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The role of nutritional lipids and antioxidants in UV-induced skin cancer

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1. ABSTRACT

Two dietary tenets of the free radical theory of cancer require refinement. The first was dietary reduction of vulnerable free-radical targets, e.g., polyunsaturated lipids. The second was the addition of one or more antioxidants to the diet. Further, it was reported in 1939 that high levels of dietary fat exacerbated UV-carcinogenesis. Both lines of enquiry (dietary lipid and antioxidant effects on UV-carcinogenesis) were investigated. Both dietary lipids and antioxidants modified carcinogenic expression. Increasing levels of omega-6 polyunsaturated fatty acids (PUFA) exacerbated UV-carcinogenesis. However, omega-3 PUFA dramatically inhibited carcinogenic expression. It is probable that the action of omega-6 and-3 PUFA rests with differential metabolic intermediates, both tumor promoting and immune-modulating, that each PUFA generates through lipoxygenase and cyclooxygenase pathways. Antioxidant supplementation with butylated hydroxytoluene or beta-carotene demonstrated that each exerted its own specific antioxidant mechanism(s). When introduced into the complex milieu of the cell with its own intricate and complex antioxidant defense system, detrimental effects may ensue. These results point to oversimplification of these dietary suggestions to reduce cancer risk and the necessity to refine these dietary recommendations.

2. INTRODUCTION

Baumann and Rusch, in 1939, were the first to demonstrate the potential influence of dietary lipid on UV-induced skin cancer in experimental animals (1). In their study, the high fat diet consisted of 30 percent hydrogenated cottonseed oil. They also demonstrated that UV-induced skin tumors were of different origin from those induced by chemical carcinogens. We now know that about 90 percent of all human nonmelanoma skin cancers (NMSC) result from UV exposure and thus many of the early dietary studies using chemical carcinogens are clinically irrelevant. Ultraviolet light is a physical carcinogenic agent and does not involve activation or detoxification of the presumed carcinogenic species; no competitive chemical inhibition; no binding to target molecules; and no transport to respective target tissues. Dietary modification, by changing the chemical milieu in which a chemical carcinogenic agent is introduced could have an impact on any of these activities. As UV is the primary causal agent of NMSC, a UV model avails a more direct examination of the underlying mechanisms of dietary modification of the carcinogenic process in skin. The relation of ultraviolet light radiation, diet, dietary modification, and antioxidants has previously been more thoroughly discussed (2). Nevertheless, this line of investigation initiated by Baumann and Rusch faded with the advent of World War II.

Denham Harman conceived the "Free Radical Theory of mutation, cancer, and aging, and the maintenance of life" in the late 1950s (3, 4). Subsequently he made dietary recommendations to protect against excessive free radical formation with an anticipated reduction of cancer risk. Indeed, free radicals are formed in skin upon UV radiation (5). A body of evidence exists, albeit circumstantial, that strongly suggests free radical involvement in UV-carcinogenesis (6). Aside from the fact that free radicals are formed in skin by UV exposure, conditions that exemplify oxidative stress (UV exposure) inhibit natural antioxidant defenses (7); conditions that increase free radical load of the host also enhance UV-carcinogenesis (8); and supplementation with

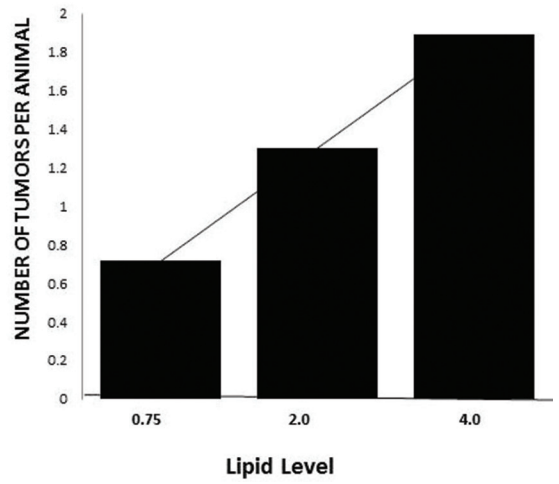

Figure 1. Relationship of dietary lipid level (omega-6 PUFA) to tumor multiplicity. Trend line shows a near linear relation of tumor expression to increasing lipid level. (Reproduced with permission from 10).

