

BOTH HIGH AND LOW LEVELS OF BLOOD VITAMIN D ARE ASSOCIATED WITH A HIGHER PROSTATE CANCER RISK: A LONGITUDINAL, NESTED CASE-CONTROL STUDY IN THE NORDIC COUNTRIES

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Vitamin D inhibits the development and growth of prostate cancer cells. Epidemiologic results on serum vitamin D levels and prostate cancer risk have, however, been inconsistent. We conducted a longitudinal nested case-control study on Nordic men (Norway, Finland and Sweden) using serum banks of 200,000 samples. We studied serum 25(OH)-vitamin D levels of 622 prostate cancer cases and 1,451 matched controls and found that both low (≤ 19 nmol/l) and high (≥ 80 nmol/l) 25(OH)-vitamin D serum concentrations are associated with higher prostate cancer risk. The normal average serum concentration of 25(OH)-vitamin D (40–60 nmol/l) comprises the lowest risk of prostate cancer. The U-shaped risk of prostate cancer might be due to similar 1,25-dihydroxyvitamin D₃ availability within the prostate: low vitamin D serum concentration apparently leads to a low tissue concentration and to weakened mitotic control of target cells, whereas a high vitamin D level might lead to vitamin D resistance through increased inactivation by enhanced expression of 24-hydroxylase. It is recommended that vitamin D deficiency be supplemented, but too high vitamin D serum level might also enhance cancer development.

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Key words: prostate cancer; 25(OH)-vitamin D₃; serum bank

Vitamin D deficiency has been implicated as risk factor for prostate cancer.¹ In cell culture studies, vitamin D metabolites have had protective action against cancer development (for review see Ylikomi *et al.*²). Normal and malignant prostate cells contain vitamin D receptor (VDR),^{3–5} which mediates the antiproliferative action of 1,25(OH)₂-vitamin D₃.⁶ In addition to the antiproliferative action of 1,25(OH)₂-vitamin D₃, it can cause apoptosis,⁷ induce differentiation,⁸ inhibit telomerase expression,⁹ inhibit tumor cell invasiveness¹⁰ and suppress tumor-induced angiogenesis.¹¹

Several epidemiologic studies have reported that high serum vitamin D levels or sunlight may protect against prostate cancer.^{3,4,12–15} Factors that affect prostate cancer include age, dark skin and environment, *e.g.*, latitude and diet.¹⁶ These factors might be linked to vitamin D availability.^{17,18} Furthermore, high fish (rich in vitamin D) consumption appears to correlate with lower prostate cancer risk.¹⁹ In addition, VDR gene polymorphism may contribute to the risk of prostate cancer.^{20–24} There is also a study showing no correlation between serum vitamin D metabolites and prostate cancer in Maryland (USA),²⁵ but the authors concluded that the power of their study was limited. In another study on U.S. male physicians, only a weak protection against prostate cancer was found with the highest quartile of serum 1 α ,25(OH)₂-vitamin D₃.²⁶ Similarly, no correlation was found in Hawaii.²⁷

There are 2 physiologically interesting metabolites of vitamin D, 1 α ,25(OH)₂-vitamin D₃, regulating calcium homeostasis for bones and muscles in extremely narrow limits, and 25(OH)-vitamin D₃, regulating target (prostate) cell proliferation and differentiation through activation to 1 α ,25(OH)₂-vitamin D₃ in the target (prostate) cell. Serum 25(OH)-vitamin D₃ is produced by liver 25-hydroxylase, the rate of the synthesis being directly proportional to vitamin D₃ serum concentration.²⁸ Therefore, serum 25(OH)-vitamin D₃ reflects vitamin D availability in the organism. Serum concentration of 25(OH)-vitamin D₃ is so high that it might possess a significant biologic activity in target cells, but it is also a precursor for the biologically more active 1 α ,25(OH)₂-vitamin D₃. Prostate as well as many other target organs can activate 25(OH)-vitamin D₃ through 1 α -hydroxylation^{29,30} and inactivate it through 24-hydroxylation.³¹

In an epidemiologic study, we found that low concentrations (< 40 nmol/l) of 25(OH)-vitamin D in serum were associated with a 1.7-fold increased risk of prostate cancer.^{3,4} Since the power of our study was limited, preventing extensive analysis of the data, and we are partners in the Nordic Specimen Banks for Cancer Causes and Control, we had an opportunity to extend our study to other Scandinavian countries located geographically at the same latitude. Our aim was to determine whether our finding could be replicated in a larger study of subjects with great variation in vitamin D exposure.

