Vitamin D Levels and Dietary Intake Among Patients with Benign Soft Tissue Tumors and Sarcomas

ASTA JUZENIENE[1], ALINA CARMEN POROJNICU[1], ZIVILE BATURAITE[1], ZOYA LAGUNOVA[1], LAGE AKSNES[2,3], ØYVIND SVERRE BRULAND[4,5] and JOHAN MOAN[1,6]

Departments of [1]Radiation Biology and [5]Oncology, Oslo University Hospital, The Norwegian Radium Hospital, Montebello, Oslo, Norway;
[2]Department of Paediatrics, Haukeland University Hospital, Bergen, Norway;
[3]Department of Clinical Science, University of Bergen, Norway;
[4]Institute of Clinical Medicine, Faculty of Medicine, and [6]Institute of Physics, University of Oslo, Oslo, Norway

Abstract. *Background: Calcitriol [1,25(OH)$_2$D] is hypothesized to lower the risk of cancer via binding to the vitamin D receptor (VDR). VDRs are also found in benign and malignant cells of mesenchymal origin. To our knowledge, vitamin D levels and dietary intake have not been previously evaluated in patients newly diagnosed with benign and malignant mesenchymal tumors. Patients and Methods: Forty-eight patients with benign soft tissue tumors and 25 patients with sarcoma had their serum 25-hydroxyvitamin D [25(OH)D], 1,25(OH)$_2$D and parathyroid hormone levels measured, vitamin D intake scored and body mass index [BMI] calculated. Results: Vitamin D deficiency [25(OH)D level <50 nmol/l] was observed in 19% and 28% of patients with benign tumor and sarcoma, respectively. Conclusion: Serum 25(OH)D, 1,25(OH)$_2$D and parathyroid hormone concentrations, BMI and daily vitamin D intake did not differ significantly between the two groups of patients. Higher vitamin D intake or UV exposure is needed to ensure that all patients achieve sufficient vitamin D levels.*

Sarcomas are an a heterogeneous group of mesenchymal neoplasms, including more than 100 different histotypes, traditionally divided into two main groups: soft tissue and bone sarcomas (1, 2). Sarcoma incidence has been slowly increasing among Scandinavians of both sexes since the 1960s (3, 4). Sarcomas represent only around 1% of all malignancies in adults, but they may be highly aggressive (1, 2, 5, 6). The 5-year survival is around 65% for the most recent period (3, 4). Current therapies are still associated with high rates of recurrence (5-7).

Accumulating evidence from experimental and epidemiological studies suggests that vitamin D deficiency might be a causal risk factor for several types of cancer (colorectal, breast, ovarian), affecting both incidence and mortality (8-12).

Calcitriol, also known as 1,25-dihydroxyvitamin D [1,25(OH)$_2$D], regulates gene expression and may play a role in cell differentiation and growth, both *in vitro* and *in vivo* (13, 14). Many epithelial cell types express vitamin D receptors [VDRs], which, when bound to 1,25(OH)$_2$D, either to promote or suppress gene expression, thereby inducing cell differentiation, inhibiting proliferation, angiogenesis, invasiveness, and metastatic potential, as well as increasing calcitriol degradation (13, 15, 16).

Measurement of serum 25-hydroxyvitamin D [calcidiol, 25(OH)D] concentration is the best indicator to assess vitamin D status resulting from both sunlight exposure and dietary vitamin D intake over long periods of time (17). Vitamin D and calcitriol have shorter half-lives (24 and 4 h, respectively) compared to three weeks for 25(OH)D, thus making the concentration in serum dependent on the most recent exposure to sunlight and vitamin D ingestion (17). The serum concentration of 1,25(OH)$_2$D is 1,000 times lower than that of 25(OH)D (17). Additionally, the production of 1,25(OH)$_2$D is tightly regulated and stimulated primarily by serum parathyroid hormone [PTH] (17).

VDRs have been found in benign and malignant cells of mesenchymal origin, and 1,25(OH)$_2$D has been shown to have antiproliferative effects in these cells (18, 19). The existence of VDR in benign soft tissue tumors and sarcomas suggests that vitamin D may play a role in the evolution of these diseases. Whether vitamin D is associated with reduced risk of benign soft tissue tumor and sarcoma remains unclear.

Correspondence to: Asta Juzeniene, Department of Radiation Biology, The Institute for Cancer Research, The Norwegian Radium Hospital, Montebello, 0310 Oslo, Norway. Tel: +47 22781200, Fax: +47 22934271, e-mail: astaj@rr-research.no

Key Words: 25-Hydroxyvitamin D, 1,25-dihydroxyvitamin D, parathyroid hormone, daily vitamin D intake, benign soft tissue tumor, sarcoma.