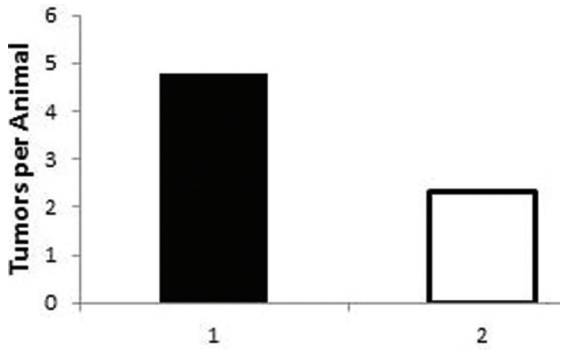

Figure 2. Influence of lipid hydrogenation on tumor multiplicity. 1, solid bar – 12% (w/w) corn oil diet. 2, Open bar – 12% corn oil diet that was 60% hydrogenated. (Reproduced with permission from 10).

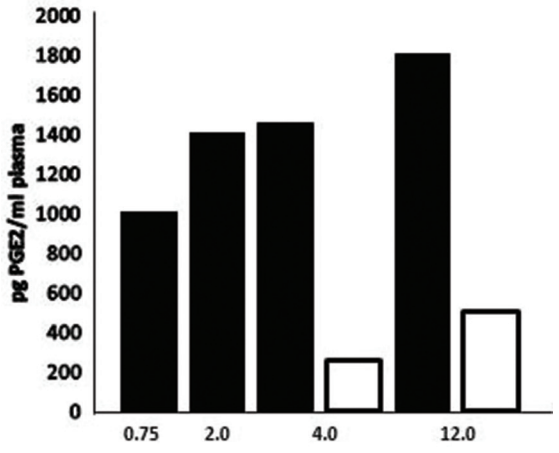

Figure 3. Relationship of dietary omega-6 and omega-3 PUFA to plasma prostaglandin E_2 levels. Solid bars, plasma PGE_2 related to the respective level of dietary omega-6 PUFA. Open bars represent the PGE_2 levels associated with omega-3 intake (4 and 12%). (Reproduced with permission from 13).

antioxidants inhibit UV-carcinogenesis (9). Accordingly, Harman's dietary recommendations included dietary reduction of vulnerable free radical targets such as polyunsaturated fatty acids (PUFA) and secondly, the addition of one or more antioxidants to the diet. A number of both dietary lipid and antioxidant supplementation studies have, on first blush, appeared to substantiate both dietary recommendations. However, in view of newly acquired knowledge and in the interest of safety, these recommendations require reassessment.

3. DIETARY LIPIDS

3.1. Experimental

It was over 40 years until the thread of evidence linking dietary lipid and UV-carcinogenesis was again pursued (10). Unlike the earlier study, nutritional parameters were closely controlled and animals were fed isocaloric diets containing various levels of omega-6 fatty acids (corn oil). Some animals received corn oil that had been partially hydrogenated. The results are shown in Figure 1. There was a near linear increase in carcinogenic expression, with respect to tumor multiplicity, as the level of dietary lipid increased. Further, there was a marked reduction in carcinogenic expression when the corn oil had been partially hydrogenated (Figure 2), suggesting that degree of saturation of dietary lipid was an important determinant for cancer expression. At this point the data seemed to confirm Harman's suggestion that reduction of dietary PUFA and degree of dietary lipid saturation could reduce cancer risk. With respect to degree of saturation, however, there are other possible reasons for our observations. First, when hydrogenating PUFA, *trans* fatty acids (TFA) and conjugated linoleic acid (CLA) are formed. It is unlikely that TFA could be responsible for a reduction of skin tumors as a recent study has demonstrated that TFA make the skin more vulnerable to UV-injury (11). Whether CLA might play a role in reduction of cancer expression is unknown, although it is reported to have anti-carcinogenic properties. The most likely explanation is simply a reduction in level of linoleic acid (omega-6) in the diet. The question of what role degree of lipid saturation plays in influencing cancer expression became moot when studies were conducted with menhaden oil – a source rich in omega-3 PUFA (12). Both omega-6 and omega-3 PUFA exhibit about the same degree of unsaturation. Whereas omega-6 fatty acid (FA) promotes carcinogenic expression (Figure 1), omega-3 FA inhibits carcinogenic expression, e.g., compare tumor latency and multiplicity for 4.0. percent omega-6 with 4.0. percent omega-3 (Table 1). Thus, degree of saturation seems to play an insignificant role in carcinogenic expression and calls for a reassessment of the general recommendation to reduce PUFA in the diet – at least as a mean to reduce vulnerable targets for free radical attack and consequently, reduce cancer risk.

