A Prospective Study of Calcium Intake and Incident and Fatal Prostate Cancer

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Abstract

Prostate cancer is the most common incident cancer and the second leading cause of cancer mortality in U.S. males. Higher milk intake has been relatively consistently associated with an increased risk of prostate cancer, especially advanced prostate cancer. Some data suggest that high intake of calcium might account for this association, but this relationship remains controversial. We hypothesized that high calcium intake, possibly by lowering $1,25(OH)_2$ vitamin D levels, is associated with poorer differentiation in prostate cancer and thereby with fatal prostate cancer. We examined calcium intake in relation to prostate cancer risk using data from the Health Professionals Follow-up Study, a prospective cohort study of 47,750 male health professionals with no history of cancer other than nonmelanoma skin cancer at baseline. We assessed total, dietary, and supplementary calcium intake in 1986, 1990, 1994, and 1998, using a validated food frequency questionnaire. We calculated the multivariable relative risk (RR) and 95% confidence intervals (95% CI) using Cox proportional hazards regression. Over 16 years of follow-up, we identified 3,544 total cases of prostate cancer, 523 advanced (extraprostatic) cases, and 312 fatal cases. Higher calcium intake was not appreciably associated with total or nonadvanced prostate cancer but was associated with a higher risk of advanced and fatal prostate cancer [for fatal prostate cancer, compared with men whose long-term calcium intake was 500-749 mg/d (excluding supplement use of <5 years); those with intakes of 1,500-1,999 mg/d had a RR, 1.87; 95% CI, 1.17-3.01; and those with ≥2,000 mg/d had a RR, 2.43; 95% CI, 1.32-4.48; P_{trend} = 0.003]. Dietary calcium and supplementary calcium were independently associated with an increased risk. For high-grade prostate cancer (Gleason ≥ 7), an association was observed for high versus low calcium intake (RR, 1.89; 95% CI, 1.32-2.71; P_{trend} = 0.005), but a nonsignificant, inverse association was observed for organ-confined, low-grade prostate cancer (RR, 0.79; 95% CI, 0.50-1.25; P_{trend} = 0.09). In a sample of this cohort, higher calcium intake was associated with lower circulating $1,25(OH)_2$ vitamin D levels. Our findings suggest that calcium intakes exceeding 1,500 mg/d may be associated with a decrease in differentiation in prostate cancer and ultimately with a higher risk of advanced and fatal prostate cancer but not with well-differentiated, organ-confined cancers. (Cancer Epidemiol Biomarkers Prev 2006;15(2):203–10)

Introduction

In many case-control studies, men consuming relatively high levels of milk and other dairy products are at an either statistically significant increased risk (1-7) or suggestively increased risk of prostate cancer (8-11) compared with low or nonconsumers, although several studies have not supported an association (12, 13). A recent meta-analysis of 11 case-control studies that met the specified inclusion criteria found a combined odds ratio (OR) of 1.68 [95% confidence interval (95% CI), 1.34-2.12] for prostate cancer comparing the highest with the lowest quantile of consumption of milk (14). Dividing the studies by number of cases, control source (hospital-based or population-based), milk type, and rates of prostate cancer incidence in the country yielded similar results. Some prospective cohort studies (15-20), but not all (21-25), also support an association between higher intake of milk or dairy products and risk of prostate cancer. In addition, countries with greater per capita consumption of milk have higher prostate cancer mortality rates (26-28).

Calcium intake has been hypothesized to be one of the factors in milk underlying this increase in risk, but the association between calcium intake and prostate cancer risk has been less studied. Several case-control (7, 29) and prospective cohort (18, 20, 25, 30) studies have found positive associations between calcium intake and prostate cancer risk, although other studies do not support (19, 24, 31, 32) or are only weakly supportive of this association (10, 11). In contrast, results from a secondary analysis of a randomized clinical trial of calcium and colorectal adenoma recurrence showed no association and, if anything, a suggestive lower risk of prostate cancer in men randomized to 1,200 mg/d of calcium (33). However, most of the cases diagnosed over the time period of this trial were nonadvanced lesions detected through prostate-specific antigen (PSA) screening, whereas in the observational studies noted above, calcium intake seemed to be associated preferentially with clinically detected or clinically advanced cancers.

In the Health Professionals Follow-up Study, based on 1,369 incident cases of prostate cancer and 201 cases of metastatic prostate cancer in 8 years of follow-up (30), we previously found that high calcium intake was associated with an increased risk of metastatic prostate cancer. With 8 more years of follow-up encompassing 3,544 total incident cases, we examined whether higher calcium intake is associated with poor differentiation, a strong determinant of progression for prostate cancer. With the additional cases, we had greater power to distinguish whether calcium had an independent role from dairy products, to better characterize the dose-response relation, and to examine fatal prostate cancer. In addition, we were able to examine the relationship between calcium intake

Received 8/1/05; revised 11/8/05; accepted 12/5/05.

Grant support: National Cancer Institute/NIH grant CA 55075; National Heart, Lung, and Blood Institute (Harvard) grant HL35464; and National Cancer Institute (Hopkins) grant CA58236.

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doi:10.1158/1055-9965.EPI-05-0586

and prostate cancer in cases diagnosed in the PSA era. Furthermore, in a sample of men, we examined whether calcium intake was associated with lower concentrations of circulating $1,25(OH)_2$ vitamin D, the vitamin D metabolite known to induce differentiation (34).

Materials and Methods

Subjects and Procedures

Study Population. In 1986, 51,529 U.S. male health professionals completed a mailed questionnaire to initiate the Health Professionals Follow-up Study cohort. The men in this cohort are predominantly Caucasian (>91%) and were 40 to 75 years old at baseline. Through the 1986 baseline questionnaire, we collected information on age, marital status, height and weight, ancestry, medications, smoking history, disease history, physical activity, and diet. Every 2 years, we collected information on new medical diagnoses and updated smoking history, body weight, and physical activities. Every 4 years, we updated dietary information. We identified >98% of the deaths in this cohort through information from family members, the postal system, and the National Death Index (35). The conduct of this cohort study and these analyses was approved by the Human Subjects Committee of the Harvard School of Public Health.

