Dairy Products, Calcium Intake, and Risk of Prostate Cancer in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial

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Abstract

Higher intakes of calcium and dairy products, a major source of dietary calcium, are reported to increase the risk of prostate cancer, potentially due to reductions in circulating vitamin D with increasing calcium intake. We prospectively examined the association of dairy product and calcium intake with prostate cancer risk in 29,509 men, including 1,910 cases, in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. We also evaluated the relation of calcium intake with serum 25-hydroxy-vitamin D [25(OH)D] and 1,25-dihydroxy-vitamin D [1,25$(OH)_2$D], in a Prostate, Lung, Colorectal, and Ovarian Trial substudy (n = 275). Dietary intake was assessed using a food frequency questionnaire. Baseline serum 1,25$(OH)_2$D was determined by RIA. Greater intake of dairy products, particularly low-fat dairy products, was weakly associated with increased risk of prostate cancer [relative risk (RR), 1.12; 95% confidence intervals (CI), 0.97-1.30; P trend = 0.06 for >2.75 versus ≤0.98 servings of total dairy/day; 1.23 (1.07-1.41) for low-fat dairy]. Greater dietary calcium intake was associated with increased risk of prostate cancer (RR, 1.34; 95% CI, 0.93-1.94; P trend = 0.02 for >2,000 versus <1,000 mg/day), but greater supplementary calcium intake was not associated with the risk. Associations of dairy product and dietary calcium intake were evident for nonaggressive disease (RR, 1.20; 95% CI, 0.99-1.46; P trend = 0.01 for dairy products; 1.64, 1.04-2.57; P trend = 0.002 for dietary calcium), but not aggressive disease (RR, 1.02; 95% CI, 0.81-1.28 for dairy products; 0.94, 0.49-1.80 for dietary calcium). Calcium intake was not associated with serum 25-hydroxy-vitamin D and 1,25$(OH)_2$D concentration. In this large prospective study in a prostate cancer screening trial, greater dietary intake of calcium and dairy products, particularly low-fat types, may be modestly associated with increased risks for nonaggressive prostate cancer, but was unrelated to aggressive disease. Furthermore, we found no relationship between calcium intake and circulating vitamin D. (Cancer Epidemiol Biomarkers Prev 2007;16(12):2623–30)

Introduction

Dietary Guidelines for Americans (1) and Dietary Reference Intakes (2) recommend two to three daily servings of dairy foods and 1,200 mg of calcium, respectively. Although there is increasing evidence that these recommended intake levels lower colorectal cancer risk (3) and improve bone health (4), through the protective effects of calcium, concerns have been raised about the potential for increased prostate cancer risk (5).

Countries with higher per capita dairy consumption have greater prostate cancer mortality (6, 7), and several (8-13), but not all (14-16), prospective studies report that greater dairy product intake is associated with increased risk of prostate cancer. Calcium, a major nutrient in dairy products, has also been associated with increased prostate cancer risk (11, 12, 17-20), although other studies do not support this association (13, 21-24).

Calcium in dairy products may increase prostate cancer risk, by reducing circulating 1,25-dihydroxy-vitamin D [1,25$(OH)_2$D] (25), the active form of vitamin D (2), as recently reported (18), and circulating 1,25$(OH)_2$D may be particularly important in relation to prostate cancer risk because prostate cancer cells lose the ability to generate 1,25$(OH)_2$D locally (26). Alternatively, a dairy product–prostate cancer association may be related to insulin-like growth factors (27) or estrogen (28) in these foods. Calcium may play an important role in prostate carcinogenesis and progression because extracellular calcium regulates prostate cancer cell growth (29, 30).

We prospectively examined the association of dairy products and calcium intake in relation to prostate cancer risk in 29,509 men, including 1,910 cases in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening

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Trial. Because calcium may impact prostate cancer risk through vitamin D–related mechanisms, we also evaluated the relation of calcium intake with circulating 1,25-dihydroxy-vitamin D [1,25$(OH)_2$D] in a PLCO Trial substudy (n = 275).

Materials and Methods

Study Population. This study was conducted in the screening arm of the PLCO Cancer Screening Trial, a randomized controlled, multisite study (Birmingham, AL; Denver, CO; Detroit, MI; Honolulu, HI; Marshfield, WI; Minneapolis, MN; Pittsburgh, PA; Salt Lake City, UT; St. Louis, MO; and Washington, DC) to evaluate selected methods for the early detection of these four cancers and to investigate etiologic factors and early markers of cancer (31, 32). Between 1993 and 2001, 38,349 men (ages 55-74 years) were randomized to the screening arm of the trial. Men in this arm received a prostate-specific antigen (PSA) test and a digital rectal exam for prostate cancer screening at study entry and for 3 years annually, followed by 2 consecutive years of screening with PSA only. Men with a PSA test result of >4 ng/mL or digital rectal exam suspicious for prostate cancer were referred to their medical care providers for follow-up. All participants were also asked to complete annual mailed end point follow-up questionnaires regarding cancer diagnoses. The study was approved by the institutional review board of the U.S. National Cancer Institute and the trial screening centers. Written informed consent was obtained from each study participant.