Omega-3 FA compete with omega-6 FA for active sites on cyclooxygenase, a major enzyme in the eicosanoid

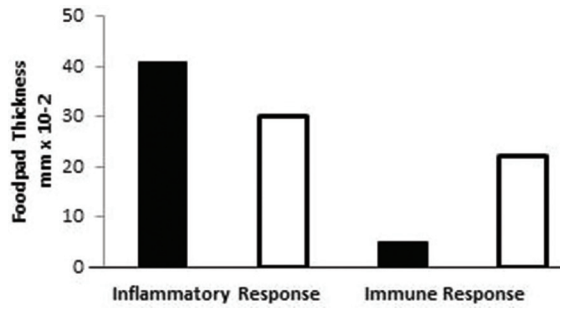

Figure 4. Effect of omega-6 and omega-3 PUFA on inflammatory and immune responses. Inflammatory response to dimethyl sulfoxide (DMSO) was determined as an increase in footpad thickness. Immune response to 2, 4-dinitrochlorobenzene (DNCB) was determined as total delayed type hypersensitivity (DTH) response minus inflammatory response. Solid bars, omega-6 FA; Open bars, omega-3 FA. (Reproduced with permission from 13).

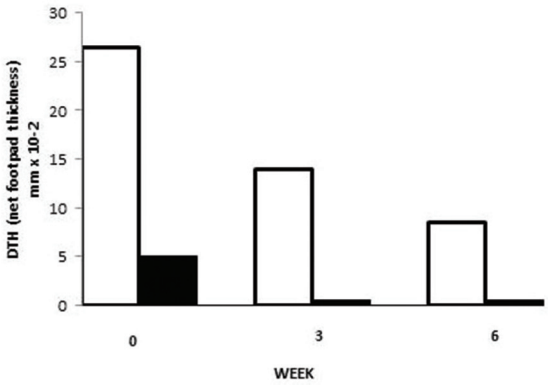

Figure 5. Effect of omega-6 PUFA dietary levels on temporal profile of the DTH response. Open bars represent low-fat (0.7.5%). Solid bars represent high-fat (12%). Zero time represents the end of a two week run-in period for the respective diets and prior to UV-irradiation. At week three of irradiation high fat has almost completely suppressed the DTH response. Suppression continued through six weeks of irradiation Comparison of the diets at time zero shows that the animals fed the low-fat diet mounted a 5-fold greater response than animals on the high-fat diet. Animals on the low-fat diet continued to mount a DTH response through six weeks of irradiation. (Reproduced with permission from 14).

Table 1. Comparison of omega-6 and omega-3 PUFA on UV-Carcinogenesis

Lipid source (% of diet)	Tumor latency (weeks)	Tumor multiplicity (tumors per animal)
0.7.5 omega-6.	21.8.8	0.4.7
4.0.0 omega-6	19.0.0	1.4.3
4.0.0 omega-3	23.2.1	0.4.1
Low levels (0.7.5%, W/W) of omega-6 FA increase tumor latent period and reduce tumor multiplicity compared to 4.0.% omega-6 FA. Omega-3 FA, at equivalent levels of omega-6 FA, reduce tumor multiplicity and increase tumor latency even further. (Reproduced with permission from 12). PUFA: polyunsaturated fatty acids		

cascade. In doing so, the level of pro-inflammatory and immune-modulating omega-6 FA metabolites is reduced. The relationship of prostaglandin E_2 (PGE_2) to dietary level of omega-6 and omega-3 FA is shown in Figure 3. As the dietary omega-6 PUFA increases, the plasma PGE_2 level increases. Omega-3 FA intake reduces PGE_2 levels approximately 7-fold in comparison to an equivalent level of omega-6 FA (13). These data are in agreement with the thesis that omega-6 and omega -3 PUFA differentially influence, not only prostaglandin E_2 levels, but other pro-inflammatory and immune-modulating intermediates of the cyclooxygenase pathway. Omega-3 FA, compared to omega-6 FA, reduced inflammation and enhanced immune responsiveness (Figure 4). Similarly, low levels of omega-6 FA, when compared to high levels of omega-6 FA, also sustain a high level of immune responsiveness both prior to UV-irradiation and after six weeks of chronic UV-irradiation (14) (Figure 5). These data certainly support the thesis that omega-6, -3 PUFA metabolism, through the lipoxygenase and cyclooxygenase pathways, leads to differential metabolites that are influential in inflammatory and immune responses involved in carcinogenesis and support the following conclusions (15, 16):

