

## Short Communication

# A Prospective Study of Total and Ionized Serum Calcium and Fatal Prostate Cancer

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## Abstract

We recently reported a significant positive association in the National Health and Nutrition Examination Survey between high levels of total calcium in serum, measured prospectively, and risk of fatal prostate cancer. To confirm this, we examined associations between total and ionized serum calcium and prostate cancer mortality in an independent cohort, the Third National Health and Nutrition Examination Survey. Twenty-five prostate cancer deaths occurred over 56,625 person-years of follow-up. Compared with men in the lowest tertile

of total serum calcium, the multivariate-adjusted relative risk for death from prostate cancer for men in the highest tertile was 2.07 (95% confidence interval, 1.06–4.04). For ionized serum calcium, the physiologically active fraction of total serum calcium, the relative risk for men in the highest tertile was 3.18 (95% confidence interval, 1.09–9.28). These findings support the hypothesis that serum calcium is a prospective biomarker of fatal prostate cancer. (Cancer Epidemiol Biomarkers Prev 2009;18(2):575–8)

## Introduction

Prostate cancer is the second most fatal cancer among men in the United States, accounting for ~28,000 deaths in 2008 (1). Considerable epidemiologic attention in prostate cancer has focused on the vitamin D–endocrine system and on calcium (2–4). Although numerous studies have investigated prostate cancer with respect to calcium intake from the diet, the subject of calcium in serum has received scarce attention, perhaps because calcium levels in serum are believed to be under strict homeostatic control. In the only study to specifically address this question, we found an ~3-fold increased risk of fatal prostate cancer in the National Health and Nutrition Examination Survey (NHANES I) Epidemiologic Follow-up Study for men with levels of total serum calcium in the upper tertile at the baseline exam (5). In this article, we confirm and extend that finding using an independent cohort, the Third National Health and Nutrition Examination Survey (NHANES III). A unique feature of NHANES III is that it included measurements of ionized serum calcium, the physiologically active fraction of total serum calcium.

## Materials and Methods

Data were derived from NHANES III, which provides public use mortality files with record linkage of

participants in NHANES III to the National Death Index (6). Eight thousand one hundred twenty-five men participated in NHANES III. We excluded men with missing information on serum calcium ( $n = 1,210$ ), a prior history of cancer (except nonmelanoma skin cancer;  $n = 199$ ), men who died from prostate cancer within 12 months of examination ( $n = 2$ ), and men without follow-up information ( $n = 4$ ). The final analytic cohort consisted of 6,710 men ages 18 to 90 years at the baseline examination in 1988 to 1994. Because of the concern that calcium levels in serum could be influenced by the presence of undetected prostate cancer, we did additional analyses excluding prostate cancer deaths that occurred during the first 3 years of follow-up ( $n = 8$ ).

We evaluated associations between prostate cancer mortality, total serum calcium, and ionized serum calcium corrected for serum pH. Approximately half of the total serum calcium is in the ionized or physiologically active state. Another 40% is bound to serum proteins, principally albumin, and the remaining 10% is bound to anions, such as lactate and phosphate. Because the binding of calcium to proteins is altered by changes in blood pH, measures of ionized calcium in blood are commonly pH corrected to a standard pH using regression analysis (7).

Person-time was computed as the number of months from initial examination to date of prostate cancer mortality (events), death from another cause, or the end of follow-up in December 31, 2000 (nonevents). We estimated relative risks adjusted for potential confounders using Cox proportional hazards. Multivariate models included age (in 1-year increments) and body mass index (BMI; as a continuous measure), as potential confounders, and variables related to NHANES III sample weights [race or ethnicity (black or white and Hispanic or

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**Table 1. Selected baseline characteristics of men in NHANES III, 1988-1994**

	Total serum calcium		
	Tertile 1	Tertile 2	Tertile 3
No. participants	2,205	2,131	2,374
Weighted population	22,801,773	23,404,681	27,861,959
Mean total calcium (mmol/L)	2.20	2.32	2.42
Mean ionized calcium (mmol/L)	1.20	1.24	1.27
Age (y)	47.7	43.1	38.3
BMI (kg/m <sup>2</sup> )	26.7	26.9	26.1
Race (% black)	8.0	10.3	11.4
Large household (% with 5 or more people)	36.6	41.9	45.6
Health (% good or better)	91.2	93.4	95.0

NOTE: Means and proportions in this table account for the complex sample design and differential probabilities of nonresponse in NHANES III and are representative of the total population of U.S. men. To convert total calcium or ionized calcium from mmol/L to mg/dL, divide mmol/L by 0.2495.

non-Hispanic), household size (number of individuals up to 10), and general health status (excellent, very good, good, fair, or poor)] to account for differential probabilities of selection and nonresponse (8). Statistical analysis used SAS (v9.1 for Linux) and Sudaan (v9 for Linux) to account for the complex sampling designs of the cohort. All statistical tests were two-sided.