Of the 38,349 men who were randomly assigned to the screening arm of the trial, we excluded men who reported having a history (prior to study entry) of cancer, other than nonmelanoma skin cancer (n = 802); men who did not have an initial PSA test or digital rectal exam (n = 2,470); men with whom there was no subsequent contact (n = 721); men who did not complete a baseline risk factor questionnaire (n = 898); and men who did not provide a dietary questionnaire (n = 6,594), missed more than seven items on the food frequency questionnaire (n = 253), had extreme values for total energy (top or bottom 1%, n = 649). We also excluded men whose initial screening examination occurred after September 30, 2002—the censor date for this analysis (n = 72). After these exclusions, the analytic cohort comprised 29,509 men (some participants were included in more than one exclusion category).

Identification of Prostate Cancer Cases. For men with suspected prostate cancer or for those who reported prostate cancer on their annual questionnaire, we requested medical records to confirm the diagnosis and to obtain stage and grade information. For deceased subjects, we also used death certificates, autopsy reports, and supporting medical/pathologic records for further confirmation. Clinical stage I/II tumors and Gleason sum <7 tumors were defined as nonaggressive. Clinical stage III/IV tumors or Gleason sum ≥7 tumors were considered aggressive. Of 1,910 total cases, 1,178 (62%) were Gleason sum <7, and 699 (37%) were Gleason sum >7. The majority of cases (n = 1,623, 85%) were clinical stage I/II, and 287 cases (15%) were stage III/IV.

Measurements

Questionnaire Variables. Dietary intake was assessed at baseline using a self-administered 137-item food frequency questionnaire, modified from the Willet and Block food frequency questionnaires. Usual frequency of intake (less than once a month, once a month, twice to thrice a month, once a week, twice a week, three to four times a week, five to six times a week, once a day, two or more times a day) and portion size (small, medium, large) were queried. The questionnaire items on dairy foods included questions on skim/low-fat milk, whole milk, sweet cream, sour cream, frozen yogurt or low-fat ice cream, ice cream, yogurt, cottage cheese, and other cheeses and cheese spreads. For the purposes of this analysis, low-fat dairy foods included skim/low-fat milk, frozen yogurt or low-fat ice cream, yogurt, and cottage cheese, whereas high-fat dairy foods were whole milk, cream, sour cream, ice cream, and other cheeses and cheese spreads. Dairy foods and their serving sizes (i.e., one cup of milk or yogurt, 1.5 oz of natural cheese, or 2 oz of processed cheese; ref. 33) were defined by Pyramid Servings Database corresponding to the 1994-1996 Continuing Survey of Food Intakes by Individuals, which uses a recipe file to disaggregate food mixtures into their component ingredients and then assigns them to food groups (34).

Nutrient intakes were derived from frequency and portion-size responses from the food frequency questionnaire, in which nutrient values per portion were multiplied by the daily frequency of intake and summed across all relevant food items. Cut points between small and medium portions and between medium and large portions correspond to the 25th and 75th percentiles, respectively, for portion sizes reported by male participants in the Continuing Survey of Food Intakes by Individuals study, 51 years or older (35). Dietary calcium or dietary vitamin D intakes were calculated from all dietary sources, without supplements, respectively. Dietary calcium was further divided according to food source, as calcium from dairy sources (dairy calcium) and calcium from nondairy sources (nondairy calcium). All nutrient intakes were adjusted for energy intake by the residual method (36).

The food frequency questionnaire also addressed multivitamin, single vitamin, and mineral supplement use with questions about current use, past use (2 and 5 years ago), dosage, and years of intake. Supplemental calcium or vitamin D intake was calculated by summing the intake from calcium (calcium, dolomite, Tums, and so forth; specific dose was assessed) or vitamin D supplements and multivitamins (162 mg calcium and 400 IU of vitamin D per one-a-day multivitamin pill; 400 IU of vitamin D per therapeutic or high-dose types), as defined by the generic multivitamins most frequently reported by participants ages 55 to 74 years in the third National Health and Nutrition Examination Survey cohort (37). If not otherwise specified, analysis of supplemental calcium was conducted for current intake (0-2 years before enrollment). Total vitamin D and calcium intake was defined as dietary plus supplemental intake.

Serum Vitamin D Measurements. Serum 25-hydroxy-vitamin D [25(OH)D] and 1,25$(OH)_2$D measurements were available for men selected as controls in another PLCO study (n = 275), as described elsewhere (38). These

men were free of cancer when they provided the blood sample (study baseline) and did not vary appreciably from other men in the cohort with respect to diet and lifestyle factors (data not shown). Most participants for this substudy were Caucasian (94%), ranging in age from 55 to 74 years (mean, 63 years). 25(OH)D and $1,25(OH)_2D$ serum values were determined in the laboratory of Dr. Bruce Hollis (Medical University of South Carolina, Charleston), by RIA using radioiodinated tracer (39). The coefficient of variation was 12.5% for $1,25(OH)_2D$ and 16.3% for 25(OH)D.

