[CANCER RESEARCH 45, 2369–2372, May 1985]

Serum Vitamin Levels and the Risk of Cancer of Specific Sites in Men of Japanese Ancestry in Hawaii[1]

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ABSTRACT

Serum specimens were obtained from over 6800 men of Japanese ancestry in Hawaii from 1971 to 1975. Since then, the following numbers of newly diagnosed cancer cases have been identified: 81 colon, 74 lung, 70 stomach, 32 rectum, and 27 urinary bladder. The stored sera of the cases and 302 controls were tested to determine their β-carotene, vitamin A, and vitamin E levels. There was no association of either vitamin A or E with any of the cancers. For serum β-carotene, there was a significant association only with lung cancer (20.0 μg/dl in cases *versus* 29.0 in controls, $P < 0.005$). The lung cancer odds ratio for men in the lowest quintile of β-carotene was 3.4 relative to men in the highest quintile. These findings suggest that a low serum β-carotene level is a predictor of increased lung cancer risk in men.

INTRODUCTION

Although a recent study found that serum levels of vitamins A and E and total carotenoids were not related to the overall incidence of cancer, there is still strong interest in the possible protective effect of these vitamins in subgroups of a population, or against particular types of cancer (22). The importance of this issue is highlighted by the fact that a large chemoprevention trial among physicians involving the use of β-carotene supplements has already begun in the United States (9).

β-Carotene is a principal component of total carotenoids and is obtained in the Western diet mainly from green-yellow vegetables and yellow-orange fruits. Many dietary studies have shown that a high intake of β-carotene is associated with reduced risk of cancer, especially lung cancer (3, 10, 14, 15, 17). Several serum studies found that cancer cases had lower serum β-carotene and vitamin A (retinol) levels than did controls (2, 11). However, the sera were collected after the cases were diagnosed, so the low levels may have been a consequence of the cancer. The levels of β-carotene in the serum and adipose tissue are directly related to the amounts ingested over recent weeks (15), which is not the case for serum retinol.

Athough a variable fraction of ingested β-carotene (provitamin A) is converted in the intestinal mucosa to vitamin A (7), dietary vitamin A is obtained from animal sources such as liver, eggs, and dairy products. In the United States, the usual foods available to the consumer are estimated to provide about one-half of the total vitamin A activity as provitamin A carotenoids, and one-half as retinol (4). Vitamin A or its synthetic analogues are potent in controlling cell differentiation, and in preventing epithelial cancers in experimental animals (18). Two community-based studies found that high serum retinol levels in prediagnostic sera were associated with reduced risk for cancer (12, 21). However, other reports in humans did not confirm this finding (19, 22).

In contrast to β-carotene and vitamin A, there have been only a few studies on vitamin E and cancer in humans (2, 22). Vitamin E has antioxidant properties which can suppress neoplasia in animals (6). It also blocks the formation of *N*-nitroso compounds which have been implicated as a causative factor for certain ancers (20).

Our community-based study has stored sera obtained from men who were free of cancer at the time of phlebotomy, but were subsequently diagnosed with their disease. Consequently, we decided to look at the association of serum β-carotene and vitamins A and E with 5 common cancer sites in our study population: the lung, stomach, colon, rectum, and urinary bladder.

MATERIALS AND METHODS

From 1965 to 1968, 8006 men of Japanese ancestry, born between 1900 and 1919, participated in the Honolulu Heart Program on the Hawaiian island of Oahu (23). Approximately 6 years later, from 1971 to 1975, 6860 of these men returned for another round of examinations. At that time, a nonfasting venous blood sample was obtained. One aliquot was tested for serum cholesterol (Auto-Analyzer Method N-24A), as well as for other biochemical measurements. The rest was stored at −75 °C. The subsequent diagnosis of cancer among these men was recorded by continuous surveillance of all general hospitals on Oahu. A computer linkage file was established with the Hawaii Tumor Registry to reduce the possibility of missing incident cases during the surveillance period. Based on a 10-year follow-up of a systematic random sample of 1685 men, it was determined that only 1.8% of the study subjects could not be found on Oahu. As a result, the surveillance for incident cancer cases should be nearly complete.

