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Diet, Vitamins, and Herbal Supplements: Do They Prevent or Alter Skin Cancer?

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Do They Prevent or Alter Skin Cancer?

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Master of Public Health Thesis

DIET, VITAMINS, AND HERBAL SUPPLEMENTS:
DO THEY PREVENT OR ALTER SKIN CANCER?

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Introduction

Skin cancer in the United States is a large and growing public health concern with an estimated one million new cases diagnosed each year. As a result, there are many modalities available for use in prevention and treatment. The efficacy of natural alternatives to prevent and treat skin cancer has become an area of great interest that is relatively unknown to physicians. Despite this, the US population has embraced complementary medicine alternatives, often as a replacement for traditional allopathic therapies, in many areas including skin cancer. For public health advocates, knowledge of the effectiveness of these alternative therapies and the evidence that support these remedies is essential.

This paper will discuss the scope of the skin cancer problem in the US including epidemiology and current methods of screening and prevention. Following this will be an extensive review of the most current information regarding the prevention and treatment of skin cancer through dietary modification and topical application of antioxidants, vitamins, and herbal supplements. The results of placebo-controlled randomized clinical trails, large human cohort studies and case-control studies have been emphasized to provide an evidence-based approach toward improving the health of our population.
Epidemiology of Skin Cancer

Incidence

Of the three major types of skin cancer, basal cell carcinoma (BCC) accounts for approximately 80%, squamous cell carcinoma (SCC) for approximately 16%, and cutaneous malignant melanoma (MM) for approximately 4%. Of skin cancer deaths, however, nearly 90% result from malignant melanoma. On average in the United States, one person dies of melanoma every hour. Early detection and education are leveling-off or decreasing the incidence, morbidity, and mortality rates of all forms of skin cancer.

Malignant Melanoma was the sixth most common cause of new cancer cases in the United States for 1999 among males and seventh among females, accounting for 4% and 3%, respectively. In the United States, MM is more common than any noncutaneous cancer among 25-29 year olds. Epidemiologic data is more precise for MM than nonmelanoma skin cancer (NMSC) because the diagnosis of the former but not the latter is routinely reported to tumor registries.

Marked increases in the incidence of MM occurred from the mid-1960s to the mid-1990s. It has been estimated that the incidence in Caucasians increased from 3% to 7% during this 30-year period. Calculations based on data from the Surveillance, Epidemiology, and End Results (SEER) program of the National Cancer Institute, showed an increase in the incidence of MM in the United States of 120.5% from 1973 to 1994. However, increased education and awareness appear to result in earlier detection and a greater increase in thin rather than thick tumors. In the United States,
a 500% increase from the period of 1973-77 to 1983-87 was reported in the diagnosis of in situ MM compared to a 52% increase in the diagnosis of invasive MM. 

Stabilization or decrease of incidence rates of MM have been reported over the past few years. For example, in the United States from 1990 to 1994, rates stabilized or declined for females younger than 60 years old and males younger than 50 years old, while rates increased for older age groups. The incidence in the United States of invasive tumors decreased two consecutive years from 14.1 per 100,000 in 1991 to 13.8 per 100,000 in 1993.

Risk Factors

Skin cancer is exceedingly more common in Caucasians than other races, although NMSC not infrequently affects Hispanics and acral lentiginous melanoma is the most common type of MM affecting Asians and blacks. All forms of skin cancer are more common in men than women. NMSC most commonly affects chronically sun-exposed sites including the head and neck, trunk, and upper extremities. MM most commonly affects sites of intermittent intense sun exposure, the trunk in men and the lower extremity in women. Risk factors for skin cancer include history of significant sun exposure; light colored eyes, skin, and hair; tendency to sunburn easily and tan poorly; personal or family history of skin cancer; male sex; advancing age; and immunodeficiency, resulting from status post organ transplantation or human immunodeficiency virus infection. There are additional unique risk factors for the development of MM such as a personal history of atypical moles or multiple moles.
Prognosis

The prognosis for BCC is excellent. Only 0.0028% of BCCs metastasize. Metastatic disease is more common in men than women and usually arises from large neglected tumors. Five year survival for metastatic BCC is less than 33%. Patients with basal cell nevus syndrome, a rare sporadic or autosomal dominant condition characterized by multiple BCCs, jaw cysts, palmoplantar pits, and a wide variety of other anomalies, may develop locally invasive or metastatic tumors.

Approximately 5% of SCCs metastasize within 5 years. Risk factors for metastases include depth greater than 4 mm; diameter greater than 2 cm; poorly differentiated histology; perineural, vascular, and lymphatic invasion; tumors located on the lip; tumors arising in osteomyelotic foci, burns, and scars; immunodeficiency. Five year survival is approximately 30% for patients with metastatic SCC. In 1998, there were approximately 1,200 deaths from SCC.

In the United States, nearly 90% of skin cancer deaths are from MM and 7,300 deaths from MM were estimated for 1999. Mortality rates for MM rose 38.9% from 1973 to 1994. Mortality rates are greater for males than for females and for older than younger individuals. Mortality rates stabilized or decreased for all groups from 1990-94, except for men 70 years or older and women 60-69 years old. Thicker tumors are associated with greater risk for advanced disease and mortality. Five year survival is 93-100% for tumors < 0.76 mm in depth and 50% or less for tumors ≥ 4 mm. For the period from 1989 to 1994, the five year survival for CMM in the United States was 88% for white patients and 69% for black patients.
Primary prevention of skin cancer involves education of the population to actively attempt to reduce the amount of their ultraviolet exposure. This includes the use of daily sunscreen application and the use of clothing and hats to prevent unnecessary sunlight exposure. Secondary prevention involves early detection of changes in the skin through specific screening methods to decrease morbidity and mortality of skin cancer.

Since BCC is so rarely fatal, early detection impacts insignificantly on mortality. However, early diagnosis of BCC is effective in minimizing local tissue destruction by the tumor and allows for less complex surgical excision of the tumor, decreased size of the surgical defect, and improved cosmesis. Similarly, early detection of SCC reduces morbidity of the disease. Additionally, early detection of SCC is likely to be associated with decreased risk for regional and distant metastatic disease and improved mortality rates.

Early detection of MM is considered essential because of the high mortality rate and the strong relationship between tumor thickness and mortality. MMs diagnosed during periodic surveillance examinations are more likely to be smaller and thinner than those diagnosed at first presentation. Skin self-examination has been suggested to reduce the mortality by 63% by reducing the incidence of CMM and the risk of advanced disease.

Physical examination of the skin is the method by which individuals are screened for skin cancer. Biopsy submitted to a dermatopathology laboratory is
essential for histologic confirmation of suspicious lesions. History of risk factors for skin cancer and of new, changing, or symptomatic lesions aids the screening process. Total body photography of patients with atypical and/or multiple melanocytic nevi, epiluminescence microscopy, and computerized digital imaging are tools the subspecialist may utilize as adjuncts to the physical examination. Detection in the peripheral blood of biochemical markers for CMM is an experimental technique which may be most useful in identifying advanced tumors and predicting response to medical therapy.
Mechanisms of Skin Cancer Development

The biochemical mechanisms responsible for cell injury and cell death are complicated and the pathways are multiple and overlapping. We will focus on the roles played by two major contributors to cellular damage, oxygen-derived free radicals and ultraviolet radiation, in mediating the pathological changes manifested clinically as NMSC and melanoma.

