# Serological Precursors of Cancer: Malignant Melanoma, Basal and Squamous Cell Skin Cancer, and Prediagnostic Levels of Retinol, β-Carotene, Lycopene, α-Tocopherol, and Selenium[1]

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## Abstract

**To determine the association between prediagnostic serum levels of retinol, β-carotene, lycopene, α-tocopherol, and selenium and the subsequent risk of malignant melanoma, and basal and squamous cell skin cancer, a nested case-control study among residents of Washington County, MD, was performed. Cases with melanoma ($n$ = 30), basal cell ($n$ = 32), and squamous cell ($n$ = 37) skin cancer who were admitted to hospital for treatment or biopsy of metastatic lesions were each matched by age, sex, and race with two controls. There were no significant associations between serum micronutrient levels and the risk of subsequent skin cancer.**

## Introduction

Basal and squamous cell cancers of the skin are major sources of morbidity in the United States, and malignant melanoma is an increasing cause of mortality. The incidence of melanoma, 12 of 100,000 among whites, has been increasing approximately 4–5% each year (1), resulting in a doubling of the overall risk every 15 years. An estimated 32,000 new cases and 6,900 deaths due to melanoma were predicted for 1994 (2). Many melanoma deaths occur prematurely in young and middle-aged adults (3). In 1984, the average cost of treating melanoma was $7,000 for initial treatment, $500/month for continuing treatment and $16,000 for terminal treatment (4). Present costs are likely to be higher.

Basal and squamous cell skin cancers are the most common malignancies in the United States. Their combined incidence was 233 of 100,000 in a 1977–1978 National Cancer Institute survey (5). An estimated 900,000–1,000,000 new cases (6) and 1,200 deaths from these tumors were predicted for 1994 (7). The majority of nonmelanoma skin cancer cases are curable but lack of early, appropriate treatment may cause significant disfigurement (8). The economic burden of nonmelanoma skin cancer has not been quantified but is likely to be high because of the associated morbidity and large number of cases (7).

Exposure to sunlight is the major risk factor for the development of melanoma, basal cell, and squamous cell skin cancer (9). UV radiation produces free radicals that may initiate carcinogenesis. Antioxidant micronutrients, in particular β-carotene, lycopene, α-tocopherol, and selenium (a cofactor for glutathione peroxidase) quench free radicals or inhibit their formation (10). Human skin contains antioxidant micronutrients (11) and retinol, a vitamin necessary for normal cell differentiation (12). In animal models, β-carotene, α-tocopherol, and selenium inhibit skin cancer induced by UV radiation (13, 14).

Only two previous nested case-control studies of melanoma using prediagnostic serum antioxidant levels have been reported. Knekt *et al.* (15), in a series of 10 cases, found that β-carotene and α-tocopherol had protective associations but retinol and selenium did not. However, Comstock *et al.* (16) in a subset of the present study population reported that β-carotene, lycopene, and α-tocopherol were not associated with protection against melanoma in 20 cases.

The results of previous nested case-control studies of basal cell and squamous cell skin cancer and prediagnostic serum antioxidant levels were also negative (16, 17), as were those from two clinical trials (18, 19), whereas a case-control (24) and prospective study (25) of selenium suggests that this nutrient may be protective. Knekt *et al.* (20–22) observed that β-carotene, retinol, α-tocopherol, and selenium were not associated with basal cell skin cancer. Comstock *et al.* (16), again in a subset of the present study population, found no association with β-carotene, α-tocopherol, and lycopene in 21 basal cell cancer cases. Wald *et al.* (17, 23) reported no association of "skin cancer" with retinol (22 cases) or with β-carotene (56 cases). Clinical trials also did not support the hypothesis that β-carotene is protective against basal or squamous cell skin cancer (18) or that isotretinoin, a form of retinoic acid, is protective against basal cell skin cancer (19). However, Clark (24, 25), using postdiagnostic blood samples, observed that selenium was associated with a lower risk of basal cell skin cancer in a case-control study of 240 subjects and a lower incidence of recurrent basal and squamous cell skin cancers in a prospective cohort study of the same subjects. Thus, evidence from epidemiological studies and clinical trials provides little consistent support for the hypothesis that antioxidant micronutrients protect against skin cancer. However, although the

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data are scant, the magnitude of the public health problem is enormous.