After a period of about 10 years, we have identified the following number of newly diagnosed cases of cancer: 81 colon; 74 lung; 70 stomach; 32 rectum; and 27 urinary bladder. Each case was confirmed by histological examination of tissue obtained by surgery or biopsy. The study was limited to epithelial tumors of the appropriate cancer sites. The histological types of lung carcinoma were classified into small cell, squamous cell, adenocarcinoma, or mixed form, according to the classification of Kreyberg (13).

Controls were randomly selected from the examined men who did not have any of the cancers under study. This sampling was stratified by age group, so as to match the age distribution of all cases combined. This was not age matching in the usual sense, but provided an efficient study design, since a single (but larger) control group served in each of the case *versus* control comparisons. In each such comparison, the median age of the cases (by cancer site) was within 2 years of the median age of controls. Of the 302 controls in the study, only 14 (4.7% of the total) had died during the surveillance period. Because of this low percentage of deaths, it is unlikely that mortality among controls could

[1] Supported by Contract N01 CP 61060 and Grant R01 CA 33644 from the National Cancer Institute, NIH, Bethesda, MD 20205.

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Received 8/27/84; revised 12/26/84; accepted 1/14/85.

be a major competing risk in this study.

The frozen sera, which had never been thawed before, were sent in dry ice to the laboratories of Hoffmann-La Roche, Basle, Switzerland, for analysis. The assay of the serum levels of β-carotene, retinol, and vitamin E was performed by normal-phase high-pressure liquid chromatography on columns of Lichrosorb Si 60 (5-μm particle size; 250 x 4 mm; Merck Hibar). The delivery of the mobile phases was isocratic. The effluents of the columns were monitored continuously, using a Waters Model 440 fixed-wavelength detector with 2 channels for β-carotene and retinol, and a Perkin-Elmer Model 650-10 LC double monochromator spectrofluorometer equipped with a xenon arc lamp for vitamin E. The operating conditions were: for β-carotene, solvent, *n*-hexane:dioxane (1000:10); flow, 0.9 ml/min; retention time, 3.7 min; detection, 436 nm; for retinol, solvent, *n*-hexane:isopropyl alcohol (970:30), flow, 1.9 ml/min; retention time, 5.0 min; detection, 313 nm; for vitamin E, solvent, *n*-hexane:ethyl acetate (1000:75); flow, 1.8 ml/min; with excitation wavelength of 290 nm and fluorescence wavelength of 330 nm.

The samples were prepared by shaking a mixture of 0.2 ml plasma, 0.2 ml water, 0.4 ml ethanol, and 0.8 *n*-hexane, centrifuging and injecting 0.1 ml of the clear supernatant. For the calibration, external standards of pure retinol, β-carotene, and pure α-tocopherol (Hoffmann-La Roche) were subjected to the above extraction procedure. The photometric or fluorometer response was checked using control samples from pooled serum. The coefficients of variation [(SD/mean) × 100%] in 10 blind frozen samples were 14.8% (10.5/70.9) for retinol, 32.6% (1.5/4.6) for β-carotene, and 13% (1.3/10.0) for vitamin E. The elevated coefficient for β-carotene may have been due to the unusually low mean level in the blind samples. According to the Hoffmann-La Roche Laboratory, the coefficient of variation for fresh samples is approximately 3% for all 3 vitamins.

We were not able to assess directly the effects of storage on the sera tests, but others have found that the reduction in serum values of vitamins with storage has been small, if any (12, 22). To indirectly measure storage effects, we examined paired samples of 20 normal men collected over an interval of approximately 8 years. The median values of the paired earlier *versus* later specimens were as follows: retinol, 57.5, 55.2 μg/dl; β-carotene, 18.5, 45.0 μg/dl; vitamin E, 9.9, 12.7 μg/ml. Although it is not known what the serum levels should be in fresh specimens obtained 8 years apart, the data suggest that β-carotene levels could be more affected by storage relative to retinol or vitamin E. Nevertheless, the occurrence of differential storage effects between cases and controls is very unlikely, because their mean duration of storage (in years) was similar: lung, 9.9; stomach, 9.9; rectum, 10.2; colon, 10.3; urinary bladder, 10.3; controls, 10.1. Furthermore, the laboratory technician could not distinguish the sera of cases from that of controls and treated them identically during analysis.