Data Analysis

Cohort Analysis. Person-years were calculated from the date of the baseline prostate cancer screen to the date of the most recently completed end point follow-up questionnaire, the date of prostate cancer diagnosis, death, or September 30, 2002, whichever came first. Between enrollment and the censor date, 10% of the cohort died or were lost to follow-up. We used Cox proportional hazards regression analysis to generate relative risks (RR) and 95% confidence intervals (CI) using the SAS PROC PHREG procedure, with age as the underlying time metric. The proportional hazards assumption was evaluated and confirmed by modeling interaction terms of time, dairy product (or calcium) variable, and other covariates. Men were categorized by quartile of dairy product intake (≤0.98, 0.99-1.67, 1.68-2.74, ≥2.75 servings/d). Calcium intake was grouped into five categories (≤750, 751-1,000, 1,001-1,500, 1,501-2,000, ≥2,001 mg/d). We chose cut points for calcium intake categories that were comparable with those used in the previous literature which incorporated a wide range of intakes (18-20).

Prostate cancer risk estimates were adjusted for the following known or suspected prostate cancer risk factors: race (Caucasian, African-American, other), study center, family history of prostate cancer (yes/no), body mass index (≤18.4, 18.5-24.9, 25-29.9, ≥30 kg/m^2), smoking status (never, current, former, pipe cigar only), physical activity (none, <1, 1, 2, 3, ≥4 h/wk), red meat intake (quintiles; g/d), total energy intake (continuous), history of diabetes (yes/no), education (<11 years, 12 years high school, post-high school or college, and college graduate or more), and total number of prostate cancer screening examinations during the follow-up period (as a time-dependent variable). In calcium analyses, all models were additionally adjusted for total phosphorous intake because phosphorous regulates calcium absorption (2). Tests for trends were conducted by assigning the median value for each category and treating this variable as continuous, using a Wald χ^2 statistic. We formally tested for interactions using log-likelihood ratio tests. All P values are based on two-sided tests. All statistical analyses were conducted using the SAS 9.1 statistical package (SAS Institute).

Calcium Intake and Serum 25(OH)D and $1,25(OH)_2D$. Least squares means of serum vitamin D [25(OH)D or $1,25(OH)_2D$] according to categories of calcium intake were calculated using linear regression. Multivariate models were adjusted for dietary vitamin D (≤100, 101-200, 201-300, 301-400, ≥401 IU/d), supplementary vitamin D (≤200, 201-400, 401-600, ≥600 IU/d), race (Caucasian, African-American, or other—as an indicator of skin pigmentation), season of blood draw, body mass index (kg/m^2—as an indicator of bioavailability of circulating 25(OH)D; ref. 40), physical activity (h/wk—as a surrogate for outdoor activity), study center (Hawaii, Alabama, Colorado, Missouri, Utah, Washington DC, Pennsylvania, Michigan, Minnesota, and Wisconsin—as an indicator for sunlight exposure), and age (continuous).

Results

Baseline Characteristics. Men with greater intake of dairy products were more likely to have a history of diabetes, they were more likely to be never smokers and Caucasian, and they consumed less red meat and alcohol compared with men with lower dairy product intakes (Table 1). Other characteristics, including the frequency of undergoing prostate cancer screening during the period of active screening, did not vary appreciably by dairy product intake. Men with greater intakes of total calcium were more likely to have a family history of diabetes, they were more likely to be never smokers and Caucasian, and they consumed less red meat, compared with men with lower calcium intakes (data not shown). Milk was the major source of dietary calcium (61% of intake), with 70% derived from low-fat and skim milk. Calcium intake was positively correlated with intake of total vitamin D (r = 0.57) and phosphorus (r = 0.47). Mean levels of PSA at study entry did not differ according to dairy product or calcium intake (data not shown).

Dairy Product Intake and Prostate Cancer Risk. With up to 8.9 years of follow-up, 1,910 men were diagnosed with prostate cancer. Greater intake of total dairy products was weakly associated with increased prostate cancer risk (P trend = 0.06; Table 2); the association was strongest for nonaggressive cancer (Gleason score <7 and stages I/II; P trend = 0.01), whereas no association was noted for aggressive disease (Gleason score ≥7 and stage ≥ III; P trend = 0.99). Overall excess risks were largely related to low-fat dairy foods (RR comparing extreme categories, 1.23; 95% CI, 1.07-1.41, P trend = 0.02), as were associations with nonaggressive disease (RR comparing extreme categories, 1.30; 95% CI, 1.09-1.55, P trend = 0.003). However, on adjusting for dietary calcium, no significant associations remained between dairy product intake and prostate cancer. When we additionally adjusted total fat intake in the model, risk estimates remained essentially unchanged and total fat intake was unrelated to prostate cancer risk (data not shown).

Calcium Intake and Prostate Cancer Risk. Total calcium intake (diet and supplement combined) was not related to prostate cancer risk (Table 3), however, risk for prostate cancer increased with greater intake of dietary calcium (P trend = 0.05). Associations with dietary calcium were most pronounced for nonaggressive prostate cancer (RR, 1.52; 95% CI, 0.94-2.47, P trend = 0.006; >2,000 versus ≤750 mg/d), and additional adjustment for dairy foods did not change this association. However, no associations were observed for aggressive (P trend = 0.88), high grade (Gleason score ≥7; P trend = 0.46), or extraprostatic cancer (stage ≥ III; P trend = 0.80). Similar associations were noted for calcium from dairy products only (data not shown).