Identification of Cases of Prostate Cancer. On the follow-up questionnaires (response rate 96%), the study participant was asked to report new diagnosis of prostate cancer. For new reports, we then recontacted the participant (or next of kin for decedents) and asked for permission to obtain relevant hospital records and pathology reports. For staging, we used information received from any procedures or tests conducted during the initial diagnosis and treatment, including prostatectomies and bone scans. From 1986 to the end of this study period (January 31, 2002), in 673,706 person years, we documented 3,544 newly incident prostate adenocarcinoma cases after excluding 71 cases (about 2% of total) of stage T_{1a} cancers (incidental histologic cancer found in ≤5% of tissue resected). We typically exclude T_{1a} cancers because these are relatively innocuous and are especially prone to detection bias. We were able to document 90% of the 3,544 cases through medical records and pathology reports. The remaining 10% of participants typically provided supporting data (e.g., evidence of treatment) for the diagnosis. Based on the pathology report, we had information on Gleason histologic grade for 2,702 cases (76.2% of the total). Fatal prostate cancer was based on a review of the medical records by a physician. We reviewed medical records blinded to any exposure information.

Food Frequency Questionnaire. We used a semiquantitative food frequency questionnaire to assess diet (previously described in detail; ref. 36). The diet questionnaire, first given in 1986, contained a list of 131 food and beverage items and included an open-ended section. We specified a commonly used unit or portion size for each item on the list and asked the participant how often, on average, over the past year, he consumed the specified amount of each item, choosing from among nine possible responses for frequencies. We also assessed current use, brand, dose, and duration of use of supplements, including calcium supplements. Information based on specific brand and type of multivitamin is taken into account. In 1990, 1994, and 1998, we administered the dietary questionnaire again (with slight modifications). We computed nutrient intakes by multiplying the consumption frequency of each unit of food by the nutrient content of the specified portions, using composition values from U.S. Department of Agriculture sources supplemented with other data.

We evaluated the validity of nutrient and food consumption measured by the questionnaire among a sample of 127 cohort members from the Boston area (36, 37). The mean intake (and SD) for total energy-adjusted calcium was 815 ± 280 mg/d based on diet records and 839 ± 346 mg/d based on the questionnaire, and their correlation was 0.61.

Assessment of PSA and Other Exposure Information. On the 1986 baseline questionnaire, the men reported their current height and weight and weight at age 21. Current weight was also assessed on each biennial questionnaire. We used body mass index (kg/m^2) to estimate total adiposity (38). To assess physical activity, we used MET-hours/wk calculated from a list of activities reported in 1986 and updated every 2 years thereafter (39). One MET-hour is the metabolic equivalent of sitting at rest for 1 hour. Detailed lifetime tobacco use was assessed at baseline and current smoking status biennially. We began asking about screening for prostate cancer by PSA beginning in 1994. In 1994 and every 2 years thereafter, we assessed whether the man had had a PSA test in the prior 2-year period. In 1990, we asked the men whether their father or any brothers had had a diagnosis of prostate cancer.

Vitamin D Assays. Plasma concentrations of $1,25(OH)_2$ vitamin D and 25(OH) vitamin D were determined by RIA in the laboratory of Dr. Bruce Hollis as previously described (40, 41). The samples, all drawn in 1993 to 1994 from the cohort, came from men without a diagnosis of cancer (42, 43). Here, we examined level of $1,25(OH)_2$ vitamin D and ratio of $1,25(OH)_2$ vitamin D to 25(OH) vitamin D by calcium intake. The mean intrapair coefficients of variation calculated from blinded quality control samples ranged from 5% to 7%.

Statistical Analysis. At baseline, we excluded men with diagnosed cancer (except for nonmelanoma skin cancer) and men who did not adequately complete a food frequency questionnaire (3% of the total). After these exclusions, each of the remaining 47,750 men accrued follow-up time beginning on the month of return of the baseline questionnaire and ending on the month of diagnosis of prostate cancer, month of death from other causes, or January 31, 2002, whichever came first. We calculated rates of total, advanced, nonadvanced, and fatal prostate cancer for men in a specific category of calcium intake by dividing the number of incident cases by the number of person-years. Advanced cases were considered those that had invaded other organs, including the seminal vesicle or lymph nodes, as well as distant organs at the time of diagnosis, or that were fatal by January 31, 2002. The remaining cases, including those with minimal extension into the prostatic capsule, were considered as nonadvanced.

For calcium intake, we used six categories to maximize contrast (<500, 500-749, 750-999, 1,000-1,499, 1,500-1,999, ≥2,000 mg/d). To assess long-term calcium intake, we used cumulative updating, which uses the 1986 diet assessment to assess risk from 1986 to 1990; the average of 1986 and 1990 assessments to assess risk from 1990 to 1994; the average of 1986, 1990, and 1994 intakes to assess risk from 1994 to 1998; and the average of 1986, 1990, 1994, and 1998 intakes to assess risk from 1998 to 2002. To better assess long-term calcium intake, we conducted alternative analyses excluding men who had used calcium supplements for <5 years at baseline. With fatal prostate cancer as the end point, we updated data up until the time of the diagnosis. We used the second lowest category of calcium intake (500-749 mg/d) as the reference group to increase statistical stability.

We computed relative risks (RR) defined as the incidence rate of disease in one category (e.g., high category of calcium intake) divided by the incidence rate in the specified category. We used Cox proportional hazards modeling to control for multiple time-varying variables simultaneously and to compute 95% CI. Age was controlled for in 1-year period and time period in 2-year intervals. The following covariables were included in the models: body mass index at age 21 years, height, cigarette pack-years in the previous 10 years, vigorous

physical activity level, family history of prostate cancer, history of diabetes mellitus, race, and intakes of total calories, red meat, fish, α-linolenic acid, and tomato sauce. We tested for trend across categories controlling for multiple covariates by modeling the median values of calcium as a continuous variable in the multivariate model. We also modeled calcium intake simultaneously with total dairy product intake or lactose intake to assess whether the association was independent and assessed calcium from food and from supplements separately in the same model. Using a case-case approach, we also assessed the OR of being diagnosed with a high-grade (Gleason ≥ 7) versus a low-grade (Gleason < 7) using multivariable logistic regression to control for potentially confounding variables. We used least square means from a regression model to measure plasma $1,25(OH)_2D$ and the $1,25(OH)_2D/25(OH)D$ ratio according to calcium intake among the subset of men with these data. We controlled for age, season of blood draw, and batch assay. All reported *P*s are two sided.