- Dietary omega-6 FA exacerbate UV-carcinogenic expression.
- Dietary omega-3 FA inhibit UV-carcinogenic expression.
- Omega-6 FA exert their principal effect upon the post-initiation, or promotion stage of carcinogenesis. Omega-3 FA exert their effect across the carcinogenic continuum (17).
- Pro-inflammatory and immunosuppressive PGE_2 levels are reduced by omega-3 FA. Omega-6 FA and UV suppress immunologic responses.
- Omega-3 FA inhibits the UV suppression of immunologic pathways as manifested in Delayed Type Hypersensitivity (DTH).

Thus, a major mode of action of dietary fat on UV- carcinogenesis is *via* the modulation of immune pathways that appear to be related to differential influence of omega-6, -3 PUFA on inflammatory and immune active products of the eicosanoid cascade. Certainly, the general indictment of PUFA as a means of reducing cancer risk is a recommendation that must be reassessed and refined.

3.2. Clinical

Experimental studies, employing a high-fat, low-fat cross-over feeding design had clearly demonstrated that the principal exacerbation of carcinogenic expression by high levels of omega-6 FA occurred during the post-initiation, or promotion, stage of carcinogenesis (17). More importantly, crossing over from a high-fat to a low-fat diet, even after a cancer causing dose of UV had been administered, negated the exacerbating influence of the high fat diet and provided a rationale for the undertaking of a clinical intervention trial. Such a trial was undertaken and has been described in more detail (2). Briefly,

133 skin patients met the inclusion criteria, of which 115 completed the two-year study. Fifty-eight were randomly assigned to the Control arm in which no dietary changes were introduced. The 57 patients randomly assigned to the Intervention arm learned how to adopt low-fat eating habits to their food preferences and lifestyles. Each patient in the Intervention arm was given a "fat gram goal" that defined the grams of fat that would provide 20% of calories as fat. At four months into the study, patients in the Intervention arm had reduced their % of calories from fat from 39% to 21% where it remained to the end of the two-year study. As this study was designed to determine the influence of dietary fat on NMSC, stability of body weight and calorie intake was maintained in order to prevent confounding effects due to these variables. The influence of dietary fat became apparent early in the study, as a significant number of actinic keratoses (pre-malignant lesions) between groups occurred (18). Patients in the control arm were found to be at 4.7. time's greater risk of having one or more actinic keratosis during the two-year period than similar patients in the low-fat Intervention arm. The influence of the reduction in calories from fat on NMSC (squamous and basal cell carcinomas) was observed after 101 patients had completed the study (19). This effect became even stronger after all 115 patients completed (20). NMSC occurrence in the control arm, when measured in 8-month intervals of the two year study, did not change significantly from the baseline period. NMSC occurrence in the intervention arm was significantly lower ($p < .02$) in the last 8-month evaluation period. The cumulative rate of occurrence of NMSC (cumulative skin cancers/patient/time period) was .21 and .19 during the first 8-month period of the study and 0.26 and 0.02 during the last 8-month evaluation period for control and intervention arms, respectively. In this study there was no effort to alter the types of fat consumed by the patient nor the type of PUFA. Effort was made to maintain the polyunsaturated/saturated fatty acid ratio (P/S ratio), however.

Experimental studies demonstrated that omega-3 PUFA dramatically *inhibited* UV-carcinogenesis, compared to the *exacerbation* of carcinogenesis resulting from high levels of omega-6 FA intake (12). Assessment of early genotoxic markers in humans indicated that omega-3 FA protected against UV-induced genotoxicity and suggested that longer term supplementation might reduce NMSC occurrence (21). A population-based case-control study found a consistent tendency for a lower risk of squamous cell carcinoma (SCC) with higher intakes of omega-3 FA (22). Their data also suggested a tendency toward reduced risk of SCC with diets containing high omega-3/omega-6 PUFA ratios. Recently, a review and meta-analysis was conducted to determine the relationship between skin cancer and dietary omega-3 intake (23). While the data were limited, the investigators reported that intake of high omega-3 FA was inversely associated with melanoma (only one estimate) and SCC, although the latter was not significant. The investigators concluded that these data were suggestive but inadequate to support the hypothesis that omega-3 FA protects against skin cancer. The most direct approach to address this issue is through intervention trials in populations with high and known risk, for NMSC. It has been proposed that a study design be adopted that is similar to that in which a reduction in the % of calories consumed as fat was shown to reduce NMSC occurrence in NMSC patients (16).

