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Low vitamin D status is associated with impaired bone quality and increased risk of fracture-related hospitalization in older Australian women

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Abstract

The vitamin D debate relates in part to ideal public health population levels of circulating 25-hydroxyvitamin D (25OHD) to maintain bone structure and reduce fracture. In a secondary analysis of 1,348 women aged 70-85 years at baseline (1998) from the Perth Longitudinal Study of Aging in Women (PLSAW, a five-year calcium supplementation trial followed by two five-year extensions), we examined the dose-response relations of baseline plasma 25OHD with hip DXA BMD at year 1, lumbar spine BMD and trabecular bone score (TBS) at year 5, and fracture-related hospitalizations over 14.5 years obtained by health record linkage. Mean baseline plasma 25OHD was 66.9±28.2 nmol/L and 28.5%, 36.4% and 35.1% of women had levels <50, 50-74.9 and ≥75 nmol/L, respectively. Generalized additive models showed that total hip and femoral neck BMD and TBS, but not spine BMD, were higher with increasing plasma 25OHD up to 100 nmol/L. Compared with those with 25OHD <50 nmol/L, women with 25OHD ≥75 nmol/L had significantly higher total hip and femoral neck BMD at year 1 (3.3-3.9%) and TBS at year 5 (2.0%), all P<0.05. During the follow-up 27.6% of women experienced any fracture-related, and 10.6% hip fracture-related hospitalization. Penalized spline regression models showed a decrease in risk with increased 25OHD levels up to 65 and 75 nmol/L for hip fracture and any fracture-related hospitalization, respectively. Cox regression grouped analyses showed that, compared with women with 25OHD <50 nmol/L, those with 25OHD levels 50-74.9 and ≥75 nmol/L had significantly lower risk for hip fracture (hazard ratio [95% CI]: 0.60 [0.40-0.91] and 0.61 [0.40-0.92], respectively), and any fracture-related hospitalization (hazard ratio [95% CI]: 0.77 [0.59-0.99] and 0.70 [0.54-0.91], respectively). In older Caucasian women 25OHD levels >50

nmol/L are a minimum public health target and 25OHD levels beyond 75 nmol/L may not have additional benefit to reduce fracture risk.

Key words: 25-hydroxy vitamin D, bone mineral density, fracture-related hospitalization, older women, longitudinal study

Introduction

For over 100 years it has been agreed that vitamin D plays an important role in bone and calcium metabolism. Persistent severe vitamin D deficiency causes rickets in children and osteomalacia in adults, whereas mild vitamin D deficiency has been reported to be associated with hyperparathyroidism and increased bone turnover (1, 2). Besides its effects on bone, vitamin D may also play a role preventing falls (3, 4), another major risk factor for fracture. However, the ideal public health population levels of circulating 25-hydroxyvitamin D (25OHD), a measure of vitamin status, for optimal bone structure and reduced fracture risk are still debated. Given the large number of genetic and environmental factors affecting bone structure and fracture risk and the heterogeneity in these factors in among communities, this is not surprising. The present study was designed to evaluate the size of the impact of baseline circulating 25OHD levels in an older population of women at high risk of fracture, where most evidence of potential benefits of increased 25OHD has been identified. In addition, we report on trabecular bone score (TBS), which has emerged as a novel grey-level texture measurement reflecting trabecular microarchitecture and had been shown to predict fracture risk independent of bone mineral density (BMD) (5).

Previous analytical processes on the association of 25OHD levels and bone outcomes mostly have used linear or grouped data, although non-linear relationship has been suggested (6). Statistical packages such as generalized additive models (GAM) and

penalized splines now offer the advantage of nonlinear curve fitting to give graphic representations of the dose-response relationship. In the Perth Longitudinal Study of Aging in Women (PLSAW), clinical data were collected over 15 years, and hospital admission record for 14.5 years after the baseline survey were obtained from the Western Australian Data Linkage System (7). Thus, in this well-characterised cohort of older women, we examined the dose-response relations between seasonally-adjusted vitamin D status with bone quality as measured by hip BMD and TBS as well as risk of incident fracture-related hospitalization to understand the shape of the relationship, and to identify the optimal threshold concentration of 25OHD for bone quality and fracture prevention in older women.

Participants and methods

This study is a secondary analysis of the data of a calcium interventional trial and its follow-up, and this article complies with the STROBE reporting guidelines for observational studies.