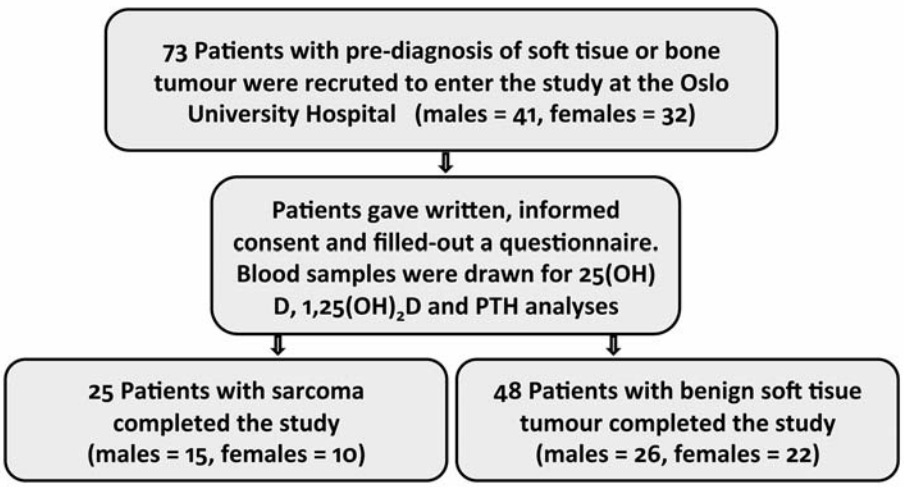

Figure 1. *Diagram of the study design. 25(OH)D: 25-hydroxyvitamin D; 1,25(OH)$_2$D: 1,25-dihydroxyvitamin D; PTH: parathyroid hormone.*

To our knowledge, no study has been published examining the vitamin D status in patients with benign soft tissue tumor or sarcoma. The goal of this study was to determine serum 25(OH)D, 1,25(OH)$_2$D and PTH concentrations and to calculate vitamin D intake in patients newly diagnosed with benign soft tissue tumor or sarcoma.

Materials and Methods

Patients. The study was approved by the Regional Ethical Committee (Ref. nr.: 2011/449), and was conducted at the Norwegian Radium Hospital which is a part of Oslo University hospital. Patients were recruited between August 1, 2011 and January 31, 2012. Any patient over 18 years of age with a soft tissue or bone tumor, either suspected as sarcoma or benign soft tissue tumor, regardless of gender or disease type at presentation, was considered eligible for the study. Patients were invited to participate in the study and during the first visit to hospital all patients gave written informed consent before providing a 10 ml blood sample and answering a questionnaire about age, weight, height, skin type, and information about food and dietary supplement intake to obtain estimates of total daily vitamin D intake.

Blood sampling and methods of analyses. Blood was sampled from each patient during the first visit to hospital (between August 1, 2011 and January 31, 2012) prior to any other surgical or medical intervention. Serum was separated from the blood cells by centrifugation and then frozen to −80˚C. All serum samples were analyzed in Haukeland University Hospital, Bergen, Norway.

The measurements of 25(OH)D and 1,25(OH)$_2$D were performed according to modified versions of the methods described by Aksnes *et al*. (20-22). The 25(OH)D and 1,25(OH)$_2$D assays were performed by a liquid chromatography–mass spectrometric method (LC/MSD SL; Agilent Technology, Santa Clara, California, USA) and a tandem-LC/MS method (Agilent 6410 Triple Quad LC/MS; Agilent Technology, Santa Clara, California, USA), respectively.

Vitamin D deficiency was defined as a serum 25(OH)D concentration <50 nmol/l, insufficiency as a serum 25(OH)D concentration of 50-75 nmol/l and sufficiency as a serum 25(OH)D concentrations ≥75 nmol/l (23).

Serum parathyroid hormone (PTH) levels were measured with an Immulite analyser (Diagnostic Products, Los Angeles, California, USA) on the basis of a two-site chemiluminescent immunometric assay. The Immulite analyser has a working range of 0.1-263 pmol/l.

Daily vitamin D intake. Daily vitamin D intake from food was computed using information from the questionnaire and the corresponding nutrient values reported in the Norwegian Food Composition Table (24). The questionnaire has previously been validated clinically in Norway (25). Current use, brand, and dosage of multivitamin or vitamin D supplements were recorded in the questionnaires, and daily vitamin D intake from supplements was calculated based on the data from the manufacturer.

Statistical analysis. The data were analyzed using SigmaPlot 12.5 (Systat SoftwareInc., San Jose, California, USA) for Windows. The values of the variables were presented as means±SE (standard error of the mean). The results were compared using unpaired t-tests. Multiple linear regression analyses were performed to assess the associations between serum 25(OH)D levels or age and levels of serum PTH and 1,25(OH)$_2$D, daily vitamin D intake and body mass index [BMI]. The associations among serum concentrations of 25(OH)D, 1,25(OH)$_2$D, age, BMI and daily vitamin D intake were examined by using Pearson correlation coefficients. The statistical significance level was set at $p<0.05$.