Reactive Oxygen Species

With a single unpaired electron in their outer orbit, free radicals or reactive oxygen species (ROS) are highly unstable chemical species that can damage lipids, proteins, and nucleic acids. Free radicals also initiate autocatalytic reactions that convert the molecules they interact with into additional free radicals. Reactive oxygen species can be generated within cells as a byproduct of mitochondrial respiration in the reduction of molecular oxygen to water, the absorption of ultraviolet light, and the donation or acceptance of free electrons by transitional metals. These processes generate hydrogen peroxide, superoxide anion radicals, and hydroxyl ions. Nitric oxide (NO), an important chemical mediator produced by endothelial cells, macrophages, and neurons, can also act as free radicals. NO can be converted to highly reactive species such as the peroxynitrite anion (ONOO⁻), NO₂⁻ and NO₃⁻. Cellular damage occurs when free radicals, in the presence of oxygen, attack membrane lipids yielding peroxides, which serve as autocatalytic propagators of free radical reactions.
Cells have an endogenous defense system to combat free radical induced cellular damage and death. There is a delicate balance between the generation of free radicals and the free radical scavenging systems. As unstable chemical species, many free radicals decay spontaneously. Additionally, there are several enzymatic and non-enzymatic pathways that help to deactivate these reactive oxygen species. Free radicals such as hydrogen peroxide, superoxide, and hydroxyl radicals are detoxified by catalase, superoxide dismutase, and glutathione peroxidase, respectively. The non-enzymatic pathway includes antioxidants such as vitamins that neutralize free radicals. When free radical formation exceeds free radical termination, cells experience oxidative stress leading to pathological transformation.\textsuperscript{13}

\textit{Ultraviolet Radiation}

The two major pathological effects of ultraviolet radiation are photodamage leading to premature aging of the skin and skin cancer. It has been postulated that ultraviolet carcinogenesis consists of two events: first, the transformation of a skin cell to a neoplastic form and second, UVB-induced immunosuppression.\textsuperscript{14} Ultraviolet radiation, specifically UVB, can cause direct damage to DNA such as the formation of pyrimidine dimers between adjacent pyrimidine bases on the same DNA strand, single-stranded breaks, and DNA-protein cross links.\textsuperscript{13} UV radiation also causes mutations in oncogenes, such as \textit{ras}, and tumor suppressor genes, such as \textit{p53}. The \textit{ras} family of proto-oncogenes mediates cell-signaling pathways for many growth factor receptors.\textsuperscript{15} \textit{Ras} may play an important role in early skin carcinogenesis as UVB rays have caused “signature mutations” in \textit{ras} genes in some human basal cell
carcinomas (BCCs). The tumor suppressor gene p53 serves as a filter for cells that have acquired mutations in the skin. The p53 gene conveys its tumor suppressive effects by initiating apoptosis of mutated progeny cells. Following exposure to UV radiation there is a transient increase in p53 levels in the skin. The elimination of mutated keratinocytes via the p53 apoptotic pathway prevents the formation of mutated keratinocyte cell colonies. This inducible reparative ability may be due to the DNA fragment thymidine dinucleotide. This DNA fragment has been shown to increase the rate of repair of DNA ultraviolet damage by 2 or 3 fold. It is thought that the induction of DNA repair mechanisms is related to the up regulation of the tumor suppressor gene p53. Cutaneous levels of p53 continue to persist several days after UV exposure. When the p53 gene becomes mutated, as a result of UVB exposure, cells fail to undergo programmed cell death. Indeed, squamous cell carcinomas have been found to have a high percentage of p53 mutations.

Additionally, UV radiation has been linked to cutaneous immunosuppression resulting in both a decrease in surveillance for UV induced tumor antigens and depressed cell mediated contact hypersensitivity response (CHS). Animal studies have shown that UV radiation exposure, prior to the application of known topical mutagens such as dinitrochlorobenzene (DNCB), decreases the normal skin response to the DNCB antigen. This subdued response includes a decrease in skin erythema, edema, and induration at the site of application for 2 to 3 days. It is thought that this immune reaction may be a result of the UV induced disturbance of the communication between the antigen presenting cells of the skin, the Langerhans cells, and antigen specific T-lymphocytes.
Dietary and Topical Antioxidant Effects on NMSC

Retinoids

The term retinoid encompasses both naturally occurring molecules that are present in low levels in the peripheral blood and also synthetically derived compounds with biological activities of vitamin A or retinol. Natural sources of vitamin A include liver, egg yolk, butter, fish, yellow and orange fruits and vegetables including carrots, tomatoes, apricots, and cantaloupe and green leafy vegetables. Vitamin A and its metabolic derivatives, retinaldehyde and retinoic acid, are fat-soluble molecules necessary for growth, differentiation, and maintenance of epithelial tissues.

Retinoids can be thought of as both vitamins and hormones. They are vitamins in that retinol is not synthesized from the body and must be obtained through the diet and they are hormones in that the body transforms retinol into substrates (9-cis RA and tRA) which bind to nuclear receptors and serve as signaling molecules to regulate epithelial cell differentiation, proliferation, growth, and metabolism. Cell growth, proliferation, differentiation and cell death are influenced by DNA transcription via the binding of retinoids to both the retinoid acid receptors (RARs) and the retinoid X receptors (RXRs).

In order to decrease the toxic effects of vitamin A and utilize its more biologically active components, the naturally occurring molecule must be modified for therapy. Three classes of synthetic retinoids are used today which include: nonaromatic retinoids such as tretinoin and isotretinoin, monoaromatic retinoids such
as etretinate and acitretin, and polyaromatic retinoids such as adapalene and tazarotene.\textsuperscript{21}

Two cell line studies by Moon et al. in 1983 and 1992\textsuperscript{22,23} have shown the influence retinoids have on cell growth and differentiation and their suppressive effects on carcinogenesis of epithelial tumors and other cancer cell lines. Earlier studies involving the chemoprotective effects of retinoids in mice and human clinical reports emphasized their effectiveness for the prevention of chemical and UV light induced skin cancer.\textsuperscript{24,25,26} Unfortunately, the human clinical studies consisted of small sample populations and the high doses of retinoids used had significant side effects.\textsuperscript{27} Larger studies involving oral administration of lower doses of retinoids (isotretinoin) resulted in poor chemoprevention against BCCs.\textsuperscript{28} Subsequently, retinoids have been more extensively studied in the chemoprevention of NMSC.

Moon et al., 1997\textsuperscript{29} conducted a randomized placebo-controlled 5 year clinical trial which examined the use of vitamin A (25,000 U) vs. placebo in 2,297 patients with a moderate risk of skin cancer development (i.e., a history of $>10$ actinic keratoses but fewer than 3 BCCs or SCCs). They found that patients who were treated with vitamin A had fewer SCCs than the placebo group; however, this was not the case for BCCs.

Several cohort and case-control studies have been undertaken during the last 5 years to examine the relationship between retinoids and chemoprevention. The van Dam et al. 2000\textsuperscript{30} study, in which a large prospective cohort of men was evaluated in terms of the relationship between their respective diets and their subsequent development of BCC, did not show a significant difference between those individuals
that consumed higher amounts of retinols in their diet than those who did not. This finding is consistent with other large randomized trials including Levine et al. 1997\textsuperscript{31} in which 400 subjects with a previous history of 4 or more skin cancers were treated with isotretinoin 10-15mg/day, vitamin A 25,000 U, or placebo. No significant difference between the treatment groups or controls was detected. These studies both support the evidence obtained by Tangrea et al., 1992\textsuperscript{28} whose multicenter study examining the possible benefit of low dose isotretinoin (10mg/day vs. placebo for 3 years) on the prevention of BCC showed no difference between the study groups.

In addition to these larger best evidence studies, several smaller trials have illustrated the potential role of oral retinoids in the prevention of NMSC.\textsuperscript{32,33} However, the most significant results were seen in niche treatment protocols for patients with a higher susceptibility to skin neoplasms. For example, patients with xeroderma pigmentosum treated with isotretinoin for 2 years had an average reduction of 63% in the number of skin cancers over a 2-year period as compared to no previous treatment.\textsuperscript{33} More recently, however, DiGiovanna 1998\textsuperscript{34} studied whether isotretinoin could be effective in chemoprevention of skin cancer in high-risk patients with xeroderma pigmentosum. A small sample of patients (of 7 patients enrolled, 5 were able to complete the study) used isotretinoin at 2mg/kg/day for 2 years. He found that there was a 63% reduction in the number of cancers these patients developed as compared with the 2 years prior to therapy. However, upon discontinuation of isotretinoin therapy, skin neoplasms developed within 2 to 3 months. These findings suggest that the chemopreventive effect of isotretinoin is limited to the active treatment period.
The development of skin cancer in immunosuppressed individuals after transplantation is particularly significant in areas of the world with high levels of ultraviolet exposure. As medical technology rapidly advances, organ transplantation and sustained immune suppression is increasingly more common. Incidences of NMSC in renal transplant recipients in Australia increased exponentially over a >9 year period: 3% within the first year, 25% at 5 years and 44% at 9 or more years.\textsuperscript{34,35}

The use of retinoids as a preventative therapy for transplant recipients has been explored in several studies. For example, Bavinck et al., 1995\textsuperscript{36} conducted a double-blind placebo controlled study in which acitretin 30mg/day or placebo pills were given to 38 renal transplant recipients for 6 months. The patients who received the acitretin developed significantly fewer SCCs than the placebo group alone (P=0.01), suggesting that acitretin may offer a chemoprotective effect against skin neoplasms.

Small studies have also been undertaken to examine the combined therapeutic effect of isotretinoin and calcitriol to treat and prevent multiple early NMSCs. Calcitriol (0.5 to 1.0 ug/kg/day) and isotretinoin (0.3 to 0.5 mg/kg/day) were given to 11 patients over a 15-month period. The authors reported a decrease in the size of most of the actinic keratoses of the 11 patients and a decrease in size of an early SCC in one patient.\textsuperscript{37} This study was both small and without placebo controls so its results may be due to chance alone. The data suggest, however, that the use of synergistic vitamin derivatives may offer possible future tumor prevention if a larger number of patients and a placebo-controlled methodology is employed.