Participants

This analysis reports data on 1,348 older postmenopausal Caucasian women from PLSAW, who had plasma 25OHD assessed at baseline (1998). PLSAW recruited 1,460 women aged 70-85 years in 1998 from the population, and this cohort first finished a five-year randomized controlled trial of calcium supplementation (Calcium Intake Fracture Outcomes Study, CAIFOS) (8), and then were recruited into two five-year extensions of follow-up without any intervention. The CAIFOS participants were recruited from the population using the Australian electoral roll, which has contact details of over 98% of women of this age, by means of a letter inviting participation in the study. The CAIFOS inclusion criteria were: aged over 70 years old, likely to survive a five-year study,

and not receiving bone active agent. There were no other specific exclusions so that the results could be generalized to the whole ambulant population. During the calcium intervention phase, women were randomized to receive calcium 1200 mg or placebo daily for 5 years. There was an excess of higher socioeconomic groups of women participated in CAIFOS, although their pharmaceutical consumption and disease burden were similar to data of the whole populations of this age (9). As this trial commenced and completed prior to the advent of the clinical trials registry, it was retrospectively registered in the Australian New Zealand Clinical Trials Registry (ACTRN12615000750583). Informed consent was obtained from each subject and the study was approved by the Human Research Ethics Committee of the University of Western Australia (approval number 05/06/004/H50). The Human Research Ethics Committee of the Western Australian Department of Health approved the data linkage study (approval number #2009/24).

Plasma 25OHD

Venous blood samples were collected between 0830 and 1030 h after overnight fasting at PLASW baseline. Plasma was separated and store at -80°C freezer. Plasma $25OHD_2$ and $25OHD_3$ concentrations were determined using a validated LC-MS/MS (Liquid Chromatography Tandem Mass Spectrometry) method at the RDDT Laboratories (Bundoora, VIC, Australia) according to published methodology (10) and summed to obtained total plasma 25OHD concentration for each individual. Between-run coefficients of variation (CVs) were 10.1% at a $25OHD_2$ mean concentration of 12 nmol/L and 11.3% at a $25OHD_3$ mean concentration of 60 nmol/L. Internal quality control (QC) test showed that the QC samples passed the acceptance criteria.

Bone parameters

Due to the restricted availability of funding and human resources, dual energy X-ray absorptiometry (DXA) assessment for total hip and femoral neck BMD was done at year 1 in 999 participants, and for lumbar spine (L1-L4) BMD at year 5 in 968 participants using a Hologic Acclaim 4500A fan beam densitometer (Hologic Corp, Waltham, MA, USA). The coefficient of variation (CV) at the total hip was 1.2%, at femoral neck 1.4% and at lumbar spine 1.1% in our laboratory (11). The TBS was obtained by direct re-analysis of the lumbar spine DXA images using TBS iNsight® Software version 1.8.2 (Med-Imaps, Bordeaux, France), and calculated as the mean value of the individual measurements for vertebrae L1 − L4. The CV for TBS was 1.1%.

Fracture ascertainment

Prevalent fractures were determined at baseline by trained research assistants obtaining a fracture history from each subject that included the site of the fracture, age at the time of fracture, and how the fracture was sustained. A prevalent fracture was included if the fracture occurred after the age of 50 years, occurred with minimal trauma as defined by falling from a height of one metre or less, and not of the face, skull, fingers, or toes.

Data on incident fracture that required hospitalization (i.e. admission to wards) were retrieved from the Western Australia Hospital Morbidity Data Collection (HMDC) for each of the study participants from 1998 when they entered the study until 14.5 years after their baseline visit via the Western Australian Data Linkage System (Department of Health Western Australia, East Perth, Australia) (7). As the HMDC provides a complete record of participants' primary diagnosis at hospital discharge using coded data from all hospitals in Western Australia (7), it allows ascertainment of fracture-related hospitalization independently of patient report with the associated problems such as loss

to follow-up unless the participant leaves Western Australia. Fracture-related hospitalizations were defined using the International Statistical Classification of Diseases and Related Health Problems, 10th Revision. Codes used for identification were S02.0-S02.9, S12.0-S12.9, S22.0-S22.9, S32.0-S32.9, S42.0-S42.9, S52.0-S52.9, S62.0-S62.9, S72.0-S72.9, S82.0-S82.9, S92.0-S92.9, M80, T02, T08, T10, T12 and T14.2. Fractures of the face (S02.2-S02.6), fingers (S62.5-S62.7), and toes (S92.4-S92.5) and fractures caused by motor vehicle injuries (external cause of injury codes V00-V99) were excluded. Fracture resulted from other kinds of trauma were not excluded, e.g. falls from more than standing height, however given the age of these women the number is likely to be very small. The ICD-10 codes used for hip fracture were: S72.0-S72.2 and S72.9. The follow-up time was from the PLSAW baseline clinic visit (1998) until either date of fracture or date of death. All women without a fracture were censored at 14.5 years from baseline.

