CLINICAL STUDY

Serum 25(OH)D₃ vitamin status of elderly Finnish women is suboptimal even after summer sunshine but is not associated with bone density or turnover

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Abstract

Objective: Concentrations of 50 and 75 nmol/l are proposed as serum 25-hydroxyvitamin D (25(OH)D) target for older people from the view of bone health. We evaluated vitamin D status of elderly Finnish women in light of these definitions, its relationship to bone mineral density (BMD) and turnover, and improvement by summer sunshine.

Design: Population-based study.

Methods: A total of 1604 ambulatory women aged 62–79 years were studied; 66% used vitamin D supplements. Serum 25(OH)D₃ was measured with HPLC before and after summer, and heel BMD in spring. In subgroups, serum parathyroid hormone (PTH) and type I procollagen aminoterminal propeptide (PINP) were analyzed.

Results: In spring, 60.3% of the women had 25(OH)D₃ ≤ 50 nmol/l, and the target of 75 nmol/l was reached by 9.1%. For supplement users, the respective numbers were 52.1 and 11.9%. Serum 25(OH)D₃ did not determine BMD or bone turnover measured by serum PINP. Summer sunshine increased serum 25(OH)D₃ by 17.4% ($P < 0.0001$), but in autumn 84% of the subjects remained under the target of 75 nmol/l. In supplement users, PTH remained stable but decreased in others during summer ($P = 0.025$).

Conclusions: Vitamin D status of elderly Finnish women is suboptimal if 25(OH)D₃ levels of 50 or 75 nmol/l are used as a threshold. It is moderately increased by supplement intake and summer sunshine. However, 25(OH)D₃ concentrations did not influence bone density in terms of serum PINP and bone turnover rate.

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Introduction

Vitamin D is essential for bone health. Severe deficiency causes rickets in children and osteomalacia in adults, while milder insufficiency contributes to the development of osteoporosis. Low vitamin D levels have been connected to an increased risk for type 1 diabetes, multiple sclerosis, rheumatoid arthritis, many cancers, and cardiovascular diseases (1).

With respect to bone health, agreement exists that serum 25-hydroxyvitamin D (25(OH)D) concentration should be above 25 nmol/l. In 2005, a quasi-consensus of vitamin D experts proposed target levels of 50 and 75 nmol/l for serum 25(OH)D concentration, at which older men and women will be at a lower risk of fracture (2). In the latest meta-analysis of intervention studies among older individuals, vitamin D supplementation of 482–770 IU daily decreased fractures; the achieved

25(OH)D levels in the treatment groups ranged from 62 to 112 nmol/l (3). In white adults, hip bone mineral density (BMD) has been shown to increase by increasing vitamin D concentration in some studies (4) but not all studies (5). The relationship between serum 25(OH)D concentration and fracture risk has remained unclear (6, 7) but a recent epidemiologic study reported a lower risk for hip fracture with 25(OH)D levels above 60 nmol/l (8).

One definition of optimal vitamin D status is based on the 25(OH)D level that reduces serum parathyroid hormone (PTH) levels to a plateau, but the estimates of the optimal level using this definition have ranged from 37.5 nmol/l in the study of Thomas *et al.* (9) up to 110 nmol/l in other studies (10–14). Also another definition of optimal vitamin D status, increase in fractional calcium absorption by vitamin repletion, is confusing: Heaney *et al.* (15) reported optimal

concentration to be 78 nmol/l, but Hansen *et al.* (16) showed only 3% increase in calcium absorption with vitamin D repletion, thus questioning the definition of vitamin D insufficiency by calcium absorption. Recently, Need *et al.* (17) reported calcium malabsorption from vitamin D deficiency occurring at the level of 10 nmol/l or below.

Discrepancies between studies defining skeletal benefits of vitamin D may be due to differences in calcium intake. Even in vitamin D-insufficient patients (defined as 25(OH)D < 60 nmol/l), vitamin D supplementation did not add any skeletal benefit (measured by bone structure, bone formation markers, and intestinal calcium absorption) over 1000 mg calcium supplementation alone (18).

