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SHORT COMMUNICATION

The relationship between serum 25(OH)D and bone density and microarchitecture as measured by HR-pQCT

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Abstract
Summary The relation between serum 25-hydroxy vitamin D [25(OH)D] and bone quality is not well understood, particularly for high levels. We measured bone microarchitecture in three groups of people stratified by their serum 25(OH)D. There was a weak association of serum 25(OH)D and microarchitecture for this cross-sectional population, suggesting possible benefits to bone quality.
Introduction Vitamin D plays an important role in bone and mineral metabolism, but the relation between serum 25(OH)D and bone quality is not well understood. Here, we present a cross-sectional study that investigated a convenience group of participants from an ongoing health initiative in Alberta, Canada, who have been receiving daily vitamin D supplementation.
Methods A total of 105 participants were organized into three groups based on their serum 25(OH)D levels: low (<75 nmol/L), medium (75–175 nmol/L), and high (>175 nmol/L). They were also assessed with 25(OH)D as a continuous variable. Average daily supplementation was 7670±438 IU, and the change in 25(OH)D ranged from 22 to 33 % during the period of receiving supplements. We used high-resolution peripheral quantitative computed tomography measurements at the radius and tibia to assess bone microarchitecture.
Results Microarchitectural parameters were not strongly associated with serum 25(OH)D. In the tibia, there were fewer trabeculae (TbN; $p=0.015$) and a non-significant trend toward thicker trabeculae ($p=0.067$) of the high group. Body mass index (BMI) was negatively associated with serum 25(OH)D levels ($p<0.001$) and PTH levels ($p<0.001$). There was no clinically significant relationship detected between high serum 25(OH)D and high serum calcium.
Conclusion These data suggest a weak relationship between serum 25(OH)D and bone microarchitecture in this population of mostly vitamin-D-sufficient participants, and there were no indications of negative effects related to the high supplementation levels. These data provided a basis to design and implement our 3-year dose-dependent randomized controlled trial investigating the effects of vitamin D supplementation on bone health outcomes.

Keywords 25(OH)D · Bone microarchitecture · Calcium · HR-pQCT · Vitamin D

Introduction

Vitamin D is necessary for a wide variety of cell functions in many tissues and organ systems, and there is an association between vitamin D deficiency and a variety of medical disorders (i.e., rheumatoid arthritis, multiple sclerosis). It is clear that vitamin D has an important influence on bone and mineral metabolism, but the optimal dose remains controversial. A recent Institute of Medicine (IOM) report [1, 2] recommends that serum levels of 25-hydroxy vitamin D [25(OH)D] between 50 and 125 nmol/L are sufficient for bone and overall health. To reach this range, the IOM recommends a vitamin D intake (all sources) of 600 international units (IU) daily for adults under age 70 and 800 IU daily for adults over age 70. The Osteoporosis Canada Guidelines recommend a dose of 400 to 1000 IU daily for adults under age 50 years and 800 to 2000 IU daily for adults over age 50 years [3].

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A problem in the area of vitamin D and bone and mineral metabolism is understanding the dose-response effect, particularly on bone outcomes. A recent meta-analysis including 23 studies, most of which involved doses less than 2000 IU daily, concluded vitamin D supplements may not increase bone density in middle-aged adults [4, 5]. Even studies with higher doses such as a randomized, controlled trial comparing 800 IU daily to 6500 IU daily that was captured in the same meta-analysis found no benefit of the high dose on bone mineral density (BMD) [6]. In fact, higher levels of vitamin D intake have been seen as problematic, with the potential to lead to higher serum calcium levels and suppression of parathyroid hormone (PTH), resulting in higher urinary calcium excretion and hypercalcaemia. On the other hand, it is notable though that no cases of vitamin D toxicity have been clearly documented with doses less than 40,000 IU daily [7].

The objective of this study was to perform a cross-sectional analysis to explore the relationship between 25(OH)D serum levels and bone quality. The assessment of bone quality was based on high-resolution peripheral quantitative computed tomography (HR-pQCT), and the cohort was derived from people who have been participating in a preventative health program in Alberta, Canada. This study served as pilot data to help inform our 3-year randomized controlled trial investigating the dose-dependent effects of vitamin D supplementation on bone health.