Results

A total of 73 patients (41 men and 32 women), aged between 24 and 91 years, were recruited. Based on the diagnosis, patients were divided into two groups (Figure 1): those with sarcoma (N=25) and those with benign soft tissue tumors (N=48). Twenty-three patients with soft tissue sarcoma and two with bone sarcoma participated in the study. Twenty-six (54%) patients with benign tumor and 15 (60%) with sarcoma were males.

Table I. *Characteristics of the study groups.*

Diagnosis	Benign soft tissue tumor		Sarcoma	
	Mean	SE	Mean	SE
Males	N=26		N=15	
Age, years	49.6	2.2	57.1	3.8
25(OH)D, nmol/l	64.2	3.8	56.1	4.1
$1,25(OH)_2D_3$, pmol/l	96.0	4.9	82.4	5.9
PTH, pg/ml	26.2	2.8	34.1	5.5
Vitamin D intake (total)	10.5	1.4	11.4	2.3
Vitamin D intake (food)	6.9	0.6	5.4	0.6
Vitamin D intake (supplemantation)	3.6	1.3	6.1	2.5
BMI (kg/m^2)	27.8	0.9	26.7	0.9
Females	N=22		N=10	
Age, years	54.8	2.7	73.2	5.1
25(OH)D, nmol/l	76.3	4.1	73.2	9.5
$1,25(OH)_2D_3$, pmol/l	105.7	5.5	101.6	8.7
PTH, pg/ml	27.6	2.9	37.2	7.3
Vitamin D intake	15.3	2.9	12.6	1.8
Vitamin D intake (total)				
Vitamin D intake (food)	5.5	0.7	6.8	1.7
Vitamin D intake (supplemantation)	9.8	2.7	5.9	1.5
BMI (kg/m^2)	25.8	1.1	25.1	1.1

BMI: Body mass index; 25(OH)D: 25-hydroxyvitamin D; $1,25(OH)_2D$: 1,25-dihydroxyvitamin D; PTH: parathyroid hormone; SE: standard error of the mean.

Table II. *p-Values calculated for differences in mean values (age, 25(OH)D, $1,25(OH)_2D$, PTH concentrations and vitamin D intakes) given in Table I between males and females in the sarcoma and benign soft tissue group, and between groups by unpaired t-test.*

	p-Values			
	Benign soft tissue tumor and sarcoma		Males and Females	
	Males	Females	Benign soft tissue tumor	Sarcoma
Age, years	0.078	0.001	0.141	0.017
25(OH)D, nmol/l	0.176	0.729	0.036	0.073
$1,25(OH)_2D_3$, pmol/l	0.091	0.691	0.163	0.069
PTH, pg/ml	0.171	0.272	0.736	0.733
Vitamin D intake (total)	0.860	0.760	0.671	0.305
Vitamin D intake (food)	0.126	0.730	0.013	0.718
Vitamin D intake (supplemantation)	0.394	0.983	0.045	0.424
BMI (kg/m^2)	0.295	0.983	0.064	0.379

BMI: Body mass index; 25(OH)D: 25-hydroxyvitamin D; $1,25(OH)_2D$: 1,25-dihydroxyvitamin D; PTH: parathyroid hormone.

Table I reports patient age, circulating 25(OH)D, $1,25(OH)_2D$ and PTH concentrations, BMI, vitamin D intake from food, supplements and total intake for males and females in both groups. Table II presents *p*-values for differences in mean values presented in Table I between males and females in the sarcoma or benign soft tissue group and between groups. The mean ages were 49.6 and 54.8 years for males and females with benign tumors respectively, and 57.1 and 73.2 years for males and females with sarcoma, respectively (Table I). Patients with sarcoma were older than patients with benign soft tissue tumors (63.5 *versus* 52.0 years, respectively, $p<0.05$) (Table II). Females with sarcoma were the oldest patients (73.2 years, Tables I and II).

There were no significant differences in serum 25(OH)D, $1,25(OH)_2D$ and PTH concentrations, nor in dietary, supplemental or total vitamin D intakes between the two groups (Tables I and II). The mean 25(OH)D concentration was 76.3±4.1 and 64.2±3.8 nmol/l for females and males with sarcoma, respectively, and 73.2±9.5 and 56.1±4.1 nmol/l for females and males with benign tumor, respectively. The only variable other than age that showed significant differences between genders was 25(OH)D concentration, with higher 25(OH)D levels recorded for females with benign tumor ($p=0.036$, Tables I and II).

The relationship of age and 25(OH)D with $1,25(OH)_2D$, PTH, total daily vitamin D intake and BMI was analyzed for both genders together (Figures 2 and 3) because of low numbers of patients in both groups due to the rarity of these types of disease (5,7,26-30).

Nine patients (19%) with benign tumor and seven (28%) with sarcoma had vitamin D deficiency (Figure 2). Vitamin D insufficiency was found in 21 (44%) and 12 (48%) patients and vitamin D sufficiency in 18 (37%) and six (24%) patients with benign tumor and sarcoma, respectively (Figures 2A and 3). A higher prevalence of vitamin D deficiency was observed in the sarcoma group than in the benign tumor group (19% *versus* 28%).

