

1 **Serum Retinol and Prostate Cancer Risk: a Nested Case-Control Study in the Prostate,**
2 **Lung, Colorectal, and Ovarian Cancer Screening Trial**

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1 ABSTRACT

2 Vitamin A (retinol) plays a key role in the regulation of cell growth and differentiation, and has
3 been studied as a potential chemopreventive agent for prostate cancer. However, findings from
4 epidemiologic studies of the association between circulating retinol concentrations and risk of
5 prostate cancer are inconsistent. We examined whether serum concentrations of retinol were
6 associated with risk of prostate cancer in a nested case-control study using 692 prostate cancer
7 cases and 844 matched controls from the Prostate, Lung, Colorectal, and Ovarian (PLCO)
8 Cancer Screening Trial. We estimated risk of prostate cancer using multivariate, conditional
9 logistic regression to calculate odds ratios and 95% confidence intervals for overall prostate
10 cancer and aggressive disease (stage 3 or 4 or Gleason 7+; n=269). Serum retinol
11 concentrations were not associated with overall prostate cancer risk; however, the highest versus
12 lowest concentrations of serum retinol were associated with a 42% reduction in aggressive
13 prostate cancer risk ($P_{\text{trend}}=0.02$), with the strongest inverse association for high-grade disease
14 (Gleason Sum7+; OR, 0.52; 95%CI, 0.32-0.84; $P_{\text{trend}}=0.01$). Our results suggest that higher
15 circulating concentrations of retinol are associated with a decreased risk of aggressive prostate
16 cancer. Further research is needed to better understand the significance of elevations in serum
17 retinol concentrations and the possible biologic mechanisms through which retinol affects
18 prostate cancer.

19

1 INTRODUCTION

2 Vitamin A is a fat-soluble vitamin that plays an essential role in the visual cycle and is
3 required in the normal growth of bone, reproduction, embryonic development, and in
4 differentiation of epithelial tissues(1). There has also been a great deal of interest in the cancer-
5 preventive potential of vitamin A (retinol), much of which stems from in vitro and animal studies
6 demonstrating a key role for retinol in regulating the growth, differentiation and apoptosis of
7 normal and malignant cells.

8 Several observational epidemiological studies and one randomized clinical trial have
9 examined the associations of vitamin A and prostate cancer, although no consistent association
10 with prostate cancer risk has been established. Prospectively designed nested case-control
11 studies have reported no association between circulating concentrations of retinol at baseline and
12 subsequent prostate cancer risk (2-6), while others have reported an increased(7) or decreased(8,
13 9) risk associated with higher retinol concentrations(Figure 1). However, many of the previous
14 studies had small sample sizes, and few examined the associations for retinol concentration and
15 prostate cancer risk by stage or grade of disease.

16 This brief report examined whether serum retinol concentrations were associated with
17 prostate cancer incidence in a large nested case-control study within the Prostate, Lung,
18 Colorectal, and Ovarian (PLCO) Cancer Screening Trial, based on healthy men aged 55 years
19 and older who were screened for prostate cancer regularly, following a standardized protocol.

20

21 MATERIALS AND METHODS

22 **Study Setting and Population.** The study setting and population has been described

1 previously(10). In brief, this nested case-control study was limited to men randomized to the
2 screening arm of the PLCO trial, who were offered prostate cancer screening by serum prostate-
3 specific antigen (PSA) at entry and annually for 5 years and digital rectal examination (DRE) at
4 entry and annually for 3 years. Men with a positive screening result (PSA >4 ng/mL or DRE
5 suspicious for prostate cancer) were referred to their medical care providers for diagnostic
6 evaluation and follow-up for diagnosis of cancer was conducted by annually mailed
7 questionnaires. Medical and pathologic records related to prostate cancer diagnoses were
8 acquired for all men suspect for prostate cancer by screening examination or annual
9 questionnaire.