Results

Age-Standardized Distribution of Covariates by Calcium Intake at Baseline. Intake of dietary calcium intake did not vary appreciably with intake of supplementary calcium (Table 1). In general, relative to men with low total calcium intakes, those with high calcium intakes were less likely to smoke, had a similar body mass index, and were more physically active. In addition, their diets tended to be lower in total fat, red meat, α-linolenic acid, and tomato sauce, and similar in fish intake. Men with higher supplementary calcium intake were more likely to take multivitamins and had higher intakes of vitamin D, zinc, and vitamin E. Dietary calcium intake was not appreciably related to supplement use, but men with high dietary calcium intake had higher vitamin D intakes because milk is a common source of both nutrients. Frequency of undergoing PSA tests did not vary appreciably by calcium intake but was slightly higher in men with higher calcium intakes.

Total Calcium Intake and Risk of Total, Nonadvanced, Advanced, and Fatal Prostate Cancer. As shown in Table 2, for total prostate cancer, no significant trend was noted for calcium intake although a statistically significant higher risk was observed for men in the highest intake category (≥2,000 mg/d) relative to those whose intake was 500 to 749 mg/d. No association was observed for nonadvanced prostate cancer, and results did not change appreciably when we excluded short-term calcium supplement users. For advanced and fatal prostate cancer, an increased risk was observed at 1,500 to 1,999 mg/d and for ≥2,000 mg/d, and the associations became stronger when men who had used calcium supplements for <5 years at baseline were excluded from the analyses. The age-adjusted results were quite similar to the multivariable results (e.g., for advanced and fatal prostate cancer, the age-adjusted RRs for ≥2,000 versus 500-749 g/d were 2.01 and 2.01, in contrast to multivariable RRs of 2.02 and 2.02, respectively); thus, only multivariable RRs are shown here. Although the number of advanced and fatal prostate cancers diagnosed in the PSA era was relatively low, the associations with calcium were compatible with the overall results.

We did not include some nutrients that may influence prostate cancer into the multivariable model because they were highly correlated with calcium intake (see Table 1). These included vitamin E, zinc, and vitamin D. Adding vitamin E, zinc, or vitamin D to the multivariable model did not change the results for calcium appreciably.

We had previously reported on cases diagnosed up to January 31, 1994. From cases diagnosed from February 1, 1994 to January 31, 2002, we had relatively few advanced (n = 179) or fatal cases (n = 80). Although the confidence intervals were wide and included one, the results for high (≥2,000 mg/d) versus low calcium (500-749 mg/d) were compatible with the previous findings (advanced prostate cancer: RR, 1.66; 95% CI, 0.65-4.22; fatal prostate cancer: RR, 2.12; 95% CI, 0.62-7.22).

Calcium Intake and Histologic Grade of Prostate Cancer. For high-grade prostate cancer (Gleason score ≥ 7), an association was observed for high versus low calcium intake (multivariable RR, 1.89; 95% CI, 1.32-2.71 for ≥2,000 versus 500-749 mg/d; P_{trend} = 0.005). For low-grade cancer, a nonsignificant inverse association was suggested (multivariable RR, 0.73; 95% CI, 0.46-1.14 for ≥2,000 versus 500-749 mg/d; P_{trend} = 0.30); a similar association was observed for low-grade and organ-confined prostate cancer (multivariable

Table 1. Mean or prevalence of age-standardized characteristics in 1986 by calcium intake (mg/d) of Health Professionals Follow-up Study

	Dietary calcium		Supplemental calcium		Total calcium intake	
	<585 (Q1)	≥933 (Q4)	0	>400	<750	≥1,500
Total calcium	609	1,289	852	1,623	577	1,878
Dietary calcium	504	1146	780	796	592	992
Supplemental calcium	35	40	0	859	1	278
Age (not standardized)	53.7	54.2	53.7	57.2	53.4	55.8
Body mass index (kg/m^2)	24.9	24.9	25.0	24.6	25.0	24.9
Leisure time activity*	18.7	21.5	20.7	26.1	19.4	23.1
Vigorous activity*	10.8	13.3	12.5	17.4	11.3	15.4
Multivitamin use (%)	38.2	44.0	39.1	82.9	32.1	66.6
Current smoker (%)	12.3	8.5	9.7	8.3	11.1	7.6
Total fat (g/d)	73.1	69.4	71.4	68.6	73.0	67.9
Saturated fat (g/d)	23.9	25.4	24.5	22.9	24.3	24.5
α-linolenic acid (g/d)	1.09	1.03	1.08	1.05	1.09	1.02
Zinc (mg/d)	19.9	22.0	19.0	58.8	16.3	45.9
Vitamin E (IU/d)	94	100	84	400	67	258
Vitamin D (IU/d)	263	495	342	734	245	713
Red meat (servings/d)	0.74	0.52	0.61	0.52	0.69	0.43
Fish (servings/d)	0.39	0.36	0.38	0.46	0.39	0.38
Tomato sauce (servings/wk)	1.0	0.88	0.96	0.99	0.99	0.84
Dairy foods (servings/d)	1.2	3.1	1.93	1.95	0.94	3.3
Lactose (g/d)	7.9	30.9	15.1	14.0	5.4	30.3
PSA test ≥1 (%) †	93.2	93.6	93.6	94.4	93.2	93.5
PSA test ≥3 (%) †	72.0	73.8	73.4	76.0	72.3	76.4

*MET-hours of vigorous leisure time physical activity.
†Men who had at least one PSA test between 1994 and 2000 and men who had at least three PSA tests between 1994 and 2000.

Table 2. RR and 95% CI of prostate cancer in relation to total calcium intake in the Health Professionals Follow-up Study (1986-2002)