Table 1. Baseline characteristics of participants, according to total dairy product consumption in the PLCO study

	Total dairy product intake (servings/day)*			
	≤0.98	0.99-1.67	1.68-2.74	≥2.75
Number of participants (%)	7,377 (25%)	7,377 (25%)	7,378 (25%)	7,377 (25%)
Age at study entry (y)	62.3	62.8	62.9	62.8
Body mass index (kg/m^2)	27.3	27.5	27.5	27.9
Physical activity (h/wk)	2.1	2.2	2.3	2.3
Family history of prostate cancer (%)	7.3	7.4	8.0	7.9
History of diabetes (%)	7.2	9.0	8.5	9.1
Daily aspirin use (%)	29.4	31.5	30.9	30.9
Number of screens/y†	0.84	0.84	0.84	0.84
Smoking status (%)				
Never	26.1	28.3	30.5	33.0
Current	12.2	9.6	9.5	10.9
Former	54.1	54.0	51.7	48.5
Cigar or pipe only	7.6	8.2	8.3	7.6
Race (%)				
Caucasian	81.2	91.3	94.4	95.9
African-American	6.7	3.2	2.1	1.4
Other	12.1	5.5	3.5	2.7
Calories (kcal/d)	1,892	2,141	2,427	2,876
Low-fat dairy (servings/d)	0.15	0.39	0.72	1.89
High-fat dairy (servings/d)	0.19	0.32	0.46	0.76
Total milk (servings/d)	0.30	0.82	1.25	2.30
Dietary calcium (mg/d)	769	897	1,029	1,387
Supplemental calcium (mg/d)	135	134	136	135
Dietary vitamin D (IU/d)	158	195	232	345
Supplemental vitamin D (IU/d)	201	199	205	207
Total phosphorus (mg/d)	1,134	1,391	1,671	2,192
Red meat consumption (g/d)	110	104	99	88
Total fat (g/d)	78	79	80	82
Total vitamin E (mg/d)	75	75	74	73
Alcohol (g/d)	18	17	15	14

NOTE: Values are expressed as means or proportions.
*Dietary intakes (except alcohol) were adjusted for total energy intake.
†Average number of prostate cancer screening examinations (prostate-specific antigen test and/or digital rectal examination) during the period of active screening (years 0-5).

Supplemental calcium contributed little to total calcium intake [mean dietary intake, 964 mg; mean supplemental intake, 135 and 320 mg (among supplement users only)] and was not associated with prostate cancer risk. This association did not vary according to years of calcium supplement use, and results did not change after adjustment for use of other multivitamins (data not shown). Associations of dietary or supplementary calcium with prostate cancer risk did not differ according to phosphorus intake, physical activity, or study site, a proxy for sunlight exposure (all P for interactions >0.1).

Dietary vitamin D was not associated with prostate cancer (data not shown) and additional adjustment for calcium and phosphorus intake did not change risk estimates. However, risks tended to decrease with greater vitamin D from supplemental sources, with a 40% reduction in men who used >600 IU of supplemental vitamin D compared with men not using vitamin D supplements (RR, 0.61; 95% CI, 0.41-0.89, P trend = 0.05). This association did not differ by tumor aggressiveness. We found no evidence of interaction between total calcium and vitamin D intake (P interaction = 0.81). Total phosphorus intake from dietary and supplemental sources was also unassociated with prostate cancer risk. The RRs for increasing quartiles were 1.00 (referent), 0.89, 0.83, and 0.90 (95% CI, 0.82-1.11).

Calcium Intake and Serum 25(OH)D and 1,25(OH)$_2$D. Associations between calcium intake and serum vitamin D [25(OH)D and 1,25(OH)$_2$D] are shown in Table 4. Mean levels of serum 1,25(OH)$_2$D did not differ according to the categories of dietary or supplemental calcium intake. Mean levels of 25(OH)D also did not differ according to the categories of dietary or supplemental calcium intake. Results were similar when we used total calcium (diet and supplement combined) or dairy product (data not shown).

Discussion

In this large prospective study in a prostate cancer screening trial, greater dietary intake of calcium and dairy products, particularly low-fat types, may be modestly associated with increased risks for prostate cancer. Risk associations were evident for nonaggressive but not for aggressive prostate cancer. When simultaneously evaluated in multivariate models, risks were related to dietary calcium, but not independently to dairy products. Furthermore, we found no relationship between calcium intake and circulating 1,25(OH)$_2$D.

Several (8-13), but not all (14-16) previous studies reported increased risks for prostate cancer with greater dairy product consumption, with two studies reporting stronger associations with low-fat dairy products (11, 12), consistent with our data. The first National Health and Nutrition Examination Epidemiologic Follow-up Study reported that low-fat milk was associated with increased

risk (RR, 1.5 comparing extreme tertiles), but whole milk was not (RR, 0.8; ref. 11). The authors argued that removal of fat from milk may remove other components with potentially cancer-protective properties, such as conjugated linoleic acid, or it could be partly due to detection bias because men of higher socioeconomic status were more likely to drink low-fat milk (11). The Physicians' Health Study also reported that only skim milk, but not whole milk, was positively related with risk among each individual dairy food tested (12). In our study, it is unlikely that low-fat dairy products were a mere marker for a low-fat diet, because total fat intake was not associated with prostate cancer risk. Low-fat milk generally contains higher levels of calcium (33), and calcium from dairy products was associated with increased risk. Also, observations on high-fat dairy products may be limited in our study because men reported generally low intake of these foods.