The induction of multiple squamous cell carcinomas following long term PUVA treatments for psoriasis has been well established.\textsuperscript{38,39} It has been proposed
that combination therapy of retinoids and PUVA may reduce the risk of skin cancer and other skin damage associated with PUVA treatment. Acitretin, the active metabolite of etretinate, has recently been approved in this country for the treatment of psoriasis. As a monotherapy, acitretin is an effective method for treatment of pustular and erythrodermic psoriasis but for plaque psoriasis, it is best used as an adjuvant therapy with UVB or with PUVA. Combining acitretin with PUVA results in a more rapid clearing of psoriatic plaques at lower doses of both treatment modalities than either therapy alone. A case report of a patient treated for over 14 years with PUVA therapy and topical steroids developed 34 SCC in total, 21 of which developed with PUVA and cyclosporin adjuvant therapy. An inhibition of tumor formation occurred with the onset of acitretin therapy (60 mg/day) and continuous treatment with acitretin has kept the patient tumor free for 4 years. Acitretin may therefore have some value in preventing the occurrence of SCCs in patients treated with long term PUVA. As acitretin thins the stratum corneum and epidermis, however, lower doses of UVB or UVA should be used as there is an increased sensitivity to ultraviolet light resulting in burning.

In summary, review of the current randomized clinical trials, large human cohort and case-control studies does not illustrate a beneficial effect of either natural dietary retinoids or synthetic retinoid supplementation for treatment or prevention of BCCs or SCCs in the general patient population. In contrast to these larger best evidence studies, several small trials have demonstrated the potential role for oral retinoids in preventing NMSCs in patients with a higher susceptibility to skin neoplasms such as patients undergoing concurrent immunosuppressive treatment
status post transplantation, patients with xeroderma pigmentosum, or patients being treated with PUVA therapy for psoriasis. Unfortunately, large cohort and randomized clinical trials have not yet been published on the relationship between retinoids, NMSC, and these specific conditions.

Selenium

Selenium is found in fish, shellfish, red meat, egg yolks, chicken, garlic, tuna, bread, cereal, mushrooms, asparagus and grain products. This trace element is necessary for the function of the detoxifying enzyme glutathione peroxidase, which helps to reduce the presence of highly reactive hydroxyl free radicals. It is thought that these hydroxyl radicals attack DNA and cause mutations. Studies in mice have shown that increased levels of dietary selenium provide protection against ultraviolet induced skin tumors. Stewart et al., 1996 examined whether the combined use of antioxidant nutrients such as selenium, Vitamin C, and Vitamin E decreased the level of UVB induced oxidative damage to mouse keratinocytes as indicated by the formation of the DNA adduct, 8-hydroxydeoxyguanosine (8OHdG). The investigators’ goal was to determine whether antioxidant supplementation could enhance the rate of DNA repair of UVB induced lesions. When keratinocytes were incubated for 2 days with the nutrients a significant decrease in the amount of oxidative damage was observed, as measured by a decrease in the number of 8OHdG adducts per pretreated cell. By increasing the concentration of antioxidants in the culture medium during cell growth there was a significant decrease in the DNA oxidative damage that occurred as a consequence of UVB irradiation.
In human trials, serum selenium levels were found to be predictive of future cancer risks in humans. A case control study by Clark et al., 1984 illustrated that skin cancer patients that were otherwise healthy had significantly lower mean serum selenium values than did controls. The most powerful study evaluating the effects of selenium supplementation for cancer prevention in patients with known carcinoma of the skin was conducted as a large, multicenter, double-blind, randomized, placebo-controlled trial by Clark et al., 1996. The purpose of this study was to determine whether the incidence of cancer could be decreased with the nutritional supplementation of selenium. A total of 1312 patients, with a previous history of BCC or SCC, from selenium deficient areas of the eastern United States, were randomized to either an oral selenium supplementation group (200ug/day) or a placebo group and followed over a 4.5-year period. They found that the selenium treated group did not have a significantly different incidence of either BCC or SCC. Thus they concluded that selenium supplementation does not protect against the development of BCCs or SCCs in the skin. This study did show, however, a 40% reduction in non-cutaneous cancers in the group receiving selenium. Although the number of cases was small, treatment with selenium was shown to reduce the incidence of cancers involving the lung, colon/rectum and prostate as well as reducing the mortality of lung cancer. Interestingly, breast cancer, bladder cancer, and leukemia-lymphoma were more frequent in the selenium group than in the placebo group. However, none of these differences was statistically significant.

In conclusion, while oral administration of selenium has been shown to reduce the incidence of several types of cancer, its direct effect in reducing BCC or SCC
incidence has not been demonstrated in human randomized clinical trials to date. The protective effect of other preparations of selenium such as topical applications has not been examined in a systematic way and may present a possibility for future studies.

**β-carotene**

β-carotene is available in food sources such as orange and red vegetables, carrots, tomatoes, beets, and berries. There are more than 600 carotenoids in the food supply but some of the most common are β-carotene, alpha-carotene, lycopene, crocetin, and fucoxanthin. In the last decade, studies have attempted to establish a relationship between NMSC risk and β-carotene, the most well studied carotene. β-carotene has been postulated to reduce free radical damage of DNA after ultraviolet exposure. Indeed, mice studies have demonstrated a decrease in the number of chemically and UV light induced skin cancers as a result of oral supplementation of β-carotene.

Data in humans showed that oral β-carotene supplementation reduced ultraviolet immunosuppression. However, when Noonan et al., 1996 examined dietary β-carotene and ultraviolet-induced immunosuppression, they were not able to show that supplementing the diet of mice with oral β-carotene altered their susceptibility to UV immune suppression. Results of these animal and small human studies on β-carotene and NMSC have not demonstrated consistent results.

In a large, randomized, placebo-controlled trial, 1805 patients with recent NMSC were given 50 mg of β-carotene/day for up to 5 years. Although the median plasma β-carotene levels increased to 8.5 times baseline, no significant effect was
found on the incidence of the first new NMSC. More recently, researchers have embarked on several large human cohorts and randomized controlled trials examining the relationship between \(\beta\)-carotene supplementation and the prevention of BCC and SCC. Van Dam et al., 2000\textsuperscript{30} examined the role of fat intake, antioxidant nutrients, retinol, folate and vitamin D as possible preventative agents against the formation of BCC in a cohort study of 43,217 men followed over an 8-year time period. At the onset of the study, the dietary habits of the male subjects were assessed via validated food frequency questionnaires. Analysis of their data, which related carotene intake in 1986 to BCC occurrence between 1990 and 1994 revealed that alpha-carotene, was associated with a slightly lower risk of BCC (i.e., \(P=0.01\) with a 95\% confidence interval and a relative risk of 0.88). \(\beta\)-carotene illustrated a less significant effect, \(P=0.03\) and a relative risk of 0.81. However, the investigators concluded that, overall, the findings do not support the hypothesis that diets low in fat or high in specific vitamins lower the risk of BCC. The findings of the van Dam et al. 2000\textsuperscript{30} study are similar to that of the Hunter et al., 1992\textsuperscript{48} large prospective cohort study in which diet (vitamins C, D, E and \(\beta\)-carotene) and the risk of BCC development was evaluated in female nurses over a 4 year follow up period with no conclusive results.

Green et al. 1999,\textsuperscript{56} in a randomized placebo-controlled trial, examined the relationship between the use of daily sunscreen application and \(\beta\)-carotene supplementation in order to prevent BCCs and SCCs. Using prospective design 1,383 patients were followed over a 4.5-year period. The patients were split into four groups accordingly: daily sun protection factor 15 sunscreen application and 30mg/day of oral \(\beta\)-carotene; sunscreen plus placebo pills, 30 mg/day of \(\beta\)-carotene only; and placebo
pills only. Results of this study illustrated that in terms of BCCs there were no statistically significant differences between the patients who used daily sunscreen and those who did not. Using daily sunscreen did, however, have a significant effect on the number of SCCs patients developed in this cohort. There was no significant difference between the group that took oral $\beta$-carotene vs. placebo pills for both BCC and SCC. These findings are consistent with an older placebo-controlled clinical trial in which 1,805 patients with a previously diagnosed NMSC were given either 50mg of oral $\beta$-carotene or daily placebo pills over a 5-year period. Neither the total number of new NMSC nor the time period in which the patients developed a new NMSC was significantly different between the two groups.\textsuperscript{54}

In conclusion, a systematic review of the large cohort and randomized clinical trials illustrates that no chemopreventive effect was demonstrated with either normal dietary consumption or oral supplementation of $\beta$-carotene.\textsuperscript{30,54,55,56} Although researchers who argue for the beneficial effects of betacarotene supplementation suggest that these benefits may only be appreciated over a lifetime of use, this idea is difficult to examine due to the length of study time that would be required.
Synergy of the Antioxidants on NMSC

Substantial evidence indicates that the antioxidant system of the skin is interlinked. Review of the current randomized and placebo controlled human trials reveals that the synergistic use of specific antioxidants proves to be more successful than with the individual components alone.