## Results

Twenty-five prostate cancer deaths occurred among 6,710 men over 56,625 person-years of follow up. Measurement of serum calcium preceded fatal prostate cancer by an average of 5.3 years (SD 2.5). The average age at baseline of prostate cancer cases was 73.4 years and their average age at death was 78.1 years. Men in the higher tertiles of total serum calcium tended to be younger, to be black, to have lower BMI, to be from a large household, and to be in good or better health. Similar relationships were observed for increasing tertiles of ionized calcium. The mean ages in tertiles 1 through 3 of ionized serum calcium were 45.2, 41.4, and 38.5 years, respectively (Table 1).

Compared with men in the lowest tertile of total serum calcium, men in the highest tertile had a multivariate-adjusted relative risk (RR) for prostate

cancer death of 2.07 [95% confidence interval (95% CI), 1.06-4.04]. This association was not materially changed after adjustment for BMI (RR, 2.02; 95% CI, 1.02-4.01). The RR for men in the second tertile was 1.39 (95% CI, 0.59-3.30). The test for linear trend was not statistically significant ( $P_{\text{trend}} = 0.26$ ). Compared with men in the lowest tertile of ionized serum calcium, the RR for fatal prostate cancer among men in the highest tertile was 3.18 (95% CI 1.09-9.28). Adjustment for BMI did not substantially alter this risk (RR, 3.12; 95% CI, 1.04-9.43). The RR for men in the second tertile was 1.82 (95% CI, 0.64-5.12). The linear trend was not statistically significant ( $P_{\text{trend}} = 0.15$ ; Table 2).

## Discussion

We found a doubling of risk for fatal prostate cancer among men in the highest tertile of total serum calcium and a tripling of risk for men in the highest tertile of ionized serum calcium. The results for total serum calcium are consistent with our previous findings for prostate cancer mortality in NHANES I in which we observed a multivariable-adjusted RR of 2.68 (5). This is the first study to examine prostate cancer risk in relation to prediagnostic levels of ionized serum calcium.

**Table 2. Relative hazards for prostate cancer mortality by tertiles of total and ionized serum calcium in NHANES III**

	Tertile of serum calcium			P trend
	Tertile 1	Tertile 2	Tertile 3	
Total serum calcium (mmol/L)				
Median (range; mmol/L)	2.22 (1.06-2.27)	2.31 (2.28-2.35)	2.41 (2.36-3.06)	
No. deaths	9	8	8	
Person-months at risk	218,964	216,797	243,725	
Age-adjusted relative hazards	1.00 (reference)	1.39 (0.59-3.30)	2.07 (1.06-4.04)	0.258
+BMI	1.00 (reference)	1.36 (0.57-3.26)	2.02 (1.02-4.01)	0.287
Ionized serum calcium (mmol/L)				
Median (range; mmol/L)	1.19 (0.99-1.21)	1.24 (1.22-1.25)	1.28 (1.26-1.60)	
No. deaths	6	8	11	
Person-months at risk	199,120	252,180	228,186	
Age-adjusted relative hazards	1.00 (reference)	1.82 (0.64-5.12)	3.18 (1.09-9.28)	0.152
+BMI	1.00 (reference)	1.81 (0.63-5.20)	3.12 (1.04-9.43)	0.160

NOTE: In addition to adjustment for age (1-y intervals) and BMI (as a continuous measure), all models are adjusted for variables used in NHANES III sample weighting; an interaction between senior status (age, >60 y) and race or ethnicity, household size, and general health status (excellent, very good, good, fair, or poor). Models account for clustering of observations in complex sampling design of NHANES III. Ionized serum calcium is pH corrected.

Each individual is believed to have his or her own set point for serum calcium that is under genetic control (9). The concentration of ionized serum calcium in a given individual normally does not deviate by >2% from its set point (10). Conversely, there is considerable variation in calcium levels between individuals, with normal levels of total serum calcium ranging from 8.5 to 10.5 mg/dL (2.1-2.6 mmol/L; ref. 11).

This study has several strengths. It is hypothesis testing, is prospective, and uses a population that is representative of the U.S. population. The greatest threat to the validity of prospective cohort studies, loss to follow-up for mortality, was very low [4 of 6,714 (0.06%)]. Measurement of calcium in serum preceded death from prostate cancer by an average of 5.3 years (SD 2.5). Because the latency between blood draw and death was relatively short, the presence of occult prostate cancer could have influenced the levels of calcium in serum (i.e., "reverse causality"). However, this possibility likely would reduce the relative risks because men with advanced prostate cancer typically are normocalcemic or hypocalcemic due to the transfer of calcium ions from serum into bony metastases (12, 13). To address this possibility, we analyzed the data after excluding fatal prostate cancers that occurred within 36 months of the baseline examination. Compared with men in the lowest tertile of ionized serum calcium, the relative hazard for fatal prostate cancer for men in the middle tertile was 2.58 (95% CI, 0.66-10.02) and for men in the highest tertile was 4.65 (95% CI, 1.40-15.49). Thus, reverse causality is an unlikely explanation for the positive association between ionized serum calcium and prostate cancer mortality.