It has been suggested from experimental data that one potential mechanism of omega-3 FA inhibition of UV- carcinogenesis is mediated through immune modulation. It was shown that plasma prostaglandin E_2 (PGE_2) levels are directly related to the intake of omega-6 FA, which, in turn, induced the greatest exacerbation of carcinogenesis (13). Omega-3 FA reduced the PGE_2 level below that of the lowest level of omega-6 FA intake. PGE_2 is known to be pro-inflammatory and immunosuppressive. Importantly, omega-3 PUFA provides striking protection against UV-induced immunosuppression (13, 24). Indeed, a preliminary double-blind, randomized controlled study of UVL suppression of nickel contact hypersensitivity in humans indicated that oral omega-3 PUFA abrogated photoimmunosuppression (25).

A considerable body of evidence has accrued that indicates the influence of omega-6,-3 PUFA on UV- carcinogenesis is predicated upon the differential metabolites of the cyclooxygenase pathway (15). The different effects upon UV- carcinogenic expression, the differences in eicosanoid intermediates, the differences in immune responsiveness of omega-6 and omega-3 PUFA precludes the general indictment of dietary PUFA in cancer risk and this recommendation must be refined, based on individual PUFA (26). In summary, the implementation of a low-fat diet and omega-3 FA supplementation show the greatest promise as dietary strategies for the management and prevention of the highly prevalent NMSC.

4. ANTIOXIDANTS

4.1. Butylated hydroxytoluene

The addition of one or more antioxidants to the diet was recommended in order to provide protection against free radical damage and ultimately reduce cancer risk. A cocktail of antioxidants containing butylated hydroxytoluene (BHT), vitamins C and E, and reduced glutathione, was shown to effectively suppress UV-carcinogenesis (9, 27) (Figure 6). This cocktail of antioxidants was also shown to inhibit epidermal lipid peroxidation (8) (Figure 7). These data were interpreted at the time to be supportive of the free radical theory of cancer and the dietary recommendations.

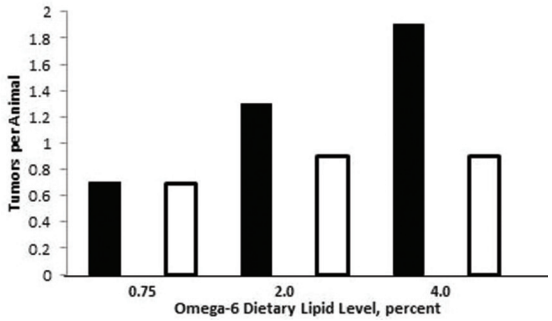

Figure 6. Influence of dietary lipid and antioxidants on UV-mediated tumor multiplicity. Dietary lipid level: solid bars. The respective dietary lipid level with antioxidant supplementation: Open bars. Antioxidants markedly inhibited tumor expression at the higher lipid levels. (Reproduced with permission from 8).

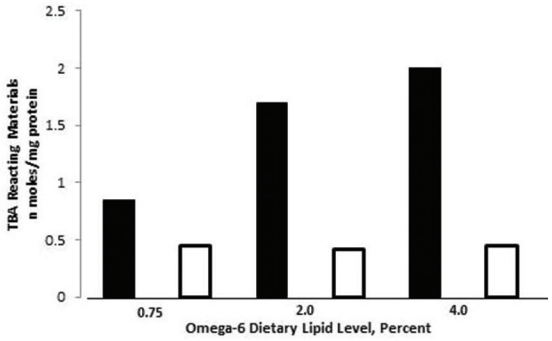

Figure 7. Influence of dietary lipid level and antioxidant supplementation on cutaneous lipid peroxidation. Solid bars, epidermal lipid peroxidation levels at the respective dietary lipid level. Open bars, antioxidant supplemented. Epidermis was prepared from animals receiving the respective diets, with and without supplementation, for 35-40 weeks and after a single UV dose. (Reproduced with permission from 8).