Methods

This study was approved by the Conjoint Health Research Ethics Board at the University of Calgary (E-25226).

Participants

All participants included in this study were enrolled with the Pure North S'Energy Foundation (PNSF), which is a not-for-profit initiative that promotes healthy living through education, as well as nutritional and lifestyle advice. Participants received daily supplements of multivitamins, along with other vitamin and mineral supplementations on the advice of a healthcare professional. Multivitamin tablets contained 1000 IU of vitamin D, while additional vitamin D supplements were given to participants as deemed necessary by a healthcare professional in the form of 1000 IU vitamin D3 drops. Participants were enrolled in the PNSF program for at least 6 months, during which time serum 25(OH)D, calcium, and PTH were monitored as part of periodic health checkups between November 1, 2012, and April 30, 2013. The laboratory testing of serum 25(OH)D was completed using an immunoassay evaluated on a DiaSorin Liaison XL system, and performance was monitored using DEQAS quality assurance samples. Laboratory testing of serum calcium was completed using a COBAS (Roche) analyzer system. PTH was measured using the DiaSorin Liaison N-TACT PTH II assay (DiaSorin, Stillwater, MN). This is a second-generation PTH assay which primarily detects the intact 84-amino-acid peptide hormone but has cross-reactivity with the 7-84 fragment. The measurements were performed by Calgary Laboratory Services, a licensed clinical laboratory which serves the Calgary Health Zone—a population of approximately 1.2 million.

Baseline serum 25(OH)D was used to divide a sub-cohort of 109 participants into three different groups: low (<75 nmol/L), medium (between 75 and 175 nmol/L), and high (>175 nmol/L) 25(OH)D. The low cutoff of 75 nmol/L was selected because it aligns with the Osteoporosis Canada and Endocrine Society threshold for optimal or sufficient 25(OH)D, and the high cutoff of 175 nmol/L was chosen to identify a subset of our unique cohort that had relatively high levels of 25(OH)D which were not likely to be attained by diet and sunlight exposure alone [8]. We also evaluated the parameters as a continuous variable, while controlling for BMI.

Bone imaging

High-resolution peripheral quantitative computed tomography (HR-pQCT; XtremeCT, Scanco Medical, Switzerland) was used to measure bone macro- and microarchitectural parameters at the distal radius of the non-dominant arm and distal tibia of the dominant leg using the standard protocol (60 kVp, 1000 mA, 100 ms integration time) [9] described in detail previously [10]. Briefly, 110 slices were acquired at the standard distal sites of radius and tibia at a nominal isotropic resolution of 82 μm. Quality control of the HR-pQCT scanner was monitored daily using a calibration phantom provided by the manufacturer.

The analysis included measures of volumetric bone mineral density (BMD, mg HA/cm^3) of the whole bone (BMD), trabecular compartment (TbBMD), and cortical compartment (CtBMD). Morphological measures included total cross-sectional area (TtAr, mm^2), trabecular number (TbN, 1/mm), trabecular thickness (TbTh, μm), and trabecular separation (TbSp, μm). An advanced analysis of the cortical region [11, 12] provided measures of cortical thickness (CtTh, mm) and cortical porosity (CtPo, %). In our lab, in vivo short-term reproducibility is less than 1 % for density and less than 4.5 % for morphologic measures [13].

Statistical analysis

Descriptive statistics were assessed for participants, reporting group means, standard deviations, and 95 % confidence intervals of this cross-sectional study. An ANOVA F test was performed on the experimental groups for HR-pQCT outcome variables, with a null hypothesis representing equality of the means and a significance level set at $p<0.05$. Due to the

exploratory nature of this cross-sectional study, trends were formally defined with a significance level of $p<0.10$. Linear regressions of 25(OH)D serum levels on bone density measures were performed with control for BMI. Serum levels from PNSF and the HR-pQCT data were combined in Microsoft Excel and analyzed using Stata 11.1 (College Station, TX).