Serum $1,25(OH)_2D$ concentrations were in the reference range [47-163 pmol/l (31)] for 97% of the patients in both groups (Figures 2C and 3B). However, the mean serum $1,25(OH)_2D$ level was 16% higher for females compared to males from both groups ($p>0.05$).

PTH levels were in the normal range (10-65 pg/ml) (32) for 89% of all patients (Figures 2B and 3A). Nevertheless, patients with sarcoma had 30% higher PTH values ($p>0.05$).

Total intake of vitamin D was similar in both sexes and in both groups (Tables I and II). However, in the benign tumor group, men obtained more vitamin D from food ($p=0.013$), while women obtained more from supplements ($p=0.045$). The mean vitamin D intake was 12.4 μg/day (500 IU/day). Equal amounts of vitamin D intake came from food and supplements (Table I). A vitamin D intake of minimum 15 μg/day was required to achieve 25(OH)D

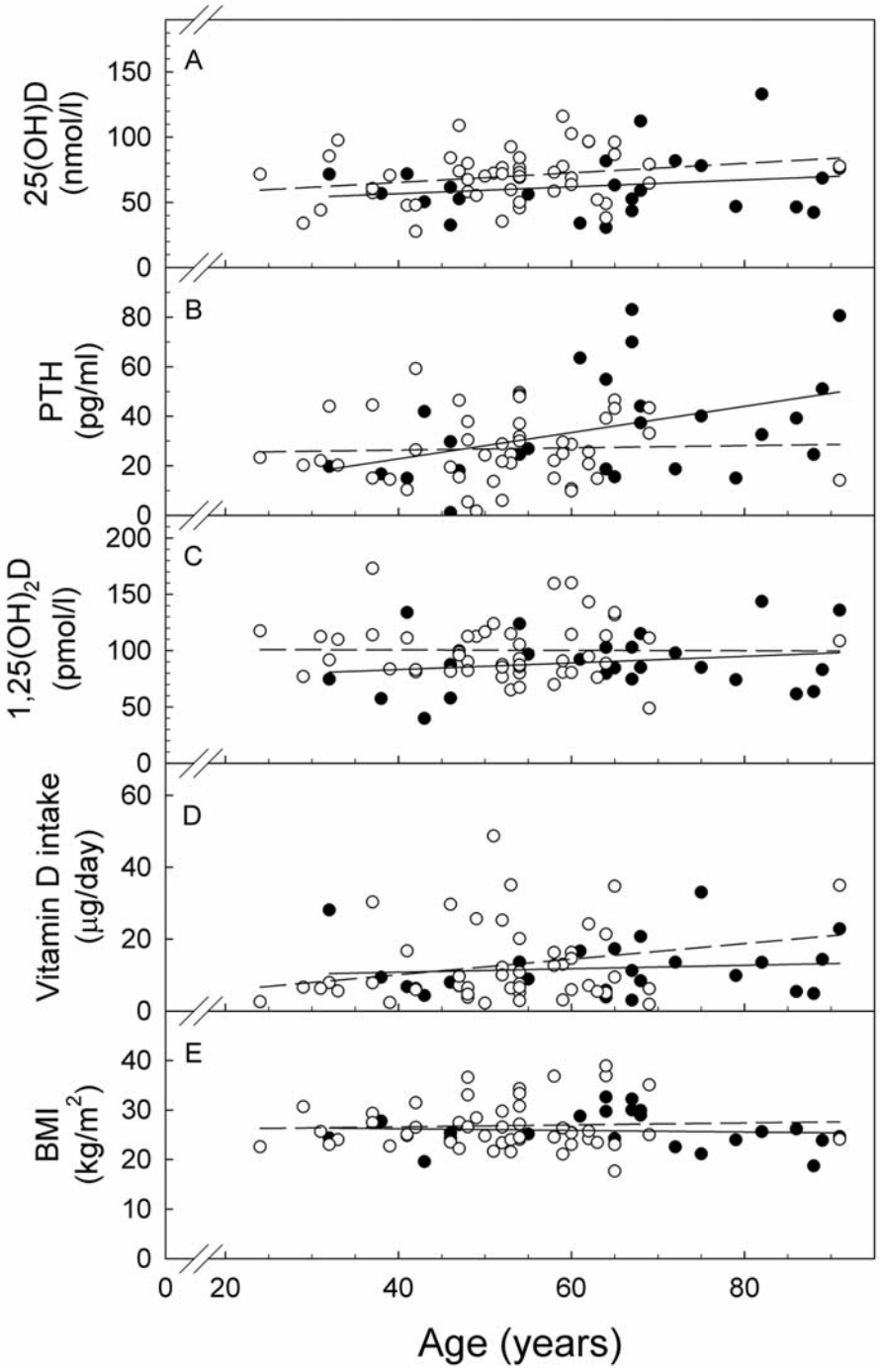

Figure 2. *Correlation of age with serum 25-hydroxyvitamin D [25(OH)D] (A), 1,25-dihydroxyvitamin D [1,25(OH)$_2$D] (B), parathyroid hormone [PTH] (C), total daily vitamin D intake (D) and body mass index [BMI] (E). Filled circles (solid line) indicate patients with sarcoma, open circles (dashed line) indicate patients with benign soft tissue sarcoma.*

levels above 75 nmol/l in both groups (Figure 3C). We observed a tendency for supplemental vitamin D intake, not dietary intake, to correlate positively with 25(OH)D level (Figure 4).