	Total calcium (mg/d)						P_{trend}
	<500	500-749	750-999	1,000-1,499	1,500-1,999	≥2,000	
Total prostate cancer							
All cases							
Cases	183	1072	1099	898	207	85	
RR (95% CI)	0.98 (0.84-1.15)	1	1.07 (0.98-1.16)	1.03 (0.94-1.12)	1.06 (0.91-1.23)	1.28 (1.02-1.60)	0.10
Excluding men using calcium supplements <5 y							
Cases	182	1040	1016	743	155	57	
RR (95% CI)	0.98 (0.84-1.15)	1	1.09 (1.00-1.18)	1.03 (0.94-1.14)	1.10 (0.93-1.31)	1.28 (0.97-1.67)	0.09
Limited to men with a PSA test (1992-2002)							
Cases	114	745	820	673	144	41	
RR (95% CI)	1.05 (0.86-1.28)	1	1.08 (0.98-1.20)	1.05 (0.95-1.17)	1.06 (0.88-1.27)	1.01 (0.73-1.38)	0.65
Nonadvanced prostate cancer							
All cases							
Cases	152	915	952	769	170	63	
RR (95% CI)	0.98 (0.83-1.17)	1	1.07 (0.98-1.17)	1.02 (0.92-1.12)	1.01 (0.86-1.20)	1.13 (0.88-1.47)	0.55
Excluding men using calcium supplements < 5 y							
Cases	151	888	879	632	125	38	
RR (95% CI)	0.98 (0.82-1.17)	1	1.09 (0.99-1.20)	1.02 (0.92-1.13)	1.03 (0.85-1.24)	1.02 (0.73-1.4)	0.80
Limited to men with a PSA test (1992-2002)							
Cases	109	674	741	611	131	32	
RR (95% CI)	1.11 (0.91-1.37)	1	1.08 (0.97-1.20)	1.06 (0.95-1.18)	1.07 (0.88-1.29)	0.87 (0.61-1.25)	0.95
Advanced prostate cancer							
All cases							
Cases	31	157	147	129	37	22	
RR (95% CI)	0.99 (0.67-1.46)	1	1.02 (0.81-1.28)	1.05 (0.83-1.33)	1.36 (0.94-1.95)	2.02 (1.28-3.19)	0.005
Excluding men using calcium supplements <5 y							
Cases	31	152	137	111	30	19	
RR (95% CI)	1.01 (0.68-1.49)	1	1.06 (0.84-1.33)	1.12 (0.87-1.43)	1.60 (1.07-2.38)	2.60 (1.60-4.23)	0.0003
Limited to men with a PSA test (1992-2002)							
Cases	5	71	79	62	13	9	
RR (95% CI)	0.48 (0.19-1.19)	1	1.09 (0.79-1.51)	1.03 (0.72-1.45)	1.03 (0.57-1.88)	2.21 (1.09-4.50)	0.09
Fatal prostate cancer							
All cases							
Cases	21	94	81	76	26	14	
RR (95% CI)	1.05 (0.65-1.69)	1	0.95 (0.70-1.28)	1.04 (0.77-1.42)	1.56 (1.00-2.43)	2.02 (1.14-3.58)	0.01
Excluding men using calcium supplements <5 y							
Cases	21	94	76	63	22	12	
RR (95% CI)	1.03 (0.64-1.66)	1	0.97 (0.71-1.31)	1.04 (0.75-1.44)	1.87 (1.17-3.01)	2.43 (1.32-4.48)	0.003
Limited to men with a PSA test (1992-2002)							
Cases	2	31	34	25	5	5	
RR (95% CI)	0.45 (0.11-1.90)	1	1.05 (0.79-1.51)	0.89 (0.64-1.72)	0.82 (0.52-1.53)	2.55 (0.96-6.76)	0.34

NOTE: Multivariable RR controlled for age, time period, body mass index at age 21, vigorous physical activity, height, cigarette pack-years in the previous 10 years, family history of prostate cancer, history of diabetes mellitus, race, and intake of total calories, red meat, fish, α-linolenic acid, zinc supplements, and tomato sauce.

RR, 0.79; 95% CI, 0.50-1.25 for ≥2,000 versus 500-749 mg/d; P_{trend} = 0.09). In an analysis limited only to those who had a prostate cancer, the multivariable OR of a man being diagnosed with a high-grade cancer relative to low-grade cancer was 2.73 (95% CI, 1.52-4.91) for ≥2,000 versus 500 to 749 mg/d. This association was even stronger when we excluded men who did not use calcium for at least 5 years (OR, 3.27; 95% CI, 1.52-7.03). For cases that were relatively rapidly fatal, we frequently had missing information for Gleason grade. If we assumed most of these were poorly differentiated and included them in the analysis with poorly differentiated prostate cancer, the results became even stronger (OR, 4.0; 95% CI, 1.93-8.44). Furthermore, when restricted to men with organ-confined prostate cancer (n = 860), this association with high grade versus low grade persisted (OR, 2.47; 95% CI, 1.04-5.90).

Calcium, Milk, and Dairy Products. An important question is whether the association with advanced prostate cancer is due to calcium per se, or whether calcium is acting as a surrogate of dairy products. We addressed this issue in several ways. First, we examined separately calcium intake from dietary and from supplementary sources together in a multivariable model, and each was independently associated with an elevated risk of advanced prostate cancer and of fatal prostate cancer (Table 3). Second, we considered simultaneously in multivariable analyses, total calcium intake and total dairy product intake, as well as simultaneously intakes of total calcium and lactose, as a surrogate of the nonfat component of dairy products (Table 4). For advanced prostate cancer, when total calcium intake and total dairy products were modeled simultaneously, a positive trend remained for calcium, but dairy products were not significantly associated with increased risk (Table 4). Similarly, when calcium intake was modeled simultaneously with lactose intake, a trend was persistent for calcium, but not for lactose intake (see Table 4), although a suggestive increased risk in the top quintile of lactose intake was observed. Similar findings were observed for fatal prostate cancer (data not shown).

Finally, we examined phosphorus, a compound highly concentrated in dairy products and correlated with total calcium (r = 0.65) and dietary calcium (r = 0.77). When controlling for phosphorus, the multivariable RR for >2,000 versus 500 to 749 mg/d of calcium was 1.56 (95% CI, 0.94-2.58) for advanced prostate cancer, 1.74 (95% CI, 0.92-3.30) for fatal prostate cancer, and 1.52 (5% CI, 1.03-2.23) for high-grade prostate cancer. After excluding short-term (<5 years) calcium supplement users, these RRs were 2.12 (95% CI, 1.23-3.64), 2.23 (95% CI, 1.12-4.42), and 1.68 (95% CI, 1.07-2.63), respectively. When adjusting for calcium intake, the phosphorus was not associated with an increased risk of advanced or fatal cancer,

Table 3. Calcium intake from dietary and supplemental sources in relation to risk of advanced and fatal prostate cancer in the Health Professionals Follow-up Study (1986-2002)

	Dietary calcium intake, quartiles (mg/d)				P_{trend}
	<585	585-731	732-932	≥933	
Advanced prostate cancer					
Cases	98	109	124	149	
RR* (95% CI)	1	1.11 (0.84-1.46)	1.22 (0.93-1.60)	1.46 (1.12-1.90)	0.003
Fatal prostate cancer					
Cases	59	68	77	84	
RR* (95% CI)	1	1.16 (0.82-1.66)	1.26 (0.89-1.78)	1.36 (0.97-1.92)	0.08
	Supplemental calcium intake (mg/d)				P_{trend}
	0	1-400	≥401		
Advanced prostate cancer					
Cases	408	15	57		
RR* (95% CI)	1	1.08 (0.64-1.81)	1.22 (0.93-1.62)		0.17
Fatal prostate cancer					
Cases	237	9	42		
RR* (95% CI)	1	1.05 (0.54-2.04)	1.51 (1.09-2.10)		0.05

*Multivariable RR controlled for age, time period, body mass index at age 21, vigorous physical activity, height, cigarette pack-years in the previous 10 years, family history of prostate cancer, history of diabetes mellitus, race, and intake of total calories, red meat, fish, α-linolenic acid, zinc supplements, and tomato sauce; excluding men using calcium supplements <5 years at baseline.

but a positive association was observed for high-grade prostate cancer (multivariable RR, 1.35; 95% CI, 1.07-1.71 for high versus low quintiles).