Results

A total of 109 people participated in this pilot study, of which four were excluded due to motion artifacts in the HR-pQCT scans. The average age of this cohort was 55 years old, and 62 % were female and had been enrolled in the program for 13 months. Details of this cohort, categorized by the serum 25(OH)D levels, are provided in Table 1. The average supplement was 7670 IU daily across all groups, and the *low* group had the lowest supplement levels. The average time in the PNSF program of 13 (±17) months was not different among the three groups. Both PTH and BMI were negatively associated with serum 25(OH)D ($p<0.001$).

The serum 25(OH)D upon entry into the PNSF program increased an average of 23 %, with the highest change in the *low* group. The average percent change of the serum calcium was less than 1 %, and the largest magnitude change was in the *low* 25(OH)D group at −3.1 %. Generally, microarchitectural parameters were similar in the three groups in this cohort (Table 2). There was a trend toward lower trabecular number (TbN, $p=0.070$) in the tibia of the *high* group, which was significant when compared to the *low* group ($p=0.015$). The decreased trabecular number coincided with thicker trabeculae (TbTh), and this trend was most apparent when comparing the *high* to *low* groups ($p=0.067$). Finally, although it was not significant, it is worth noting that total BMD was lowest in the low group at the tibia and radius.

A regression of volumetric total BMD at the radius and tibia against the serum 25(OH)D for the entire cohort (Fig. 1) found no significant correlation of BMD with 25(OH)D with this population at both the radius ($r=0.053$, $p=0.59$) or tibia ($r=0.069$, $p=0.48$). However, trabecular BMD and TbTh at the tibia were positively associated with 25(OH)D in our regression model that included BMI and controlled for age and sex (Table 3; $p<0.05$).

Figure 1 also shows a significant negative association was found in the regression of serum calcium against total BMD at the radius ($r=-0.260$, $p<0.01$) and tibia ($r=-0.357$, $p<0.01$). When considering the entire cohort, there was a negative association between 25(OH)D and serum calcium (Fig. 2; $p=0.05$), although a 100 nmol/L change was estimated to lower serum calcium by only 0.037, which is not likely clinically significant.

Discussion

This study explored the relationship between serum 25(OH)D and bone microarchitecture in a unique convenience cohort of people who have been participating in an ongoing health initiative in Alberta, Canada, receiving high levels of vitamin D supplementation. Grouping participants by their serum 25(OH)D levels, these data suggest a weak association with bone microarchitecture, which is likely confounded by the heterogeneity of the participants. As it was not a controlled trial, there was significant variance in the participants' duration of participation in the program, and the levels of their supplements were high compared to the general population. Considering the serum calcium, we found it was not strongly associated to the serum 25(OH)D, even when those levels were high. Clearly, this is a small cross-sectional study, and performing a prospective study would be much better powered to elicit effects; however, these data suggest that high

Table 1 The sex, age, time in program, daily supplementation of vitamin D and calcium, PTH, and BMI of participants in three groups based on serum 25(OH)D: high (>175 nmol/L), medium (75–175 nmol/L), and low (<75 nmol/L)

	All	High 25(OH)D >175 nmol/L	Medium 25(OH)D 75–175 nmol/L	Low 25(OH)D <75 nmol/L	*p* values
Sex (% female)	62 %	57 %	65 %	70 %	0.635
Age (years)	55±15	56±14	56±16	53±13	0.853
Time in program (months)	13±17	13±14	14±18	9±18	0.735
Supplement vit D (IU/day)	7670±4359	7000±4090	8531±4565	5875±3682	0.050**
Supplement calcium (mg/day)	325±277	348±328	296±241	366±200	0.589
PTH (ng/L)	38±20	31±15	40±18	59±32	<0.001***
BMI	27±6	25±5	27±5	33±6	<0.001***
N	105	44	51	10	

Data are presented for mean and standard deviation (SD)

*$p\leq0.10$; **$p\leq0.05$; ***$p\leq0.01$

Table 2 he mean and 95 % confidence interval for serum 25(OH)D and serum Ca, and percent change from entry into the study and time of bone scan