Imprecision of different 25(OH)D assays makes the definition of optimal vitamin D level difficult. It is well known that the results produced by different immunological assays are variable, and even by the same assay from different laboratories (19, 20). Accurate methods to measure serum 25(OH)D are absolutely necessary. HPLC and liquid chromatography–mass spectrometry have become the reference methods to assay 25(OH)D (20–23).

The present study was undertaken to evaluate vitamin D status of Finnish postmenopausal women with an HPLC assay. We studied a population-based cohort of 1604 who were participants or controls in an ongoing study aiming at preventing fractures by an intervention program. Vitamin D status was related to serum concentrations of PTH and type I procollagen aminoterminal propeptide (PINP), in the first place a marker of bone formation, but also through the coupling phenomenon of bone resorption, and of heel BMD measured by dual-energy X-ray absorptiometry (DXA). We also describe the changes in 25(OH)D₃ levels over a sunny summer at a high latitude.

Subjects and methods

Subjects and study design

The subjects are participating in an ongoing controlled study aiming at preventing fractures by an intervention program that affects lifestyle. For the basic study, inclusion criteria were female gender, 60–70 years of age, and living in the Uusimaa region (in southern Finland, latitude 60–61°north). From 1996 to 2000, we recruited 2181 women (52% of those initially invited) from the population to register randomly as the intervention and control groups. For the intervention group, 400 IU of vitamin D was recommended as a daily supplement during winter. From 2003, this recommendation has been 800 IU/day over the year. Calcium intake of 1000 mg/day from diet or supplements was recommended. The control group would get information through media and health

care system. Any fracture is an end point during the ten-year follow-up.

All participants and controls were asked to provide a serum sample from February to May 2002 and again from September to November 2002, and had a heel BMD measurement in spring 2002. Altogether 1604 women, 820 (82%) in the intervention group and 784 (67%) in the control group participated in this vitamin D study 2–6 years after entering the main study.

This analysis comprises serum 25(OH)D₃ measurements for 1543 women in spring and for 1401 women in autumn. Among these women, 1340 had pair samples in both spring and autumn. In a subgroup of 353 women, serum PTH was assayed in pair samples, and out of these, serum PINP measurements were performed in 292 women. The Ethics Committee of Department of Medicine, Helsinki University Hospital approved the study. All participants gave informed written consent.

Lifestyle factors

Current use of vitamin D supplements was assessed by a questionnaire, which addressed the use of fish products scaled as irregular, regular with 1–2 times or 3 or more times/week, and the use of margarine (yes or no), which was the only food fortified by vitamin D in 2002. Sunlight exposure was estimated as daily outdoor stay during summertime (yes or no). Calcium intake was calculated on the basis of the supply from dairy products using the following estimations: 1 dl of milk, sour milk, and yogurt contains 120 mg of calcium, and one slice of cheese 100 mg. Smoking status was assessed by asking whether the participants had ever smoked, were ex-smokers or current smokers. Physical activity was assessed by asking how many times/week the subjects spent at least half an hour on walking, gymnastics, swimming, bicycling, or other sports. Regarding estrogen use, the women were divided into current users, ex-users, or never users.

Laboratory assessments

Serum 25(OH)D₃ was determined by the HPLC method published earlier (21) with minor modifications of the chromatography. The separation of 25(OH)D₃ and 25(OH)D₂ was achieved by using a Discovery HS F5 column (Supelco, Bellefonte, PA, USA). The column dimensions were 250 × 4 mm with 5 µm particle size, and the mobile phase was 80% methanol in water. The sensitivity for both metabolites is 3 nmol/l (signal-to-noise ratio 1:3), and exact detection limit is 10 nmol/l (signal-to-noise ratio 10:1). Within the concentration range of 2–167 nmol/l, within-assay coefficient of variation (CV) was <5.8% and total CV was <7.4%. A few samples contained detectable amounts of D₂ form, although about 60% of subjects used vitamin D

supplements, and the sensitivity of our HPLC method for 25(OH)D₂ was adequate. Therefore, only the results of 25(OH)D₃ were taken into account in calculations. Negligible 25(OH)D₂ levels are in accordance with the fact that most vitamin D supplements in Europe contain cholecalciferol instead of ergocalciferol, used in the United States.