Our results could be influenced by misclassification and by confounding. The use of total serum calcium as an exposure variable is subject to misclassification because ~50% of the total calcium in serum is non-ionized and is physiologically inert (11). The potential misclassification associated with ionized serum calcium, the physiologically active fraction of total serum calcium, is far smaller. This is consistent with our finding of a substantially higher relative risk of prostate cancer in relation to ionized serum calcium than in relation to total serum calcium (RR, 3.2 versus 2.1, respectively).

Fatal prostate cancer was measured using record linkage between NHANES III and the National Death Index. To preserve the privacy of participants in NHANES III, an algorithm applied to the Public Use Mortality Data files randomly reassigned the cause of death for some participants, a procedure that may have introduced some misclassification. Because the misclassification was nondifferential with respect to exposure, it should underestimate the true association between serum calcium and prostate cancer mortality. Finally, several epidemiologic studies have implicated vitamin D deficiency as a risk factor for prostate cancer (2). Vitamin D deficiency is unlikely to confound the observed association between prostate cancer and high serum calcium because vitamin D deficiency is associated with normocalcemia or hypocalcemia (11).

In persons without cancer, most cases of serum calcium that are elevated outside the reference range are caused by elevated serum levels of parathyroid hormone; (PTH), i.e., primary hyperparathyroidism (11). Similarly, some persons with serum calcium levels

that are elevated but within the reference range may have "high normal" levels of serum PTH. It is therefore unclear whether the mechanism for the increased risk of fatal prostate cancer that we observed in association with elevated serum calcium involves elevated serum calcium, elevated serum PTH, or both factors. There are plausible mechanisms by which either high levels of calcium or PTH could each increase prostate cancer mortality (14). For example, prostate cancer cells express the calcium-sensing receptor and the common receptor for PTH and parathyroid hormone-related protein (PTHrP). At physiologic levels, both calcium and PTH promote the proliferation and metastasis of prostate cancer cells (15, 16).

Relatively small differences in the levels of ionized calcium in serum were associated with large differences in the risk of fatal prostate cancer. The effects of small differences in the levels of ionized serum calcium could be magnified at sites of bony metastases via feed-forward mechanism. As shown by Sanders et al., ionized calcium in serum induces prostate cancer cells to synthesize PTHrP, which causes local osteolysis of bone, resulting in greatly elevated levels of extracellular calcium within the bony microenvironment (16). Whereas ionized calcium in serum normally circulates at 1.1 to 1.3 mmol/L, levels of extracellular calcium in the bony microenvironment of metastatic prostate cancer cells may reach 8 to 40 mmol/L (17). These high levels of extracellular calcium then elicit further secretion of PTHrP by prostate cancer cells which exacerbates the progression of osteolytic and osteoblastic metastases (18).

Our results may help to resolve some discrepant findings in the literature on dietary calcium and prostate cancer risk (4, 19, 20, 21). Several studies have reported a positive association between high levels of dietary calcium, from food and from supplements, and advanced and/or fatal prostate cancer. For example, in the Health Professionals Follow-up Study, Giovannucci et al. found a significantly increased risk of advanced and fatal prostate cancer at calcium intakes of 1,500 to 1,999 mg/d with greatest risk at intakes >2,000 mg/d (20). (The recommended allowance for calcium for men ages 51 years and older is 1,200 mg/d.) Conversely, most epidemiologic studies reporting more moderate intakes of dietary calcium have not found significantly elevated risks (19).

Calcium levels in serum are tightly controlled over a wide range of dietary calcium and generally are not correlated with dietary intake of calcium (22, 23). However, the consumption of large quantities of calcium (e.g., via self-medication with calcium supplements, such as calcium-containing antacids) can cause hypercalcemia in some individuals (24). Thus, we hypothesize that calcium intakes that exceed the recommended allowance may increase the risk of fatal prostate cancer because these intakes may increase the levels of calcium in serum.

Our results are based on a small number of deaths ( $n = 25$ ) and the confidence intervals around the relative risks are relatively large. Thus, these findings require confirmation by other prospective studies. If confirmed, our finding that prediagnostic levels of ionized calcium in serum strongly predict risk of fatal prostate cancer has important implications for clinical decision making and for prostate cancer prevention. For example, most incident prostate cancers that are detected using

prostate-specific antigen screening would not prove fatal if untreated. Thus, deciding which screen-detected cancers need treatment is intensely problematic (25). High levels of serum calcium significantly predicted fatal but not incident prostate cancer in NHANES I and significantly predicted fatal prostate cancer in NHANES III (for which data on incident cancers are not available). This suggests that in addition to other clinical variables, the knowledge of a man's serum calcium level could contribute to informed decisions about whether his cancer is likely to be life-threatening and therefore require therapy. Because the relative risks associated with elevated serum calcium are high and because levels of calcium in serum can be changed (13), further study of the role of serum calcium in the natural history of prostate cancer should be a research priority.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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