		All	High 25(OH)D	Normal 25(OH)D	Low 25(OH)D	*p* values	High v. normal	High v. low	Normal v. low
Serum	25(OH)D at entry [nmol/L]	137 [114, 161]	199 [151, 247]	102 [84, 119]	49 [41, 57]	<0.001***	<0.001***	0.005***	0.012**
	25(OH)D at scan	169 [152, 185]	243 [219, 267]	125 [117, 133]	65 [55, 75]	<0.001***	0.01***	<0.001***	<0.001***
	25(OH)D % change	23 %	22 %	23 %	33 %				
	Ca at entry [nmol/L]	2.47 [2.44, 2.50]	2.42 [2.38, 2.47]	2.50 [2.46, 2.53]	2.57 [2.42, 2.73]	0.006***	<0.001***	0.008***	0.128
	Ca at scan	2.46 [2.42, 2.49]	2.45 [2.39, 2.50]	2.48 [2.42, 2.51]	2.49 [2.32, 2.66]	0.735	0.547	0.455	0.634
	Ca % change	–0.4 %	1.2 %	–1.6 %	–3.1 %				
Radius	BMD [mg/cc]	304 [289, 318]	310 [285, 335]	304 [284, 323]	274 [221, 327]	0.389	0.672	0.204	0.223
	TbBMD [mg/cc]	155 [145, 165]	160 [143, 177]	150 [137, 164]	157 [113, 200]	0.698	0.394	0.875	0.730
	CtBMD [mg/cc]	935 [922, 947]	934 [916, 952]	941 [923, 960]	902 [849, 955]	0.197	0.557	0.139	0.091*
	TbN [1/mm]	1.99 [1.92, 2.06]	2.00 [1.88, 2.12]	1.96 [1.88, 2.06]	2.06 [1.80, 2.32]	0.745	0.675	0.665	0.424
	TbTh [μm]	64 [61, 67]	65 [61, 70]	63 [58, 68]	62 [51, 72]	0.802	0.618	0.534	0.794
	CtTh [mm]	0.90 [0.87, 0.94]	0.92 [0.86, 0.98]	0.91 [0.85, 0.96]	0.81 [0.68, 0.94]	0.293	0.744	0.131	0.158
	CtPo [%]	2.6 % [0.024, 0.029]	2.8 % [2.4, 3.1]	2.5 % [2.1, 2.9]	2.8 % [1.7, 3.8]	0.605	0.347	0.965	0.569
	Tt.Ar [mm^2]	127 [120, 135]	130 [117, 142]	123 [112, 134]	137 [113, 160]	0.554	0.460	0.622	0.315
Tibia	BMD [mg/cc]	286 [274, 299]	289 [269, 310]	288 [268, 307]	265 [222, 309]	0.585	0.905	0.310	0.339
	TbBMD [mg/cc]	164 [156, 173]	168 [154, 181]	161 [149, 173]	166 [133, 200]	0.740	0.447	0.944	0.703
	CtBMD [mg/cc]	846 [806, 886]	874 [827, 921]	821 [750, 892]	847 [781, 913]	0.454	0.227	0.590	0.754
	TbN [1/mm]	1.74 [1.68, 1.80]	1.70 [1.62, 1.78]	1.73 [1.65, 1.82]	1.94 [1.78, 2.10]	0.070*	0.586	0.015**	0.055*
	TbTh [μm]	78 [75, 81]	81 [77, 86]	77 [73, 82]	71 [60, 83]	0.145	0.202	0.067*	0.283
	CtTh [mm]	1.25 [1.19, 1.31]	1.25 [1.15, 1.34]	1.27 [1.19, 1.36]	1.15 [0.94, 1.36]	0.498	0.670	0.367	0.240
	CtPo [%]	6.3 % [0.058, 0.069]	6.1 % [5.3, 6.8]	6.6 % [5.7, 7.4]	6.5 % [4.4, 8.7]	0.700	0.412	0.621	0.978
	TtAr [mm^2]	287 [273, 301]	290 [269, 311]	279 [258, 300]	315 [260, 371]	0.345	0.474	0.318	0.169
	N	105	44	51	10				

