immunoregulatory, and anti-tumorigenic effects and have shown to be safe and tolerable. Once systemic photoprotection is achieved by carotene or PLE supplementation, photoprotection will always be present and will homogenously affect the whole skin. Effective systemic photoprotection would bridge the gap that currently exists in sunscreen use and allow for increased protection against the development of UVR-associated damage. This proposal and subsequent study are steps towards increased photoprotection, with the intention of complementing topical application of sunscreens, provide all-day protection against UVR, and establish superiority between commonly used phytonutrients. Several studies have established a relationship between an increase in MED and carotenoids and PLE consumption. However, our study will be the first to compare the two phytonutrients, and therefore better guide clinicians when recommending appropriate and effective systemic agents.
References


Appendices

Appendix A. Sample HIC Consent Form

CONSENT FOR PARTICIPATION IN A RESEARCH PROJECT

YALE UNIVERSITY SCHOOL OF MEDICINE

Study Title: Comparison of Oral Carotenoid Supplementation vs Oral Polypodium Leucotomas Extract Supplementation in Preventing Photodamage
Principal Investigator: Suguru Imaeda, MD; Jazmin Martinez, PA-SII
Funding Source: Yale University School of Medicine: Physician Associate Program

Invitation to Participate and Description of Project

We are inviting you to participate in a research study designed to look at the effectiveness of supplementation with oral carotenoids compared to supplementation with oral Polypodium Leucotomas extract in preventing photodamage in adults with Fitzpatrick skin types II-IV. You have been asked to participate because you are an adult between the age of 20-40 with Fitzpatrick skin type II, III, or IV.

In order to decide whether or not you wish to be a part of this research study you should know enough about its risks and benefits to make an informed decision. This consent form gives you detailed information about the research project, which a member of the research team will share with you. This discussion should go over all aspects of this research: its purpose, the procedures that will be performed, any risks of the procedures, and possible benefits. Once you understand the study, you will be asked if you wish to participate; if so, you will be asked to sign this form.

Description of Procedures

If you agree to participating in this study, you will be randomly assigned to (a) the control group, which will receive the standard of care and placebo OR (b) the intervention group that will receive the standard of care and oral carotenoid supplement OR (c) the intervention group which will receive the standard of care and oral Polypodium Leucotomas extract supplement. The study has a total duration of 15 weeks.
If you are pregnant, become pregnant, are breastfeeding, have a personal or family history of skin cancer, known photosensitivity disorders, pre-existing intense skin tanning response, abnormal response to sunlight, dry skin or rash, or special diet, you cannot participate in this study. If you are participating in any other trials or taking any drugs known to interact with carotenoids or Polypodium Leucotomas extract you will also be excluded. If you have regular sun exposure behavior or indoor tan you will be excluded.

If you agree to enrolled in this study, a baseline serum antioxidant level and complete metabolic panel (CMP) will be taken and your information reviewed to ensure that you meet the inclusion/exclusion criteria. These same tests will be performed at every 4-week follow-up visit for safety monitoring and possible side effects.

The intervention groups will receive either an oral carotenoid capsule 15 mg or oral Polypodium Leucotomas extract capsule 180 mg twice daily for the duration of the study. The control group will receive a capsule identical in appearance to the intervention groups. All groups will be instructed to take the capsule with the same frequency and return for the same regularity of monitoring including blood tests and total body skin checks. All groups will also be instructed to apply sunscreen as recommended by the CDC.

A description of this study is available on [http://www.ClinicalTrials.gov](http://www.ClinicalTrials.gov), as required by U.S. Law. This website will not include information that can identify you. The purpose of this database is to allow everyone to see information on what studies are being done, and what studies have been done. At most, the website will include a summary of the results. You can search this website at any time.

You will be told of any significant new findings that are developed during the course of your participation in this study that may affect your willingness to continue to participate. Research results will not be returned to your doctor. If your research results are published, your name and other personal information will not be given.