This investigation was undertaken to further explore the relationship between an array of antioxidant micronutrients and skin cancer. It extends the previous nested case-control study by Comstock *et al.* (16) by increasing the number of cases of melanoma and basal cell skin cancer and including cases of squamous cell skin cancer. The objective of this study is to examine the association between prediagnostic serum levels of retinol, β-carotene, lycopene, α-tocopherol, and selenium and the risk of developing melanoma, basal cell, and squamous cell skin cancer in residents of Washington County, MD.

## Materials and Methods

Washington County is a semirural community in western Maryland. In 1974, from August through November, a mass campaign collected 15-ml blood samples from 25,620 volunteers for a serum bank. In 1975, a private census enumerated about 90% of the total population and an additional 182 blood samples were collected. Among the 62,222 persons over age 18 years who were enumerated in the census, 30% had donated blood for the serum bank. Participation was somewhat greater among females, persons ages 35–64 years and those who were better educated. Subsequent cases of melanoma and basal and squamous cell carcinomas occurring among Washington County residents who participated in the blood donation program were identified from the Washington County cancer register. Information for this register comes from death certificates and discharge records of Washington County Hospital, which has an active well-equipped oncology service. It is the only general hospital in the county.

All of the cases in this study had been admitted to hospital either for treatment of the cancer or for biopsy of suspected metastases. As a result, all represent relatively serious disease and all have had pathological confirmation of the diagnosis. To be eligible for this study, cases were excluded from the study if there had been a diagnosis of cancer before the date of blood donation. Cases of basal and squamous cell cancer were also excluded if a second, different cancer had been diagnosed after diagnosis of skin cancer but before the time serum was withdrawn from the bank for assays. The melanoma and squamous cell cancer cases represent all eligible cases in the register before the time when sera were sent to the laboratory. Among the 98 basal cell cancer cases that developed in the population who donated blood for the serum bank, a sample of 32 cases was selected by a systematic scheme based on the year of diagnosis. The sample was weighted toward including cases diagnosed within the first 3 years of follow-up. The characteristics of the sample were virtually identical with those of all eligible basal cell cancer cases with respect to the characteristics in Table 1, except for the weighting toward earlier years of diagnosis and for a considerable excess in persons over the age of 65 years.

Two controls were selected for each case, matched for race (all were white), sex, and age (usually within a few months). Potential controls were excluded from the study if they had died before the time of diagnosis of the case to which they were matched or if they were registered as cancer cases during the follow-up period.

In the 1974 blood donation campaign, blood was collected in 15-ml vacutainers (Becton Dickinson, Rutherford, NJ), allowed to stand at room temperature for 30 min, and then refrigerated at 4°C until centrifugation in the laboratory, usually within 3–4 h and always within 24 h. Serum was then placed in two 5-ml Nunc tubes (Nunc, Roskilde, Denmark), and frozen at −70°C until removed for aliquotting in preparation for assays. Under dim yellow light, tubes were thawed in ice water and aliquots were prepared for shipment to the laboratory in insulated boxes containing sufficient dry ice to insure the arrival of the serum in a frozen condition. Case-control status of the samples was not known to laboratory personnel.

Because of financial constraints and the necessity to give first priority to studies for which specific funding had been provided, assays were done in batches at different times. For melanoma, 23 case-control sets were assayed in 1988 and another 7 were assayed in 1994. For basal cell carcinoma, 16 sets were assayed in 1986 and another 16 were assayed in 1991, and 14 sets with a case of squamous cell carcinoma were assayed in 1986, and 23 more were performed in 1994. Sera in each case-control set were assayed on the same day with the same reagents and by the same technician. In this way, cases and controls were also matched on duration of storage and circumstances of the assays. Serum retinol, α-tocopherol, β-carotene, and lycopene were assayed by high-pressure liquid chromatography (26, 27) and selenium was assayed by neutron activation analysis (28). Not all case-control sets of melanoma and basal cell skin cancer were assayed for selenium. Laboratory accuracy for high-pressure liquid chromatography, based on internally and externally prepared quality controls, is 2% for retinol, <5% for tocopherols, and <12% for the individual carotenoids. For selenium, the external quality control specimens were found to have a concentration of 1.06 ± 0.015 ppm compared to the certified concentration of 1.1 ± 0.01 ppm.