Baseline measurements

Height and weight were measured with the subjects in light clothing and without shoes at baseline. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. Nutrient intakes were determined from a self-administered semi-quantitative food frequency questionnaire (FFQ) (12, 13). Alcohol intake was also collected using the FFQ and calculated as 1 unit of alcohol equals 10 g of pure alcohol in a drink. Physical activity level was assessed by a questionnaire (9, 14), and activity levels were calculated in kcal/day using a validated method utilizing body weight, questions on the number of hours and type of physical activity and energy costs of such activities (15, 16). Ever smoking was defined as smoking of at least 1 cigarette per day for at least 3 months at any time. The Timed Up and Go (TUG) test was performed in which the patient is timed

while rising from a chair, walking 3 meters, turning, returning to sit on the chair (17). Subjects were allowed to practise once then timed. The inter-observer CV error was 6% for the TUG test in our laboratory. The subject's postcode area was recorded at baseline and an index of socioeconomic disadvantage (SES, range 1-6, where a score of 1 indicates the top 10% most highly disadvantaged, and a score of 6 indicates the top 10% least disadvantaged) was derived according to the Australian Bureau of Statistics method (18). Medication data were collected using a self-report dairy returning to the study centre every 4 months from baseline to 5 years.

Data analysis

Descriptive statistics are reported as mean ± SD for all variables unless otherwise stated. The

normality of continuous variables was checked through the construction of histograms and variables that were not normally distributed (TUG and physical activity level) were log-transformed. Generalized additive model (GAM) is a semi-parametric extension of generalized linear model (GLM), which makes assumptions that link functions are additive and components are smooth thus offer a greater flexibility to represent the relations between the dependent variable and predictor variables compared with linear regression. R package "mgcv"(19) was used to generate GAM graphic representations of the dose-response relations between plasma 25OHD and the bone structural variables BMD and TBS, adjusted for covariates including season of blood sampling (as summer/autumn and winter/spring), baseline age, BMI, calcium intake, ever smoking, consumption of ≥ 3 unit/day of alcohol, physical level, SES and treatment group during the intervention phase, with method = REML and knots = NULL. In addition participants were grouped into three vitamin D status groups according to serum 25OHD

concentration as lower (<50 nmol/L), intermediate (50 to 74.9 nmol/L), and higher (≥75 nmol/L), using the sufficient level recommended by the United States Institute of Medicine Committee (50 nmol/L) (20), and the Endocrine Society (75 nmol/L) (21) as cut-offs. Baseline characteristics between subjects in the three vitamin D status groups were compared using analysis of variance (ANOVA) and Chi-square test where appropriate. Comparisons on total hip and femoral neck BMD measured at year 1 and lumbar spine BMD and TBS measured at year 5 between the three vitamin D status groups were made by ANOVA and analysis of covariance (ANCOVA) adjusted for covariates as in the GAM. Post hoc comparisons were made by Tukey's HSD test for ANOVA and Bonferroni test for ANCOVA. The dose-response relations between serum 25OHD and fracture outcomes were examined with penalized spline using R package "survival" (22) with df = 4 and using 75 nmol/L as the reference level, adjusted for covariates as in the Model 2 for Cox regression models below. Cox regression models were used for evaluating the relationship of 25OHD (both as continuous per SD (28 nmol/L) change and in selected categories) and first fracture or hip fracture-related hospitalization since PLASW baseline (1998), and the estimated hazard ratios (HRs) with 95% confidence interval (CI) are reported after four levels of adjustment for potential confounders. Model 1 adjusted for season of blood sampling, baseline age and BMI, treatment group during the intervention phase and fracture history, Model 2 was Model 1 further adjusted for other risk factors for fracture including TUG (23), calcium intake, ever smoking, consumption of ≥ 3 unit/day of alcohol, physical activity level (24) and SES (n = 1322), Model 3 was Model 2 further adjusted for femoral neck BMD at year 1 (n = 981), and Model 4 was Model 2 further adjusted for TBS at year 5 (n = 953). The proportional hazards assumption was tested for each covariate and no violations were detected. For the primary analysis, we treated deaths as censored. This cause-specific

approach meant that the hazard ratios could be interpreted as the risk of fracture-related hospitalizations for any time during follow-up assuming that the participant stayed alive for that duration of time. In addition, a competing risk analysis with death was also performed for plasma 25OHD categories and fracture-related hospitalizations for Models 1 and 2. A P value of less than 0.05 in two tailed testing was considered significant. The statistical analyses were performed using IBM SPSS (version 22; IBM, Chicago, IL), and R (version 3.5.2, R Foundation for Statistical Computing, Vienna, Austria).