β-carotene in Synergy

The protective effect of oral supplementation with β-carotene and vitamin E against the development of erythema in humans was recently examined alone and in combination. Serum β-carotene and vitamin E concentrations were found to increase with oral supplementation. After 12 weeks of treatment with a carotenoid supplement (25 mg) or carotenoid plus vitamin E (500 IU), the erythema reaction after UV irradiation was significantly diminished (p<0.01) on dorsal (back) skin. The authors found that the suppression of erythema was greater when the antioxidants were used in combination than when the carotenoids were used alone. Weaknesses of this study include the lack of a placebo group and the absence of blinding of the investigators to the identity of the experimental groups.

Another proposed mechanism for the prevention of skin cell carcinogenesis is to protect cells from the attack of the free radicals, NO₂ and peroxynitrite anion (OONO⁻). In a study by Bohm et al., 1998, human lymphoid cells were taken before and after patients consumed a 2-week regimen combination of oral β-carotene (150mg/day), vitamin C (1000mg/day), and vitamin E (α- tocopherol 800mg/day).
All cells were then exposed to the free radicals NO$_2$ and OONO$^-$, in sequential experiments. Cell staining with eosin was used to show which cells had membrane destruction leading to cell death. The cells taken from patients after the antioxidant treatment showed cell staining of 6% while cells taken from patients before antioxidant treatment had a cell staining of 61.4%. The authors concluded that the use of β-carotene with vitamins C and E offered synergistic cell protection against both the NO$_2$ and OONO$^-$ radicals with a greater protective effect against NO$_2$.

Vitamin C and Vitamin E alone and in Synergy

Studies suggest that the topical application of vitamin C combined with vitamin E offers a more significant photoprotective effect than either agent alone. On a molecular level, this may be due to the capacity of vitamin C to regenerate vitamin E from its free radical form.$^{59,60}$ This premise has been supported by other investigations that examined the antioxidant interactions on membrane lipid oxidation.$^{61,62}$ In effect, by combining vitamin C and E, the free radical scavenging capabilities of each may be extended, thereby increasing the total antioxidant capacity of the skin.

Vitamin C, or ascorbic acid, is a cofactor for the enzymes that are responsible for the hydroxylation of proline and lysine in collagen, which helps to stabilize collagen’s helical structure.$^{62}$ The body does not produce this antioxidant; instead it must be consumed in the form of citrus fruits, strawberries, tomatoes, cantaloupe, potatoes, and dark green leafy vegetables. Studies by Shindo et al. in 1993 and 1994$^{64,65}$ in murine epidermis and dermis have shown that UV exposure leads to the
depletion of vitamin C. As a result, the skin appears to be susceptible to damage by reactive oxygen species. Inasmuch as vitamin C is a known antioxidant, it has been studied as a therapeutic means to protect the skin against free radical damage.

Porcine studies examining the use of vitamin C as a topical photoprotectant have shown it to concentrate in the skin while decreasing erythema and UVB phototoxic damage. Studies in mice have also shown vitamin C to offer a photoprotective effect against UV induced chronic skin damage.

Miyai, 1996 examined the ability of a stable derivative of ascorbic acid, ascorbic acid 2-O-alpha-glucoside (AA-2G), to induce resistance against UVB cell injury in a human keratinocyte cell line established from squamous cell carcinoma. Cells were preincubated in the AA-2G solution, exposed to UVB radiation and then reincubated in the same solution for 24 hours. Miyai found that the stable ascorbic acid derivative AA-2G had a significant preventive effect against UVB cellular damage and this effect had greater significance with increasing concentrations from 0.1 to 1mM (p<0.01, p<0.001, p<0.0001) when compared with ascorbic acid or controls, thereby offering a photoprotective effect against UVB-induced damage in human epithelial cells.

However, few human double-blind placebo-controlled studies have shown either topical or oral administration of vitamin C alone to offer photoprotection against damaging UV radiation. One small study by Murray et al., abstract 1991, examined the effect of UVB radiation on the forearms of 10 human volunteers who were pretreated with 10% topical vitamin C solution or placebo. The areas of skin that were
pretreated with the vitamin C solution demonstrated a less intense erythematous response while demonstrating a significant increase in the minimal erythema dose.

Vitamin E is found in foods such as nuts, vegetable oils, shortening, margarine, whole grains, olives, asparagus, spinach, and mayonnaise. Vitamin E is the main lipid soluble antioxidant that has been shown to protect cell membrane lipids from peroxidation by scavenging free radicals. The topical application of alpha-tocopherol, the most active form of vitamin E, has been shown to prevent skin cancer and immunosuppression induced by UVB irradiation in mice. However, alpha-tocopherol has limited stability at room temperature in comparison with the thermostable esters of vitamin E, alpha-tocopherol acetate and alpha-tocopherol succinate. Commercially, the most common form of vitamin E, alpha-tocopherol acetate, is a popular ingredient added to skin lotions, sunscreens, and cosmetic preparations. It should be noted that the topical application of the different forms of vitamin E (including alpha-tocopherol, alpha-tocopherol acetate, alpha-tocopherol methyl ether, gamma-tocopherol, and delta-tocopherol) has demonstrated varied results in animal models. For example, mice topically treated with a 1% alpha-tocopherol and exposed to UVB had 43% less formation of thymine dimers than controls. Other forms of vitamin E (including alpha-tocopherol acetate, alpha-tocopherol methyl ether, gamma-tocopherol, and delta-tocopherol) also inhibited DNA thymine dimer formation but were 5 to 10 fold less potent than alpha-tocopherol.

Additionally, Mc Vean et al., 1999 compared the efficacy of alpha-tocopherol to other vitamin E compounds (alpha-tocopherol acetate, alpha-tocopherol methyl ether, gamma-tocopherol, delta-tocopherol) and 3 commercially available
sunscreens for their ability to inhibit DNA photodamage in mouse skin *in vivo*. They found that application of a 5% solution of either alpha-tocopherol, gamma-tocopherol, or delta-tocopherol each produced a significant inhibition of thymine dimer formation whereas both alpha-tocopherol acetate and alpha-tocopherol methyl ether did not. In terms of the commercial sunscreens, only the sunscreen agent octylmethoxycinnamate inhibited dimer formation while the other two agents tested (ethylhexyl salicylate and oxybenzone) did not. The incorporation of vitamin E into sunscreen products confers protection against procarcinogenic DNA photodamage. However, the specific type of tocopherol appears to be determinate of the magnitude of the prophylactic effect.

Berton et al., 1998⁷³ studied the effect of alpha-tocopherol acetate, applied before or after UV exposure on the inhibition of UV carcinogenesis in the hairless mouse compared with vehicle controls. They found that mice that received the alpha-tocopherol acetate preparation had delayed tumor formation and yield for the first 20 weeks of the study but that this effect was lost by week 30. The telomerase activity of carcinomas in the mice treated with vitamin E was significantly lower than vehicle controls. Additionally, cyclobutane dimer repair was greater in the alpha-tocopherol acetate treated groups and tumor suppressor gene p53 expression was maximally higher after less UV exposure in this group as well. This study therefore supports the mitigating role of alpha-tocopherol acetate in the initial events of skin carcinogenesis associated with UV irradiation such as DNA damage. However, it has limited potential in preventing UV-induced proliferation and tumor formation.
Gensler, 1996\textsuperscript{70} examined whether the thermostable esters of vitamin E, alpha-tocopherol acetate and alpha-tocopherol succinate prevented skin cancer or immunosuppression in mice. They found that neither of the esters significantly prevented photocarcinogenesis nor prevented the induction by UV radiation of immunosusceptibility to implanted antigenic UV-induced tumor cells. Additionally, they noted that at the concentrations of 12.5mg alpha-tocopherol acetate and 25mg of alpha-tocopherol succinate, photocarcinogenesis was actually enhanced (p=0.0114). They concluded that these esterified forms (alpha-tocopherol acetate and alpha-tocopherol succinate) do not prevent carcinogenesis and may actually enhance skin cancer development and growth in a UVB carcinogenesis mouse model.