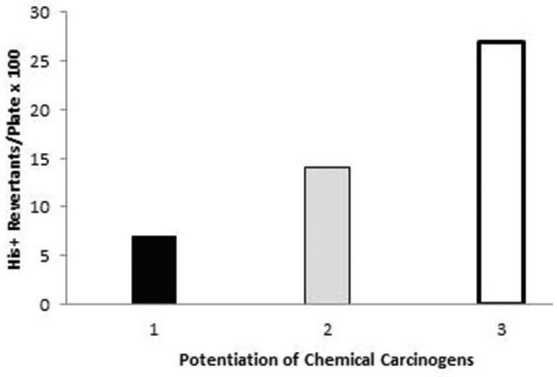

Figure 8. Potentiation of chemical carcinogen activation by diet and antioxidants. 1, solid bar, Mutation frequency ((hepatic activation of N-2-fluorenyacetamide (2-AAF)) from microsomal fractions obtained from animals receiving a closed-formula ration. 2, Shaded bar, carcinogen activation from animals receiving a semi-defined diet containing 4% omega-6 PUFA (corn oil). 3, Open bar, animals receiving the same diet as (2) but containing a BHT supplement. Data indicate that both diet and antioxidant supplements can influence chemical carcinogen activation. (Reproduced with permission from 34).

Butylated hydroxytoluene, at concentrations employed in the cocktail, was shown to be the principal ingredient of the cocktail responsible for suppression of tumors (28, 29). The mode of action of BHT's protective effect was determined to be one of diminution of UV dose to target layers of the epidermis. Forward transmission studies of epidermis and stratum corneum found that transmission of the carcinogenic wavelengths of UV was 65% greater through non-supplemented tissues than those tissues from BHT supplemented animals (30, 31). It was suggested that BHT retards the oxidation of keratin in the stratum corneum. (a non-living tissue) and thus prevents its natural differentiation, i.e., oxidation. In retarding the formation of S-S bridges, the optical properties of keratin are altered and BHT's mode of action in preventing UV-carcinogenesis is one of UV-dose diminution.

Butylated hydroxytoluene is an effective systemic photoprotectant and antioxidant. It significantly increases tumor latent period and decreases tumor multiplicity (28); provides a 2X increase in erythema threshold (32); results in a marked reduction in cutaneous lipid peroxidation (8) and inhibits UV-induction of ornithine decarboxylase activity (33). All of these effects could be attributed to UV-dose diminution. BHT was originally GRAS (Generally Recognized As Safe) approved. However, questions began to arise regarding its safety. Because the phenol evokes a number of physiological responses including hepatomegaly and induction of hepatic Phase I and II microsomal activation/detoxification enzymes, there is concern that BHT might predispose the host to other types of cancer. Indeed, BHT has been shown to potentiate chemical carcinogen activation (34) (Figure 8). Thus, this effective systemic photoprotectant may behave as a double-edged sword – creating a risk to the very host it is intended to serve.

4.2. Beta-carotene

Beta-carotene is one of about 100 carotenoids found in human foods. Chemically it is a tetraterpenoid consisting of eight isoprenoid residues. It strongly absorbs light in the 400-500 nm range. Beta-carotene is widely distributed in fruits and vegetables, especially green leafy and yellow vegetables. It is an important micronutrient that functions as a precursor for vitamin A synthesis.

Beta-carotene is an efficient quencher of singlet oxygen and exhibits good radical-trapping capacity at low oxygen partial pressures. At greater oxygen partial pressures beta-carotene loses its antioxidant capacity and shows autocatalytic pro-oxidant effects (35). The radical reactions of beta-carotene and their potential influence on UV-carcinogenesis have been reviewed previously (36).

An epidemiologic study in 1981 found that individuals that consumed greater levels of green

Table 2. Influence of diet on beta-carotene mediated UV-carcinogenesis

Diet	Median tumor time (weeks)	Tumor multiplicity (tumors per animal)
Closed-Formula		
Control	20.6.	0.5.2
0.0.7% beta-carotene	20.0.	0.6.0
Semi-Defined		
Control	19.5.	0.6.0
0.0.7% beta-carotene	17.2.*	1.6.3*

*data that are significantly different from the respective Semi-defined Control and Closed-formula Control and Supplemented groups. Beta-carotene significantly shortened the tumor latent period and increased the tumor multiplicity in the semi-defined diet. (Reproduced with permission from 43)

Table 3. Effect of varying levels of vitamins C and E on beta-carotene-mediated tumor multiplicity