Power calculation

With an event rate of 28% for fracture-related hospitalisation and 11% for hip fracture-related hospitalisation, our study has 80% power to detect hazard ratios of 0.70 and 0.56 for the lower 25OHD group compared with the intermediate or the higher group for total and hip fracture-related hospitalization, respectively, at 5% level of significance.

Results

Characteristics of study participants

The characteristics of study participants by their vitamin D status groups are presented in **Table 1**. The mean plasma total 25OHD was 66.9 ± 28.2 nmol/L, with 384 (28.5%) women had lower plasma 25OHD concentration <50 nmol/L, 491 (36.4%) had intermediate levels 50-74.9 nmol/L and 473 (35.1%) had higher levels ≥75 nmol/L. There were no significant differences between the three groups in terms of baseline age, height, calcium intake, percentage that received calcium treatment during the intervention phase, ever smoked, consumption of alcohol ≥3 unit per day or had prevalent fracture, and the percentage reported bisphosphonates use from baseline to 5 years. However, compared to women with lower 25OHD, those with intermediate and/or

higher 25OHD had lower body weight and BMI, better TUG performance and higher levels of physical activity, and those with intermediate level had lower SES score. In the higher 25OHD group, more participants had blood sampled in fall. These variables were used in covariate-adjusted analysis to reduce the effects of these differences.

Associations of plasma 25OHD with BMD and TBS

Figure 1 provides a graphic presentation of the dose-response relationship between plasma 25OHD and BMD of total hip and femoral neck at year 1, as well as BMD and TBS of lumbar spine L1-L4 at year 5 obtained using the generalized additive models. For the aforementioned analyses, covariates included season of blood sampling, baseline age, BMI, calcium intake, ever smoking, alcohol consumption, physical level and SES, and treatment group during the intervention phase. The values for total hip and femoral neck BMD and TBS increased with higher plasma 25OHD until approximately 100 nmol/L, but with wide confidence intervals around this value (**Figure 1A, 1B and 1D**). There was no significant trend for the association between plasma 25OHD and spine BMD (**Figure 1C**).

In the categorical analyses, there were no significant differences between the three groups in total hip and femoral neck BMD measured at year 1 in the unadjusted analysis. After accounting for covariates, women with higher vitamin D status had significantly higher total hip (3.3%) and femoral neck (3.9%) BMD than those with lower vitamin D status (**Table 2**). In the unadjusted analysis, both the intermediate and the higher vitamin D status groups had significantly higher TBS at year 5 compared with the lower vitamin D status group (2.0% and 2.8%, respectively), and in the covariates adjusted analysis, the difference between the higher and lower vitamin D status groups remained significant

(2.0%) (Table 2). Further adjustment of bisphosphonates use at 5 years in the model had little influence on the results (1.232 ± 0.006 vs 1.208 ± 0.007, P = 0.015).

Fracture-related hospitalization

Over the 14.5 years of follow-up, a total of 372 (27.6%) participants experienced any fracture-related (including 338 non-vertebral and 34 vertebral fracture), and 143 (10.6%) experienced hip fracture-related hospitalization. The actual incidence of any fracture in participants with baseline 25OHD <50 nmol/L was 32.3% compared to 25.7% in those with 25OHD ≥50 nmol/L. The actual incidence of hip fracture in participants with baseline 25OHD <50 nmol/L was 14.1% compared to 9.2% in those with 25OHD ≥50 nmol/L.

Figure 2 shows the estimated adjusted HRs for each level of 25OHD vs a level of 75 nmol/L as estimated from the penalized spline regression model. There was a decrease in risk with increase in 25OHD levels up to 65 and 75 nmol/L for hip fracture and any fracture-related hospitalization, respectively (**Figure 2**). Compared with participants with a 25OHD level of 75 nmol/L, those with 25OHD <50 nmol/L had a significantly higher risk of any fracture and hip fracture-related hospitalization (i.e. 95% CI is entirely above 1.00). The analyses show an increasing gradient of risk for each outcome as baseline 25OHD fell below 65-75 nmol/L, with steeper slope for hip fracture. However, at higher levels no further reduction in risk was identified.