Our findings of an increased risk with higher calcium intake are consistent with several (11, 12, 17-20), but not all (13, 21-24), existing studies. Of note, an increased risk of prostate cancer was noted among men with very high calcium intakes (i.e., >1,500 mg/d), which is also consistent with other studies (17-19). Studies that lacked data above this level or used a lower cutoff value for calcium intake (13, 21, 22, 24) could have missed an association.

We found stronger associations with nonaggressive tumors, in contrast to two previous studies reporting stronger associations with aggressive or fatal disease (18, 41). Although there is evidence from epidemiologic studies that greater calcium intake is related to increased risk for prostate cancer, the critical role of calcium in prostate cancer progression remains uncertain. Because intracellular calcium regulates prostate cancer cell growth (30), calcium may delay tumor progression, resulting in a greater proportion of nonaggressive tumors, consistent with our findings. Further studies on calcium and prostate cancer development are warranted.

Our findings of no association with supplemental calcium is consistent with other (11, 19), but not all (18) observational studies. A recent randomized trial of supplemental calcium (1,200 mg/d) also showed no effect (42); however, the follow-up period might have been too brief to observe such effects. A null association with supplemental calcium may be due to its relatively small contribution to overall calcium intake in men in our population. Alternatively, measurement error of calcium from supplements may play a role if dietary calcium intake is more constant than supplement use, over the long-term. In any case, the association of supplemental calcium with prostate cancer risk did not vary according to years of calcium supplement use.

Table 2. Multivariate RRs and 95% CIs of prostate cancer, according to categories of dairy food consumption in the PLCO study

	Dairy product intake (servings/day)*				P_{trend} †
Total dairy	≤0.98	0.99-1.67	1.68-2.74	≥2.75	
Total cases	434	451	521	504	
RR (95% CI) ‡	1.00 (referent)	0.99 (0.86-1.13)	1.13 (0.99-1.30)	1.12 (0.97-1.30)	0.06
RR (95% CI) ‡ + diet calcium	1.00 (referent)	0.98 (0.85-1.13)	1.11 (0.95-1.30)	1.06 (0.88-1.30)	0.44
Nonaggressive cases§	236	238	305	310	
RR (95% CI) ‡	1.00 (referent)	0.94 (0.78-1.13)	1.17 (0.98-1.40)	1.20 (0.99-1.46)	0.01
RR (95% CI) ‡ + diet calcium	1.00 (referent)	0.93 (0.77-1.12)	1.14 (0.92-1.40)	1.06 (0.82-1.38)	0.48
Aggressive cases	190	207	207	187	
RR (95% CI) ‡	1.00 (referent)	1.07 (0.87-1.31)	1.08 (0.88-1.33)	1.02 (0.81-1.28)	0.99
RR (95% CI) ‡ + diet calcium	1.00 (referent)	1.06 (0.86-1.31)	1.08 (0.85-1.38)	1.07 (0.79-1.45)	0.73
Low-fat dairy	≤0.08	0.09-0.41	0.42-0.99	≥1.00	
Total cases	424	483	478	525	
RR (95% CI) ‡	1.00 (referent)	1.14 (1.00-1.30)	1.12 (0.98-1.28)	1.23 (1.07-1.41)	0.02
RR (95% CI) ‡ + diet calcium	1.00 (referent)	1.14 (1.00-1.30)	1.11 (0.96-1.28)	1.19 (1.00-1.41)	0.13
Nonaggressive cases§	239	263	259	328	
RR (95% CI) ‡	1.00 (referent)	1.08 (0.90-1.29)	1.04 (0.87-1.25)	1.30 (1.09-1.55)	0.003
RR (95% CI) ‡ + diet calcium	1.00 (referent)	1.08 (0.90-1.29)	1.03 (0.85-1.24)	1.20 (0.96-1.50)	0.12
Aggressive cases	177	213	211	190	
RR (95% CI) ‡	1.00 (referent)	1.23 (1.01-1.51)	1.22 (0.99-1.49)	1.12 (0.90-1.39)	0.93
RR (95% CI) ‡ + diet calcium	1.00 (referent)	1.23 (1.00-1.51)	1.22 (0.98-1.51)	1.17 (0.90-1.53)	0.66
High-fat dairy	≤0.10	0.11-0.25	0.26-0.52	≥0.53	
Total cases	466	496	478	470	
RR (95% CI) ‡	1.00 (referent)	1.09 (0.96-1.24)	1.04 (0.91-1.20)	1.07 (0.92-1.23)	0.65
RR (95% CI) ‡ + diet calcium	1.00 (referent)	1.09 (0.95-1.24)	1.04 (0.91-1.19)	1.05 (0.91-1.21)	0.82
Nonaggressive cases§	262	282	274	271	
RR (95% CI) ‡	1.00 (referent)	1.06 (0.89-1.26)	1.01 (0.84-1.20)	1.03 (0.85-1.24)	0.99
RR (95% CI) ‡ + diet calcium	1.00 (referent)	1.06 (0.89-1.26)	1.00 (0.84-1.20)	1.00 (0.83-1.21)	0.80
Aggressive cases	198	205	195	193	
RR (95% CI) ‡	1.00 (referent)	1.11 (0.91-1.36)	1.08 (0.88-1.34)	1.13 (0.91-1.42)	0.42
RR (95% CI) ‡ + diet calcium	1.00 (referent)	1.11 (0.91-1.36)	1.08 (0.88-1.33)	1.13 (0.91-1.42)	0.43