Additionally there has been further research into whether Vitamin E in the form of alpha-tocopherol acetate was converted in human skin to the active and photoprotective alpha-tocopherol. In a double-blind study, 19 patients >30 years of age who had a least three actinic keratoses on their forearms were randomly assigned to the treatment group (alpha-tocopherol acetate) or vehicle control group. Both groups applied the creams to their arms twice daily for three months. Blood samples, photographs, and punch biopsies were taken before the start of the study and after the study was completed. Plasma and skin concentrations of free alpha-tocopherol and alpha-tocopherol acetate were calculated. The investigators found that while alpha-tocopherol acetate was substantially absorbed in the skin, there was no evidence of cutaneous or systemic conversion to the active alpha-tocopherol form of vitamin E\textsuperscript{74}.

Additionally, the authors surveyed commercially available sunscreens to assess their alpha tocopherol content and found that of 191 sunscreens sampled, 119 (62\%)
contained some form of alpha-tocopherol, 69 (36%) contained no alpha-tocopherol, and only three (2%) disclosed the active ingredients.

In light of the Gensler, 1996\textsuperscript{70} and the Alberts et al. 1996\textsuperscript{74} studies, it is evident that alpha-tocopherol acetate, the most commercially available form of topical vitamin E, confers less photoprotective effects in mice models than other forms of vitamin E such as alpha-tocopherol and may even promote carcinogenesis. Additionally, alpha-tocopherol acetate is substantially absorbed in the skin and is not converted to its active form. These findings underscore the importance of determining which forms of vitamin E inhibit the UV induced lesions involved in photocarcinogenesis and which may actually lead to cancer formation.

Similarly to topical vitamin E, the potencies of several forms of oral vitamin E vary as well. Dosage is therefore usually expressed in terms of international units (IU), based on terms of activity. For example, 1 mg d-alpha tocopherol = 1.49 IU. In humans, a small double-blind placebo-controlled study of 12 patients given oral vitamin E (not otherwise specified) 400 IU/day for 6 months was not shown to reduce the MED or the number of sunburn cells when the treatment group was compared to controls.\textsuperscript{75} Additionally, Vural, 1999\textsuperscript{76} investigated whether levels of certain plasma *antioxidant alpha-tocopherol, ascorbic acid, total thiol groups, ceruloplasmin, urate, albumin, and erythrocyte glutathione, were altered in patients with actinic kerotoses (AKs) or BCCs. Plasma samples from 13 patient with AKs, 12 patients with BCCs and 16 healthy controls were compared. Plasma levels of ascorbic acid (p<0.001), alpha-tocopherol (p<0.05) and RBC glutathione (p<0.05) were significantly lower in patients with AKs and BCCs. The investigators suggested that plasma levels of
certain antioxidants were decreased in patients with AKs or BCCs due to the long exposure of UV irradiation.

In a double-blind placebo-controlled study, Eberlein-Konig et al., 1998 assessed the photoprotective effect of systemic vitamins C and E in humans. Ten subjects were randomly assigned to the vitamin group and given daily doses of vitamin C (ascorbic acid 2g) and Vitamin E (d-alpha-tocopherol 1000 IU) for 8 days, while the other randomly assigned ten subjects, matched to the first group by skin type, were given the placebo. The sunburn reaction was assessed by determining the MED and by measuring cutaneous blood flow of irradiated skin and nonirradiated skin before and after administration of the oral vitamins or the placebo. The results of the study illustrated that the patients given the oral vitamin combination had significant increase in their MED (p<0.01) vs. patients in the placebo group. Cutaneous blood flow was significantly decreased in the treatment group, whereas it was increased in the placebo group (p<0.05). The authors concluded that the combined use of oral vitamin C and vitamin E created a synergistic effect to reduce sunburn reactions significantly.

A second prospective, randomized and placebo controlled human trial by Fuchs and Kern, 1998 examined whether oral supplementation with vitamin E (d-alpha-tocopherol) and vitamin C (L-ascorbic acid) alone or in combination influenced the UV radiation induced skin inflammation in volunteers before and 50 days after supplementation. A dose-response curve of UV induced erythema was generated and the MED was determined by visual grading before and after supplementation. The dose response curve showed a significant flattening while the MED showed a
significant increase for the group that received combined antioxidant therapy as opposed to the groups that received either the vitamins alone or the placebo. The authors concluded that the antioxidant vitamins E and C acted synergistically in the suppression of the sunburn reaction.

Dreher et al. 1998 recently investigated the possible photoprotective effect achieved when topical vitamin C, vitamin E, and melatonin were combined in a small randomized, double-blind human study. Twelve patients of Fitzpatrick skin type II or III were selected and all patients served as their own respective controls. All patients received combinations of the antioxidant mixtures and vehicle controls, which were applied to the lower backs of the patients in a randomized double-blind manner. Thirty minutes after treatment, all patients were subjected to UV radiation and their response was measured in terms of Frosch and Kligman (1979) erythema scale. The authors found that the topical application of the hormone melatonin alone resulted in a dose-dependent inhibition of the erythema formation. The use of vitamin C or E alone had only a slight effect on the amount of erythema demonstrated by the patients. The combination of vitamin C and E showed a more prominent effect in erythema prevention while the combination of vitamin C, E, and melatonin had a significant inhibitory effect on erythema.

In conclusion, the skin has an endogenous complex antioxidant defense system that scavenges reactive oxygen species and combats UV-induced oxidative skin damage. This defense system, however, can become overwhelmed with excessive UV exposure, which leads to cutaneous UV damage, premature aging and skin cancer. Supporting the naturally occurring antioxidant system of the skin via
delivery of antioxidants either topically or orally may be a successful strategy for photoprotection.
Effects of Fat Intake on NMSC

Black and colleagues, 1994\textsuperscript{81} studied the relationship between fat intake and the development of actinic keratoses (AKs) and NMSC. In an initial randomized clinical trial 76 patients with a history of NMSC were asked to eat their regular diets (control group) or a modified diet with 20\% of their total calories derived from fat (the experimental group).\textsuperscript{81} For a total of 24 months the patients were examined by a physician, blinded to the designation of the study groups, for the development of actinic keratoses. At four months of dietary intervention the control group maintained their fat intake at 40\% of their total calories while the experimental group had lowered their fat intake to 20\% of their total calories. The total number of AKs per patient over the study period was significantly fewer in the experimental group than in the control group.

A further clinical trial by Black et al. 1998\textsuperscript{82} involving 115 patients who were randomly assigned to either the control or experimental group examined the relationship between a low fat diet and the prevention of actinic keratoses and NMSC over a two-year period. In this study the patients in the experimental group were educated in detail regarding dietary changes in order to reduce their fat intake to 20\% of their total calories and maintain this change over the entire two-year period. The results of this study were similar to the Black et al., 1994 study, in that there was a significant decrease in the skin cancer occurrence of the experimental group as compared with controls (p<0.01). The cumulative numbers of new AKs per patient over the 2 years was significantly (p<0.001) fewer in the experimental group than in
the control group. There were no significant changes in the numbers of NMSC skin cancers per patient in the control group over the 2-year period. However, in the experimental group the numbers of NMSC decreased significantly over the last 8 months of the study (p<0.02). The authors did not postulate a specific reason for this interesting finding in the study’s final 8 months instead of at earlier time periods during the study.
Herbal and Alternative Therapies for NMSC

Anticarcinogenic Effects of Tea

Chemoprevention by means of phytochemicals has been the focus of many cancer prevention studies within the last decade. In many cultures, the most popular beverage in the world, next to water, is tea. Many animal organ specific studies have shown that certain components of tea offer anti-carcinogenic properties. These constituents are known as polyphenols, which serve as antioxidants to prevent oxidative damage induced by free radicals. Studies have focused on the phytogenic properties of green tea polyphenolic (GTPs) antioxidants and have recently been reviewed in Katiyar et al., 2000. The major and most preventive ingredient thought to confer the cancer chemopreventive properties of green tea is known as (-)-epigallocatechin-3-gallate (EGCG).

