An association of serum vitamin D concentrations < 40 nmol/L with acute respiratory tract infection in young Finnish men[1–3]

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ABSTRACT

Background: The effects of vitamin D in regulating bone mineralization are well documented. The action of vitamin D as a key link between Toll-like receptor activation and antibacterial responses in innate immunity has recently been shown. The data suggest that differences in the ability of human populations to produce vitamin D may contribute to susceptibility to microbial infection.

Objective: We aimed to explore whether an association exists between vitamin D insufficiency and acute respiratory tract infection in young Finnish men.

Design: Young Finnish men ($n = 800$) serving on a military base in Finland were enrolled for this study. Their serum 25-hydroxyvitamin [25(OH)D] concentrations were measured in July 2002. They were followed for 6 mo, and the number of days of absence from duty due to respiratory infection were counted.

Results: The mean ($\pm$ SD) serum 25(OH)D concentrations were 80.2 ± 29.3 nmol/L ($n = 756$). Subjects with serum 25(OH)D concentrations < 40 nmol/L ($n = 24$) had significantly ($P = 0.004$) more days of absence from duty due to respiratory infection (median: 4; quartile 1–quartile 3: 2–6) than did control subjects (2; 0–4; $n = 628$; incidence rate ratio 1.63; 95% CI: 1.15, 2.24). We found a significant ($P = 0.004$) association between serum 25(OH)D concentrations and the amount of physical exercise before induction into military service. We also found significantly ($P < 0.001$) lower serum 25(OH)D concentrations in subjects who smoked (72.8 ± 26.6 nmol/L; $n = 192$) than in control subjects (82.9 ± 29.7 nmol/L; $n = 537$).

Conclusion: Clinical trials of vitamin D supplementation are needed to investigate whether it enhances immunity to microbial infections. *Am J Clin Nutr* 2007;86:714–7.

KEY WORDS Vitamin D, respiratory infection, insufficiency, men, public health, 25-hydroxyvitamin D

INTRODUCTION

Vitamin D is produced in the skin through a photolytic reaction of 7-dehydrocholesterol induced by ultraviolet B (UVB) radiation (290–315 nm). It also occurs naturally in foods. The metabolite formed in the skin and the vitamin D absorbed in the gut must be hydroxylated in the liver to form 25-hydroxyvitamin D [25(OH)D] and then hydroxylated in the kidney to form 1α,25-dihydroxyvitamin D [1,25$(OH)_2$D] (1). After these transformations, vitamin D is a biologically active substance, a hormone that is chemically akin to steroid hormones. The main function of vitamin D in the body is to regulate calcium and phosphorous homeostasis, a process essential for bone mineralization (2). Vitamin D deficiency is known to lead to secondary hyperparathyroidism, which causes rickets in children and osteomalacia and osteoporosis in adults (3).

To determine vitamin D status, the serum concentration of 25(OH)D, the major circulating form of the hormone, must be measured (4). Vitamin D insufficiency is usually determined by parathyroid hormone (PTH) secretion, which increases when serum 25(OH)D concentrations fall below 78–100 nmol/L (5, 6). There is a growing scientific consensus that vitamin D insufficiency is reached at serum 25(OH)D concentrations < 80 nmol/L (7, 8).

In northern climates, diet is the most important source of vitamin D in the winter months, because exposure to sunlight is limited, which results in inadequate endogenous production of vitamin D. In Finland, with its geographical location between 60° and 70° N, vitamin D insufficiency is common in all age groups between October and March (9, 10).

New, diverse roles of vitamin D have also been discovered, including its antiproliferative, differentiative, and apoptotic effects on prostate cancer cells in vitro and its effects on cancer invasion and angiogenesis. Recently, Liu et al (11) showed that the action of vitamin D was a key link between Toll-like receptor (TLR) activation and antibacterial responses in innate immunity. They showed a dose-dependent up-regulation of one known antimicrobial peptide (cathelicidin) in human monocytes. The addition of 1,25$(OH)_2$D to primary human macrophages infected with *Mycobacterium tuberculosis* reduced the number of viable bacilli. The addition of either 25(OH)D or TLR alone had

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Received January 19, 2007.
Accepted for publication April 20, 2007.

no effect, but their simultaneous addition up-regulated cathelicidin mRNA. Furthermore, the specific inhibition of 1-hydroxylase enzyme reduced this antimicrobial activity by 70%, which suggests that $1,25(OH)_2D$ is the active form of vitamin D. Liu et al also found that the induction of cathelicidin mRNA was significantly lower in serum from African Americans, which contained less 25(OH)D, than in the serum from white persons. However, 25(OH)D supplementation of serum from African Americans restored the TLR induction of cathelicidin mRNA (11).