Due to the heavily skewed serum vitamin distributions (especially β-carotene), nonparametric methods of statistical analysis were used. Simple correlations were of the Spearman rank type. To perform the multiple comparisons of interest (each case group *versus* controls), we used the nonparametric rank sum method of Dunn (5), which does not require equal sample sizes. Odds ratios by serum vitamin levels were derived from logistic regression models, with and without adjustment for relevant covariates. The odds ratio estimates the risk of disease in a particular group relative to the risk of some reference group (namely, men in the highest β-carotene quintile). Lung cancer odds ratios were adjusted for age and current smoking (cigarettes/day). Tests for linear trend in the logit of risk (16) were derived from logistic models using continuous, ungrouped vitamin variables and covariates. All logistic models were fitted using iterative maximum likelihood methods (8).

RESULTS

The mean ages of the cancer cases and their controls at time of examination were as follows: lung, 62.5 years; stomach, 63.7; colon, 62.3; rectum, 64.7; urinary bladder, 61.9; and controls, 62.4.

There was no significant association of either serum retinol or vitamin E with the specific cancers in this study, as shown in Table 1. For serum β-carotene, there was a strong negative association only with lung cancer. Stomach and colon cancer cases had reduced β-carotene levels, but the difference compared with controls was not significant at $P < 0.05$.

Serum cholesterol was correlated with β-carotene (0.21), retinol (0.21), and vitamin E (0.39). It also tended to vary by cancer site, but adjustment for serum cholesterol in the analysis did not change the pattern of the findings seen in Table 1.

When the lung cancer patients were separated by histological types (25 squamous cell, 18 adenocarcinoma, and 12 small cell), the median level of β-carotene of each type was uniformly lower than that of the controls. They were 19.5 μg/dl for squamous cell ($P < 0.05$), 21.3 μg/dl for adenocarcinoma ($P > 0.50$), and 15.3 μg/dl for small cell ($P < 0.05$). The histological type for the remaining 19 lung cancer cases was either mixed form or unknown.

The odds ratios of lung cancer risk by approximate quintiles of serum β-carotene concentration are presented in Table 2. The quintiles were determined from the 302 controls only. The unadjusted odds ratios show the presence of a high risk for lung cancer, especially in subjects with β-carotene levels at 25.0 μg/dl or less. With adjustment for age and current number of cigarettes smoked per day, the magnitude of the odds ratios was decreased, but was still suggestive of a dose-response relationship. Simultaneous multiple adjustment for age, current

Table 1

Median values of serum β-carotene, retinol, and vitamin E among cancer patients and controls

Cancer site	No. of cases	β-Carotene (μg/dl)		Retinol (μg/dl)		Vitamin E (μg/ml)	
		Median	P[a]	Median	P[a]	Median	P[a]
Lung	74	20.0	<0.005	63.8	>0.50	12.8	>0.50
Stomach	70	22.8	>0.10	60.4	>0.50	12.2	>0.50
Colon	81	23.5	>0.30	62.1	>0.50	12.2	>0.50
Rectum	32	27.8	>0.50	58.8	>0.50	11.6	>0.40
Bladder	27	29.0	>0.50	57.5	>0.50	12.7	>0.50
Controls	302	29.0		59.6		12.3	

[a] For significance of the difference between each site-specific distribution and the distribution for controls, by the nonparametric multiple comparisons procedure of Dunn (5).

Table 2

Odds ratios of lung cancer by quintiles of serum β-carotene concentration

β-Carotene concentration (μg/dl)	No. of cases	No. of controls	Unadjusted Odds ratio	Unadjusted 95% confidence interval	Adjusted[a] Odds ratio	Adjusted[a] 95% confidence interval
57.1–311.5	7	60	1.0		1.0	
34.6–57.0	12	60	1.7	0.6, 4.7	1.5	0.5, 4.1
25.1–34.5	10	59	1.5	0.5, 4.1	1.2	0.4, 3.5
15.1–25.0	21	62	2.9	1.1, 7.3	2.4	0.9, 6.2
0–15.0	24	61	3.4	1.4, 8.4	2.2	0.8, 6.0
Total	74	302	$P^b = 0.004$		$P^b = 0.040$	

[a] Adjusted for age and smoking (current number of cigarettes/day), using multiple logistic regression analysis.

[b] Test for linear trend in values of: $\log_e$ [risk/(1 − risk)], known as the logit of risk.