Volumetric density parameters (total, trabecular, and cortical) and morphological parameters are presented for the radius and tibia. Data is presented for all participants, and for each of the groups based on serum 25(OH)D: high (>175 nmol/L), medium (75–175 nmol/L), and low (<75 nmol/L)

*$p \leq 0.10$; **$p \leq 0.05$; ***$p \leq 0.01$

Table 3 Regression coefficients for bone microarchitectural parameters against serum 25(OH)D and BMI, controlling for age and sex

		Vit D	BMI
Radius	BMD [mg/cc]	0.0233	0.2070
	TbBMD [mg/cc]	0.0108	1.2034
	CtBMD [mg/cc]	0.0395	–2.1400*
	TbN [1/mm]	0.0000	0.0154*
	TbTh [μm]	–0.0040	–0.0268
	CtTh [mm]	–0.0001	–0.0017
	CtPo [%]	–0.0004	0.0500
	TtAr [mm^2]	–0.0311	0.4622
Tibia	BMD [mg/cc]	0.0990	1.1398
	TbBMD [mg/cc]	0.0988*	1.9216**
	CtBMD [mg/cc]	0.3343	4.7497
	TbN [1/mm]	–0.0001	0.0208***
	TbTh [μm]	0.0498*	0.0710
	CtTh [mm]	–0.0002	0.0037
	CtPo [%]	0.0000	0.0007
	TtAr [mm^2]	–0.0021	3.1057***

*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$

levels of supplementation were not detrimental to bone microarchitecture and may have modest beneficial effects.

As this was a cross-sectional study design, rigorous control was not maintained over all supplement intakes due to the multivitamins participants received. It is possible that the effects on bone microarchitecture are confounded by other

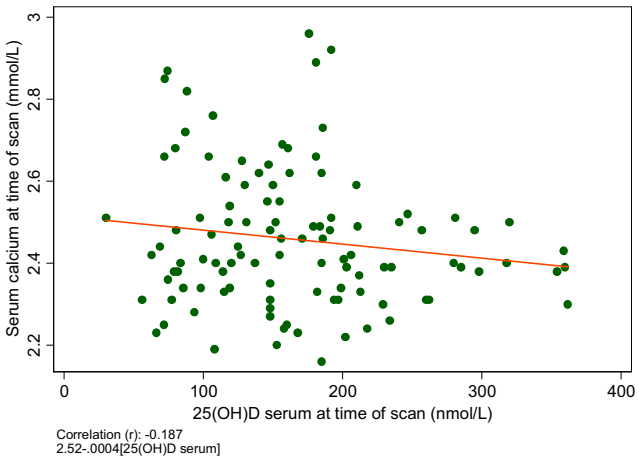

Fig. 2 The relationship between serum 25(OH)D and serum calcium for the entire cohort ($N=105$). The *ordinate axis* shows serum calcium measured in nmol/L

factors (e.g., other vitamins, sunlight exposure, physical exercise). Furthermore, the supplemental intake of daily vitamin D varied for participants over the course of their participation in the PNSF program. Consistently, the average supplemental vitamin D intake for all participants was high compared to IOM recommendations, and our cohort included participants with a wide range of serum 25(OH)D levels albeit weighted toward sufficient or high levels.

The relationship between serum 25(OH)D and bone microarchitecture was weak, and although there was a trend toward higher BMD being associated with high serum