There are increasing amounts of experimental evidence both in animal and human studies that indicate that both topical and systemic extracts of green and black tea can inhibit carcinogenesis. Animal studies have shown both chemically and UV induced cutaneous carcinogenesis has been inhibited by the phytochemicals available in tea. Agarwal et al. 1992 studied the effect of topical application of EGCG to the skin of SENCAR mice pretreated with TPA, a tumor promoter. They found that there was a dose-dependant reduction in epidermal inhibition of ornithine decarboxylase, an enzyme responsible for clonal expansion of skin tumors. Studies involving black tea polyphenols have elicited similar results in the inhibition of the tumor promoter TPA. Green tea polyphenols (GTP), applied to DMBA/TPA...
induced cutaneous papillomas have been shown to inhibit the conversion of benign neoplasms to malignancies in SENCAR mice. Mice with pre-existing papillomas were exposed to additional free radical generating compounds such as benzoyl peroxide or 4-nitroquinoline-N-oxide to initiate a malignant conversion. The formation of squamous cell carcinomas from papillomas was significantly reduced with administration of GTP 30 minutes prior to application of the free radical generating compounds.91

In an in vivo study by Gensler et al. 199692, topical EGCG was applied 3 times per week to BALB/cAnNHsd mice in doses of 0mg, 10mg, or 50mg for 28 weeks while they concurrently received 2.1x 10^6 J/m^2 of UVB light 5 times per week. The incidence, multiplicity and volume of UV-induced skin tumors were significantly reduced in a dose dependant manner in these animals.

The protective effect of topically applied GTP against UV induced erythema has been studied in human volunteers by Mukhtar et al., 1996.93 Different concentrations of green tea polyphenol preparations, as well as a placebo dose, were applied to the backs of volunteers who were then subjected to UV radiation at twice the minimal erythema dose (MED). Sites that were pretreated with the GTP solutions exhibited significantly less erythema than the controls. The photoprotective effect of GTP was dependent on the strength of the dose applied with the most effective protection seen at dose of 200µl of a 5% solution.

A significant protective effect against MED enhancement of sunburn cell formation was also reported. A more recent human study by Katiyar et al., 199994 examined whether topical treatment with EGCG would offer protection against UVB-
induced infiltration of leukocytes, a possible source of oxygen radical generation and
prostaglandin metabolites which have been shown to play a prominent role in skin
carcinogenesis. The authors found that application of topical EGCG prior to UVB
exposure significantly blocked the infiltration of leukocytes, led to decreased
erythema, and produced fewer inflammatory prostaglandin metabolites. Additionally,
the topical application of GTPs before UVB exposure of human skin led to a
decreased formation of cyclobutane pyrimidine dimers in DNA, which have been
implicated as initiators in the process of UV induced mutagenesis and
carcinogenesis.\textsuperscript{84,94}

As described above, there is much animal and human evidence pointing to the
use of tea as a phytogen to help to boost the endogenous antioxidant defense system of
the skin. Randomized clinical placebo-controlled trials on human subjects are now
required to evaluate the efficacy of naturally occurring polyphenols found in tea.
Current Phase I Clinical Trials are now underway at M.D. Anderson Cancer Center
and Memorial Sloan-Kettering Cancer Center on the therapeutic effect of green tea on
advanced solid tumors.\textsuperscript{85}

\textit{Other Herbal and Alternative Treatments for NMSC}

A myriad of small studies examining the relationship between herbal and
alternative remedies for the treatment and prevention of NMSC have been published
in the oncology literature and are summarized in table 1.
Table 1. Herbal and alternative remedies for the treatment and prevention of NMSC.

<table>
<thead>
<tr>
<th>Study</th>
<th>Plant, Seed or Herb</th>
<th>Active Ingredient</th>
<th>Experimental Milieu</th>
<th>Mechanism of Action</th>
<th>Findings</th>
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<tbody>
<tr>
<td>1</td>
<td>Hibatallah et al., 1999&lt;sup&gt;55&lt;/sup&gt;</td>
<td>Ginkgo biloba</td>
<td>33% extract of Gingko flavone gycosides</td>
<td>In vitro and In vivo (human models)</td>
<td>Gingko extract significantly inhibited the cutaneous blood flow by 37% (which reflects the skin inflammatory level)</td>
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<td>2</td>
<td>Lin &amp; Chang, 1997&lt;sup&gt;56&lt;/sup&gt;</td>
<td>Ginkgo biloba</td>
<td>Extract in 50% alcohol</td>
<td>In vivo (mouse models)</td>
<td>Ginkgo biloba pre-treated skin showed significant protection against UVB damage</td>
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<tr>
<td>3</td>
<td>Guevara et al. 1999&lt;sup&gt;57&lt;/sup&gt;</td>
<td>Seeds of the Philippin e Plant-Moringa oleifera Lam “Horse radish”</td>
<td>Niazimicin</td>
<td>In vivo (mouse models)</td>
<td>Niazimicin has a potent antitumor promoting activity against the two stage mouse tumor carcinogenesis model (DMBA as a tumor initiator and TPA as a tumor promoter)</td>
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<tr>
<td>4</td>
<td>Keum et al., 2000&lt;sup&gt;58&lt;/sup&gt;</td>
<td>Ginseng</td>
<td>Methanol extract of heat-processed ginseng</td>
<td>In vivo (mouse models)</td>
<td>TPA-induced enhancement of epidermal ornithine decarboxyase activity and mRNA expression was abolished</td>
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Table 1. Herbal and alternative remedies for the treatment and prevention of NMSC.

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<td>5</td>
<td>Srivastava &amp; Shukla, 1998&lt;sup&gt;99&lt;/sup&gt;</td>
<td>Cruciferous vegetables: cabbage, cauliflower, and brussel sprouts</td>
<td>Indole-3-carbinol</td>
<td>A single dose of the tumor initiator DMBA, followed by the tumor promoter, TPA twice per week. Half of the mice received 250 ug indole-3-carbinol. Tumor development was significantly inhibited in indole-3-carbinol-supplemented animals in terms of cumulative numbers of tumors and average tumors per mouse. 44% of the male mice and 29% of female mice remained tumor free by the end of the experiment. A significant delay in tumor induction time was also observed in indole-3-carbinol supplemented animals.</td>
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<td>6</td>
<td>Yasukawa, 1998&lt;sup&gt;100&lt;/sup&gt;</td>
<td>Rice bran</td>
<td>Cycloartenol ferulate</td>
<td>Pharmacological activities of the ferulate components of rice bran include: improves peripheral blood flow, possesses anti-inflammatory effect, possesses anti-tumor promoting effect. Dose dependant inhibition of DMBA/TPA tumor induction and inflammation in mice.</td>
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Table 1. Herbal and alternative remedies for the treatment and prevention of NMSC.

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<th>Herbal</th>
<th>Alternative</th>
<th>Model</th>
<th>Mechanism</th>
<th>Effect</th>
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<td>7</td>
<td>Ichihashi, 2000&lt;sup&gt;101&lt;/sup&gt;</td>
<td>Extra virgin olive oil</td>
<td>Extra virgin olive oil</td>
<td>In vivo (mouse model)</td>
<td>Possibly by decreasing reactive oxygen species induced 8-OhdG, which is responsible for gene mutation. 8-OHdG formation in mice was inhibited.</td>
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<tr>
<td>8</td>
<td>Zhao et al., 1999&lt;sup&gt;102&lt;/sup&gt;</td>
<td>Grape seeds</td>
<td>Grape seed polyphenols (GSP)</td>
<td>In vitro SENCAR mouse skin</td>
<td>The polyphenols isolated significantly inhibited epidermal lipid peroxidation. The observed antitumor-promoting effects of GSP were dose dependent and resulted in a reduction of tumor incidence, multiplicity and volume.</td>
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<tr>
<td>9</td>
<td>Gensler et al., 1999&lt;sup&gt;103&lt;/sup&gt;</td>
<td>Niacin</td>
<td>Vitamin B3</td>
<td>In vivo mouse model</td>
<td>Niacin supplementation elevated skin NAD content, which is known to modulate the function of DNA strand scission surveillance proteins p53 and poly(ADP-ribose) polymerase, two proteins critical in cellular responses to UV-induced DNA damage. A dose-dependent preventive effect of oral niacin on photocarcinogenesis and photo-immunosuppression.</td>
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<td></td>
<td>Herbal and alternative remedies for the treatment and prevention of NMSC</td>
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<td><strong>10</strong> Huang et al., 1994</td>
<td>Rosemary, Carnusol/ursolic acid, Curcumin</td>
<td>In vivo mouse model, Not specified</td>
<td>Topically applied rosemary inhibits skin tumor initiation by DMBA, tumor promotion by TPA</td>
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<td><strong>11</strong> Limtrakul et al., 1997</td>
<td>The plant <em>Curcuma longa</em>, Curcumin, the yellow pigment used as a spice and food coloring agent</td>
<td>In vivo mouse model, Not specified</td>
<td>Dietary administration of curcumin significantly inhibited the number of tumors per mouse (p&lt;0.05) and the tumor volume (p&lt;0.01)</td>
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<tr>
<td><strong>12</strong> Lahiri-Chatterjee et al., 1999</td>
<td>Milk Thistle, The flavonoid Silymarin</td>
<td>SENCAR mouse model, Not specified</td>
<td>Application of silymarin prior to DMBA/TPA significantly reduced tumor incidence (p&lt;0.001), tumor multiplicity (p&lt;0.001) and tumor volume (p&lt;0.001)</td>
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Melanoma Skin Cancer