10 Men in this study had no prior history of prostate cancer and at least one blood collection and
11 one valid prostate cancer screen prior to October 1, 2001 (the censor date). All men were
12 followed from their initial prostate cancer screen (PSA and/or DRE), to occurrence of prostate
13 cancer, loss-to-follow-up, death (by National Death Index), or censor date, whichever came first.
14 Cases were men diagnosed with adenocarcinoma of the prostate. Aggressive cancers were
15 defined as clinical stages III or IV (regionally invasive or distant metastatic disease) or biopsy
16 Gleason Sum \geq 7 based on the pathologic report.

17 1,320 prostate cancers were identified, from which non-Hispanic Black cases and cases
18 diagnosed in the first year after blood draw were excluded, leaving a total of 803 cases. Controls
19 were frequency-matched by age at entry (5-year intervals), time since initial screening (1-year
20 intervals), year of blood draw, and race/ethnicity using incidence-density sampling(11) with a
21 case-control ratio of 1:1.2. Serum collected at study entry was available for 692 (86.2%) cases
22 and 844 (88.9%) controls.

23 **Assessment of Questionnaire-Based Covariates.** At enrollment, all participants were asked

1 to complete a questionnaire about sociodemographic factors, medical history, and risk factors for
2 cancer. Usual dietary intake over the 12 months prior to enrollment was assessed with a 137-item
3 food frequency questionnaire including 14 additional questions about intake of vitamin and
4 mineral supplements and 10 additional questions on meat cooking practices(12). Daily dietary
5 nutrient intake was calculated by multiplying the daily frequency of each consumed food item by
6 the nutrient value of the sex-specific portion size, using the nutrient database from the U.S.
7 Department of Agriculture(13). Total vitamin and mineral intakes were calculated using the sum
8 of dietary and supplement intakes.

9 **Laboratory Analysis.** Nonfasting blood specimens collected at the clinical centers were
10 processed and frozen within 2 hours of blood draw and stored at -70°C. Serum concentrations of
11 total retinol (bound and unbound) were determined using reversed-phase high-performance
12 liquid chromatography, with UV detection(14). Cases and their matched controls were analyzed
13 in the same batch. Blinded quality control samples were randomly inserted into each batch. The
14 coefficient of variation estimated from 171 blinded duplicates was 5.1%. To investigate the
15 reproducibility of serum retinol concentrations over time we also included a second serum
16 sample drawn 1 year after study entry in the same sample batch as the subject-paired serum
17 sample collected at study entry for a subset of 46 controls. Serum concentrations of selenium and
18 cholesterol were measured for another nested case-control study, and the methods have been
19 described previously(15). Serum cholesterol was measured enzymatically by a standard
20 procedure at 37°C on a Hitachi 912 analyzer.

21 **Statistical Analysis.** Conditional logistic regression was used to estimate ORs and 95% CIs
22 of prostate cancer, with serum retinol concentrations categorized as quintiles, based on the
23 distribution among controls. Tests for linear trend were based on quintile-specific median values

1 expressed as a continuous variable. Analyses were conditioned on matching factors (age, time
2 since initial screening, and year of blood draw), and adjusted for study center. In addition, we
3 evaluated potential confounders based on a priori hypotheses for prostate cancer risk factors
4 (PSA, DRE screening, family history of prostate cancer, educational attainment, physical
5 activity, body mass index (BMI), aspirin and ibuprofen use, history of diabetes, smoking, intakes
6 of alcohol, energy, fat, red meat, heterocyclic amines from meat (2-amino-1-methyl-6-
7 phenylimidazo[4,5-*b*]pyridine, PhIP), fruits, vegetables, cruciferous vegetables, vitamin E,
8 calcium, serum selenium, serum cholesterol, month of blood draw); however, none were
9 included in the analyses because the factors, either separately or together, did not change the risk
10 estimates by more than 10%. To assess effect modification we performed stratified analyses and
11 evaluated the statistical significance of multiplicative interaction terms by comparing the -2 log-
12 likelihood statistics of the main effect model and the model including the interaction term.
13 Spearman correlation coefficients were calculated to measure the correlation between serum
14 retinol concentrations obtained one year apart in 46 controls and between serum concentrations
15 and dietary vitamin A intake in all controls. Correlation coefficients were adjusted for month of
16 blood draw, serum cholesterol concentration, smoking, body mass index, age, and energy intake.
17 All P values are two-sided and significance set at the 0.05 level.