The coefficients of correlation between variables are shown in Table III. Among patients with benign tumors, 25(OH)D and 1,25(OH)$_2$D levels correlated negatively with BMI, and daily vitamin D supplement intake was positively associated

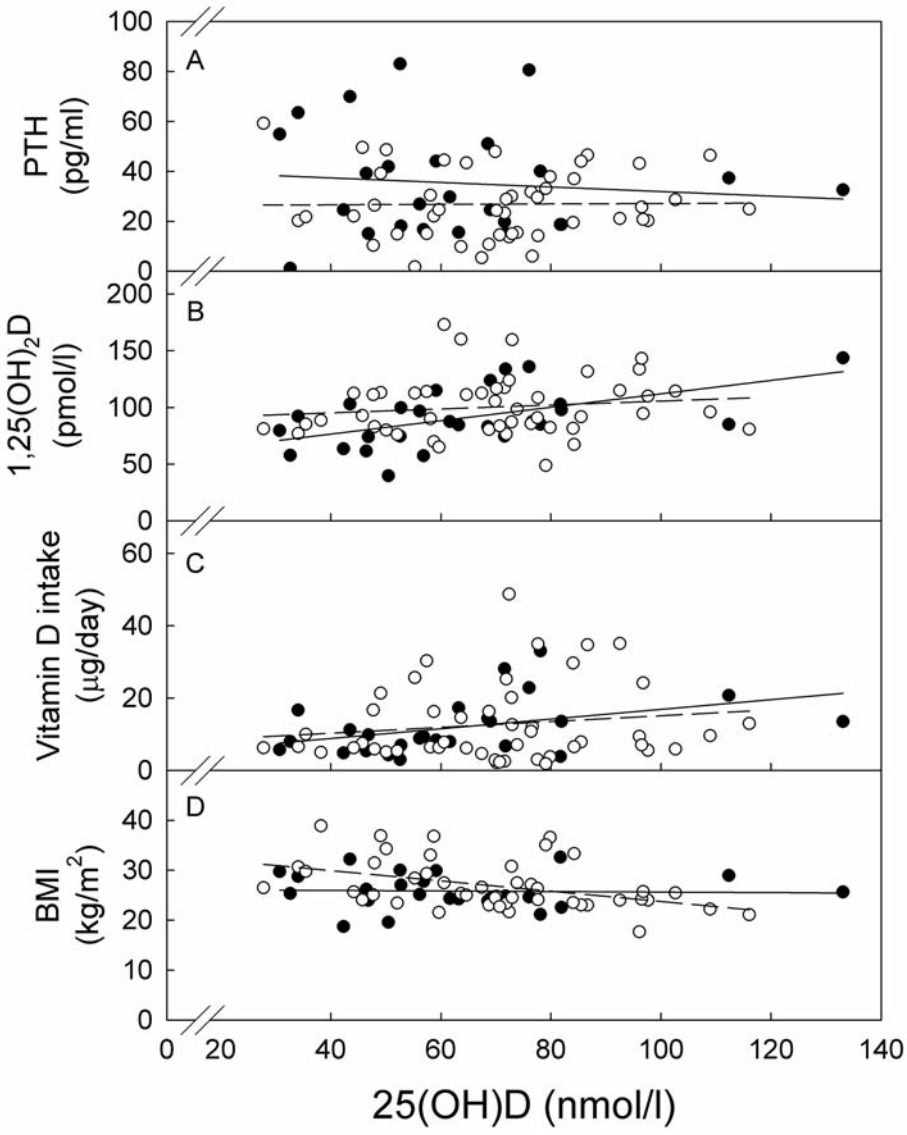

Figure 3. *Relationship of serum 25-hydroxyvitamin D [25(OH)D] with parathyroid hormone [PTH] (A), 1,25-dihydroxyvitamin D [1,25(OH)$_2$D] (B) total daily vitamin D intake (C) and body mass index [BMI] (D). Filled circles indicate patients with sarcoma (solid line), open circles indicate patients with benign soft tissue sarcoma (dashed line).*

with total vitamin D intake (Table III). Vitamin D supplements correlated negatively with BMI. Among patients with sarcoma, age, 25(OH)D level and daily supplemental vitamin D intake correlated positively with PTH, 1,25(OH)$_2$D level and total vitamin D intake, respectively (Table III).

Blood samples were collected between August 1, 2011 and January 31, 2012. In August and September, 25(OH)D values were around 16 nmol/l (25%) higher than in other months in both groups of patients, but no statistical significance was found (Figure 5A). Monthly variations of serum 1,25(OH)$_2$D and PTH concentrations were not observed (Figure 5B and C).