Evidence supports the concept that UV radiation induces DNA damage that leads to melanoma in both animal and human skin.\textsuperscript{107} For example, patients with xeroderma pigmentosum have a decreased ability to repair DNA damage caused by UV radiation and have a greater risk of developing both melanoma and non-melanoma skin cancer. Additionally, it was recently shown that melanoma could be induced via UV radiation exposure on human skin in an experiment conducted with human skin xenographically attached to immunologically naïve mice.\textsuperscript{108} The incidence of melanoma skin cancer increases exponentially with age.\textsuperscript{109} The opportunities for the generation of tumors over time are increased in addition to the decrease in the ability to repair DNA.\textsuperscript{110}

UVB radiation is significantly responsible for the formation of the main DNA lesions and also the formation of photoproducts such as cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidone photoproducts.\textsuperscript{107} The incorrect repair of these principal DNA lesions leads to mutations. A significant contributor to the formation of melanoma is intense intermittent exposure to UV radiation\textsuperscript{107}. In accordance with this hypothesis, although melanoma can occur at any location on the body, it most commonly affects areas of intermittent sun exposure such as the upper backs of men and the lower legs of women.\textsuperscript{107,110,111}

Melanocytes, unlike keratinocytes, do not experience the same level of apoptotic screening. Although a first high dose of UV radiation will cause significant damage to melanocytes, they are much more likely to mutate and survive than
keratinocytes. In contrast, after prolonged UV exposure, the most severely
damaged keratinocytes undergo apoptosis, leaving the less damaged keratinocytes to
up-regulate DNA repair capacity and restore themselves back to their baseline. With
each continued exposure the most damaged cells are removed and the cells with only
minimal damage remain. It has been previously illustrated that these sub-lethal hits to
the DNA of keratinocytes actually promote the DNA repair mechanisms of the
cells.

**Phytochemicals for MM Prevention**

Mice studies have examined a variety of dietary supplements that may prove to be helpful in the prevention of cutaneous melanoma and the metastasis of melanoma. Plant estrogens or phytoestrogens have been investigated for their anticancer effects. Recently, Li et al. 1999 looked at the importance of lignans, a group of phytoestrogens, which are converted in humans to enterodiol and enterolactone by the action of the bowel microflora. Lignan precursors are found in oilseeds, whole grains, legumes and certain vegetables, but flaxseed is the richest source. Evaluation of the effect of dietary supplementation with a flaxseed derivative known as secoisolariciresinol diglycoside (SDG) on the metastasis of melanoma in mice demonstrated a reduction in the volume and number of pulmonary melanoma metastases as compared to the control group (P< 0.01).

Studies of the murine melanoma cell line B16 and specific phytochemicals have illustrated a tumor growth-suppressive effect. Mo and Elson 1999 examined the relationship between the isoprenoids, β-Ionone (a cyclic analog of β-carotene) and
gamma-tocotrienol (a less potent form of vitamin E) isolated from rice bran oil on the murine melanoma B16 cell line. They found that the addition of $\beta$-ionone and gamma-tocotrienol to the tumor cell lines led to a 23% inhibition in growth ($p<0.001$ for 15umol/L) and a 56% inhibition in growth ($p<0.001$ for 150umol/L) of the melanoma cells, respectively. The study also illustrated a synergistic and additive growth-suppressive action on the melanoma cells when these phytochemicals were combined.\textsuperscript{114}

\textit{Vitamin D}

Vitamin D is found in fortified milk, fish (herring, mackerel, salmon, sardines, shrimp, tuna, cod), fish oil, and is also produced in the skin by UV induced conversion of previtamin D. Vitamin D is then hydroxylated in the liver and kidney respectively to its most active form 1,25-dihydroxyvitamin D3 (VD3). This active steroid hormone binds to the vitamin D receptor (VDR) to exert its actions, including calcium homeostasis.\textsuperscript{115} VDR has also been detected in certain cancer cell lines including melanoma.\textsuperscript{116} A panel of eight human melanoma cell lines was assessed for the level of VDR expression and the growth inhibitory effects of VD3 by Evans et al. 1996.\textsuperscript{115} VDR expression was illustrated in the various melanoma cell lines. Furthermore, VD3 was a significant ($P<0.05$) melanoma growth inhibitor in cells with a high concentration of the VDR receptor. The authors concluded that melanoma cell growth inhibition might be mediated through the VDR receptor concentration.

Additionally, the role of VD3 and its derivatives on the induction of programmed cell death, or apoptosis, for human melanoma cells has been examined
by Danielsson et al., 1998.\textsuperscript{117} VD3 has been shown to induce apoptosis in human breast cancer and leukemic cell lines.\textsuperscript{118} In spite of the fact that VD3 has therapeutic potential, its side effects such as hypercalcemia, hypercalciuria and soft tissue calcification make it impractical to use clinically.\textsuperscript{117,119} VD3 analogs with molecular modifications have therefore been created. The VD3 analog CB1093 has been examined as a future treatment against specific types of melanoma inasmuch as it was previously found to induce apoptosis of rat mammary tumors \textit{in vivo} at a lower concentration than other VD3 analogs with fewer calcemic side effects.\textsuperscript{118} Danielsson et al., 1998\textsuperscript{117} reported that in the early melanoma stage cell line WM1341 apoptosis was induced by CB1093 up to twentyfold after 5 days of treatment at a tenfold lower concentration than the natural hormone (VD3). In contrast, CB1093 was not effective against the advanced stage MeWo melanoma cell line. In conclusion, the 1,25-dihydroxyvitamin D3 analog, CB1093, may serve as a promising therapeutic intervention for in vivo regression of the early stages of melanoma without the severe calcemic side effects of its natural vitamin D counterpart.

In further studies, the role of vitamin D and select retinoids on the induction of apoptosis of human melanoma cells has been examined by Danielsson et al., 1999.\textsuperscript{120} The combined treatment of Vitamin D3 with the select retinoid receptor ligand CD437 resulted in a synergistic induction of apoptosis for the specific human melanoma cell line WM1341. In contrast, combination therapy did not induce apoptosis in the MeWo cell line. The authors concluded that each type of melanoma cell line has an individual response to combined treatment with vitamin D and select retinoids.
Reactive Oxygen Species

As previously discussed, the delicate balance between free-radical generation and free radical scavenging systems is monitored by the skin’s endogenous defense system which combats free radical induced cellular damage and death. An imbalance in antioxidant levels was found in certain cultures of melanoma cells and normal melanocytes from patients with melanoma. This disequilibrium included higher levels of polyunsaturated fatty acids, a decreased level of catalase activity, and increased concentrations of superoxide dismutase and vitamin E in cells from melanoma patients.

In a recent study by Grammatico et al., the intracellular levels of catalase, superoxide dismutase, and vitamin E in normal melanocytes from 11 patients with melanoma were compared with normal melanocytes from 11 controls. The percentage of unsaturated fatty acids of cell membranes after exposure to a peroxidizing agent was measured as a means of determining the amount of free radical lipid peroxidation damage. A significant decrease (P<0.001) in the catalase level was associated with an increase in the vitamin E concentration in 5 out of the 11 cultures from melanoma patients as compared with the controls. No significant change of superoxide dismutase activity was found. The percentage of unsaturated fatty acids of cell membrane peroxidation was affected by the ratio of superoxide dismutase to catalase and increased in melanocytes from melanoma patients vs. controls. The authors concluded that an imbalance in the free radical scavenging system of the skin could be present in normal melanocytes from melanoma patients and this may be the...
reason for the increased susceptibility to pro-oxidizing agents such as reactive oxygen species.