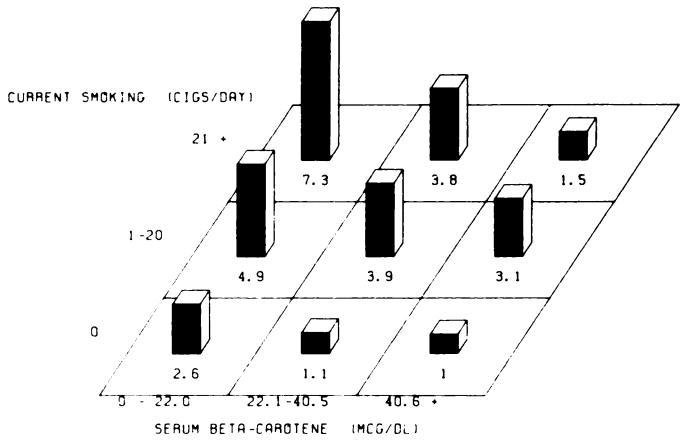

Chart 1. Age-adjusted lung cancer odds ratios by level of serum β-carotene and by current cigarette smoking status. Numbers of cases and controls per cell range from 4 to 14 and 11 to 77, respectively, except for the highest-carotene/greatest-smoking category which had 1 case, 7 controls.

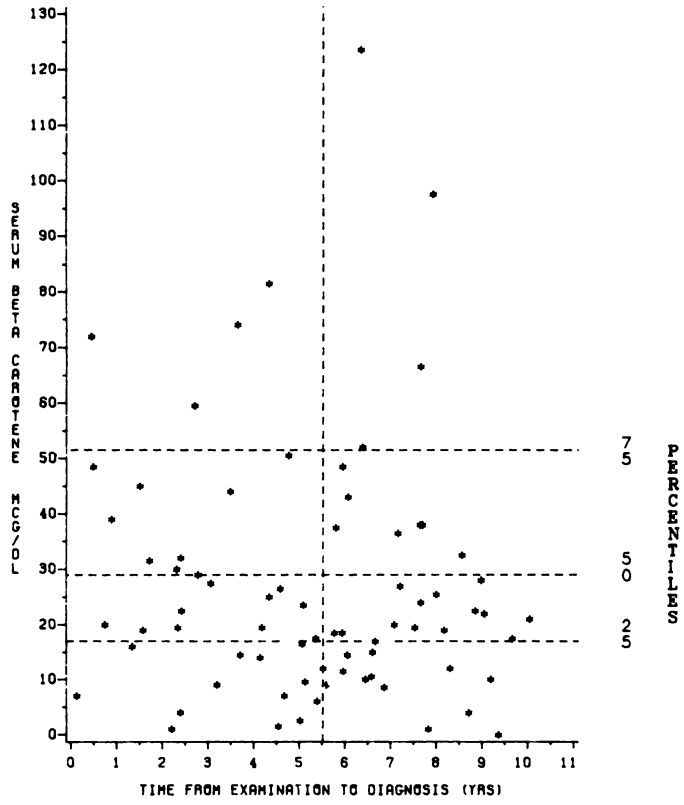

Chart 2. Serum β-carotene levels for 74 lung cancer cases according to time interval from phlebotomy to diagnosis. The β-carotene percentiles are based on the value of controls. *Vertical line*, median time interval (5.52 years) for the 74 men.

cigarettes per day, alcohol intake, serum cholesterol, serum retinol, and serum vitamin E was also done. It did not meaningfully alter the estimated odds ratios of lung cancer in Table 2. The revised odds ratio for men in the lowest quintile of β-carotene was 2.0 relative to men in the highest quintile.

In order to study further the association of lung cancer with cigarette smoking and serum β-carotene, we separated the subjects into 3 groups according to current smoking status and into tertiles of β-carotene. The results are shown in Chart 1. The age-adjusted odds ratio was 7.3 for heavy smokers in the lowest tertile of β-carotene (14 cases and 21 controls) relative to nonsmokers in the highest β-carotene group (8 cases and 77 controls). A negative, monotonic risk relationship with serum β-carotene was seen in each smoking category.