18 **RESULTS**

19 The distribution, overall and by retinol concentrations, of demographic and health-related
20 covariates possibly linked to prostate cancer risk is shown in Table 1. The average age at study
21 entry among controls was 64.7 years and did not vary by retinol concentration. Compliance with
22 the PLCO screening protocol was very high and did not differ by serum retinol concentrations, as
23 shown by the average number of cancer screens (PSA or DRE; Table 1). Men with higher serum

1 retinol concentrations were less likely to have a personal history of diabetes, and had higher
2 serum cholesterol concentrations, were less obese, more likely to smoke, more physically active
3 and more likely to use aspirin. Men with higher serum retinol concentrations also had a lower
4 intake of fat and red meat, and higher intakes of PhIP, vitamin D, supplemental vitamin E, and
5 alcohol. The average time between blood collection and prostate cancer diagnosis (among cases)
6 was 2.4 + 1.4 years. The correlation between serum retinol concentrations measured in 46 men
7 at study entry and 1 year later was modest ($r=0.38$; $p=0.009$), and the correlation between serum
8 retinol and vitamin A intake from diet and supplements among controls was weak ($r=0.08$,
9 $p=0.06$).

10 Median serum retinol concentrations did not differ significantly between cases and controls
11 (67.11 versus 68.52 ug/dl, $p=0.22$). In multivariate analyses comparing men in the highest to
12 lowest quintiles, serum retinol was associated with a non-significant 20% reduction in risk of
13 prostate cancer with no indication of a linear trend ($P_{\text{trend}} 0.11$, Table 2). When restricted to
14 aggressive prostate cancers (Gleason Sum ≥ 7 or clinical stage III or IV), high concentrations of
15 serum retinol were associated with a significant reduction in risk (OR, 0.58; 95% CI, 0.36-0.92
16 for highest versus lowest quintile; $P_{\text{trend}}=0.02$; Table 2), with the strongest association for high-
17 grade disease (Gleason Sum ≥ 7 ; OR, 0.52; 95% CI, 0.32-0.84 for highest versus lowest quintile;
18 $P_{\text{trend}}=0.01$; Table 2). For both aggressive and high-grade prostate cancers, the greatest
19 decrease in risk occurs between the first and second quintiles, with risk being similar for
20 quintiles 2 through 5.

21 Similar associations between serum retinol and overall prostate cancer and aggressive disease
22 were observed for subgroups characterized by year of diagnosis (<4 and > 4 years), age (<65 and

1 ≥ 65 years), smoking (ever versus never), family history of prostate cancer (yes versus no),
2 alcohol (0-2.9, 3-14.9 and ≥ 15 g/day), and serum vitamin D (<49.9, 50.0-68.7 and ≥ 68.8 nmol/L)
3 (data not shown). Furthermore, ORs did not vary when these analyses were limited to the years
4 of active screening (years 1-5) or when the second year of follow-up was excluded (the first year
5 of follow-up was already excluded by design; data not shown).

6 **DISCUSSION**

7 In this prospective analysis of 692 incident cases, we observed a decreased risk of
8 aggressive prostate cancer, particularly high-grade disease, with increased concentrations of
9 serum retinol. This association was not modified by age, smoking, family history, alcohol
10 intake, or vitamin D.

11 Our findings of a decreased risk of aggressive prostate cancer with increased
12 concentrations of serum retinol are supported by results from two earlier cohort studies; in the
13 National Health and Nutrition Examination Survey (NHANES) I Epidemiologic Follow-up
14 Study, high retinol concentrations were associated with a decreased risk of prostate cancer(8) and
15 in CLUE I a non-significant inverse association between serum retinol and prostate cancer was
16 reported(9), although this finding was not confirmed on further follow-up(6).