MATERIAL AND METHODS

We used data from prospective cohorts of more than 200,000 men who had donated blood samples stored in biobanks in Finland, Norway and Sweden.

Grant sponsor: Nordic Cancer Union; Grant sponsor: Nordic Academy of Advanced Studies; Grant sponsor: Finnish Cancer Foundation; Grant sponsor: Academy of Finland; Grant sponsor: University Hospital of Tampere; Grant sponsor: Swedish Cancer Society.

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Received 28 January 2003; Accepted 30 April 2003

DOI 10.1002/ijc.11375

The cohorts

In Finland, the cohort consisted of approximately 19,000 men who attended the first screening visit within the Helsinki Heart Study. In this clinical trial, the effect of gemfibrozil, a drug that modulates lipid levels, was investigated with regard to coronary heart disease.^{3,4,32} The participants, middle-aged (40–58 years at onset) employees in 2 governmental agencies and 5 industrial companies, were recruited between 1981 and 1982. A blood sample was drawn from participants in the morning, and serum samples were stored at -20°C . Most samples were collected during the winter months.

The Janus Project in Norway was started in 1973 and contains blood samples from about 160,000 men.³³ Samples were collected from men who participated in county health examinations, mostly for cardiovascular diseases, and from blood donors. Participants were recruited from several counties in Norway. Blood donors were from the Red Cross Blood Donor Center in Oslo. Blood collection took place during office hours, and serum samples were stored at -25°C .

In Sweden, men in The Northern Sweden Health and Disease Cohort are recruited through the Västerbotten Intervention Project (VIP), and the northern Sweden part of the WHO study for Monitoring of Trends and Cardiovascular Disease Study (MONICA).³⁴ The VIP started in 1985 and is still ongoing; each year all residents of Västerbotten County aged 40, 50 and 60 years are invited by letter to participate in a health-promoting project with the aim of reducing cardiovascular disease and cancer. MONICA recruited participants in 1986, 1990 and 1994. Together these 2 projects have recruited about 30,000 men as a representative population sample from the counties of Västerbotten and Norrbotten. For the large majority of participants, blood collection took place in the morning, and plasma samples were stored at -80°C .

All participants signed an informed consent form, and the study was approved by each respective local ethical committee.

Case ascertainment and control selection

All incident cases of prostate cancer and all cases of death were identified through linkage with national cancer registries in 1997, using a nationwide individual identification number as the identity link. If several samples were available for the same case subject, the first sample was chosen. The study design was that 4 control subjects for each case subject were selected within each cohort from all members alive and free of cancer at the time of diagnosis of the case, by matching on age (± 2 years) and date (± 2 months, in Norway ± 6 months) of the blood sampling, country and the region inside the country. Some of the stored frozen Finnish samples were accidentally thawed and matched for thawing.^{3,4} Controls were randomly selected within each group of eligible subjects if more than 4 subjects matched the case were identified. If 4 control subjects were not found in this procedure, fewer were accepted. Due to great seasonal variation in vitamin D levels, it was additionally required that the blood from the case subject and its controls should be sampled during the same season of the year. This restriction entailed that from the Finnish cohort 132 case subjects from originally 140, from the Norwegian cohort 404 from 575 and from the Swedish cohort 86 from 87 were available for study. Altogether, 622 case subjects and 1,451 matched control subjects were available for analysis.

Serum concentrations of 25-hydroxyvitamin D₃/D₂ were analyzed by radioimmunoassay (Incstar, Stillwater, MN). Samples were analyzed blinded, without knowledge of case-control status, during the same day and using the same lot of the assay kit. The coefficients of intra- and interassay variations for the 25(OH)-vitamin D assay were 8.5% and 16%, respectively. Cut-off points of the quartiles of 25(OH)-vitamin D concentration were based on the total original cohort.