Discussion

Epidemiological studies reveal a relationship between insufficient 25(OH)D levels and increased risk of some types of cancer (9, 33-38). This may also be of importance in benign soft tissue tumors and sarcoma. There exist experimental evidence supporting the existence of VDR in both benign and malignant cells of mesenchymal origin, and 1,25(OH)$_2$D has been shown to have antiproliferative effects in such cells (18, 19).

Despite a clear histogenetical relationship, sarcomas are, by definition, the malignant part of soft tissue or bone tumors. If vitamin D plays a role in their prevention, we

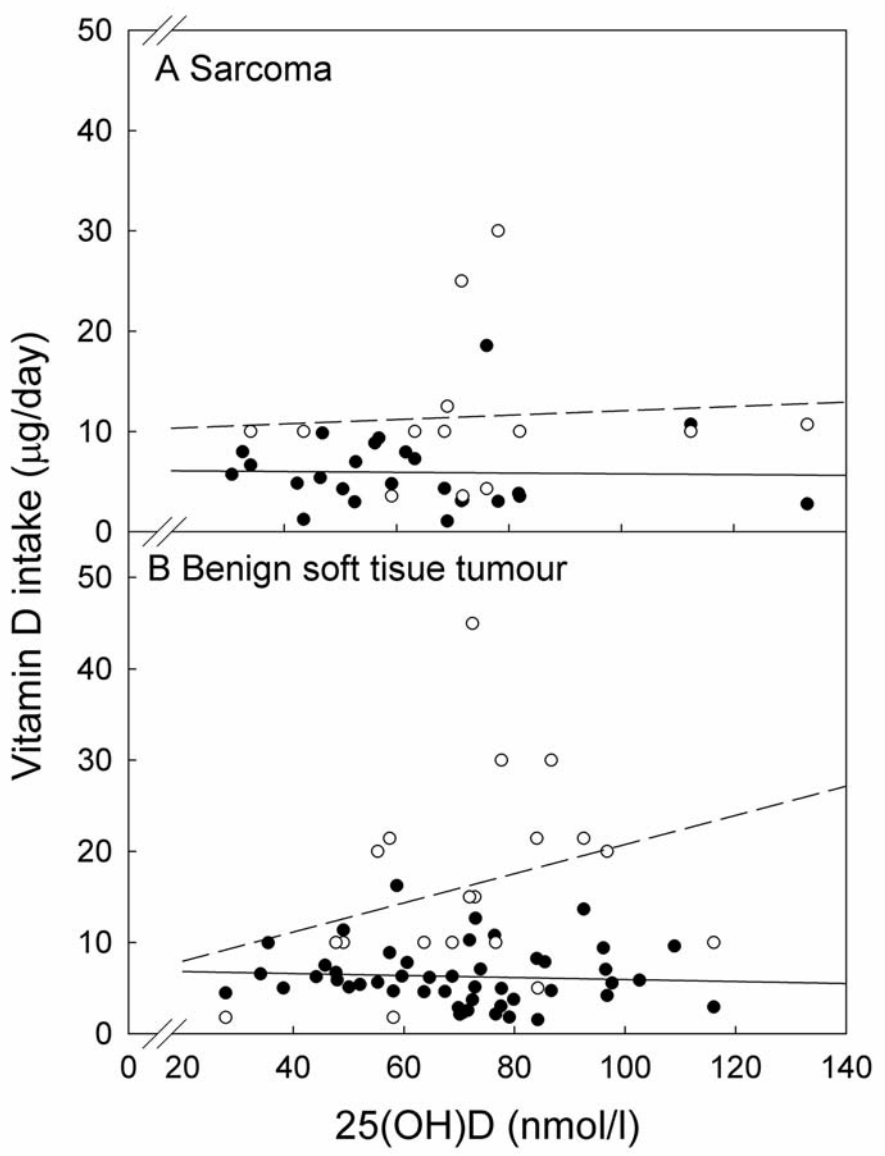

Figure 4. *Relationship of dietary (filled circles; solid line) and supplemental (open circles; dashed line) vitamin D intake with serum 25-hydroxyvitamin D [25(OH)D] in patients with sarcoma (A) and with benign soft tissue tumors (B).*

would expect to find lower vitamin D values in the sarcoma group. However, there were no statistically significant differences in serum 25(OH)D, 1,25(OH)D and PTH concentrations, nor in supplemental or dietary vitamin D intakes between patients with sarcoma and those with benign tumors (Table II). We further compared our results for vitamin D status in the general healthy Caucasian population living in Norway. Healthy Norwegians had similar 25(OH)D values (around 50-74 nmol/l) (39-44) to patients with sarcoma and benign soft tissue tumors (Table I). The prevalence of vitamin D deficiency [serum 25(OH)D levels lower than 50 nmol/l] are also similar in all groups: 14-41% in healthy Norwegians (39, 40, 43-45), 19% among patients with benign tumor and 28% among patients with sarcoma.