*Dairy food consumption categorized as quartiles.
†*P* trend was based on the medians of the categories.
‡RRs adjusted for age, race, study center, family history of prostate cancer, body mass index, smoking status, physical activity, history of diabetes, red meat intake, total energy intake, education, and number of screening examinations during the follow-up period.
§Nonaggressive cases were defined as Gleason score <7 and stage I/II. Thirty cases were deleted due to missing data on Gleason sum or tumor stage. Aggresive cases were defined as Gleason score ≥7 or stage ≥ III

Table 3. Multivariate RRs and 95% CIs of prostate cancer, according to categories of calcium intakes in the PLCO study

	Calcium intake (mg/day)					P_{trend}*
Total calcium	≤750	751-1,000	1,001-1,500	1,501-2,000	≥2,001	
Total cases	240	513	792	280	85	
RR (95% CI)†	1.00 (referent)	0.85 (0.72-1.01)	0.95 (0.79-1.15)	1.04 (0.83-1.30)	0.89 (0.66-1.19)	0.51
RR (95% CI)† + total dairy	1.00 (referent)	0.83 (0.70-0.99)	0.89 (0.73-1.08)	0.96 (0.76-1.22)	0.82 (0.61-1.11)	0.98
Nonaggressive cases‡	134	285	445	165	60	
RR (95% CI)†	1.00 (referent)	0.87 (0.69-1.09)	0.95 (0.74-1.22)	1.06 (0.78-1.42)	1.08 (0.75-1.56)	0.24
RR (95% CI)† + total dairy	1.00 (referent)	0.84 (0.67-1.05)	0.87 (0.67-1.13)	0.95 (0.70-1.31)	0.98 (0.67-1.43)	0.58
Aggressive cases	102	218	334	114	23	
RR (95% CI)†	1.00 (referent)	0.83 (0.64-1.07)	0.97 (0.73-1.30)	1.07 (0.76-1.51)	0.61 (0.37-1.02)	0.89
RR (95% CI)† + total dairy	1.00 (referent)	0.81 (0.62-1.05)	0.92 (0.68-1.25)	1.02 (0.71-1.47)	0.58 (0.34-1.02)	0.71
Dietary calcium	≤750	751-1,000	1,001-1,500	1,501-2,000	≥2,001	
Total cases	332	662	726	153	37	
RR (95% CI)†	1.00 (referent)	0.89 (0.76-1.03)	1.07 (0.89-1.28)	1.17 (0.90-1.52)	1.22 (0.83-1.79)	0.05
RR (95% CI)† + total dairy	1.00 (referent)	0.86 (0.73-1.00)	0.97 (0.78-1.19)	1.07 (0.79-1.44)	1.12 (0.75-1.69)	0.32
Nonaggressive cases‡	183	363	416	102	25	
RR (95% CI)†	1.00 (referent)	0.91 (0.75-1.12)	1.13 (0.88-1.45)	1.45 (1.03-2.03)	1.52 (0.94-2.47)	0.006
RR (95% CI)† + total dairy	1.00 (referent)	0.88 (0.72-1.09)	1.02 (0.77-1.35)	1.31 (0.89-1.94)	1.40 (0.84-2.34)	0.05
Aggressive cases	143	288	299	50	11	
RR (95% CI)†	1.00 (referent)	0.86 (0.68-1.09)	1.01 (0.76-1.34)	0.89 (0.58-1.35)	0.83 (0.42-1.64)	0.88
RR (95% CI)† + total dairy	1.00 (referent)	0.83 (0.65-1.05)	0.92 (0.67-1.27)	0.81 (0.50-1.30)	0.77 (0.38-1.55)	0.54
Supplement calcium	No supplement	≤400	401-800	≥801		
Total cases	1,145	516	209	40		
RR (95% CI)†	1.00 (referent)	0.97 (0.87-1.08)	0.95 (0.82-1.11)	0.94 (0.68-1.29)		0.46
RR (95% CI)† + total dairy	1.00 (referent)	0.99 (0.89-1.10)	0.96 (0.83-1.12)	0.96 (0.70-1.31)		0.59
Nonaggressive cases‡	640	309	119	21		
RR (95% CI)†	1.00 (referent)	1.03 (0.90-1.18)	0.96 (0.79-1.17)	0.88 (0.57-1.36)		0.58
RR (95% CI)† + total dairy	1.00 (referent)	1.05 (0.92-1.21)	0.98 (0.80-1.19)	0.90 (0.58-1.39)		0.72
Aggressive cases	485	199	89	18		
RR (95% CI)†	1.00 (referent)	0.90 (0.76-1.07)	0.97 (0.77-1.22)	1.02 (0.63-1.63)		0.77
RR (95% CI)† + total dairy	1.00 (referent)	0.91 (0.77-1.08)	0.98 (0.78-1.23)	1.03 (0.64-1.65)		0.84

*P trend was based on the medians of the categories.
†RRs adjusted for age, race, study center, family history of prostate cancer, body mass index, smoking status, physical activity, history of diabetes, red meat intake, total energy intake, education, number of screening examinations during the follow-up period, and total phosphorus intake.
‡Nonaggressive cases were defined as Gleason score <7 and stage I/II. Thirty cases were deleted due to missing data on Gleason sum or tumor stage. Aggressive cases were defined as Gleason score ≥7 or stage ≥ III.