17 Analyses in many of these studies were limited to total prostate cancer, and therefore,
18 differences in associations according to stage or grade of disease may have been obscured(2, 4-6,
19 8, 9). For prostate cancer, this is particularly important since the disease is heterogeneous and
20 the significance of early-stage (PSA-detected) disease is unclear(16). Two larger prospective
21 studies (n=578 and n=966 cases) analyzed non-aggressive and aggressive cancers separately,
22 although neither found an association between retinol concentrations and risk of aggressive

1 (Stage III or IV or Gleason sum \geq 7)(7) or advanced (T3 or T4, N1+, M1 or metastatic)(3)
2 disease, and one reported an elevated risk for high levels of retinol that was limited to non-
3 aggressive(Stage I or II and Gleason sum<7) disease(7). In the current study, the association of a
4 decreased risk with increasing retinol concentrations was limited to aggressive disease and was
5 strongest for high-grade disease. This association did not differ by year of diagnosis (<4 versus
6 > 4), suggesting that a reverse-causality effect on retinol is unlikely. These findings could
7 indicate that retinol has a specific effect on disease progression(17), although it is also possible
8 that this association is due to chance as our study is the only one reporting an inverse association
9 with aggressive disease specifically.

10 Our findings are supported by several *in vitro* and animal models which demonstrate a
11 key role for retinol in regulating the growth, differentiation and apoptosis of normal and
12 malignant prostate cells(18, 19). In experimental models, retinoids suppress transforming effects
13 of carcinogens, inhibit growth of premalignant cells, enhance differentiation of malignant cells
14 and induce apoptosis(20). These regulatory effects are mediated through the activation of two
15 families of retinoid nuclear receptors, retinoic acid receptors (RARs) and retinoid X receptors
16 (RXRs). Prostate cancer tissue has lower levels of retinoic acid(21), and low retinol levels could
17 also directly alter the expression of retinoic acid and retinoid X receptors(22), which have been
18 shown to be selectively reduced in prostate cancers(23).

19 It is unlikely these findings can be solely attributed to differences in dietary or
20 supplemental retinol intake, given that serum retinol concentrations are under tight homeostatic
21 control(24), and neither dietary intake nor supplements is strongly correlated with retinol
22 concentrations(25, 26). However, it should be noted that the range of retinol concentration in
23 this study population is wide (27.4 to 262.6 ug/dl), and other factors such as BMI, physical

1 activity, and dietary fat and alcohol intake are correlated with retinol concentrations, suggesting
2 that in a well-nourished population, these factors influence retinol concentrations to some degree.

3 Lower concentrations of retinol may reflect insufficient vitamin A stores in the liver, although
4 this is unlikely given the healthy study population. Alternatively, low circulating retinol
5 concentrations could result from underlying aberrations in cellular retinol binding protein
6 (CRBP) metabolism(27). CRBP plays an essential role in the maintenance of hepatic retinol
7 stores and the synthesis of retinoic acid from retinol(24), and the reduction or loss of CRBP1
8 expression has been demonstrated in prostate carcinomas(28).

9 A limitation of this study and previous observational studies is the use of a single baseline
10 measure of serum retinol. Although one examination of the repeatability of serum retinol
11 concentrations 15 years apart showed moderate correlation ($r=0.58$; 95%CI:0.46-0.67)(29); the
12 correlation between serum retinol concentrations 1 year apart in this study was somewhat lower
13 ($r=0.38$; $p=0.009$) and thus, a single measure of serum retinol may not be representative of long-
14 term retinol exposure. Nonetheless, such non-differential measurement error in serum retinol
15 generally leads to an attenuation of the estimated association, and the true protective effect
16 should be stronger than that found in our study. A further limitation is the relatively short follow-
17 up of 8 years.

18 The strengths of this study include standardized procedures for prostate cancer screening
19 and very high compliance with the screening protocol, availability of pre-diagnostic serum
20 samples, a large sample size, and detailed diagnostic data which allowed stratification by stage
21 and grade and simultaneous adjustment for multiple confounders.

22 In summary, our results support a protective association between serum retinol
23 concentrations and risk of aggressive prostate cancer. Further research to better understand the

- 1 significance of elevations in serum retinol concentrations and possible effects of aberrations in
- 2 CRBP metabolism on prostate cancer risk would be useful.