Control	Beta-carotene supplemented	Beta-carotene minus vit c	Beta-carotene minus vitamin c, Reduced vitamin E
1.0.5	3.2.0	3.4.5	5.9.0

There is a three-fold increase in tumor multiplicity when beta-carotene supplemented semi-defined diet is compared to semi-defined Control diet. There is no significant effect on tumor multiplicity when vitamin C is deleted from the semi-defined diet. A near six-fold increase in tumor multiplicity, compared to Control, occurs when vitamin C is deleted from the diet and vitamin E level is reduced. These data suggest an interaction of beta-carotene with vitamin E, but not with Vitamin C. (Reproduced with permission from 48, 49)

leafy vegetables exhibited a lower cancer risk (37). Because these foods are rich in beta-carotene, and as the carotenoid was known to be an efficient singlet oxygen quencher and to terminate free radical reactions, those anti-cancer effects attributed to those foods were subsequently attributed to beta-carotene. Indeed, Mathews-Roth and colleagues reported that beta-carotene supplementation could inhibit UV-carcinogenesis (38, 39). However, the role of beta-carotene as an anti-cancer agent began to be questioned when clinical trials failed to show any effect on NMSC (40). A second clinical trial found no reduction in the incidence of lung cancer in male smokers after 5-8 years of beta-carotene supplementation (41). Alarmingly, an excess cumulative incidence of lung cancer occurred after 18 months of beta-carotene supplementation and increased progressively thereafter – resulting in an 18 per cent increase in incidence by the end of the study.

In 1998, experimental studies not only failed to demonstrate a protective effect of beta-carotene supplementation to UV-carcinogenesis but found a significant exacerbation (42). This result was particularly disturbing and experimental variables between the earlier studies of Mathews-Roth and our later studies were carefully examined. The one variable that attracted attention was diet. The earlier studies in which protective effects were observed employed closed-formula diets (an example would be a commercial rodent chow) whereas the latter study employed a semi-defined diet in which purified macro nutrients (such as casein as protein source) were compounded. Indeed, when beta-carotene supplementation studies were conducted with these two types of rations there was no effect of beta-carotene with the closed formula diet, but exacerbation occurred in the semi-defined diet (43) (Table 2).

In an effort to explain the exacerbative effect of beta-carotene on UV-carcinogenesis, Truscott demonstrated that the carotenoid was highly reactive with peroxy radicals (RO_2*) and that the resulting reaction could proceed *via* electron transfer resulting in the formation of the carotenoid radical cation (CAR*+) (44). The carotenoid radical cation exhibits a reduction potential of about 1000 mv and is, itself, a strong oxidizing agent (45). It could act as a pro-oxidant and do considerable tissue damage as a pro-carcinogenic agent if not repaired (Eq. 1).

$$RO_2^* + CAR \rightarrow RO_2 + CAR^{*+} \qquad \text{(Eq. 1)}$$

Based upon one-electron transfer rate constants between various carotenoids and interactions between vitamins E (TOH) and C (Asch), a mechanism was proposed that would ultimately result in repair of the beta-carotene radical cation (46, 47). Vitamin E (TOH) would repair a peroxyl radical to yield a tocopherol radical cation (TOH*+) (Eq. 2).

$$RO_2^* + TOH \rightarrow TOH^{*+} + ROOH \qquad \text{(Eq. 2)}$$

Vitamin E radical cation (TOH^{*+}) would then be repaired by beta-carotene (Car), producing the beta-carotene radical cation (Car^{*+}) (Eq. 3).

$$TOH^{*+} + Car \rightarrow TOH + Car^{*+} \qquad \text{(Eq. 3)}$$

It was proposed that vitamin C (Asch) would repair the carotenoid radical cation at the aqueous membrane interface and the resulting vitamin C radical cation (Asc^{*+}) would be cleared (Eq. 4).

$$Car^{*+} + Asch \rightarrow Car + Asc^{*+} \qquad \text{(Eq. 4)}$$

Nonetheless, when vitamin C was either eliminated from the semi-defined diet or increased 6-fold, compared to usual levels, beta-carotene exacerbation of UV-carcinogenesis was not affected (48,49) (Table 3). Ten-fold increases in vitamin E levels did not influence beta-carotene exacerbation of UV-carcinogenesis.