However, the mean serum $1,25(OH)_2D$ concentration seem to be slightly lower in patients than in the healthy population: 90 pmol/l in the sarcoma group and 100 pmol/l in the benign tumor group (Table I) *versus* around 120 pmol/l in healthy persons (19, 40-42, 44).

The present study has several limitations. Firstly, only few patients were included due to the rarity of disease (1, 5). Even if benign soft tissue tumors are diagnosed almost 100-fold more often than soft tissue sarcomas (28), only 48 patients

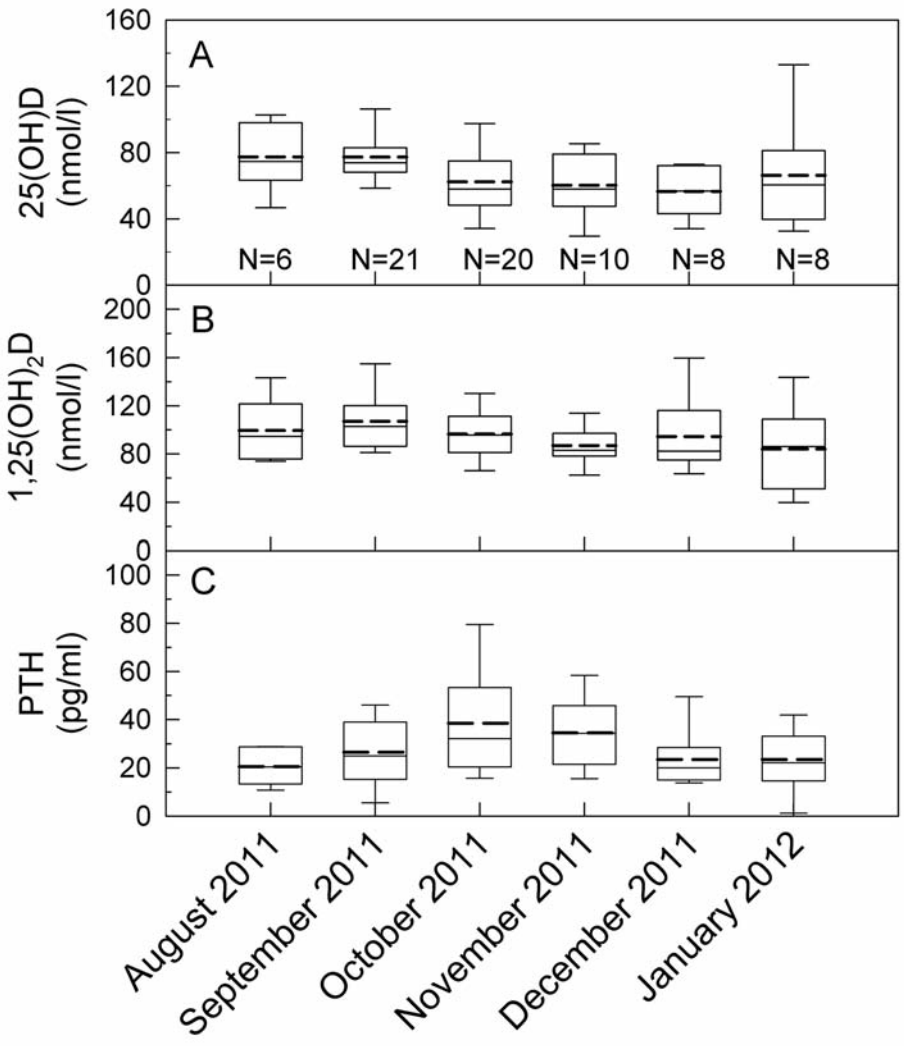

Figure 5. *Distribution of serum 25-hydroxyvitamin D [25(OH)D] (A), 1,25-dihydroxyvitamin D [1,25(OH)$_2$D] (B) and parathyroid hormone [PTH] (C) values by month in the patients from both groups and both sexes. The bottom of the box represents the 25th percentile, the dashed line represents the mean, the solid line represents the median, the top of the box represents the 75th percentile, and the whiskers represent the 5th and 95th percentiles; N is the number of patients.*

with benign tumors (~50% more than patients with sarcoma, Table I) participated in the study.