Serum PTH was quantitated with an automatic immunoanalyzer (IMMULITE 2000 Intact PTH, DPC, Los Angeles, CA, USA). Detection limit of the assay was 3 ng/l. Intra-assay CV was <7% in the range 70–660 ng/l, and inter-assay CV was <9% in the range 50–390 ng/l. Serum PINP was determined by a competitive RIA with a commercial kit (Intact PINP RIA) from Orion Diagnostica, Oulunsalo, Finland. Analytical sensitivity of this assay was 2 µg/l, and intra- and inter-assay CV ranged from 2 to 6%.

DXA measurement

Heel BMD was measured using a densitometer from GE Medical Systems, LUNAR, PIXI (Wisconsin, MA, USA). The precision of the method (CV) was 2.0%.

Definition for vitamin D status

Vitamin D deficiency was defined as serum 25(OH)D₃ lower than 25 nmol/l, and vitamin D insufficiency was defined as 25(OH)D₃ between 25 and 50 nmol/l.

The target of 75 nmol/l was taken from the expert meeting, where five out of six experts proposed this threshold (2).

Sun exposure

The Finnish Meteorological Institute provided the number of sunshine hours at Helsinki–Vantaa observational station (60° North) during summer 2002 and the long-term mean values for the same area.

Statistical methods

Variables are reported as medians with ranges or interquartiles. The participants were divided into two categories based on the use of vitamin D supplements (users and nonusers). Comparisons between the groups and between spring and autumn measurements were performed using nonparametric Mann–Whitney *U* test. χ^2 test was used to compare the proportions. Heel BMD was analyzed using a multiple regression model by adjusting for age, height, weight, smoking, exercise, calcium intake, and estrogen use together with either 25(OH)D₃ or PTH or PINP. In addition, a multiple regression model was used when 25(OH)D₃ was handled as a categorical factor, using cutoff limits of 20, 37.5, 50, or 75 nmol/l. All tests were performed as two-sided with a significance level of 0.05. Analyses were done with SPSS 13.0 version (Chicago, IL, USA) and or with SAS System (Cary, NC, USA; version 9.13 for Windows).

Table 1 Characteristics (median with range or number with percentage) of the whole study population and of users and nonusers of vitamin D supplements in spring.

	All (<i>n</i> =1604)	Nonusers (<i>n</i> =550)	Users (<i>n</i> =1054)	<i>P</i> value
Age (years)	68.0 (62.0–78.9)	67.9 (62.0–78.9)	68.1 (62.1–76)	0.22
Height (cm)	162 (143–182)	162 (143–178)	162 (144–182)	0.25
Weight (kg)	69 (36–128)	70 (42–128)	68 (36–115)	<0.0001
Calcium intake (mg/day)	830 (0–5100)	780 (0–2720)	840 (0–5100)	0.013
Physical activity (times/week)	5 (0–7)	5 (0–7)	5 (0–7)	0.06
Alcohol consumption ^a (g/week)	42 (0–441)	42 (0–367)	42 (0–441)	0.67
Heel bone mineral density (g/cm ²)	0.488 (0.175–0.918)	0.495 (0.182–0.918)	0.486 (0.175–0.878)	0.059
Use of estrogens				
No	789 (49)	310 (56)	479 (45)	<0.0001
Ex-user	283 (18)	82 (15)	201 (19)	
User	532 (33)	158 (29)	374 (35)	
Smoking				
No	1174 (73)	400 (73)	774 (73)	0.65
Ex-smoker	284 (18)	95 (17)	189 (18)	
Smoker	146 (9)	55 (10)	91 (9)	
Use of fish (times/week)				
3 or more	236 (15)	65 (12)	171 (16)	0.03
1–2 times	1003 (63)	364 (66)	639 (61)	
Irregular use	363 (23)	120 (22)	243 (23)	
Use of margarines				
Yes	1207 (75)	412 (75)	795 (75)	0.82
No	397 (25)	138 (25)	259 (25)	
Outdoors daily				
Yes	1494 (93)	509 (93)	985 (94)	0.52
No	105 (7)	39 (7)	66 (6)	

P values for comparisons between nonusers and users of vitamin D supplements.