Clarification of the role of vitamin D in relation to infections, such as acute respiratory tract infections, deserves a high priority (12). The aim of the present study was to explore whether an association exists between vitamin D insufficiency and acute respiratory tract infections in young Finnish men.

SUBJECTS AND METHODS

Subjects

Young Finnish men serving on a military base in southwestern Finland ($n = 800$) in July 2002 were enrolled for this study. When they entered military service, their medical examination found them to be healthy. Conscripts taking vitamin D supplements or having traveled in sunny countries during the preceding 3 mo were excluded from the study. The study population and the military conditions with respect to physical activity, nutrition, clothing, living quarters, and exposure to sunlight were homogenous.

All volunteers provided written informed consent. The Ethics Committee of Pirkanmaa Hospital District approved the study.

Identification of respiratory infections

Medical records for all participants covering the first 6 mo of military service were abstracted, and physician-diagnosed respiratory tract infections (ie, sinusitis, tonsillitis, otitis, bronchitis, pneumonia, pharyngitis, and laryngitis) were recorded, and the number of days of absence from duty due to respiratory tract infection were counted.

Background information

We used a structured questionnaire to elicit information on age, smoking, amount of physical exercise before military service (h/wk), physician-diagnosed lactose intolerance, education, geographical location (northern or southern Finland), and area of residence (urban or rural). The height and weight were measured to determine the body mass index (BMI; in kg/m^2).

Serum 25-hydroxyvitamin D concentrations

Blood specimens for the measurement of serum 25(OH)D concentrations were drawn from 800 male conscripts at their entry into military service in the beginning of July 2002. Because of failed samples, incomplete follow-up data, and terminated military service, the total study population comprised 756 subjects. After coagulation at room temperature for 1 h, the samples were centrifuged at $2000 \times g$ for 20 min at room temperature for serum separation. The serum samples were frozen and stored at −20 °C for later analysis. Total serum 25(OH)D concentrations were measured with the OCTEIA enzyme immunoassay (Immunodiagnostic Systems Inc, Fountain Hills, AZ). According to the manufacturer, the cross-reactivities obtained for $25(OH)D_3$ and $25(OH)D_2$ are 100% and 75%, respectively (13).

Statistical analysis

Serum 25(OH)D concentration were expressed as means ± SDs. Differences in means between independent samples were tested by using Student's *t* test or one-way analysis of variance (ANOVA). The number of days of absence from duty due to respiratory infection was expressed as medians and lower and upper quartiles (Q1 and Q3, respectively) because the distribution was skewed. Poisson regression analysis was used to explain this variable plus 0.5, because the variable included zeros. Overdispersion was estimated by the deviance divided by its df. Serum 25(OH)D concentrations were categorized as <40 nmol/L and ≥40 nmol/L for the regression analysis.

The limit for statistical significance was set to be equal to 0.05 for a 2-sided test. We used SAS for WINDOWS software (version 8.2; SAS Institute, Cary, NC) for Poisson regression analysis and SPSS for WINDOWS software (version 11.0; SPSS Institute, Chicago, IL) for other data analysis.

RESULTS

In July 2002, the mean serum 25(OH)D concentration was 80.2 ± 29.3 nmol/L ($n = 756$). Serum 25(OH)D concentrations < 40 nmol/L were found in 3.6% of the subjects. The subjects with 25(OH)D concentrations < 40 nmol/L ($n = 24$) had significantly ($P = 0.004$) more days of absence from duty due to respiratory infections (median: 4; Q1–Q3: 2–6) than did control subjects ($n = 628$) (2; 0–4; incidence rate ratio: 1.63; 95% CI: 1.15, 2.24). The model was adjusted for smoking ($n = 169$) (**Figure 1**).

We found a significant ($P = 0.004$) association between serum 25(OH)D concentrations and the amount of previous physical exercise. Subjects who exercised > 5 h/wk before induction into the military service had higher serum 25(OH)D concentrations (85.8 ± 30.6 nmol/L) than did subjects reporting no preinduction physical exercise (71.3 ± 32.0 nmol/L). There was no significant association between the amount of physical exercise before induction and the number of days of absence from duty due to respiratory infection ($P = 0.388$; $n = 264$) (**Table 1**).