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Summary of Prospective Studies on Serum or Plasma Retinol and Prostate Cancer Risk

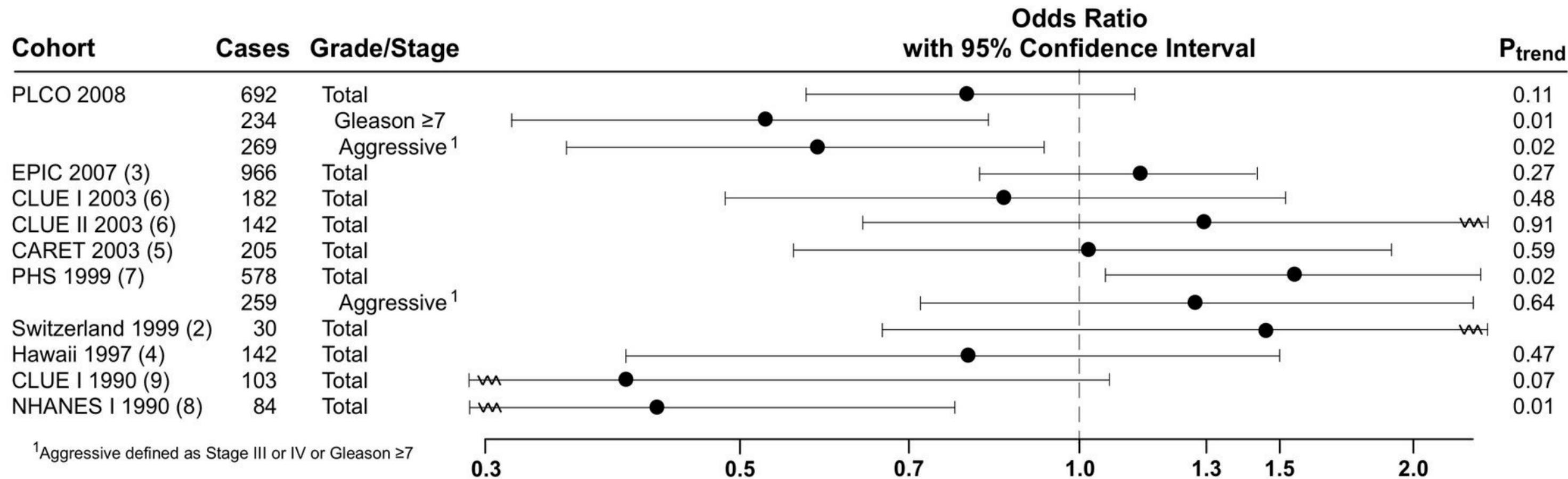


Table 1. Description of Baseline Characteristics Overall and According to Quintiles of Serum Retinol ¹