^aForty-seven percent of nonusers and 53% of users of vitamin D supplements answered the question.

Results

Table 1 summarizes the characteristics of participants in spring 2002. All women were Caucasians and 66% reported use of vitamin D supplements. Vitamin D supplement users weighed less ($P<0.0001$), reported higher calcium intake ($P=0.013$), and used estrogens ($P<0.0001$) and fish products ($P=0.03$) more frequently than nonusers.

Vitamin D status in spring

The median 25(OH)D₃ level for the study group ($n=1543$) was 45 nmol/l (range <10–124), lower in the supplement nonusers 38 nmol/l (range <10–101) than in the supplement users 49.5 nmol/l (range <10–124) ($P<0.0001$). The prevalence of vitamin D deficiency (25(OH)D₃ ≤ 25 nmol/l) was 8.6% and insufficiency (25(OH)D₃ 25–50 nmol/l) was 51.7%. Among nonusers and users of supplements, the prevalence of vitamin D deficiency was 16.2 and 4.8% and that of vitamin D insufficiency was 60.3 and 47.3% respectively. The target level of 75 nmol/l was reached by 9.1% of all women, 3.7% of the nonusers, and 11.9% of the users of supplements.

Heel BMD

There existed no correlation between heel BMD and 25(OH)D₃ ($r=-0.04$, $P=0.15$) or PTH ($r=-0.01$, $P=0.79$), but heel BMD was inversely related to serum PINP ($r=-0.19$, $P=0.0007$). In a multiple regression model, significant negative determinants of heel BMD were age ($P<0.0001$), smoking ($P=0.022$), and serum PINP ($P=0.029$), and positive ones were weight ($P<0.0001$) and estrogen use ($P<0.0001$). Serum 25(OH)D₃ ($P=0.54$), height ($P=0.13$), calcium intake ($P=0.17$), exercise ($P=0.87$), or PTH ($P=0.51$) did not affect heel BMD at all. Adjusted heel BMD was similar below or equal to and above different cutoff points of vitamin D adequacy (Table 2).

Bone turnover

There was no relationship between 25(OH)D₃ and bone turnover in terms of serum PINP.

Table 2 Heel bone mineral density (g/cm²) adjusted for age, height, weight, calcium intake, smoking, exercise, and estrogen use below or equal to and above different cutoff limits of vitamin D adequacy (least square means (S.E.M)).

Cutoff limit (nmol/l)	Below or equal to	Above	P
25	0.474 (0.007)	0.488 (0.004)	0.23
37.5	0.485 (0.005)	0.488 (0.004)	0.59
50	0.489 (0.004)	0.484 (0.005)	0.79
75	0.487 (0.004)	0.492 (0.009)	0.55

Figure 1 The relationship between serum 25(OH)D₃ and PTH concentrations in spring.

Serum PTH

There existed an inverse correlation between 25(OH)D₃ and PTH both in spring ($r=-0.217$, $P<0.0001$; Fig. 1) and in autumn ($r=-0.169$, $P<0.0001$). PTH was related to PINP in spring ($r=0.164$, $P=0.005$), but not in autumn.

Effect of summer

Solar radiation In 2002, the mean number of sunshine hours was 296 in June, 294 in July, and 317 in August, totalling 907 h. During the reference period 1971–2000, the respective average was 767 h. Thus, in 2002, the average was exceeded by 18%.

Vitamin D status Among the 1340 participants with pair samples, median 25(OH)D₃ level was 8 nmol/l (+17.4%) higher in autumn than in spring ($P<0.0001$; Table 3). Among users ($n=902$) and nonusers ($n=438$) of supplements, the respective changes over summer were 6 nmol/l (+12.0%) ($P<0.0001$) and 13 nmol/l (+34.2%) ($P<0.0001$).

Among all the available participants in autumn ($n=1401$), the median 25(OH)D₃ level was 54 nmol/l (range <10–123), lower in supplement nonusers 51 nmol/l (range <10–121) than in supplement users 56 nmol/l (range 11–123) ($P<0.0001$). The prevalences of vitamin D deficiency and insufficiency were 3.6 and 38.4%. In autumn, 16% were at level 75 nmol/l or more. Below the target of 75 nmol/l were 87.7% of nonusers and 81.9% of users of supplements after summer.

