

## *Null Results in Brief*

# Lack of Association between Serum Levels of 25-Hydroxyvitamin D and the Subsequent Risk of Prostate Cancer in Finnish Men

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## Introduction

Ecologic studies support an inverse association between sunlight exposure and incidence of prostate cancer (1-3). Because sun exposure is the major source of human vitamin D production, several authors have hypothesized that the link between reduced prostate cancer incidence and increased sun exposure may be increased vitamin D levels (4, 5). Laboratory studies show that both the circulating form of vitamin D, 25-hydroxyvitamin D and the hormonal form 1,25-dihydroxyvitamin D, can inhibit the proliferation and invasiveness of prostate cancer cells in culture (6, 7). Nevertheless, few large prospective epidemiology studies have supported the hypothesis that circulating levels of 25-hydroxyvitamin D are associated with the development of prostate cancer (8-14). Of the studies that have shown an inverse association, two were conducted in Nordic countries, where a significant percentage of the population is vitamin D deficient as measured by serum 25-hydroxyvitamin D levels (9, 14). In this study, we examined this relationship in a nested case-control study of Finnish male participants of a large intervention trial, the  $\alpha$ -Tocopherol,  $\beta$ -Carotene Prevention Study.

## Materials and Methods

**Study Population.** The  $\alpha$ -Tocopherol,  $\beta$ -Carotene Prevention Study enrolled 29,133 males residing in south-

western Finland in 1985 to 1988 who were 50 to 69 years old and current smokers (i.e., at least five cigarettes per day) at entry. This study was a randomized, placebo-controlled, double-blind trial that examined the effect of daily supplementation of either  $\alpha$ -tocopherol (50 mg),  $\beta$ -carotene (20 mg), both, or placebo for 5 to 8 years (mean duration, 6.1 years) on incidence of lung and other cancers. The  $\alpha$ -Tocopherol,  $\beta$ -Carotene Prevention Study was approved by the institutional review boards of the U.S. National Cancer Institute and the National Public Health Institute of Finland.

The rationale, design, and main findings of the  $\alpha$ -Tocopherol,  $\beta$ -Carotene Prevention Study have been published elsewhere (15-17). A fasting serum sample was collected at baseline, as was health, demographic, and life-style information. Postintervention, the cohort was followed for cancer incidence through the national Finnish Cancer Registry. A nested case-control sample set was constructed from the 29,133 men who provided a baseline fasting blood sample. Of those prostate cancer cases diagnosed during the intervention or follow-up period, 296 were randomly selected and, using incidence density sampling, matched 1:1 to controls on age ( $\pm 1$  year), study clinic, treatment group, and date of blood draw ( $\pm 28$  days). The medical records of each case were centrally reviewed by two study oncologists to confirm diagnosis and stage. Both histologic and cytologic gradings were determined by a central review of prostate cancer specimens. Of the cases, 30.2% were diagnosed with grade I disease, 46.8% with grade II, and 23.0% with grade III. Additionally, 26.6% of cases were diagnosed with stage I disease, 39.8% with stage II, 13.5% with stage III, and 20.1% with stage IV.

**25-Hydroxyvitamin D Serum Assay.** Serum levels of 25-hydroxyvitamin D were assessed using the OCEIA 25-hydroxyvitamin D direct ELISA kits (IDS, Inc.). Matched case and control samples were included on the same plate (placed side by side in adjacent wells) and assayed in duplicate. In addition, quality control serum aliquots ( $n = 49$ ) were obtained from a pool of sera from a sample of noncases in the  $\alpha$ -Tocopherol,  $\beta$ -Carotene Prevention cohort and placed throughout the ELISA plates. Laboratory personnel were blind to both the

Cancer Epidemiol Biomarkers Prev 2007;16(12):2784-6

Received 7/25/07; revised 8/24/07; accepted 10/9/07.

**Grant support:** National Cancer Institute Division of Cancer Prevention and Center for Cancer Research (J.M. Faupel-Badger), National Cancer Institute Center for Cancer Research Intramural Research Program, and National Cancer Institute Public Health Services contract N01-CN-45165, N01-RC-45035, and N01-RC-37004 (ATBC Study).

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**Note:** Dr. Faupel-Badger is a fellow in the National Cancer Institute Cancer Prevention Fellowship Program.