Significantly ($P < 0.001$) lower serum 25(OH)D concentrations were found in subjects who smoked (72.8 ± 26.6 nmol/L; $n = 192$) than in control subjects (82.9 ± 29.7 nmol/L; $n = 537$), and there was a trend toward a significant association ($P = 0.065$) between smoking ($n = 169$) and number of days of absence from duty due to respiratory infection (incidence rate ratio: 1.18; 95% CI: 0.988, 1.40; $n = 629$).

There was a nonsignificant association between BMI and serum 25(OH)D concentration, but no association was found between serum 25(OH)D concentration and lactose intolerance. No differences were found in vitamin D status between subjects from southern and northern Finland or between subjects living in urban and rural areas. Education level was not associated with vitamin D status (Table 1).

DISCUSSION

Our study contains several major findings. In July 2002, vitamin D insufficiency was identified in 3.6% of young Finnish men

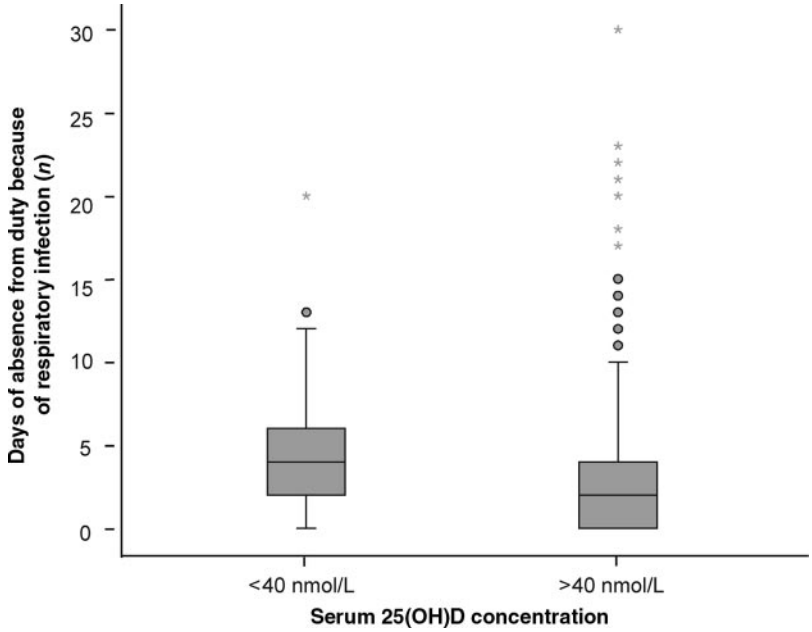

FIGURE 1. The subjects with serum concentrations of 25-hydroxyvitamin D [25(OH)D] < 40 nmol/L (n = 24) had significantly (P = 0.004) more days of absence from duty due to respiratory infections (median: 4; quartile 1–quartile 3: 2–6) than did controls (2; 0–4; incidence rate ratio: 1.63; 95% CI: 1.15, 2.24; n = 628). The model was adjusted for smoking (n = 169) (Poisson regression analysis).

TABLE 1
Summary of the associations of serum 25-hydroxyvitamin D [25(OH)D] concentrations with BMI, smoking, alcohol use, physical exercise, lactose intolerance, location of residence, area of residence, and education

	Subjects	25(OH)D concentration (nmol/L)	P
	n		
BMI (kg/m^2)			0.092
<20.0	63	76.5 ± 31.0[1]	
20.0–24.9	345	83.0 ± 30.9	
25.0–30.0	127	79.7 ± 25.8	
>30.0	29	71.4 ± 21.8	
Smoker			<0.001[2]
Yes	192	72.8 ± 26.6	
No	537	82.9 ± 29.7	
Alcohol use			0.306
<1 time/mo	39	74.2 ± 28.1	
1 time/mo	99	82.3 ± 35.6	
>1 time/mo	152	77.6 ± 27.5	
Amount of physical exercise before military service			0.004[2]
>5 h/wk	99	85.8 ± 30.6	
≤5 h/wk	96	79.6 ± 27.6	
No physical exercise	92	71.3 ± 32.0	
Lactose intolerance diagnosed			0.474
Yes	30	82.9 ± 33.5	
No	255	78.7 ± 30.4	
Location of residence			0.632
Northern Finland	35	81.1 ± 28.4	
Southern Finland	255	78.4 ± 30.9	
Area of residence			0.275
Urban	208	80.0 ± 30.1	
Rural	82	75.6 ± 31.6	
Education			0.716
No high school	127	79.3 ± 32.6	
High school or more	154	78.0 ± 29.4	

[1] $\bar{x}$ ± SD (all such values).
[2] Significantly associated with serum 25(OH)D concentrations (Student's t test and ANOVA).

who were followed for 6 mo during military service. Furthermore, the findings of the present study showed a significant negative association of serum 25(OH)D concentration with the number of days of absence from duty due to physician-diagnosed respiratory tract infection, a negative association with smoking, and a positive association with physical exercise.