Statistical methods

All analyses of the association of 25(OH)-vitamin D level and risk of prostate cancer were performed using conditional logistic regression analysis so that the matching status could be maintained. In this kind of analysis, the differences between the case's and the control's value of the explanatory variable (here, vitamin level) are considered instead of the actual vitamin levels. This is an advantage when strong potential confounders are matched for. As the vitamin D levels differed between the national study groups, we also used pooled data to investigate the number of cases in different intervals of vitamin D level. Analyses were performed using the SAS program package, version 8.1 (SAS Institute, Cary, NC).

RESULTS

Study characteristics

The distribution of the study characteristics is shown in Table I. The Swedish men were the oldest, and the Norwegians (mean age) were the youngest. The lag time between sampling and diagnosis was shortest among Swedish (≤ 9 years) and Finnish (≤ 14 years) cases, while in 71% of the Norwegian study group it was > 14 years. Tumor stage distribution was 65.8% localized, 24.6% non-localized and 9.6% not determined. The age distribution of men at diagnosis of prostate cancer was similar in all countries.

Serum 25(OH)-vitamin D concentrations

There was large seasonal variation in vitamin D levels, the highest concentrations being observed in the summer and the lowest concentrations in the spring (Table II). Mean levels of vitamin D were, in the Finnish material, 42 nmol/l; in the Swedish, 53 nmol/l; and in the Norwegian, 55 nmol/l.

Serum vitamin D levels and risk of prostate cancer

First, prostate cancer risk in relation to serum levels of vitamin D was analyzed using quintiles of serum vitamin D levels. In the Finnish study group, an increased risk was seen for the lowest

TABLE I—CASES WITH PROSTATE CANCER BY STUDY CHARACTERISTICS AND COUNTRY

Characteristic	Country			
	All countries (n = 622)	Norway (n = 404)	Finland (n = 132)	Sweden (n = 86)
Age at serum sampling (years)				
<40	16	16	0	0
40–44	121	109	11	1
45–50	292	248	35	9
51–60	185	31	86	68
>60	8	0	0	8
Lag between sampling and diagnosis (years)				
<5	90	16	12	62
5–9	93	40	29	24
10–14	154	63	91	0
15–19	178	178	0	0
≥ 20	107	107	0	0
Time of serum sampling				
Before	378	378	0	0
1980				
1980–1984	135	3	132	0
1985–1989	31	22	0	9
After 1989	78	1	0	77
Tumor stage				
Localized	409	282	64	63
Nonlocalized	153	105	34	14
Unknown	60	17	34	9
Age at diagnosis (years)				
<50	15	13	1	1
50–54	36	24	7	5
55–59	88	51	33	4
60–64	247	147	46	54
65–69	204	143	44	17
≥ 70	32	26	1	5

TABLE II – MEANS AND SDS OF 25(OH)-VITAMIN D (NMOL/L) BY SEASON AND COUNTRY

Season (month)	Country							
	All countries		Norway		Finland		Sweden	
	Number	Mean (SD)	Number	Mean (SD)	Number	Mean (SD)	Number	Mean (SD)
Winter (12, 1, 2)	727	47 (18.5)	258	50 (19.8)	352	43 (17.4)	117	51 (16.2)
Spring (3–5)	563	44 (17.7)	200	46 (17.8)	218	39 (17.9)	145	49 (15.1)
Summer (6–8)	143	62 (19.7)	111	61 (20.1)	—	—	32	64 (18.4)
Autumn (9–11)	640	59 (17.6)	508	59 (18.0)	18	70 (12.3)	114	58 (16.2)
All seasons (1–12)	2,073	51 (19.4)	1,077	55 (19.5)	588	42 (18.2)	408	53 (16.6)