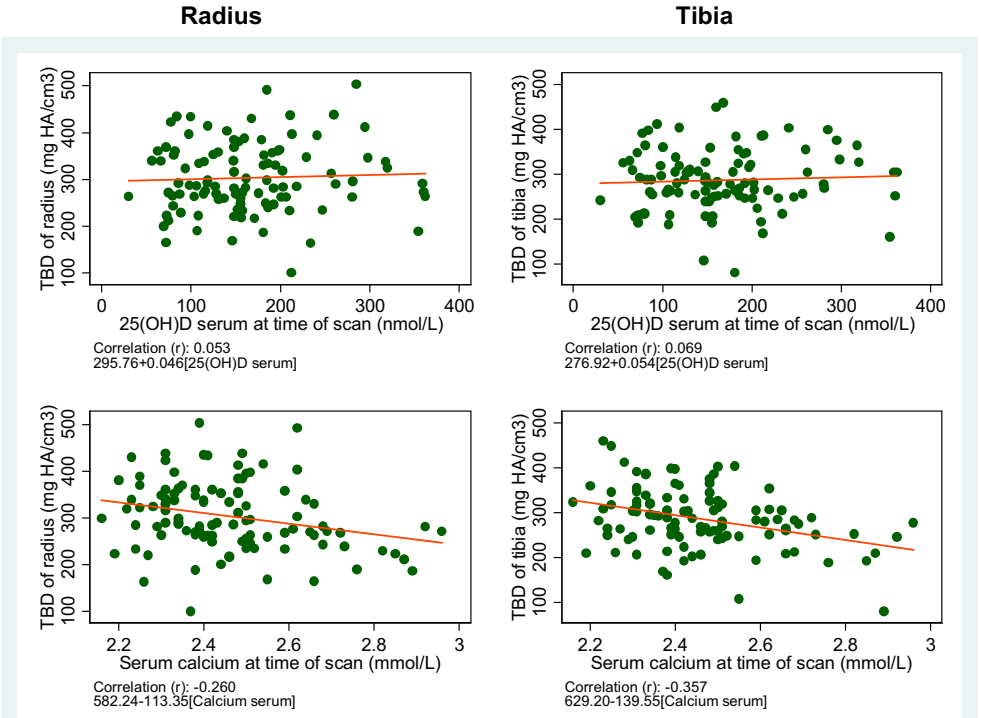

Fig. 1 The relationship between radius bone mineral density (*left*) and tibia bone mineral density (*right*) and the serum 25(OH)D at the time of the HR-pQCT scan (*top*) and serum calcium (*bottom*). The *ordinate axis* shows BMD measured in mg HA per cc

25(OH)D, it was not significant. The biggest effect was on the lower number of trabecular in the tibia in the group with high serum 25(OH)D, which also had correspondingly thicker trabeculae—this trend toward fewer but thick trabeculae may be advantageous to the bone microarchitecture. While it is notable that for nearly all parameters measured in the radius and tibia, the *high* and *medium* serum 25(OH)D groups appeared to be associated with the best microarchitecture, the relationships were not strong. Nevertheless, these data suggest that high vitamin D supplementation did not result in *reduced* bone quality on this cohort that was taking supplements for a time frame of 1 to 2 years. Whether there is a clinically relevant relationship will be better ascertained in our prospective controlled trial. Our finding here that there were no detectable differences in serum calcium among the groups despite many participants having very high serum 25(OH)D is encouraging as it suggests at least that there were not strong negative effects. The decreased PTH and BMI associated with the highest serum 25(OH)D is consistent with the findings of others.

Some limitations have already been discussed, including that there was a wide variation in the duration of the participants in the PNSF program, which ranged from six months up to 5 years. Also, due to the fact that this convenience cohort comprised people who were all taking very high levels of supplementation, the number of participants classified as *low* was small, which may have reduced our ability to detect effects on bone quality. We did not include dual-energy X-ray absorptiometry (DXA) in this study because our focus was on bone microarchitecture, and it is unlikely it would have detected BMD trends that were not picked up by HR-pQCT. However, future studies will benefit from using both DXA and HR-pQCT to measure multiple skeletal sites, and by studying a highly controlled prospective cohort, we will be able to maximize the sensitivity to detect bone quality changes.

In conclusion, this cross-sectional study demonstrated there may be benefits of vitamin D supplementation on bone microarchitecture and supports that a longitudinal dose-dependent trial will be important to establish whether there is a beneficial effect of high levels of vitamin D supplementation on bone health. The data we present here suggests that the large daily supplementation of vitamin D did not pose a hazard to the health of this small cohort by the measures we performed.

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Conflicts of interest None.

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