Influence of Calcium on Plasma Vitamin D Concentrations. Finally, we assayed $1,25(OH)_2$ vitamin D and 25(OH) vitamin D in a subset of men, free from cancer when they provided a blood sample in 1993 to 1994 and compared the adjusted means across levels of calcium intake. Across increasing intakes of total calcium, the circulating $1,25(OH)_2$ vitamin D concentration and the $1,25(OH)_2$ vitamin D/25(OH) vitamin D ratio decreased (Table 5).

Discussion

In this cohort, we previously reported that higher intake of calcium was associated with an increased risk of

Table 4. RR and 95% CI of prostate cancer in relation to dairy food and lactose intake in the Health Professionals Follow-up Study (1986-2002)

	Dairy food intake, quintiles (median servings/d)					P_{trend}
	0.50	1.05	1.57	2.28	3.72	
Total prostate cancer						
Cases	648	713	696	771	716	
Multivariable model						
RR (95% CI)	1.00	1.04 (0.93-1.15)	1.02 (0.91-1.14)	1.11 (0.99-1.24)	1.07 (0.95-1.20)	0.20
Multivariable model + calcium						
RR (95% CI)	1.00	1.03 (0.92-1.15)	1.00 (0.89-1.13)	1.09 (0.96-1.23)	1.05 (0.91-1.21)	0.44
Advanced prostate cancer						
Cases	81	116	90	111	125	
Multivariable model						
RR (95% CI)	1.00	1.25 (0.94-1.67)	0.95 (0.70-1.29)	1.08 (0.80-1.46)	1.21 (0.89-1.64)	0.39
Multivariable model + calcium						
RR (95% CI)	1.00	1.25 (0.93-1.69)	0.93 (0.67-1.29)	1.04 (0.74-1.45)	1.08 (0.75-1.55)	0.99
	Lactose intake, quintiles (median g/d)					P_{trend}
	3.8	7.7	12.0	17.5	31.1	
Total prostate cancer						
Cases	608	656	720	766	794	
Multivariable model						
RR (95% CI)	1.00	1.02 (0.91-1.14)	1.07 (0.96-1.19)	1.11 (0.99-1.23)	1.13 (1.02-1.26)	0.01
Multivariable model + calcium						
RR (95% CI)	1.00	1.02 (0.91-1.15)	1.07 (0.95-1.21)	1.12 (0.98-1.27)	1.18 (1.02-1.35)	0.02
Advanced prostate cancer						
Cases	82	103	94	109	135	
Multivariable model						
RR (95% CI)	1.00	1.14 (0.85-1.52)	0.99 (0.73-1.34)	1.08 (0.80-1.44)	1.35 (1.02-1.79)	0.03
Multivariable model + calcium						
RR (95% CI)	1.00	1.15 (0.84-1.56)	1.01 (0.72-1.40)	1.10 (0.78-1.55)	1.36 (0.95-1.97)	0.09

NOTE: Multivariable RR controlled for age, time period, body mass index at age 21, vigorous physical activity, height, cigarette pack-years in the previous 10 years, family history of prostate cancer, history of diabetes mellitus, race, and intake of total calories, red meat, fish, α-linolenic acid, zinc supplements, and tomato sauce. Dairy foods based on servings/day of skim/low fat milk, whole milk, cream, sour cream, sherbet ice milk, ice cream, yogurt, cottage cheese, cream cheese, other cheese.

Table 5. Mean plasma $1,25(OH)_2D$, 25(OH)D, and $1,25(OH)_2D/25(OH)D$ ratio according to calcium intake

	Total calcium (mg/d)						P_{trend}
	<500	500-749	750-999	1,000-1,499	1,500-1,999	≥2,000	
n	90	243	276	230	58	28	
Plasma $1,25(OH)_2D$	35.5	34.5	33.9	32.9	32.6	31.2	0.002
Plasma 25(OH)D	23.0	22.5	23.3	24.5	25.1	23.9	0.002
Plasma $1,25(OH)_2D/25(OH)D$	1.83	1.68	1.62	1.46	1.42	1.46	<0.0001

NOTE: Mean of plasma vitamin D is based on least square means from the regression model adjusting for age, season of blood drawn, and year of assay (1996 or 1998). Calcium intake is from 1994 food frequency questionnaire because blood samples were drawn in 1993-1994.

advanced-stage prostate cancer (30). With further follow-up, we were able to extend these findings to fatal prostate cancer and to poorly differentiated (higher grade) cancers. For organ-confined and moderately or well-differentiated cancers, we observed no increased risk. Results for advanced and fatal prostate cancer were substantially stronger when we excluded men who used calcium supplements for <5 years. Our results are compatible with the pattern that high calcium intake leads to more poorly differentiated prostate cancers, that over time, are more likely to progress and lead to fatality.

We considered several potential noncausal interpretations of our findings. Chance is an unlikely explanation because the results persisted over time and were consistent for clinically advanced, fatal, and for poorly differentiated prostate cancers even when limited to organ-confined cancers. The potential for bias was minimized because dietary information was ascertained prospectively, and follow-up rates were high. Less frequent screening for prostate cancer could have led to a more advanced stage of diagnosis and poorer prognosis, but men with higher calcium intake had actually had PSA tests slightly more frequently than those with lower intakes.