Findings for the combined analysis of dietary calcium and dairy products indicated that calcium may be an important nutrient in the dairy product/prostate cancer association. However, given that we did not observe any association between supplemental calcium and prostate cancer risk, this should be interpreted cautiously, and findings could be confounded by other components in dairy products: dairy products could carry insulin-like growth factors and contaminant hormones, the influence of which cannot be convincingly excluded.

We found that dietary vitamin D intake was unrelated to risk of prostate cancer. However, accurate estimates of dietary vitamin D intake are not available, largely because the vitamin D composition of fortified foods is highly variable and a significant portion of vitamin D is generated in the skin by UV exposure (2). Also, residual

Table 4. Least square mean of baseline serum 25(OH)D and 1,25(OH)$_2$D by calcium intake among 275 men in a PLCO substudy

	N	25(OH)D (nmol/L)		1,25(OH)$_2$D (pmol/L)	
		Least squares mean (95% CI)*	P	Least squares mean (95% CI)*	P
Dietary calcium (mg/d)					
<750	82	53.28 (39.43-67.13)	0.87	78.72 (65.04-92.41)	0.34
571-1,000	76	49.91 (36.70-63.12)		84.12 (71.07-97.18)	
1,001-1,500	67	51.56 (37.97-65.14)		76.26 (62.81-89.71)	
≥1,500	47	53.29 (38.79-67.79)		80.24 (65.89-94.59)	
Supplementary calcium (mg/d)					
0	147	49.33 (33.91-64.75)	0.55	78.04 (62.78-93.30)	0.67
1-399	83	51.47 (38.49-64.45)		82.49 (69.63-95.33)	
≥400	42	55.25 (41.40-69.11)		78.03 (64.31-91.75)	

*Least squares means of serum 25(OH)D and 1,25(OH)$_2$D were adjusted for vitamin D, supplemental vitamin D, race, season of blood draw, body mass index, physical activity, study center, and age.

confounding by other nutrients in dairy products remains possible, but candidate agents have not been identified.

Although high calcium intake has been reported to influence circulating $1,25(OH)_2D$ (25), we found no such relationship, consistent with its relatively tight homeostatic regulation (2). Limiting our analysis, calcium intake was measured as regular use, whereas serum measurements were based on one sample time point. However, a supplementation trial also showed little effect of calcium on serum $1,25(OH)_2D$ (42), with $1,25(OH)_2D$ levels changed from 42.9 to 41.2 pg/mL with 1,200 mg/d of calcium supplementation (42). Thus, calcium intake is unlikely to have a substantial influence on prostate cancer risk through its effect on serum $1,25(OH)_2D$, although subtle effects remain possible.

Because our study was conducted in the screening arm of a randomized controlled trial, the majority of prostate cancer cases were PSA or digital rectal exam screen-detected, and results may not generalize to all cases. Kristal et al. (43) found that calcium supplement use was associated with a reduced PSA velocity, and The Cancer Prevention Study cohort (19) reported an association with calcium primarily in men without PSA testing, indicating that PSA testing may be less likely to detect cases associated with high calcium intake. On the other hand, in this trial case, detection patterns should have largely been unrelated to diet and other behavioral covariates. Other strengths include the large study size, prospective design, and detailed dietary data with a substantial range in dairy and calcium intake. Finally, we had a rare opportunity to evaluate the relation of dietary intake with serum $1,25(OH)_2D$.

In summary, our study provides evidence that higher dairy product and dietary calcium intakes are modestly related to increased risk for prostate cancer, particularly nonaggressive disease. Our study does not support the hypothesis that a positive association of calcium intake with prostate cancer risk is due to reduced circulating $1,25(OH)_2D$. Although biological mechanisms are not known, greater dairy product and dietary calcium intake may be related to increased risk for some forms of prostate cancer.