**Risks and Inconveniences**

Antioxidant supplementation, including carotenoids Polypodium leucotomas, have been studied in several clinical trials for many conditions. The safety of oral carotenoids and Polypodium leucotomas, have been studied and it appears they have no significant toxicity at any doses over any length of time. The recommended dose for total carotenoids falls in the range of 17.7-39.8 mg/d, making the dose used in this study safe to use over an extended period of time. The recommended dose for Polypodium leucotomas falls in the range of 360-480 mg, also making the dose used in this study safe to use.
If you are pregnant, become pregnant, are breastfeeding, have a personal or family history of skin cancer, known photosensitivity disorders, pre-existing intense skin tanning response, abnormal response to sunlight, dry skin or rash, or special diet, you cannot participate in this study. If you are participating in any other trials or taking any drugs known to interact with carotenoids or *Polypodium Leucotomas* extract you will also be excluded. If you have regular sun exposure behavior or indoor tan you will be excluded.

Other risks from participating in the study include the breach of confidentiality about your health status and participation in the study. This is very unlikely to occur, as all study investigators are trained and certified in research privacy and this is a double blinded study.

We will also ask you to have your baseline antioxidant serum level and complete metabolic panel (CMP) done at every 4 week follow-up visit to monitor your adherence and possible side effects. The risks involved in drawing blood from a vein include, but are not limited to, momentary discomfort at the site of the blood draw, possible bruising, redness, and swelling around the site, bleeding at the site, feeling lightheadedness when the blood is drawn, and rarely, an infection at the site of the blood draw. There are no major risks associated with these procedures.

**Benefits**

There are potential benefits resulting from the study including an increased level of photoprotection, measured through the minimal erythema dose (MED) compared to those not receiving oral supplementation. This research may also lead to new preventative therapies in the future.

**Economic Considerations**

The carotenoid, *Polypodium leucotomas*, or placebo will be provided free of charge. There are no other costs associated with your participation in the study. Parking will be provided free of charge.

**Confidentiality and Privacy**

Any identifiable information that is obtained in connection with this study will remain confidential and will be disclosed only with your permission or as required by U.S. or State law. Examples of information that we are legally required to disclose include abuse of a child or elderly person, or certain reportable diseases. Information will be kept confidential by using only identification numbers on study forms, storing signed forms in locked cabinets, and password
protecting data stored on a computer. When the results of the research are published or discussed in conferences, no information will be included that would reveal your identity unless your specific permission for this activity is obtained.

We understand that information about your health is personal, and we are committed to protecting the privacy of that information. If you decide to be in this study, the researcher will get information that identifies your personal health information. This may include information that might directly identify you, such as his or her name, address, telephone number, and email address, or mobile phone number. This information will be de-identified at the earliest reasonable time after we receive it, meaning we will replace your identifying information with a code that does not directly identify you. The principal investigator will keep a link that identifies you and your coded information, and this link will be kept secure and available only to the principal investigator or selected members of the research team. Any information that can identify you will remain confidential. Information will be kept confidential; by using only identification numbers on study forms, storing signed forms in locked cabinets, and password protecting data stored on a computer. The research team will only give this coded information to others to carry out this research study. The link to your personal information will be kept for 5 years, after which time the link will be destroyed and the data will become anonymous. The data will be kept in this anonymous form indefinitely.

The information about your health that will be collected in this study includes:

- Research study records
- Records about phone calls made as part of this research
- Records about your study visits

Information about your health which might identify your child may be used by or given to:

- The U.S. Department of Health and Human Services (DHSS) agencies
- Representatives from Yale University, the Yale Human Research Protection Program and the Yale Human Investigation Committee (the committee that reviews, approves, and monitors research on human subjects), who are responsible for ensuring research compliance. These individuals are required to keep all information confidential.
- Those individuals at Yale who are responsible for the financial oversight of research including billings and payments
- The principal investigator (Dr. Suguru Imaeda)
- Co-investigators and other investigators
- Study Coordinator and Members of the Research Team

By signing this form, you authorize the use and/or disclosure of the information described above for this research study. The purpose for the uses and disclosures you are authorizing is to ensure
that the information relating to this research is available to all parties who may need it for research purposes.