Although we controlled for known or suspected risk factors for prostate cancer, the possibility that higher calcium intake was a marker of another behavior (confounder) that directly increased risk cannot be definitively excluded. Men who consumed more calcium on average were less likely to smoke, exercised more, consumed less total fat and red meat, and more multivitamins and vitamin E, and were screened more frequently, suggesting men who achieved high calcium intake did so primarily because they perceived this as a healthful choice. Although the evidence for these factors with regard to prostate cancer risk is not definitive, for the most part, they are believed to be associated with lower risk; thus, residual confounding due to measurement error in controlling for these factors would mostly likely attenuate any direct association between calcium intake and prostate cancer risk. Some men could have been taking calcium supplements because of a concern for osteoporosis; however, higher bone density has been shown to be positively related to prostate cancer risk (44), and men at greater concern for osteoporosis would likely be those with lower bone density. Thus, any use of calcium for osteoporosis would have tended to attenuate rather than accentuate the association between calcium and prostate cancer risk. Use of high-dose zinc supplements has been associated with a higher risk of advanced prostate cancer in this cohort (45), but controlling for zinc supplement use did not influence the association with calcium intake.

An important question is whether the increased risk of advanced prostate cancer is due to calcium or to other consequences of milk consumption, such as an increase in insulin-like growth factor (46). For advanced and fatal prostate cancer, we found positive associations independently for both dietary and long-term supplemental calcium (see Table 3). The maximal relative risk for either high dietary or supplemental calcium was about 1.5. Most men achieving very high total calcium intakes (e.g., >1,500 mg) tended to have relatively high dietary calcium intake plus they took supplements; these men experienced 2- to 2.5-fold elevations in advanced prostate cancer and 3.5- to 4-fold elevations in high-grade/fatal prostate cancer. The finding that calcium from dietary and from supplemental sources both independently increased risk of advanced prostate cancer suggests calcium is the critical component. Furthermore, when we modeled simultaneously total calcium intake with total dairy products or lactose intakes, only the association with calcium remained. However, there was a suggestive increase in risk with high lactose or phosphorus intakes, perhaps as an indicator of the nonfat component of milk. These nutrients were highly correlated with dietary calcium (phosphorus: $r = 0.77$; lactose, $r = 0.91$); thus, these possibly were acting as a surrogate of dairy calcium.

A novel finding is that calcium intake was preferentially associated with high-grade versus low-grade prostate cancer, even among cancers diagnosed at a nonadvanced (organ confined) stage. To our knowledge, this relationship has not been examined previously. Interestingly, among all dietary factors in our cohort shown to be associated with advanced prostate cancer, including total energy, red meat, α-linolenic acid, zinc intake, and low lycopene intake, only calcium intake was preferentially associated with high-grade prostate cancer. Our results suggest that the mechanism of calcium's action may be through a reduction in differentiation.

PSA screening has clearly altered the clinical presentation and course of prostate cancer, decreasing the percentage of men with metastatic prostate cancer at the time of diagnosis to about ≤10% (47). In studies conducted entirely or partly in the PSA era, associations with calcium have tended to be observed only for advanced cancers or non–PSA-detected cancers (7, 25, 30), whereas before PSA screening, associations with milk or calcium intake were generally observed for total prostate cancer (1-4, 6, 11, 15, 16, 18-20, 29). Conceivably, frequent PSA screening, by leading to diagnosis (and treatment) before advanced stages, could minimize the potential deleterious effects of high calcium intakes. However, if high calcium intake decreases differentiation, perhaps rate of recurrence and progression could still be higher, as Gleason score predicts metastasis and mortality many years later even in men treated for apparently organ-confined cancer (48).

One hypothesized mechanism between milk or calcium intake and prostate cancer risk has been the $1,25(OH)_2$ vitamin D lowering effect of high dietary calcium intakes (49). Most human prostate cancer cells contain vitamin D receptors, which when activated by $1,25(OH)_2$ vitamin D (50, 51), induces differentiation and inhibits proliferation, invasiveness, angiogenesis, and metastatic potential (34, 52-54). Although many cells have 1-α-hydroxylase activity to convert 25(OH) vitamin D to $1,25(OH)_2$ vitamin D, prostate cancer cells generally lose 1-α-hydroxylase activity (55), which could limit their responsiveness to 25(OH) vitamin D and increase their dependence on circulating $1,25(OH)_2$ vitamin D. In a subset of our population, high calcium intakes were associated with decreased $1,25(OH)_2$ vitamin D and a decreased $1,25(OH)_2$ vitamin D/25(OH) vitamin D ratio. Interestingly, in two prospective studies, risk of clinically aggressive prostate cancer was highest for men with low $1,25(OH)_2$ vitamin D and

high 25(OH) vitamin D (56, 57), a pattern characteristic of high calcium and dairy product intake. However, the relationship between circulating $1,25(OH)_2$ vitamin D and prostate cancer risk remains unsettled (58), and alternative mechanisms are possible.

In contrast to most observational studies, in a secondary analysis of a randomized clinical trial of calcium supplementation (1,200 mg/d) and adenoma risk, men randomized to calcium had a nonsignificant lower risk of prostate cancer (33 prostate cancer cases in the calcium-treated group and 37 in the placebo-treated group; rate ratio, 0.83; 95% CI, 0.52-1.32; ref. 33). This study was conducted largely in the PSA era in the United States; thus, most cases were organ confined, and the mean Gleason score was 6.2. Interestingly, when we limited our analysis to organ-confined, low-grade prostate cancers, our findings for high versus low calcium were similar to those of the trial (RR, 0.79; 95% CI, 0.50-1.25). These findings suggest that high calcium intakes may have different if not opposing effects on low-grade, organ-confined prostate cancer and high-grade, aggressive prostate cancer. Another possibility is that if high calcium intake reduces differentiation in a prostate cancer, the proportion of well-differentiated and moderately differentiated cancers will be reduced. Alternatively, it is possible that calcium intake may not increase risk, or may even be protective, and that calcium in epidemiologic studies is acting as a surrogate of other factors in dairy products.

An NIH Consensus Development Panel on Optimal Calcium Intake convened in 1994 concluded that in men over 65 years of age, calcium intake of 1,500 mg/d seems prudent (59). Our results, as well as those from several other studies (7, 18, 20, 25, 29, 30) suggest that some caution may be warranted for such high levels of calcium intake, especially in older men, for whom the incidence of prostate cancer is high. Because the food frequency questionnaire does not perfectly capture absolute intakes, the apparent threshold of 1,500 mg/d for prostate cancer risk should be taken as an approximation. However, the means and SDs of energy-adjusted calcium intake based on diet records (815 $\pm$ 280 mg/d) and questionnaires (839 $\pm$ 346 mg/d) were comparable.