To estimate the number of melanoma cases expected among the study population requires that the population at risk during the midpoint of the period 1975–1991 be calculated, along with the sex-age specific rates at that point. As the result of determining the vital and residential status in 1985 of a 5% sample of the 1975 private census population, it is possible to estimate the study population at risk at the midpoint of the study period by age and sex. Similarly, the expected melanoma rates at the midpoint can be estimated from the published SEER[3] rates for the periods 1973–1975 and 1988–1990 (1). Both calculations assumed a straight line relationship for the intervening years.

Nonparametric tests were used to determine the statistical significance of differences in levels of nutrients because distributions were skewed toward higher values (29). To determine whether there was a trend in skin cancer risk according to serum micronutrient level, the distributions for each micronutrient were divided into thirds based on values among controls. ORs and CIs for middle and high *versus* low thirds were estimated by conditional logistic regression (30). When ORs by thirds were compatible with a dose-response relationship, the significance of observed trends was evaluated by using likelihood ratio tests from regressions (30). Micronutrients were coded as single, quantitative variables, and median levels were used as exposure scores for each third.

## Results

As far as can be ascertained from available resources, Washington County does not appear to be unusual with respect to

[3] The abbreviations used are: SEER, Surveillance, Epidemiology, and End Results Program; CI, confidence interval; OR, odds ratio.

*Table 1* Percentage distribution of descriptive characteristics among skin cancer cases and controls in Washington County, MD, in 1974

| | Melanoma | | Basal | | Squamous | |
|---|---|---|---|---|---|---|
| | Case ($n$ = 30) | Control ($n$ = 60) | Case ($n$ = 32) | Control ($n$ = 64) | Case ($n$ = 37) | Control ($n$ = 74) |
| Sex | | | | | | |
| Male | 36.7 | 36.7 | 53.1 | 53.1 | 73.0 | 73.0 |
| Female | 63.3 | 63.3 | 46.9 | 46.9 | 27.0 | 27.0 |
| Age (yr) | | | | | | |
| ≤24 | 20.0 | 20.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 25–34 | 6.7 | 6.7 | 0.0 | 0.0 | 2.7 | 2.7 |
| 35–44 | 16.7 | 16.7 | 3.1 | 3.1 | 5.4 | 5.4 |
| 45–54 | 26.7 | 25.0 | 21.9 | 21.9 | 27.0 | 27.0 |
| 55–64 | 20.0 | 21.7 | 18.8 | 18.8 | 29.7 | 31.1 |
| 65–74 | 6.7 | 6.7 | 43.8 | 43.8 | 32.4 | 31.1 |
| ≥75 | 3.3 | 3.3 | 12.5 | 12.5 | 2.7 | 2.7 |
| Month of blood draw | | | | | | |
| September | 40.0 | 46.7 | 37.5 | 42.2 | 29.7 | 33.8 |
| October | 43.3 | 38.3 | 46.9 | 43.7 | 32.4 | 27.0 |
| November | 16.7 | 15.0 | 15.6 | 14.1 | 37.8 | 39.2 |
| Hours since last meal | | | | | | |
| 0–1 | 13.3 | 21.7 | 21.9 | 25.0 | 29.7 | 28.4 |
| 2–3 | 50.0 | 45.0 | 46.9 | 35.9 | 37.8 | 35.1 |
| 4–5 | 20.0 | 21.7 | 25.0 | 32.8 | 13.5 | 16.2 |
| ≥6 | 16.7 | 11.7 | 6.3 | 6.3 | 18.9 | 20.3 |
| Cigarette smoking | | | | | | |
| Never | 66.7 | 45.0 | 43.8 | 42.2 | 43.2 | 39.2 |
| Past | 13.3 | 25.0 | 31.3 | 40.6 | 37.8 | 36.5 |
| Current | 20.0 | 30.0 | 25.0 | 17.2 | 18.9 | 24.3 |
| School (yr) | | | | | | |
| <12 | 43.3 | 45.0 | 46.9 | 48.4 | 54.1 | 50.0 |
| 12 | 40.0 | 36.7 | 28.1 | 29.7 | 29.7 | 28.4 |
| >12 | 16.7 | 18.3 | 25.0 | 20.3 | 16.2 | 21.6 |
| Not stated | 0.0 | 0.0 | 0.0 | 1.6 | 0.0 | 0.0 |
| Yr of diagnosis[a] | | | | | | |
| 1975–1977 | 20.0 | | 50.0 | | 24.3 | |
| 1978–1980 | 33.3 | | 18.8 | | 5.4 | |
| 1981–1983 | 13.3 | | 12.5 | | 5.4 | |
| 1984–1986 | 10.0 | | 15.6 | | 35.1 | |
| 1987–1989 | 13.3 | | 3.1 | | 29.7 | |
| 1990–1992 | 10.0 | | 0.0 | | 0.0 | |