All health care providers subject to HIPAA (Health Insurance Portability and Accountability Act) are required to protect the privacy of your information. The research staff at the Yale School of Medicine are required to comply with HIPAA and to ensure the confidentiality of your information.

If you choose to participate in this study, the research assistants will check your electronic medical record at Yale (EPIC) to make sure you qualify. Any access to your electronic medical record will be done consistent with HIPAA regulations.

Some of the individuals or agencies listed above may not be subject to HIPAA and therefore may not be required to provide the same type of confidentiality protection. They could use or disclose your information in ways not mentioned in this form. However, to better protect your health information, agreements are in place with these individuals and/or companies that require that they keep your information confidential.

You have the right to review and copy your health information in your medical record in accordance with institutional medical record policies. This authorization to use and disclose your health information collected during your participation in this study will never expire.

**Voluntary Participation and Withdrawal**

You are free to choose not to participate in this study. Your health care outside the study, the payment for your health care, and your health care benefits will not be affected if you do not agree to participate. However, you will not be able to enroll in this research study and will not receive study procedures as a study participant if you do not allow use of your information as part of this study. You do not give up any of your legal rights by signing this form.

**Withdrawing From the Study**

If you do become a subject, you are free to stop and withdraw from this study at any time during its course.

To withdraw from the study, you can call a member of the research team at any time and tell them that you no longer want to take part. This will cancel any future appointments.

The researchers may withdraw you from participating in the research if necessary. This will only occur if you do not attend the assigned weekly sessions.
If you choose not to participate or if you withdraw it will not harm your relationship with your own doctors or with the Yale School of Medicine and Yale New-Haven Hospital.

**Withdrawing Your Authorization to Use and Disclose Your Health Information**

You may withdraw or take away permission to use and disclose your health information at any time. You do this by calling or sending written notice to the Principal Investigator, Dr. Suguru Imaeda Department of Dermatology, Yale School of Medicine.

When you withdraw your permission, no new health information identifying you will be gathered after that date. Information that has already been gathered may still be used and given to others until the end of the research study, as necessary to ensure the integrity of the study and/or study oversight.

**You do not give up any of your legal rights by signing this form.**

**Questions**

We have used some technical terms in this form. Please feel free to ask about anything you don’t understand and to consider this research and the permission form carefully - as long you feel is necessary before you make a decision.

**Authorization**

I have reach (or someone has read to me) this form and have decided to participate in the project described above. Its general purposes, the particulars of my involvement and possible hazards and inconveniences have been explained to my satisfaction. My signature also indicates that I have received a copy of this consent form.

Name of Subject: ____________________________________

Signature: __________________________________________

Relationship: _______________________________________

Date: __________

____________________________________
Signature of Principal Investigator

Date

or

Signature of Person Obtaining Consent

Date

If you have further questions about this project or if you have a research-related problem, you may contact the Principal Investigator, Dr. Suguru Imaeda at 203-415-9221.

If, after you have signed this form you have any questions about your privacy rights, please contact the Yale Privacy Officer at 203-432-5919. If you would like to talk with someone other than the researchers to discuss problems, concerns, and questions you may have concerning this research, or to discuss your rights as a research subject, you may contact the Yale Human Investigation Committee at (203)-785-4688.
Appendix B: Sample Size Calculation

Alpha: 0.05

Power: 80%

1-tailed hypothesis

N= 153 per treatment group, 100 control group

3:3:2 assignment

Factoring in an unexpected 15% dropout rate the sample size is n= 176 per treatment group, n=115 control group

Effect size was calculated with data from Ito et al. These numbers were then used to calculate the estimated sample size using Power and Precision 4 Software. See chapter 2 for full citations and description of the study.
Bibliography


31. Toti E, Chen CO, Palmery M, Villaño Valencia D, Peluso I. Non-Provitamin A and Provitamin A Carotenoids as Immunomodulators: Recommended Dietary Allowance,