The cases consistently had reduced serum β-carotene levels throughout the surveillance period, as shown in Chart 2. Twenty five of 37 (67.6%) lung cancer men diagnosed during the first 5.5 years of follow-up, and 26 of 37 (70.2%) diagnosed during the rest of the surveillance period had β-carotene values below the 50th percentile of controls. In all, 27 of 74 (36.5%) cases had serum β-carotene values below the 25th percentile of controls.

DISCUSSION

Observational studies have strongly suggested that dietary β-carotene can protect against lung cancer. Three prospective dietary studies (3, 10, 17) found that cases consumed substantially less green-yellow vegetables and other sources of β-carotene than did noncases. In one serum case-control study of 26 lung cancer cases, a nonsignificant negative association with β-carotene was found (2) but to our knowledge there has not been any previous study specifically relating serum β-carotene to lung cancer risk in prediagnostic serum.

The findings from the present study suggest that a low level of serum β-carotene is a risk factor for lung cancer. Even after considering the strong effects of current cigarette smoking on lung cancer, reduced serum β-carotene levels still seem to accentuate lung cancer risk. This was particularly evident in heavy smokers (Chart 1). Previous dietary studies have also observed that the effects of a low carotene intake were independent of cigarette smoking, and especially increased the frequency of lung cancer in heavy smokers (3, 10, 17). Further analysis of our data showed that the increased lung cancer risk in men with low β-carotene levels persisted up to 10 years after their serum was obtained. This made it less likely that the low levels of serum β-carotene resulted from the effects of the disease process. It is possible that β-carotene exerts a protective effect by enhancing immunological function (15), or by quenching singlet oxygen and trapping free radicals, which may cause DNA damage and can be found in high levels in cigarette smoke (1).

Our findings on serum vitamin A support the observation from past studies using prediagnostic serum that it is not associated with total cancer risk (19, 22). One of the 2 earlier studies which found a low level of serum vitamin A in cancer cases failed to confirm its finding with new cases (22). In the other study, a total of 86 cancer cases had lower retinol levels in prediagnostic serum than in controls (21). Although it is useful to study all cases of cancer combined, it is more informative to look at individual cancer sites.

When site-specific cancers were considered in previous studies, they did not show a consistent pattern. One investigation found a negative association of serum vitamin A in 17 cases of stomach cancer (19), a second study observed it in 14 cases of lung cancer (21), and a third study found it in 11 cases of gastrointestinal cancer (22). Because of this lack of consistency by cancer site and the relatively small numbers of cases in each study, these may be chance findings due to multiple site-specific comparisons. In either case, further work is needed in this area. We also need to learn more about the determinants of vitamin A level in the serum, since it does not appear to be related directly

to dietary vitamin A intake (15), or correlated with serum β-carotene levels ($\rho = -0.06$) in our data.

In spite of the fact that vitamin E appears to inhibit induced carcinogenesis in experimental animals (6), we found no suggestion of any protective effect of vitamin E against specific cancers. Others have also observed a similar lack of any association in human studies (2, 22). Until stronger evidence is available supporting the concept that the antioxidant and antinitrosating properties of vitamin E can protect against cancer formation, it cannot be promoted as an anticancer agent.

In order to put the association of β-carotene with lung cancer into proper perspective, it should be noted that the factor of greatest importance for lung cancer in this study population continues to be cigarette smoking. Subjects who smoked more than one pack of cigarettes a day had 5.5 times the risk of never-smokers of developing lung cancer. Although β-carotene seems to exert an effect independent of cigarette smoking, further work should be done to clarify the relationship among cigarette smoking, serum β-carotene, and dietary intake of β-carotene. There was a significant negative correlation ($\rho = -0.28$) between current cigarettes smoked per day and serum β-carotene levels in this study, but these data sources were collected simultaneously. Longitudinal metabolic studies need to be done to determine the effects of cigarette smoking on serum β-carotene. Furthermore, it is not known if cigarette smokers can reduce their risk for lung cancer by increasing their intake of dietary β-carotene.

ACKNOWLEDGMENTS

The authors thank the following institutions for their helpful cooperation: Castle Medical Center, Kaiser Medical Center, Leahi Hospital, Queen's Medical Center, St. Francis Hospital, Straub Clinic and Hospital, Tripler Medical Center, Wahiawa General Hospital, and the Hawaii Tumor Registry.

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