[a] Controls had no skin cancer and, therefore, no year of diagnosis.

skin cancer. During the 3 decades from 1950 to 1980, the socioeconomic area that includes Washington County had death rates from melanoma and from other skin cancers that were neither in the upper 10% of the United States' rates nor were they significantly low (31). On the basis of age-sex-specific rates from the SEER registers, the expected number of melanoma cases among the study population is 32.1; there were 33 observed cases. Data are not available to calculate the expected number of other skin cancer cases that would come to attention through a review of hospital discharge records.

Characteristics of cases and controls are presented in Table 1. None of the case-control differences was statistically significant. Within each type of skin cancer, case-control sets had similar distributions for sex, age, and years of schooling. Most melanoma cases were female, and most squamous cell skin cancer cases were male. Melanoma cases were younger than basal and squamous cell cases; 43% were <45 years of age. Fewer melanoma cases than controls were past or current smokers.

Mean prediagnostic levels of serum micronutrients are shown for melanoma in Table 2 and for basal cell and squamous cell skin cancer in Table 3. There were no significant differences between cases and matched controls for any serum micronutrients in any skin cancer group except for retinol, which was significantly higher in basal cell skin cancer cases than controls ($P$ = 0.02). There were no meaningful interactions of the micronutrients with sex, age, or smoking status (data not shown).

Table 4 shows the relative odds of developing melanoma and basal and squamous cell skin cancer by thirds of serum nutrients. There were no significant dose-response relationships. Adjustment for smoking, education (an indicator of socioeconomic status), and hours since the last meal did not substantially change the results.

All analyses were repeated on the subset of cases and matched controls diagnosed 4 years or more after their blood was drawn in 1974 because cancers existing at the time of blood drawing might be associated with altered serum micronutrient concentrations. This did not change the results.

*Table 2* Mean prediagnostic levels of serum micronutrients among melanoma cases and matched controls in Washington County, MD

| Nutrient | Melanoma | | | | |
|---|---|---|---|---|---|
| | Case-control sets (*n*) | Mean levels | | % of difference[a] | *P* value[b] |
| | | Cases | Controls | | |
| Retinol (μg/dl) | 30 | 60.8 | 63.7 | −4.6 | 0.23 |
| β-carotene (μg/dl) | 30 | 18.9 | 21.2 | −10.8 | 0.23 |
| Lycopene (μg/dl) | 30 | 40.8 | 38.6 | +5.7 | 0.57 |
| α-tocopherol (mg/dl) | 30 | 1.17 | 1.14 | +2.6 | 0.67 |
| Selenium (μg/dl) | 23 | 11.6 | 11.5 | +0.9 | 0.79 |

[a] (Case mean − control mean)/(control mean) × 100.
[b] Wilcoxon sign rank test.

*Table 3* Mean prediagnostic levels of serum micronutrients among basal and squamous cell skin cancer cases and matched controls in Washington County, MD.

| Nutrient | Case-control sets (*n*) | Mean levels | | % of difference[a] | *P* value[b] |
|---|---|---|---|---|---|
| | | Cases | Controls | | |
| Basal cell skin cancer | | | | | |
| Retinol (μg/dl) | 32 | 71.4 | 63.9 | +11.7 | 0.02 |
| β-carotene (μg/dl) | 32 | 21.3 | 21.3 | 0.0 | 0.84 |
| Lycopene (μg/dl) | 32 | 30.9 | 29.0 | +6.6 | 0.52 |
| α-tocopherol (mg/dl) | 32 | 1.55 | 1.27 | +22.0 | 0.12 |
| Selenium (μg/dl) | 17 | 11.5 | 11.7 | −1.7 | 0.51 |
| Squamous cell skin cancer | | | | | |
| Retinol (μg/dl) | 37 | 71.2 | 67.2 | +6.0 | 0.19 |
| β-carotene (μg/dl) | 37 | 26.3 | 24.5 | +7.3 | 0.67 |
| Lycopene (μg/dl) | 37 | 28.1 | 27.0 | +4.1 | 0.93 |
| α-tocopherol (mg/dl) | 37 | 1.30 | 1.23 | +5.7 | 0.43 |
| Selenium (μg/dl) | 37 | 11.3 | 11.4 | −0.9 | 0.49 |

[a] [(Case mean − control mean)/(control mean)] × 100.
[b] Wilcoxon sign rank test.