Dietary Supplements

The relationship between diet and the risk of developing melanoma has been examined in human case-control studies over the last decade. For example, a study conducted at the Massachusetts General Hospital included 165 melanoma patients and 209 controls. Both populations were controlled for age, hair and eye color, and family history of melanoma. No significant associations were found between the total amount of vitamin D intake from food, supplements or milk and the risk of developing melanoma in the case versus control populations.123

Kirkpatrick et al. 1994124 assessed intakes of vitamin A, dietary antioxidants, and other dietary nutrients and their relationship to the risk of developing melanoma in a case-control study. Patients with a history of melanoma were randomly selected from the Seattle-Puget Sound cancer registry and 234 cases were matched to 248 controls for age, sex, and county. All subjects completed a telephone interview and mailed in food questionnaires in which they were asked to estimate their food intake 7 years prior to diagnosis for melanoma patients and a similar time period for controls. They found that the level of vitamin E obtained from food was inversely related to the risk of developing melanoma at an age, education, and energy intake adjusted odds ratio (OR= 0.34, and P=0.01). Additionally, zinc from food supplements was associated with a decreased risk of melanoma (OR=0.46, and P=0.01). There were no correlations found with the levels of vitamin A, retinoids, carotenoids and the risk of
melanoma. There was also no increase in the risk of developing melanoma with increased alcohol or polyunsaturated fat consumption. In contrast, body mass index was significantly related to melanoma risk as melanoma patients were more obese than controls after both populations were age, sex and education adjusted (OR= 1.90, and P=0.02). The authors point out the important confounders of this study including a possible recall bias of the cases who may have been hypervigilant in recalling their diets previous to diagnoses of their respective melanomas. In addition, the validity of a food questionnaire is always a concern in epidemiological studies; however, other researchers have substantiated dietary questionnaires of this type. These findings were similar to the Stryker et al., 1990 case-control study of 204 melanoma patients compared with 248 controls in terms of the plasma levels of retinol, vitamin E, and B-carotene and the lack of correlation of melanoma. However, the Stryker et al., 1990 study found a significant correlation between the amount of alcohol consumed and the risk of developing melanoma (OR=1.8, 95% confidence interval and P=0.03).

Another case-control study examining the correlation between prediagnostic serum levels of retinol, B-carotene, vitamin E, and selenium and the subsequent risk of developing melanoma was conducted by Breslow et al., 1995. Serum retinol, B-carotene, vitamin E, and selenium were assayed to determine the concentration of these nutrients in case and control samples. Results showed no significant differences in the serum concentration between the cases or controls. No beneficial influence of serum nutrients was elicited on the risk of developing melanoma.

The dietary factors and the risk of developing melanoma have also been studied in a prospective large human cohort study on 50,757 Norwegian men and
women. All patients in the study completed self-administered dietary questionnaires previously tested for their validity and reproducibility. The questionnaire did not focus on the intake of nutrients such as carotenoids, retinoids or vitamin C or E, hence no broad range of vegetables or fruits were taken into account. The study focused more on foods such as milk, potatoes, bread, jam, cheese, meat, fats on bread, fats in cooking, fish, cakes, eggs, oranges, porridge, cod liver oil and vitamin pills. They found that over the study period from 1977 to 1992, 108 cases of cutaneous melanoma were identified. Of the 47 cases found in men, most were found on the trunk, whereas of the 67 cases found in women, most were on the lower limbs. This study found a higher incidence of the development of melanoma in women who had a higher intake of polyunsaturated fats and cod liver oil supplements. The intake of caffeine for women was inversely related to the development of melanoma. There were no significant correlations for the men in the study. As the authors point out, the strengths of this study are the high number of participants, the high response rates of the participants and the prospective design including thorough follow-up. The results were somewhat surprising as cod-liver oil is high in omega-3 fatty acids, which have been previously linked to the inhibition of UV-carcinogenesis, the decreased risk of lung cancer, and a protective effect against breast and colon cancer. It should be noted, however, that no other study has examined the relationship of cod liver oil to melanoma.

In agreement with the Veierod et al., 1997 study, one Danish case-control study illustrated a decreased incidence of melanoma with increased coffee intake. Other studies examining this relationship have not concurred with these results.
Similar discrepancies exist among studies examining the relationship between melanoma and polyunsaturated fats.\textsuperscript{134,135,136}
Discussion

Primary and secondary prevention of skin cancer remain two essential ways to curb the increasing incidence of skin cancer in our population. Physical examination of the skin and biopsy of all suspicious lesions is still the mainstay of decreasing the morbidity and mortality of skin cancer.

Of note, in the last decade, the patient population has shown an increased interest in the use of dietary supplements such as vitamins and herbal preparations to attempt to prevent cancer. In a recent mail survey of 1,035 people, 40% of respondents reported the use of some form of alternative health care during the prior year. Of the four alternative treatments most commonly used, dietary changes or changes in “lifestyle diet” were the second most frequently reported.

Public health advocates need evidence-based information about the effects of dietary intake and topical applications of antioxidants, vitamins, and herbal remedies in order to make sound treatment decisions for the care of their patients. In terms of NMSC, review of recent randomized clinical trials, large human cohort studies, and case-control studies does not substantiate a beneficial effect for either natural dietary retinoids or synthetic retinoid supplementation for treatment or prevention of BCCs or SCCs in the general population. Select populations with defective DNA repair mechanisms, immunosuppression, or psoriasis receiving PUVA have been shown to significantly benefit from systemic retinoid treatments. Additionally, while oral administration of selenium has shown promise for reducing the incidence of several types of cancer, its direct effect in reducing BCC or SCC incidence has not been
demonstrated. No protective effect for NMSC was achieved with either normal dietary consumption or oral supplementation of β-carotene\textsuperscript{23,47,48,49}.

However, substantial evidence indicates that the cutaneous antioxidant system is both synergistic and multifactorial. Review of the current randomized human clinical trials reveals that the synergistic use of specific antioxidants proves to be more successful than with the individual components alone. The use of β-carotene with vitamins C and E offered synergistic cell protection against both the NO\textsubscript{2} and OONO- radicals\textsuperscript{51}. In a double-blind placebo-controlled study, the combined use of oral vitamin E (d-alpha-tocopherol) and vitamin C (L-ascorbic acid) created a synergistic effect to reduce sunburn reactions significantly\textsuperscript{52,70,71}. In summary, the skin has an endogenous complex antioxidant defense system which can become overwhelmed. Delivery of antioxidants in combination may serve as a successful strategy for enhancing the natural antioxidant system of the skin.

The most commercially available form of an antioxidant may not necessarily be the best form for the treatment of patients. This fact has been highlighted by the Gensler, 1996\textsuperscript{63} and the Alberts et al., 1996\textsuperscript{67} studies which emphasize the importance of determining which forms of vitamin E can inhibit photocarcinogenesis and which forms may actually lead to cancer formation. Moreover, in order to gain the photoprotective benefits from vitamin C, it is essential that the type of ascorbic acid used must be able to penetrate the skin and deliver L-ascorbic acid. Patients should be advised that many cosmetic vitamin C based products available in the commercial market contain ineffective derivatives and analogs of ascorbic acid.\textsuperscript{153}
In terms of herbal remedies for NMSC, the phytogens available in tea have been shown to help boost the endogenous antioxidant defense system of the skin in \textit{in vitro} mouse and human cell lines and in \textit{in vivo} mouse models. However, randomized clinical placebo-controlled trials on human subjects are required to evaluate the efficacy of the naturally occurring polyphenols. Also, many single studies in mice and humans have elaborated potential new antioxidants for the treatment and prevention of NMSC including ginkgo biloba, ginseng, horseradish, rosemary, and milk thistle.

Results from human case-control and large cohort studies examining dietary intake or vitamin supplementation for the treatment and prevention of melanoma have not been consistent. In studies with specific melanoma cell lines there appears to be promise for the 1,25-dihydroxyvitamin D3 analog, CB1093, as a therapeutic intervention for \textit{in vivo} regression of the early stages of melanoma without the severe calcemic side effects of its natural vitamin D counterpart\textsuperscript{110}. 
Conclusion

The increasing incidence of skin cancer in our population has reached epidemic proportions and has highlighted the need for public health awareness and intervention. Methods to curtail the rise of skin cancer in the US include the use of natural alternatives to prevent and treat NMSC and MM. The effectiveness of these alternative therapies may be supported with further human randomized clinical trials providing an evidence-based approach toward improving the health of our population.

In this paper, the use of dietary interventions and herbal remedies relating to the treatment and prevention of skin cancer has been discussed. For the general population, the natural or synthetic dietary retinoids, selenium or β-carotene do not seem to be a clinically protective against NMSC. Current clinical trials reveal that the synergistic use of specific antioxidants has greater efficacy than the use of individual components alone.

In terms of herbal remedies for NMSC, the active herbal extractions available in tea, ginkgo biloba, ginseng, horseradish, rosemary, and milk thistle have not yet been shown to be effective for humans.

Dietary intake or dietary supplementation for the treatment and prevention of melanoma, the vitamin D analog, CB1093 may have promise as a therapeutic intervention.

Enthusiasm for the use of dietary derived modalities for the treatment and prevention of skin cancer and is shared by physicians, patients, and the pharmaceutical industries but needs to be supported by further evidence based studies.
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