TABLE III – OR AND 95% CI OF PROSTATE CANCER BY 25(OH)-VITAMIN D LEVEL AND COUNTRY

Vitamin D level (nmol/l)	All countries		Norway		Finland		Sweden	
	Number of cases	OR (CI)	Number of cases	OR (CI)	Number of cases	OR (CI)	Number of cases	OR (CI)
≤ 19	19	1.5 (0.8–2.7)	5	0.9 (0.3–2.8)	13	2.4 (1.1–5.1)	1	1.3 (0.1–12.5)
20–39	169	1.3 (0.98–1.6)	89	1.2 (0.9–1.7)	68	1.9 (1.1–3.1)	12	0.7 (0.3–1.4)
40–59 (ref.)	229	1	155	1	29	1	45	1
60–79	138	1.2 (0.9–1.5)	98	1.2 (0.8–1.7)	18	1.4 (0.7–2.8)	22	1.0 (0.5–1.8)
≥80	67	1.7 (1.1–2.4)	57	1.8 (1.1–2.8)	4	1.2 (0.4–3.8)	6	1.5 (0.5–4.4)

compared to the highest quintile [odds ratio (OR) = 1.9, 95% confidence interval (CI) 0.97–3.7], whereas in the Norwegian and Swedish study groups, the pattern of risk was reversed: increased risks were seen for the highest compared to the lowest quintiles (OR = 1.4, 95% CI 0.9–2.1 and OR = 1.7, 95% CI 0.7–3.9, respectively).

As the distribution of vitamin D concentration (as well as quintile limits) differed largely between the national study groups and the limits within the 3 middle groups were physiologically rather small, we analyzed the full study group using the same 5 intervals of vitamin D serum level for each country and fixing the third interval (40–59 nmol/l) as the reference group (Table III). In the full study group, a U-shaped prostate cancer risk was observed, with an increasing trend of risk (ORs = 1.3 and 1.5) when the vitamin D level decreased from the reference level of 40–59 nmol/l. Again, when the vitamin D level increased from the reference level, risk increased (ORs = 1.2 and 1.7). In the Finnish study group, significantly increased risks (ORs = 1.9 and 2.4) were seen with the 2 lowest serum vitamin D level groups; also, with a high vitamin D serum concentration, the cancer risk increased, but the increase was not statistically significant. In the Norwegian study group, a significantly increased risk was seen at the highest vitamin D serum values (OR = 1.8, 95% CI 1.1–2.8). In the Swedish study group, no statistically significant differences were seen, but the trend was similar to other groups. The association of the high and low vitamin D concentration with prostate cancer was similar for localized and nonlocalized cancers.

Lag time

To explore the direction of causality, we repeated the analysis after excluding cases with a short lag time and their matched controls (Table IV). The Swedish material was excluded since it contained only short lag times. ORs associated with high levels of vitamin D did not decrease with the longer lag time, but those associated with low vitamin D levels decreased, suggesting that cancer existing at the time of serum sampling may affect serum vitamin D level.

DISCUSSION

In this large longitudinal nested case-control study within 3 Nordic biobanks, we observed that both low and high serum concentrations of 25(OH)-vitamin D appear to be risk factors for prostate cancer. The U-shaped risk of prostate cancer cannot be found in a small sample or when the variation of vitamin D serum concentrations is small. This may explain why some earlier epidemiologic studies failed to show any correlation between serum

vitamin D and prostate cancer risk. Furthermore, in most studies, high and low vitamin D serum concentrations are compared, whereas we used the normal average concentration (40–60 nmol/l) as the reference group.

It is possible that vitamin D serum concentrations are associated with some other factors influencing prostate cancer risk. Because vitamin D interacts strongly with calcium and phosphate, which are known to be prostate cancer risk factors,^{35,36} it is possible that vitamin D acts indirectly. Since a portion of vitamin D comes from dietary sources, it could be associated with dietary factors affecting prostate cancer risk.³⁷

Methodologic considerations

We used data from biobanks in Norway, Finland and Sweden, with serum samples from more than 200,000 subjects. This is the largest study so far on vitamin D and prostate cancer risk, identifying 622 prostate cancer cases and comparing them to 1,451 matched controls. Our study shows that without simultaneous availability of data from different populations it would not be possible to detect the U-shaped association. Our study also shows that research on the vitamin D–prostate cancer association based on a separate homogenous population might yield a positive or negative or “no association” result, all depending on how the vitamin D values of that population are centered. Indeed, both association between prostate cancer risk and serum vitamin D^{3,4,12,14,15,38} and no association^{25–27} have been found. On the basis of the present results, the prostate cancer risk is U-shaped; therefore, the reference serum level should be the normal average level (40–60 nmol/l).