However, as seen in Table 3, reduced levels of vitamin E augmented beta-carotene exacerbation of UV-carcinogenesis. These data do not support a role for Vitamin C in beta-carotene radical cation repair but do suggest a vitamin E and beta-carotene interaction.

Overall, the dietary studies indicate that diet can have a profound influence upon beta-carotene modulated UV-carcinogenesis. They also suggest that the repair of the beta-carotene radical cation indicated in the previous schema is dependent on other factors that would be present in closed formula rations, e.g., other carotenoids, or their isomers, or other phytochemicals as yet unidentified. These natural compounds would be absent in the semi-defined diet. Their identification remains to be elucidated (Eq. 5):

$$Car^{*+} + \mathbf{?} \rightarrow Car + \mathbf{?} \qquad \text{(Eq. 5)}$$

Pryor and colleagues have cited some of the difficulties in understanding the physiologic responses evoked when supplementing the diet with antioxidants (50). For example, determining the rate of absorption of the antioxidant by the target tissue; target tissue concentrations; interactions between water and lipid soluble antioxidants; turnover rates in the respective target tissue; regeneration and recycling; and determination of rate constants within the target tissue are but some of the difficulties. When supplementing a highly complex and intricate natural antioxidant defense system, with a high level of one type of antioxidant, the stoichiometry of the antioxidant pathways may be altered – driving the reaction from an antioxidant to a pro-oxidant or pro-carcinogenic state. This could account for the conflicting reports of the benefits and risks of antioxidant supplementation (51). Regardless, in the case of beta-carotene, the International Agency for Research on Cancer Working Group on the Evaluation of Cancer Preventive Agents has recommended: "Until further insight is gained, beta-carotene should not be recommended for use in cancer prevention in the general population and it should not be assumed that beta-carotene is responsible for the cancer protecting effects of diets rich in carotenoid containing fruits and vegetables" (52).

5. CONCLUSIONS

The preceding discussion highlights two dietary recommendations of the Free Radical Theory of Cancer that require reassessment. Although earlier UV-carcinogenesis studies appeared to support these recommendations, i.e., increasing levels of omega-6 PUFA increased tumor multiplicity and reduced tumor latency of UV-induced skin tumors, later studies demonstrated that omega-3 PUFA inhibited UV-carcinogenesis. Indeed, omega-3 PUFA can have a number of beneficial effects on inflammatory and immune responses associated with reduction of UV-induced cancer risk. Thus, the general indictment of all PUFA was premature and clearly demonstrated that the recommendation to reduce PUFA in the diet as a mean to reduce free radical attack on vulnerable tissue targets and, consequently, reduce cancer risk, needs refinement.

The recommendation to add one or more antioxidants to the diet also requires close scrutiny. BHT, or a cocktail of antioxidants of which BHT is the active constituent, has been shown to be an efficient photoprotectant to UV-carcinogenesis. However, in a mutagenesis test, concern arose regarding its potential to predispose the host to chemical carcinogenesis at sites other than skin. Thus, enthusiasm for this effective systemic photoprotectant was diminished. Beta-carotene has been shown, under some dietary conditions, to exacerbate UV-carcinogenesis. While the IARC (International Agency for Research on Cancer) has made a specific recommendation to avoid the use of beta-carotene as a mean to reduce cancer risk, the World Cancer Research Fund/American Institute for Cancer Research has concluded "A general recommendation to consume supplements for cancer prevention might have unexpected adverse effects" and thus, "Dietary supplements are not recommended for cancer prevention" (53).

The experience with beta-carotene and BHT clearly make the widespread recommendation for antioxidant supplements no longer tenable. This recommendation for the general public, as a mean to reduce cancer risk, must be reassessed. It is likely that antioxidant therapy, in some personalized cases, may be indicated. However, new algorithms for efficacy and safety testing must be developed. One potential approach has been described (54). In those cases where an effective photoprotectant to NMSC has been identified, one might test for other adverse cancer effects against environmental or occupational carcinogenic agents to which the individual is exposed using a mutagenesis test, e.g., a modified Ames test. The cancer risk for those agents to which the individual is exposed would be determined and used in an overall risk-benefit analysis, weighing the risk of one form of cancer against the benefit of the photoprotectant to NMSC. Until safety and evaluation methods for antioxidant supplementation have been developed, the best recommendation for maintaining a balanced and effective antioxidant defense system remains the consumption of a balanced diet containing adequate fruit and green leafy and yellow vegetables that are known to be rich in a broad range of antioxidants.

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