Important strengths of our study include the prospective design, the use of multiple validated dietary questionnaire over time to better assess long-term and consistent intake, the high follow-up rates, the large number of cases, and the ability to examine various prostate cancer end points. A limitation is that our cohort was comprised predominantly of White men of relatively high socioeconomic status. Although the make-up of our cohort is a disadvantage in terms of generalizing results to other groups, such as African-American men, the relatively homogeneity is advantageous in limiting the possibility for uncontrolled confounding. Although we had to rely on pathology reports for information on Gleason grade, misclassification of grade is unlikely to differ by calcium intake. For this reason, we may have underestimated rather than overstated the association between calcium and high-grade prostate cancer.

In conclusion, we found that high calcium intake is associated with an increased risk of poorly differentiated, clinically advanced, and fatal prostate cancer but not with the generally moderate to well-differentiated cancers diagnosed in the PSA era. Although our findings seem to implicate calcium, we could not entirely exclude a role of additional factors in the nonfat component of dairy products. In addition, we confirm a plasma $1,25(OH)_2$ vitamin D lowering effect of high calcium intakes. These results suggest a degree of caution in recommending high intakes of calcium in middle age to older men. Our findings also suggest that future studies of calcium and dairy products and prostate cancer risk, particularly those conducted in populations with widespread PSA screening, focus on indicators of aggressive behavior, such as Gleason grade, or metastatic or fatal prostate cancer rather than total prostate cancer.

Acknowledgments

We thank Jill Arnold, Stacey De Caro, Elizabeth Frost-Hawes, Mira Kaufman, Siobhan Saint Surin, Laura Sampson, Barbara Vericker, and Olga Veysman for their continuing help in the Health Professionals Follow-up Study.

References

1. Talamini R, La Vecchia C, Decarli A, et al. Nutrition, social factors, and prostatic cancer in a Northern Italian population. Br J Cancer 1986;53:817–21.
2. Talamini R, Franceschi S, La Vecchia C, et al. Diet and prostatic cancer: a case-control study in Northern Italy. Nutr Cancer 1992;18:277–86.
3. Mettlin C, Selenskas S, Natarajan NS, Huben R. Beta-carotene and animal fats and their relationship to prostate cancer risk: a case-control study. Cancer 1989;64:605–12.
4. La Vecchia C, Negri E, D'Avanzo B, et al. Dairy products and the risk of prostatic cancer. Oncology 1991;48:406–10.
5. Jain MG, Hislop GT, Howe GR, Ghadirian P. Plant foods, antioxidants, and prostate cancer risk: findings from case-control studies in Canada. Nutr Cancer 1999;34:173–84.
6. De Stefani E, Fierro L, Barrios E, Ronco A. Tobacco, alcohol, diet and risk of prostate cancer. Tumori 1995;81:315–20.
7. Kristal AR, Cohen JH, Qu P, Stanford JL. Associations of energy, fat, calcium, and vitamin D with prostate cancer risk. Cancer Epidemiol Biomarkers Prev 2002;11:719–25.
8. Rotkin ID. Studies in the epidemiology of prostatic cancer: expanded sampling. Cancer Treat Rep 1977;61:173–80.
9. Schuman LM, Mandel JS, Radke A, et al. Some selected features of the epidemiology of prostatic cancer: Minneapolis-St. Paul, Minnesota case-control study, 1976–1979. In: Magnus K, editor. Trends in cancer incidence: causes and practical implications. Washington (DC): Hemisphere Publishing Corp; 1982. p. 345–54.
10. Hayes RB, Ziegler RG, Gridley G, et al. Dietary factors and risks for prostate cancer among blacks and whites in the United States. Cancer Epidemiol Biomarkers Prev 1999;8:25–34.
11. Tzonou A, Signorello LB, Lagiou P, et al. Diet and cancer of the prostate: a case-control study in Greece. Int J Cancer 1999;80:704–8.
12. Ewings P, Bowie C. A case-control study of cancer of the prostate in Somerset and East Devon. Br J Cancer 1996;74:661–6.
13. Deneo-Pellegrini H, De Stefani E, Ronco A, Mendilaharsu M. Foods, nutrients and prostate cancer: a case-control study in Uruguay. Br J Cancer 1999;80:591–7.
14. Qin LQ, Xu JY, Wang PY, et al. Milk consumption is a risk factor for prostate cancer: meta-analysis of case-control studies. Nutr Cancer 2004;48:22–7.
15. Snowdon DA, Phillips RL, Choi W. Diet, obesity, and risk of fatal prostate cancer. Am J Epidemiol 1984;120:244–50.
16. Le Marchand L, Kolonel LN, Wilkens LR, et al. Animal fat consumption and prostate cancer: a prospective study in Hawaii. Epidemiology 1994;5:276–82.
17. Michaud DS, Augustsson K, Rimm EB, et al. A prospective study on intake of animal products and risk of prostate cancer. Cancer Causes Control 2001;12:557–67.
18. Chan JM, Stampfer MJ, Ma J, et al. Dairy products, calcium, and prostate cancer risk in the Physicians' Health Study (comment). Am J Clin Nutr 2001;74:549–54.
19. Schuurman AG, van den Brandt PA, Dorant E, Goldbohm RA. Animal products, calcium and protein and prostate cancer risk in the Netherlands Cohort Study. Br J Cancer 1999;80:1107–13.
20. Tseng M, Breslow RA, Graubard BI, Ziegler RG. Dairy, calcium, and vitamin D intakes and prostate cancer risk in the National Health and Nutrition Examination Epidemiologic Follow-up Study cohort. Am J Clin Nutr 2005;81:1147–54.
21. Hsing AW, McLaughlin JK, Schuman LM, et al. Diet, tobacco use, and fatal prostate cancer: results from the Lutheran Brotherhood Cohort Study. Cancer Res 1990;50:6836–40.
22. Mills PK, Beeson WL, Phillips RL, Fraser GE. Cohort study of diet, lifestyle, and prostate cancer in Adventist men. Cancer 1989;64:598–604.
23. Veierod MB, Laake P, Thelle DS. Dietary fat intake and risk of prostate cancer: a prospective study of 25,708 Norwegian men. Int J Cancer 1997;73:634–8.
24. Chan JM, Pietinen P, Virtanen M, et al. Diet and prostate cancer risk in a cohort of smokers, with a specific focus on calcium and phosphorus (Finland). Cancer Causes Control 2000;11:859–67.
25. Rodriguez C, McCullough ML, Mondul AM, et al. Calcium, dairy products, and risk of prostate cancer in a prospective cohort of United States men. Cancer Epidemiol Biomarkers Prev 2003;12:597–603.
26. Grant WB. An ecologic study of dietary links to prostate cancer. Altern Med Rev 1999;4:162–9.
27. Armstrong B, Doll R. Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. Int J Cancer 1975;15:617–31.
28. Rose DP, Boyar AP, Wynder EL. International comparisons of mortality rates for cancer of the breast, ovary, prostate, and colon, and per capita food consumption. Cancer 1986;58:2263–71.
29. Chan JM, Giovannucci E, Andersson SO, et al. Dairy products, calcium,