The sera were stored for several years at freezing temperatures. We have not found any significant effect of the storage time on vitamin D values, when samples are stored properly and protected against UV light. Indeed, our preliminary study in Finland suggests that serum concentrations of vitamin D are lower today than 20 years ago. Some of the Finnish samples were accidentally thawed during storage, but this had an insignificant effect on vitamin D concentration.^{3,4}

Factors influencing vitamin D concentration in serum

The Norwegian and Swedish men in our study had significantly higher serum vitamin D values than the Finnish men, most probably due to the higher consumption of fish by Norwegians and Swedes and fish liver oil by Norwegians than Finns. Since all Nordic countries have had vitamin D–fortified, low-fat milk products for a long time, this cannot explain the differences between countries; but the explanation is most likely the other sources of

TABLE IV – OR AND 95% CI OF PROSTATE CANCER BY 25(OH)-VITAMIN D LEVEL, COUNTRY AND LAG TIME (FROM SAMPLING TO DIAGNOSIS)

Vitamin D level (nmol/l)	All countries				Norway				Finland			
	Lagtime ≤ 10 years		Lagtime > 10 years		Lagtime ≤ 10 years		Lagtime > 10 years		Lagtime ≤ 10 years		Lagtime > 10 years	
	Number of cases	OR (CI)	Number of cases	OR (CI)	Number of cases	OR (CI)	Number of cases	OR (CI)	Number of cases	OR (CI)	Number of cases	OR (CI)
≤ 19	10	2.6 (1.1–6.2)	9	1.0 (0.4–2.2)	1	3.1 (0.2–53.2)	4	0.8 (0.2–2.6)	8	4.2 (1.4–12.5)	5	1.4 (0.4–4.3)
20–39	56	1.2 (0.8–1.9)	113	1.3 (0.9–1.8)	19	1.7 (0.8–3.9)	70	1.1 (0.8–1.7)	25	2.1 (0.9–4.7)	43	1.7 (0.9–3.3)
40–59 (ref.)	81	1	148	1	25	1	130	1	11	1	18	1
60–79	43	1.1 (0.7–1.8)	95	1.2 (0.9–1.7)	13	1.2 (0.5–2.9)	85	1.2 (0.8–1.7)	8	1.9 (0.6–5.5)	10	1.2 (0.5–2.9)
≥80	15	1.4 (0.7–2.7)	52	1.8 (1.1–2.9)	7	1.6 (0.5–5.1)	50	1.8 (1.1–3.0)	2	1.0 (0.2–5.3)	2	1.4 (0.3–7.4)

vitamin D. High fish consumption has been associated with a reduced risk of prostate cancer.¹⁹ Furthermore, fish consumption is highest in northern Norway and the prostate cancer incidence is lower in northern Norway compared to southern Norway, which is the opposite of the prostate cancer distribution by latitude in the other parts of the world.¹⁴ However, serum concentrations of 25(OH)-vitamin D₃ >75 nmol/l can be reached commonly during summer through UV-B radiation from the sun. After the initial increase of vitamin D levels, the skin and adipose tissue begin isomerization and storage of excess vitamin D and serum concentration levels decrease.³⁹ Only a small proportion of the high vitamin D values in Norwegians can be explained by the fact that more Norwegian serum samples were collected during the summer. A benefit of the present study is that the material was collected from countries with similar sun exposure. Our results from Nordic men cannot be compared to those from men living in southern areas.²⁷