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References

1. Available from: http://www.health.gov/dietaryguidelines/.
2. Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, fluoride. National Academies Press; 1999.
3. Cho E, Smith-Warner SA, Spiegelman D, et al. Dairy foods, calcium, and colorectal cancer: a pooled analysis of 10 cohort studies. J Natl Cancer Inst 2004;96:1015–22.
4. Welten DC, Kemper HC, Post GB, van Staveren WA. A meta-analysis of the effect of calcium intake on bone mass in young and middle aged females and males. J Nutr 1995;125:2802–13.
5. Gao X, LaValley MP, Tucker KL. Prospective studies of dairy product and calcium intakes and prostate cancer risk: a meta-analysis. J Natl Cancer Inst 2005;97:1768–77.
6. Grant WB. An ecologic study of dietary links to prostate cancer. Altern Med Rev 1999;4:162–9.
7. 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;58:2363–71.
8. Kesse E, Bertrais S, Astorg P, et al. Dairy products, calcium and phosphorus intake, and the risk of prostate cancer: results of the French prospective SU VI MAX (Supplementation en Vitamines et Mineraux Antioxydants) study. Br J Nutr 2006;95:539–45.
9. Le Marchand L, Kolonel LN, Wilkens LR, Myers BC, Hirohata T. Animal fat consumption and prostate cancer: a prospective study in Hawaii. Epidemiology 1994;5:276–82.
10. Snowdon DA, Phillips RL, Choi W. Diet, obesity, and risk of fatal prostate cancer. Am J Epidemiol 1984;120:244–50.
11. 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.
12. Chan JM, Stampfer MJ, Ma J, Gann PH, Gaziano JM, Giovannucci EL. Dairy products, calcium, and prostate cancer risk in the Physicians' Health Study. Am J Clin Nutr 2001;74:549–54.
13. 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.
14. Severson RK, Nomura AM, Grove JS, Stemmermann GN. A prospective study of demographics, diet, and prostate cancer among men of Japanese ancestry in Hawaii. Cancer Res 1989;49:1857–60.
15. Mills PK, Beeson WL, Phillips RL, Fraser GE. Cohort study of diet, lifestyle, and prostate cancer in Adventist men. Cancer 1989;64:598–604.
16. 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.
17. 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.
18. Giovannucci E, Liu Y, Stampfer MJ, Willett WC. A prospective study of calcium intake and incident and fatal prostate cancer. Cancer Epidemiol Biomarkers Prev 2006;15:203–10.
19. 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.
20. Mitrou PN, Albanes D, Weinstein SJ, et al. A prospective study of dietary calcium, dairy products and prostate cancer risk (Finland). Int J Cancer 2007;120:2466–73.
21. 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.
22. Koh KA, Sesso HD, Paffenbarger RS, Jr., Lee IM. Dairy products, calcium and prostate cancer risk. Br J Cancer 2006;95:1582–5.
23. Rohrmann S, Platz EA, Kavanaugh CJ, Thuita L, Hoffman SC, Helzlsouer KJ. Meat and dairy consumption and subsequent risk of prostate cancer in a US cohort study. Cancer Causes Control 2007;18:41–50.
24. Severi G, English DR, Hopper JL, Giles GG. Re: Prospective studies of dairy product and calcium intakes and prostate cancer risk: a meta-analysis. J Natl Cancer Inst 2006;98:794–5.
25. 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.
26. Giovannucci E. The epidemiology of vitamin D and cancer incidence and mortality: a review (United States). Cancer Causes Control 2005;16:83–95.
27. Gunnell D, Oliver SE, Peters TJ, et al. Are diet-prostate cancer associations mediated by the IGF axis? A cross-sectional analysis of diet, IGF-I and IGFBP-3 in healthy middle-aged men. Br J Cancer 2003;88:1682–6.
28. Qin LQ, Wang PY, Kaneko T, Hoshi K, Sato A. Estrogen: one of the risk factors in milk for prostate cancer. Med Hypotheses 2004;62:133–42.
29. Liao J, Schneider A, Datta NS, McCauley LK. Extracellular calcium as a candidate mediator of prostate cancer skeletal metastasis. Cancer Res 2006;66:9065–73.
30. Legrand G, Humez S, Slomianny C, et al. Ca2+ pools and cell growth. Evidence for sarcoendoplasmic Ca2+-ATPases 2B involvement in human prostate cancer cell growth control. J Biol Chem 2001;276:47608–14.
31. Prorok PC, Andriole GL, Bresalier RS, et al. Design of the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. Control Clin Trials 2000;21:273–309S.
32. Hayes RB, Sigurdson A, Moore L, et al. Methods for etiologic and

early marker investigations in the PLCO trial. Mutat Res 2005;592:147–54.
33. U S. Food supply database. U S. Department of Agriculture, Center for nutrition and policy promotion, Beltsville, MD;2003. Available at: http://www.ars.usda.gov/ba/bhnrc/ndl. 2006.
34. Design and operation: the Continuing Survey of Food Intakes by Individuals and the Diet and Health Knowledge Survey, 1994–96. Nationwide Food Surveys Rep No 96–1. U.S. Department of Agriculture, Agricultural Research Service, 1998.
35. Subar AF, Midthune D, Kulldorff M, et al. Evaluation of alternative approaches to assign nutrient values to food groups in food frequency questionnaires. Am J Epidemiol 2000;152:279–86.
36. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 1986;124:17–27.
37. Centers for Disease Control and Prevention: the Third National Health and Nutrition Examination Survey (NHANES III 1988–94) Reference Manuals and Reports. Bethesda (MD): National Center for Health Statistics; 1996.
38. Peters U, Hayes RB, Chatterjee N, et al. Circulating vitamin D metabolites, polymorphism in vitamin D receptor, and colorectal adenoma risk. Cancer Epidemiol Biomarkers Prev 2004;13:546–52.
39. Hollis BW. Quantitation of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D by radioimmunoassay using radioiodinated tracers. Methods Enzymol 1997;282:174–86.
40. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. Am J Clin Nutr 2000;72:690–3.
41. 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.
42. 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.
43. Kristal AR, Chi C, Tangen CM, Goodman PJ, Etzioni R, Thompson IM. Associations of demographic and lifestyle characteristics with prostate-specific antigen (PSA) concentration and rate of PSA increase. Cancer 2006;106:320–8.

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