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doi:10.1158/1055-9965.EPI-07-0672

**Table 1. Odds ratios (95% confidence intervals) of prostate cancer by quartile of baseline serum 25-hydroxyvitamin D (ng/mL)**

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	<i>P</i> <sub>trend</sub>
Cases/controls	83/75	69/73	57/74	87/75	
Range, 25-hydroxyvitamin D (ng/mL)	≤14.79	14.80-18.82	18.83-23.98	>23.98	
Unadjusted odds ratio (95% confidence interval)	1.00	0.81 (0.45-1.47)	0.57 (0.30-1.06)	0.87 (0.49-1.57)	0.99
Adjusted* odds ratio (95% confidence interval)	1.00	0.88 (0.48-1.61)	0.59 (0.31-1.11)	0.89 (0.49-1.62)	0.97

\*Adjusted for age at randomization, body mass index, and pack-years of smoking.

case-control status of the samples and to the plate location of the quality control aliquots. The average intrabatch coefficient of variance for this assay was 5.3%, and the interbatch coefficient of variance was 8.4%.

**Statistical Analysis.** Baseline characteristics of cases and controls were compared by the *t* test or Wilcoxon rank sum test for continuous variables and the  $\chi^2$  test for categorical variables. Conditional logistic regression was used to estimate odds ratios and 95% confidence intervals for the association between baseline serum 25-hydroxyvitamin D levels and prostate cancer in relation to quartiles of 25-hydroxyvitamin D with the lowest quartile as the reference category. Quartile cut points were determined by examining the distribution of 25-hydroxyvitamin D levels among the controls. Confounders were included in the multivariate model if they changed the 25-hydroxyvitamin D estimate by >10%. To test effect modification on a multiplicative scale, the cross-product interaction term was included in the multivariate regression model and the variable also was examined by stratified analysis. Among the cases, we also examined the association of 25-hydroxyvitamin D levels with disease stage, grade, time from blood collection to diagnosis of prostate cancer, and disease-specific survival. A two-sided  $\alpha$  with a *P*-value cutoff of 0.05 and 95% confidence intervals were used to test statistical significance. The study had 95% power to detect a 12% difference in mean baseline serum 25-hydroxyvitamin D levels between cases and controls and 80% power to detect a 12% risk decrease across quartiles of baseline serum 25-hydroxyvitamin D serum levels. Statistical analyses were done using Statistical Analysis Systems software package PC SAS 8.2 (SAS Corp.).

## Results and Conclusions

Cases and controls did not differ by baseline 25-hydroxyvitamin D serum levels (cases 18.54 ng/mL, controls 18.73 ng/mL; *P* = 0.80). The mean serum concentration among the controls was slightly lower than that reported for a similar U.S. study population (13) and >25% of our study population would be considered vitamin D deficient even by conservative standards (<15 ng/mL). As seen in Table 1, there was no significant association of prostate cancer incidence with quartile of 25-hydroxyvitamin D. In multivariate analysis, adjustment for age, body mass index, and smoking pack-years did not change the estimate. Because others have reported a stronger association of 25-hydroxyvitamin D levels with prostate cancer among younger patients (9), we evaluated age as an effect modifier but

found no significant effect. In addition, among cases, 25-hydroxyvitamin D levels were not associated with tumor grade, stage, days from blood collection to diagnosis, or disease-specific survival (data not shown). This study population has the advantage of being nested within a large prospective cohort with complete disease ascertainment (through the study and the Finland Cancer Registry) and over 19 years of active follow-up, with an average of 9.26 person-years for individuals included here. In addition, the vitamin D status of this Finnish population is skewed to the low end with >25% deficient by conservative standards. In spite of this, we did not observe a significant association between serum 25-hydroxyvitamin D and prostate cancer risk. Our results are in contrast to other similar reports from Finland (9, 14). Ahonen et al. showed increased risk of prostate cancer for men with 25-hydroxyvitamin D levels below the median of 40 nmol/L or 16 ng/mL, with even greater risk for men <52 years (9), whereas Touhima et al. detected increased risk only for men with 25-hydroxyvitamin D <7.6 ng/mL or >32 ng/mL (14). The median of 25-hydroxyvitamin D levels in our study are similar to those of Ahonen (9), yet we did not detect an association or modification of the association with age. Our results are in agreement with most of the other large studies that have examined an association between 25-hydroxyvitamin D and prostate cancer incidence and/or tumor characteristics (8, 10-13). These findings and the inconsistency of this relationship as reported in the literature cast doubt on the hypothesis that low vitamin D status is etiologically associated with prostate cancer.

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