All Finnish men are required to complete a 6-, 9-, or 12-mo military service between the ages of 18 and 29 y. (Military service is voluntary for women.) Each year, on average, 26 500 conscripts and 500 enlisted women undergo military training. Our study population of 756 conscripts represents homogeneity with respect to age and the conditions for physical activity, nutrition, clothing, living area, and exposure to sunlight in the military environment. Because the recruits live in close quarters, respiratory infections are common in garrisons.

The accuracy of the outcome data (the number of respiratory infections identified) with respect to absence from duty can be considered a strength of this study. Despite the fact that a relatively small number of the men had vitamin D concentrations < 40 nmo/L, we were able to show a statistically significant effect on the absence from duty due to infectious disease. Our results show that the statistical power was sufficient. Furthermore, we were able to adjust for the effect of smoking on respiratory infections. The validity of our findings was also enhanced by the comparability of the conditions during the study for all subjects.

However, the present study had several potential limitations. Because we obtained only one vitamin D measurement, the persistence of differences in vitamin D status during the study was not evaluated. The present study was not randomized, and the validity requires comparability of the groups with different concentrations of vitamin D. Although vitamin D was also correlated with the amount of previous physical exercise, the latter was not associated with infections and therefore did not confound the results.

There is also strong evidence that, to avoid secondary hyperparathyroidism and the resulting increase in calcium release and bone turnover rate, serum 25(OH)D concentrations should be > 100 nmol/L (5, 9, 14, 15). Only 20.1% of the subjects in the present study population fulfilled that criterion in July 2002. The evidence that serum 25(OH)D concentrations decrease somewhat with aging is suggestive of a potential risk of osteoporosis later in life (9, 16).

According to the findings from the present study, it seems likely that, in terms of adequate sunlight exposure, cutaneous synthesis of vitamin D is sufficient in Finland during the summer. In the winter months, however, vitamin D insufficiency in Finland is very common (9, 10). Since February 2003, on the basis of recommendations of the Ministry of Social Affairs and Health, vitamin D has been added to commercial milk and dairy products (0.5 μg/100 mL) and to margarine (10 μg/100 g) in Finland. This fortification is part of a national health policy aimed at increasing vitamin D intake through diet to 280 IU/d. In a recent study, we (17) showed that the mean wintertime serum 25(OH)D concentrations increased by 50% after implementation of the vitamin D fortification recommendations. Correspondingly, the prevalence of serum 25(OH)D concentrations < 40 nmol/L decreased by 50%, from 78% to 35%. Despite these efforts, however, vitamin D concentrations remained low in one-third of young Finnish men during the winter months. It should be noted that the present study was conducted before the fortification recommendations were implemented.

On the basis of the present study's finding that low vitamin D status at initial entry into military service and subsequent respiratory infections are statistically significantly related, it seems evident that vitamin D insufficiency contributes to proneness to these diseases. Taking into account the geographical position of Finland, which extends from the 60th to the 70th northern parallel, we expected to see regional differences in the subjects' vitamin D concentrations. However, we found no significant differences in serum 25(OH)D concentrations between subjects from northern and southern Finland.

The findings from the present study contribute to the diversity of consequences already known to result from vitamin D insufficiency and recognized as carrying significant global public health implications. In the context of immune function, clarification of the role of vitamin D in relation to infections, such as acute respiratory tract infections, represents a high priority for future research. Furthermore, consideration must be given to clinical trials of vitamin D supplementation to investigate whether it enhances immunity to microbial infections.

We thank the conscripts who agreed to participate in the study and Hilkka Mäkinen for excellent technical assistance.

The authors' responsibilities were as follows—IL (principal investigator): the idea for the study, preparation of the study protocol, and writing the draft of the manuscript; IL, JR, and HP: data collection; IL, JR, HP, PT, and TY: coordinated the study and obtained funding; RH and AA: statistical expertise; TY, HP, JR, RH, and AA: contributed to the writing and revision of the manuscript; and HP and TY: study supervision study. None of the authors had a personal or financial conflict of interest.

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