Characteristic	Quintile of Serum Retinol					Overall
	1	2	3	4	5	
Controls, n	168	169	168	169	168	842
Quintile range (ug/dl)	27.4-54.7	54.8-64.3	64.4-72.8	72.9-85.3	85.4-262.6	27.4-262.6
Mean age at study entry, years (SD)	65.1 (0.4)	64.6 (0.4)	64.5 (0.4)	64.6 (0.4)	64.8 (0.4)	64.7 (0.2)
Average no. of screens /yr ²	0.96	0.96	0.97	0.95	0.97	0.96
Family history of prostate cancer, %	5.6	3.4	5.7	4.5	6.3	5.3
History of diabetes, %	11.2	10.6	6.9	8.2	5.8	8.3
Cholesterol, mmol/l (SD)	5.4 (0.1)	5.7 (0.1)	5.7 (0.1)	6.2 (0.1)	7.8 (0.1)	6.1 (0.1)
Mean current BMI, kg/m ² (SD)	27.8 (0.3)	27.9 (0.3)	27.3 (0.3)	26.9 (0.3)	26.9 (0.3)	27.3 (0.1)
Smoking history, %						
Never	34.3	25.0	27.1	27.8	30.7	30.0
Current	8.8	9.3	9.7	8.9	9.4	9.1
Former	46.7	52.8	46.1	56.9	51.6	51.5
Pipe/Cigar	8.7	11.6	15.9	4.9	6.8	9.4
Mean physical activity, hours/week (SD)	2.7 (0.1)	2.8 (0.1)	2.8 (0.1)	3.2 (0.1)	3.1 (0.1)	2.9 (0.1)
Aspirin use, ≥ 1 times/week %	42.6	46.4	44.9	52.0	53.7	47.6
Mean intake (SD)						
Energy, kcal/day	2361 (73)	2333 (72)	2439 (73)	2284 (73)	2350 (72)	2347 (32)
Total fat, g/day	82.0 (1.3)	81.7 (1.3)	79.4 (1.4)	77.2 (1.3)	78.0 (1.3)	79.2 (1.4)
Fruit, servings/2000 kcal/day	3.2 (0.2)	3.4 (0.2)	3.5 (0.2)	3.4 (0.2)	3.5 (0.2)	3.5 (0.1)
Vegetables, servings/2000 kcal/day	5.5 (0.2)	5.2 (0.2)	5.5 (0.2)	5.5 (0.2)	5.4 (0.2)	5.5 (0.1)
Red meat, g/day	114.8 (4.5)	100.5 (4.5)	101.4 (4.5)	95.7 (4.5)	96.0 (4.5)	97.7 (2.6)
PHIP, ng/day	236 (43)	228 (43)	226 (43)	243 (43)	284 (43)	220 (19)
Calcium, mg/day	1125 (35)	1160 (35)	1132 (35)	1215 (35)	1184 (35)	1166 (21)
Vitamin D, IU/day	350 (25)	428 (25)	406 (25)	448 (25)	463 (25)	420 (12)
Supplement vitamin E use, % ever ³	36.5	46.1	49.8	56.7	58.9	48.6
Alcohol %						
≥ 0 and < 3 g/d	50.2	50.0	46.2	38.3	39.6	45.7

>=3 and <15g/d	22.1	28.2	23.2	19.6	18.0	21.5
>=15g/d	27.7	21.6	31.0	41.9	42.5	32.8

¹ All values other than age were directly standardized for age. Intakes of total fat, fruit, vegetables, red meat, PhIP, calcium, vitamin D, and supplement vitamin E were also standardized for energy intake.

² Average number of prostate cancer screening examinations (PSA or DRE) up to diagnosis of prostate cancer (cases) or selection as a control. Maximum period is limited to period of active screening (years 0-5).

³ Includes both single supplement and multivitamin use

Table 2. Odds Ratio (OR) of Prostate Cancer According to Quintile of Serum Retinol and Stage of Disease Progression at Diagnosis

	Serum Retinol										<i>p</i> -trend
	Cases	<u>Q1</u> ¹ OR (95% CI) ⁴	Cases	<u>Q2</u> OR (95% CI) ⁴	Cases	<u>Q3</u> OR (95% CI) ⁴	Cases	<u>Q4</u> OR (95% CI) ⁴	Cases	<u>Q5</u> OR (95% CI) ⁵	
All Cases ²	155	1.00	154	0.97(0.71,1.34)	124	0.77 (0.55,1.07)	130	0.79 (0.57,1.10)	129	0.80 (0.57,1.11)	0.11
Non-Aggressive ³	94	1.00	96	1.22 (0.84,1.79)	71	0.86 (0.58,1.29)	79	0.95 (0.64,1.41)	83	0.97 (0.66,1.43)	0.53
Aggressive	68	1.00	57	0.69 (0.44,1.07)	52	0.64 (0.40,1.00)	49	0.57 (0.36,0.91)	43	0.58 (0.36,0.92)	0.02
Stage III & IV	18	1.00	20	0.87 (0.42,1.79)	19	0.83 (0.40,1.73)	19	0.74 (0.35,1.56)	14	0.69 (0.32,1.51)	0.33
Gleason Sum \geq 7	66	1.00	48	0.58 (0.37,0.92)	42	0.50 (0.31,0.82)	39	0.45 (0.27,0.73)	39	0.52 (0.32,0.84)	0.01

CI confidence interval

¹Reference category

²Numbers of Non-Aggressive and Aggressive do not add up to All Cases due to missing information on stage or grade

³Aggressive defined as Gleason Sum \geq 7 or stage III or IV

⁴Adjusted for age, time since initial screening, year of blood draw, and study center