Secondly, due to the low numbers, the obtained data were not adjusted for season, age, gender, BMI, recent holidays to southern countries, regular solarium visits (one person reported the use of sunbeds), exposure to sun when outdoors, sunscreen use, skin pigmentation, *etc*. Many studies report a significant seasonal variation of 25 (OH)D levels due to ultraviolet B (280-315 nm) exposure with the highest concentrations of 25(OH) observed in July to September (41,44,46). Our patients also had slightly higher levels in August and September than in later months (Figure 5). Regular dietary and supplemental vitamin D intake may lower seasonal fluctuations of 25(OH)D (43,47-49). The mean levels of 25(OH)D in our study and in Norway are within the range of means found in Denmark and Sweden (22, 43, 44, 47, 48, 50, 51). However, the levels are higher than those reported from Germany, Belgium, Italy and Spain even though UV exposure levels are higher in these countries (52-54). This indicates a relatively high vitamin D intake in the Scandinavian population. The official recommended dose of vitamin D intake in Norway is 10 μg/day (400 IU/day) for adults, but only around 40% of our patients achieved this level (Figure 4). The upper tolerable limit of vitamin D intake (the dose that does not pose any risk of hypercalcaemia/hypercalciuria) for adults is 50 μg/day (2000 IU/day) (55, 56). Many researchers suggest that the daily recommended and upper tolerable vitamin D intake is too low to achieve a

Table III. *Pearson's correlation coefficients between age (years) and serum 25(OH)D (nmol/l), 1,25(OH)$_2$D (pmol/l), PTH (pg/ml), vitamin D intake (μg/day), and BMI (kg/m2) in patients with benign soft tissue tumor and in those with sarcoma.*

Variable	25(OH)D	1,25(OH)$_2$D$_3$	PTH	Vitamin D intake (total)	BMI
Patients with benign soft tissue tumor					
Age	0.223	–0.010	0.041	0.244	0.051
25(OH)D		0.136	0.012	0.149	–0.431**
1,25(OH)$_2$D$_3$			–0.078	0.206	–0.333*
PTH				–0.277	0.110
Total vitamin D intake					–0.184
Food vitamin D intake	–0.067	0.158	0.027	0.282	0.043
Supplemental vitamin D intake	0.300	–0.432	–0.276	0.962***	–0.470*
Patients with sarcoma					
Age	0.191	0.192	0.417*	0.106	–0.080
25(OH)D		0.534**	–0.099	0.410*	–0.036
1,25(OH)$_2$D$_3$			0.147	0.195	0.237
PTH				0.093	0.323
Total vitamin D intake					–0.278
Food vitamin D intake	–0.024	–0.005	0.096	0.153	–0.031
Supplemental vitamin D intake	0.072	–0.551	–0.216	0.807***	–0.409

BMI: Body mass index; 25(OH)D: 25-hydroxyvitamin D; 1,25(OH)$_2$D: 1,25-dihydroxyvitamin D; PTH: parathyroid hormone. Significance of correlation: *p<0.05; **p<0.01; ***p<0.001.

sufficient vitamin D level in the general population (55-57). Actual toxicity is not seen below serum 25(OH)D values of 250 nmol/l, a value that would be reached only with regular intakes of 250 μg/day (10000 IU/day) (55-57). The recommendations for a daily intake of 50 μg/day (2000 IU/day) are proposed to achieve 25(OH)D level above 75 nmol/l (55-57).

Higher concentrations of 25(OH)D and lower 1,25(OH)$_2$D and PTH levels are usually observed in older (>50 years) rather than younger (<50 years) persons in Norway (41, 44). However, in many other countries, older persons have much lower concentrations of 25(OH)D (58-60). We have observed the tendency that 25(OH)D level increases with age in both groups (Figure 2A), PTH levels increased only in the sarcoma group (Figure 2B) while 1,25(OH)$_2$D levels were stable (Figure 2C). The increase of 25(OH)D level with age can be explained by the likely possibility that older persons have slightly higher intakes of vitamin D (Figure 2D), and that vitamin D intake correlates to some extent with serum 25(OH)D level (Figure 3C).

Persons with higher BMI have lower levels of 25(OH)D (41, 46, 61). We observed a similar trend in patients with benign tumor but not in patients with sarcoma (Figure 3B). Additionally, vitamin D supplement intake correlated negatively with BMI (Table III) and weight (data not shown) in patients with benign tumor, which supports recent findings that higher vitamin D intake may be associated with lower body weight (62). The reasons for these discrepancies between the two groups may be related to a lower number of patients in the sarcoma group. Exposure to UV radiation from sun or artificial sources, sunscreen use and skin pigmentation influence vitamin D levels (63), but these factors were not taken into account in the present study.

A third limitation is that the patients were from different regions in Norway, which extends over 13 degrees of latitude, from 58 to 71 degrees north. UV level decreases with increasing latitude. The annual UV exposure is approximately 60% higher at 56˚N than at 70˚N (64).

A fourth limitation is that vitamin D intake was determined through a self-report, which increases the possibility of inaccuracies.

Conclusion

Patients with sarcoma and benign tumors have similar 25(OH)D levels to healthy persons in Norway. The proportions of patients with 25(OH)D concentrations below 50 nmol/l were 28% and 19% in the sarcoma and benign soft tissue tumor group, respectively. More patients with benign soft tissue tumors had sufficient vitamin D levels than did patients with sarcoma (48% *versus* 24%). Vitamin D intake from food and supplements was similar in both groups (12.4 μg/day). Higher vitamin D intake or UV exposure is required to ensure that all patients achieve sufficient vitamin D levels.

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