There existed a negative correlation between serum 25(OH)D₃ concentration in spring and its change during summer ($r=-0.427$, $P<0.0001$). Overall, serum PTH concentrations decreased during summer

Table 3 Serum 25-hydroxyvitamin D₃ concentrations (median with range, nmol/l) in 1340 women with pair samples in spring and autumn.

	Spring	Autumn	% Change over summer	P
Whole group	46.0 (<10–124)	54.0 (<10–123)	+17.4	<0.0001
Users of vitamin D supplements (n=902)	50.0 (<10–124)	56.0 (11–123)	+12.0	<0.0001
Nonusers of vitamin D supplements (n=438)	38.0 (<10–101)	51.0 (<10–121)	+34.2	<0.0001

($P=0.012$ for time), but there were no statistically significant differences between the users and nonusers of supplements ($P=0.26$ for the interaction of group $\times$ time). However, when looking at within-group changes, the decrease for nonusers ($P=0.025$) but not for users ($P=0.24$) of supplements was statistically significant (Table 4). Serum PINP levels declined across the summer ($P=0.0013$ for time) in both nonusers ($P=0.018$) and users ($P=0.025$) of supplements. The interaction of time $\times$ group (=users and nonusers of supplements) was not significant ($P=0.53$), albeit in terms of medians the change appeared to be highest in nonusers of supplements (Table 4).

Weight and 25(OH)D₃ inversely correlated in both spring ($r=-0.180$, $P<0.0001$) and in autumn ($r=-0.186$, $P<0.0001$).

Discussion

In terms of the target level of 75 nmol/l for serum 25(OH)D, 9.1% of elderly Finnish women had optimal vitamin D status in spring and 16% in autumn. If the threshold was reduced to 50 nmol/l, 40% had optimal vitamin D concentration after winter in spring. Depending on the season, vitamin D supplement users had 10–30% higher concentrations than nonusers, and depending on supplement use, summer sunshine improved circulating vitamin D level by 12–34%, benefiting most those who had the lowest level in spring. Numerous epidemiological studies have previously indicated that vitamin D inadequacy is common worldwide (24–28).

Our findings do not necessarily imply that to optimize only the bone health characteristics (=muscle function not taken into account) the serum 25(OH)D level of 75 nmol/l (measured by HPLC) should be reached. We did not find any association between serum 25(OH)D₃ and heel BMD, albeit bone density was expectedly related to age, weight, estrogen use, and smoking. We were not capable of measuring central BMD, but several studies have shown the peripheral BMD to predict a fracture as well as the central measurements (29, 30). Although denied by some (31, 32), a positive relationship between 25(OH)D and BMD has been found by many authors (4, 33–38). Interestingly, in the OFELY study, there was no significant difference in BMD, radius bone loss, bone markers, or incidence of fracture between postmenopausal women with 25(OH)D levels below or above 75, 50, or 30 nmol/l (5). Among 615

community-dwelling postmenopausal women, vitamin D explained only 0.5% of the variance in total hip BMD, although 25(OH)D levels ranged from 10 to 337 nmol/l (38). In a recent Dutch study, Kuchuk *et al.* (28) did not find any association between serum 25(OH)D and heel ultrasound parameters, but total hip and femoral trochanter BMD as well as total body bone mineral content were 7–10% lower among those with 25(OH)D levels <25 nmol/l versus more than 75 nmol/l but not among those with 25(OH)D levels ranging from 25 to 75 nmol/l (28). Recently, Dawson-Hughes concluded that the effect of vitamin D on bone mass is small (1–2%) and probably not alone, but in combination with the improved muscle performance vitamin D supplementation reduces fracture risk in the elderly (39).

We reported that the use of vitamin D supplement abolished the seasonal perturbation of PTH, which has been used as one sign of vitamin D adequacy (11) and this occurred at the median 25(OH)D₃ levels of 50 nmol/l in spring and 56 nmol/l in autumn. One study indicated a change in the rate at which PTH increases, to occur when serum 25(OH)D drops below 44 nmol/l, but importantly the rate of bone loss was similar below and above this threshold (40), questioning also this concept of basing the optimization of vitamin D status on the scattergrams of PTH versus 25(OH)D.