phosphorous, vitamin D, risk of prostate cancer. Cancer Causes Control 1998;9:559–66.
30. Giovannucci E, Rimm EB, Wolk A, et al. Calcium and fructose intake in relation to risk of prostate cancer. Cancer Res 1998;58:442–7.
31. Berndt SI, Carter HB, Landis PK, et al. Calcium intake and prostate cancer risk in a long-term aging study: the Baltimore Longitudinal Study of Aging. Urology 2002;60:1118–23.
32. Tavani A, Gallus S, Franceschi S, La Vecchia C. Calcium, dairy products, and the risk of prostate cancer. Prostate 2001;48:118–21.
33. Baron JA, Beach M, Wallace K, et al. Risk of prostate cancer in a randomized clinical trial of calcium supplementation. Cancer Epidemiol Biomarkers Prev 2005;14:586–9.
34. Peehl DM, Skowronski RJ, Leung GK, et al. Antiproliferative effects of 1,25-dihydroxyvitamin D_3 on primary cultures of human prostatic cells. Cancer Res 1994;54:805–10.
35. Stampfer MJ, Willett WC, Speizer FE, et al. Test of the National Death Index. Am J Epidemiol 1984;119:837–9.
36. Rimm EB, Giovannucci EL, Stampfer MJ, et al. Reproducibility and validity of a expanded self-administered semiquantitative food frequency questionnaire among male health professionals. Am J Epidemiol 1992;135:1114–26.
37. Feskanich D, Rimm EB, Giovannucci EL, et al. Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. J Am Diet Assoc 1993;93:790–6.
38. Rimm EB, Stampfer MJ, Colditz GA, et al. Validity of self-reported waist and hip circumferences in men and women. Epidemiology 1990;1:466–73.
39. Chasan-Taber S, Rimm EB, Stampfer MJ, et al. Reproducibility and validity of a self-administered physical activity questionnaire for male health professionals. Epidemiology 1996;7:81–6.
40. Hollis BW, Kamerud JW, Selvaag SR, et al. Determination of vitamin D status by radioimmunoassay using I^{125}-labeled tracer. Clin Chem 1993;39:529–33.
41. Hollis BW, Kamerud JQ, Kurkowski A, et al. Quantification of circulating 1,25-dihydroxyvitamin D by radioimmunoassay with 125I-labeled tracer. Clin Chem 1996;42:586–92.
42. Platz EA, Leitzmann MF, Hollis BW, et al. Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and subsequent risk of prostate cancer. Cancer Causes Control 2004;15:255–65.
43. Platz EA, Rimm EB, Willett WC, et al. Racial variation in prostate cancer incidence and in hormonal system markers among male health professionals. J Natl Cancer Inst 2000;92:2009–17.
44. Zhang Y, Kiel DP, Ellison RC, et al. Bone mass and the risk of prostate cancer: the Framingham Study. Am J Med 2002;113:734–9.
45. Leitzmann MF, Stampfer MJ, Wu K, et al. Zinc supplement use and risk of prostate cancer. J Natl Cancer Inst 2003;95:1004–7.
46. Chan JM, Stampfer MJ, Ma J, et al. Insulin-like growth factor-I (IGF-I) and IGF binding protein-3 as predictors of advanced stage prostate cancer. J Natl Cancer Inst 2002;94:1099–106.
47. Smart CR. The results of prostate carcinoma screening in the U.S. as reflected in the surveillance, epidemiology, and end results program. Cancer 1997;80:1835–44.
48. Pound CR, Partin AW, Eisenberger MA, et al. Natural history of progression after PSA elevation following radical prostatectomy. JAMA 1999;281:1591–7.
49. Giovannucci E. Dietary influences of $1,25(OH)_2$ vitamin D in relation to prostate cancer: a hypothesis. Cancer Causes Control 1998;9:567–82.
50. Skowronski RJ, Peehl DM, Feldman D. Vitamin D and prostate cancer: 1,25 dihydroxyvitamin D_3 receptors and actions in human prostate cancer cell lines. Endocrinology 1993;132:1952–60.
51. Miller GJ, Stapleton GE, Ferrara JA, et al. The human prostatic carcinoma cell line LNCaP expresses biologically active, specific receptors for 1 alpha,25-dihydroxyvitamin D3. Cancer Res 1992;52:515–20.
52. Schwartz GG, Oeler TA, Uskokovic MR, Bahnson RR. Human prostate cancer cells: inhibition of proliferation by vitamin D analogs. Anticancer Res 1994;14:1077–81.
53. Schwartz GG, Wang M-H, Zhang M, et al. 1α,25-dihydroxyvitamin D (calcitriol) inhibits the invasiveness of human prostate cancer cells. Cancer Epidemiol Biomarkers Prev 1997;6:727–32.
54. Lokeshwar BL, Schwartz GG, Selzer MG, et al. Inhibition of prostate cancer metastasis *in vivo*: a comparison of 1,23-dihydroxyvitamin D (calcitriol) and EB1089. Cancer Epidemiol Biomarkers Prev 1999;8:241–8.
55. Chen TC, Wang L, Whitlatch LW, et al. Prostatic 25-hydroxyvitamin D-1alpha-hydroxylase and its implication in prostate cancer. J Cell Biochem 2003;88:315–22.
56. Corder EH, Guess HA, Hulka BS, et al. Vitamin D and prostate cancer: a prediagnostic study with stored sera. Cancer Epidemiol Biomarkers Prev 1993;2:467–72.
57. Gann PH, Ma J, Hennekens CH, et al. Circulating vitamin D metabolites in relation to subsequent development of prostate cancer. Cancer Epidemiol Biomarkers Prev 1996;5:121–6.
58. Giovannucci E. The epidemiology of vitamin D and cancer incidence and mortality: a review (United States). Cancer Causes Control 2005;16:83–95.
59. Anonymous. NIH Consensus Conference. Optimal calcium intake. NIH Consensus Development Panel on Optimal Calcium Intake. JAMA 1994;272:1942–8.