Influence of vitamin D deficiency on prostate cancer risk

A dominating hypothesis has been that vitamin D deficiency increases prostate cancer risk.^{1,3,4,12,14,15,38} This has been supported by *in vitro* studies demonstrating that 1 α ,25(OH)₂-vitamin D₃ is a potent antiproliferative hormone.^{2,40,41} In prostate and breast cancer cell cultures, the effect of 1 α ,25(OH)₂-vitamin D₃ is concentration-dependent so that low concentrations are mitogenic whereas high ones are antiproliferative.^{40,41} Several epidemiologic studies suggest that low serum vitamin D is associated with an increased prostate cancer risk.^{1,3,4,12,14,15,38} However, this association should be interpreted with caution as, in the present study, the lag time had a significant effect on ORs. In our study, the ORs associated with low levels of vitamin D were high if the lag time between blood sampling and diagnosis was ≤10 years but considerably lower if the lag time was >10 years. One possible explanation would be that existing preclinical prostate cancer could lower vitamin D levels by changing vitamin D metabolism.^{29,42} Another explanation could be that low vitamin D levels influence more the late than the early stages of carcinogenesis. This idea is also supported by experimental data that low vitamin D concentration enhances dedifferentiation, cancer invasion and angiogenesis.^{8,10,11} Therefore, it is possible that low serum vitamin D enhances accumulation of mutations during the progression of the cancer.

Influence of high vitamin D supply on prostate cancer risk

The increased prostate cancer risk associated with high vitamin D concentration is unexpected and difficult to explain on the basis of experimental results. It is interesting that, in contrast, the association of high vitamin D levels with prostate cancer risk was not dependent on the lag time between serum sampling and diagnosis. This suggests that low and high vitamin D levels may exert their effects *via* quite different mechanisms. There are no experimental data supporting the idea that high vitamin D concentration en-

hances cancer development. In contrast, many studies suggest that the beneficial effects of vitamin D against cancer are more prominent with increasing vitamin D concentrations.² High vitamin D supply may be associated with other risk factors, eliminating the putative protective effect of vitamin D. Some food sources of lipid-soluble vitamin D are also rich in lipid-soluble vitamin A, a risk factor for prostate cancer.⁴³ It is also possible that a high concentration of vitamin D may itself be a risk factor for prostate cancer. High vitamin D concentrations may affect vitamin D metabolism within the target tissue, leading to increased 24-hydroxylation.³¹ This means reduced concentration of the biologically active 1 α ,25(OH)₂-vitamin D₃ in the prostate, which in turn leads to weak antiproliferative action. Since prostate tissue and cells are able to activate 25(OH)-vitamin D₃^{29,30} and inactivate all metabolites *via* 24-hydroxylation,³¹ local vitamin D metabolism is crucially important for the final response to serum 25(OH)-vitamin D₃. Therefore, it is necessary to define 2 vitamin D endocrine systems: one based on the kidney hormone 1 α ,25(OH)₂-vitamin D₃, regulating calcium homeostasis, and the second based on the liver hormone 25(OH)-vitamin D₃, regulating target cell differentiation and proliferation. In this model, target cells regulate their own vitamin D balance, and therefore, both low and high concentrations of circulating vitamin D can cause imbalance. Prostate normal and cancer cells might have increased vitamin D inactivation by 24-hydroxylase,³¹ and thus, vitamin D is not able to control mitotic activity. The significance of 24-hydroxylase on cancer progression is evident, and amplification of the gene has shown that it is oncogenic.⁴⁴ The final result of the increased activity of 24-hydroxylase will be local vitamin D deficiency and resistance.

Implications for clinical use of vitamin D

Since there are plans for prostate cancer prevention with vitamin D supplementation alone or combined,⁴⁵ the present findings might be an important contribution to the strategy because very high serum vitamin D levels may not be the appropriate goal. Very high 25(OH)-vitamin D serum levels can be reached without any significant side effects for short periods.²⁸ Recommendations for vitamin D supplements have been put forward, and the recommended vitamin D doses are steadily increasing. However, synthetic vitamin D derivatives are recommended for cancer prevention because of their low calcemic effects. Because they are slowly inactivated in the living organism and may increase vitamin D exposure, their use in cancer prevention should be considered with caution; our study suggests that moderately high levels of vitamin D for long periods may have adverse effects on prostate cancer risk. Therefore, other carefully planned studies on vitamin D and prostate cancer risk are needed to conclusively elucidate this issue.

ACKNOWLEDGEMENTS

This is study number 25 from the Nordic Biological Specimen Bank Working Group on Cancer Causes and Control.

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