In the present study, vitamin D status did not influence bone turnover rate in terms of serum PINP, which in a multiple regression model was an independent negative predictor of heel BMD. PINP levels declined over summer, but the changes in 25(OH)D₃ and PINP were not related to each other. Serum PTH and PINP were related in spring, and this relationship disappeared during summer, but the changes in PTH and PINP over summer did not correlate (data not shown).

Table 4 Seasonal variation in serum parathyroid hormone (PTH) and type I procollagen aminoterminal propeptide (PINP) concentrations (median with interquartile ranges) in nonusers and users of vitamin D supplements and all combined.

	n	Spring	Autumn	P
PTH (ng/l)				
All subjects	353	44.2 (32.8–60.6)	41.1 (30.9–57.4)	0.012
Non-users	119	48.7 (34.6–62.3)	40.8 (32.0–59.1)	0.025
Users	234	42.4 (31.7–59.0)	41.7 (30.5–55.6)	0.24
PINP (µg/l)				
All subjects	292	39.0 (28.0–54.0)	38.0 (25.0–52.0)	0.0013
Nonusers	99	45.0 (27.0–60.0)	40.0 (24.2–55.0)	0.018
Users	193	38.0 (28.0–52.5)	37.0 (26.0–51.5)	0.025

Hence, the seasonal variation in serum PINP was not explained by vitamin D status or changes in PTH, and had to be ascribed to other factors.

Our findings do not support the view that from bone characteristics point of view only 25(OH)D₃ levels of elderly Finnish women should be raised, albeit a recent meta-analysis pointed out that vitamin D in daily doses of over 400 IU prevents bone fractures (3). Reduction of falls is one benefit of vitamin D supplementation (41). In a Dutch study 25(OH)D concentrations below 50 nmol/l were associated with poorer physical performance and a greater decline in performance in older persons (42). Thus, the reduction in fracture risk might be ascribed rather to extraskeletal than to skeletal effects of vitamin D.

There is a general belief that vitamin D status of the Finns is much improved by summer, but this has been documented only in young people (43, 44). The exceptionally high amount of sunshine in summer 2002 raised median 25(OH)D₃ level by 8 nmol/l (17%), benefiting more nonusers (34%) than users (12%) of vitamin D supplements. Keeping in line with previous findings (45), the change over summer was the higher, the lower serum 25(OH)D₃ level was in spring. Importantly, 25(OH)D₃ concentrations in supplement users were at a safe level over summer, indicating that summer is not any excuse to interrupt supplementation. Furthermore, in autumn, the median 25(OH)D₃ level was only 54 nmol/l in the whole study group (56 nmol/l in supplement users), far away from the threshold of 75 nmol/l of vitamin D adequacy. These findings together with the previous ones (46, 47) support the idea that sunshine does not play a central role in providing vitamin D adequacy for postmenopausal women at the population level.

Our study has several limitations. The exact amount of vitamin D content of the supplements is unknown. Although heel BMD predicts fractures, it correlates only modestly with hip and spine BMD. Consequently, our findings do not preclude an association between 25(OH)D₃ and central BMD. Although PINP is a general marker of bone turnover, measuring a direct marker of bone resorption would have added the value of our study. The strengths of our investigation are its population-based nature, a great number of participants, and a reliable HPLC method used to assay serum 25(OH)D₃ concentrations.

In conclusion, independent of whether a concentration of 50 or 75 nmol/l of serum 25(OH)D is used as a threshold of vitamin D adequacy, vitamin D status of elderly Finnish women is suboptimal. It can be moderately improved by summer sunshine and use of vitamin D supplements. Approximately a half of the present population reached the level of 50 nmol/l, which in line with a very recent Dutch study (28) might be enough in terms of bone health characteristics only. More rigorous use of vitamin D supplements and fortification of food than that started in February 2003,

which already has appeared insufficient (48), are warranted to improve vitamin D status among Finnish elderly women.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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