## Discussion

This study evaluated the possible beneficial influence of serum levels of several micronutrients on the risk of subsequent melanoma, basal cell, and squamous cell skin cancer. No important associations were observed between any of the micronutrients and these skin cancers. This conclusion was unaltered after accounting for the possible effects of latent skin cancer, smoking, socioeconomic status, and the time between the most recent meal and serum donation.

The strengths of this nested case-control study are that three types of skin cancer were investigated in the same study population, the role of multiple micronutrients was evaluated, and prediagnostic blood was utilized. In this study, the cases are representative of all cases in a defined group of persons who were free of other major cancers and who were admitted to hospital for treatment of skin cancer or for biopsy of suspected metastatic sites. The latter restriction insured that all cases were pathologically confirmed. The number of melanoma cases in this study was almost identical to the number expected on the basis of rates from the SEER register (1). Although there are no data from which to make such a calculation for basal or squamous cell cancers, a number of calculations over the years have consistently shown that the numbers of cases of a variety of cancers in this study population approximate 90% of the expected numbers. Although some controls must have had less serious skin cancers that did not require hospitalization, we know of no reason why prediagnostic micronutrient levels should not be similar for cancers that require and do not require major surgical intervention.

It is unfortunate that this study, like its predecessors, was not able to produce evidence on several risk factors for skin cancers, such as exposure to sunlight, family history, predisposing lesions, or occupational exposures. A finding that has some slight indirect bearing on the issue of sunlight exposure differences between melanoma cases and controls is that in 23 of the case-control sets in this study, the serum concentrations of 1,25-dihydroxyvitamin D were 4.8% lower among cases than controls (32). In any case, unless these missing risk factors are also associated with micronutrient serum levels, they will not affect the study findings.

This study lends some support to the scanty epidemiological evidence in failing to find an important role for antioxidant micronutrients in the prevention of melanoma, basal, or squamous cell skin cancer. Efforts to curtail the melanoma epidemic and the increasing incidence of basal cell and squamous cell cancer in this country should focus on

*Table 4* Association between serum micronutrients and the development of melanoma, basal and squamous cell skin cancers by thirds of serum nutrients, Washington County, MD

| Thirds | Retinol | β-Carotene | Lycopene | α-tocopherol | Selenium |
|---|---|---|---|---|---|
| | OR 95% CI | OR 95% CI | OR 95% CI | OR 95% CI | OR 95% CI |
| Melanoma | | | | | |
| Low[a] | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Mid | 1.0 (0.4–2.9) | 0.5 (0.2–1.7) | 1.2 (0.4–3.5) | 1.9 (0.5–6.9) | 0.3 (0.1–1.1) |
| High | 0.4 (0.1–1.6) | 0.8 (0.2–2.3) | 1.1 (0.4–3.2) | 2.0 (0.5–8.4) | 0.9 (0.3–2.5) |
| *P* | 0.23 | NA[b] | NA | 0.35 | NA |
| Basal cell | | | | | |
| Low | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Mid | 0.5 (0.1–2.1) | 1.2 (0.3–4.1) | 0.2 (0.3–4.1) | 2.4 (0.7–8.2) | 2.3 (0.5–10.3) |
| High | 3.3 (0.9–11.6) | 1.3 (0.4–4.0) | 1.4 (0.4–4.0) | 2.6 (0.7—9.2) | 0.8 (0.1–4.5) |
| *P* | NA | 0.72 | NA | 0.23 | NA |
| Squamous cell | | | | | |
| Low | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Mid | 1.7 (0.6–5.0) | 0.9 (0.4–2.4) | 2.1 (0.7–5.9) | 1.3 (0.4–4.0) | 0.6 (0.2–1.5) |
| High | 1.8 (0.6–5.8) | 1.4 (0.5–4.0) | 1.0 (0.3–3.1) | 1.5 (0.5–4.6) | 0.6 (0.2–1.5) |
| *P* | 0.35 | NA[b] | NA | 0.48 | 0.23 |

[a] Reference category.
[b] NA, not applicable.

reducing the risk by limiting exposure to sunlight, the major risk factor.

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