In the Cox regression analysis where plasma 25OHD was analyzed as a continuous variable, increased 25OHD levels associated with reduced risk for any fracture and hip fracture-related hospitalization in Models 1 and 2, but the associations were attenuated in Models 3 and 4 when femoral neck BMD or TBS was adjusted in the model (**Table 3**). In the categorical Cox regression analyses (Table 3), participants with intermediate and

higher 25OHD had significantly lower risk compared with those in the lower category for both any fracture and hip fracture-related hospitalization in Models 1 and 2 (Model 2 intermediate vs lower: HR=0.77, 95% CI 0.59, 0.99 for any fracture, and HR=0.60, 95% CI 0.40, 0.91 for hip fracture; higher vs lower: HR=0.70, 95% CI 0.54, 0.91 for any fracture, and HR=0.61, 95% CI 0.40, 0.92 for hip fracture). In Model 3 where Model 2 further adjusted for femoral neck BMD, the association was attenuated for any fracture but remained significant for hip fracture-related hospitalization (intermediate vs lower: HR=0.59, 95% CI 0.37, 0.94; higher vs lower: HR=0.58, 95% CI 0.36, 0.95). In Model 4 where Model 2 further adjusted for TBS, again the association was attenuated for any fracture but remained significant for hip fracture-related hospitalization (intermediate vs lower: HR=0.58, 95% CI 0.36, 0.93; higher vs lower: HR=0.62, 95% CI 0.38, 0.99), (Table 3). The risk for fracture outcomes of those with intermediate 25OHD level was not significantly different from those with higher 25OHD level (data not shown).

Other analyses

Only 29 (2.2%) women had detectable levels of plasma $25OHD_2$, and the mean $25OHD_3$ was 66.4 ± 28.2 nmol/L. Repeating the above analyses for bone and fracture outcomes using $25OHD_3$ values yielded similar results (data not shown). A competing risk analysis for Models 1 and 2 was also performed (**Supplemental Table 1**), and the associations were only slightly attenuated (Model 2 intermediate vs lower: sub-distribution hazard ratios (SHR) = 0.79, 95% CI 0.60, 1.00 for any fracture and SHR=0.62, 95% CI 0.41, 0.92 for hip fracture; higher vs lower: SHR=0.73, 95% CI 0.56, 0.96 for any fracture and SHR=0.64, 95% CI 0.42, 0.97 for hip fracture).

Discussion

Data from the current study identifies 25OHD levels of 50–75 nmol/L as the public health target in older Caucasian women to reduce fracture risk. Lower circulating 25OHD (<50 nmol/L) levels are associated with impaired bone structure and increased fracture risk; higher levels do not confer further benefit on fracture risk reduction, although bone density or TBS may be improved.

Other population based studies have been inconsistent regarding the influence of 25OHD on bone structure. Such results are possibly related to differences in study design, study population and covariates included in the analyses. Given the importance of age, gender and BMI on the epidemiology of bone structure and fracture risk, studies must consider these important covariates. For example in our study the association with total hip and femoral neck BMD was only significant after accounting for BMI, as BMI negatively associated with serum 25OHD (Table 1), but positively associated with BMD. Three studies have reported the dose-response relationship between 25OHD and bone structure. In a Scandinavian study of 5003 women aged 56 to 80 years, no increase in BMD was noted above a serum 25OHD concentration of 50 nmol/L (25). The Longitudinal Ageing Study of Amsterdam (LASA) reported an increase in BMD of total hip, trochanter and total body that reached a plateau at serum 25OHD of ~50 nmol/L in older individuals (26). An American study including older men and women, in which gender was apparently not a significant covariate, reported that in participants aged over 50 years, serum 25OHD levels were associated with higher BMD up to 90-100 nmol/L in all ethnic groups (27), but confidence intervals were not presented. In contrast, a French study did not identify any association between 25OHD and bone structure, however a relationship between low 25OHD and raised parathyroid hormone was reported (28).

Overall, data from the present study and others suggest a benefit on bone structure of aiming for a population level of 25OHD of at least 50 nmol/L in older women. This is perhaps related to improved bone mineralization associated with increased circulating level of 25OHD, given that subclinical osteomalacia has been reported to be common (43%) amongst individuals aged 15 to 95 years with low 25OHD (<50 nmol/L) in a German study (2). It has been reported that a 5% difference in BMD is associated with a 20% difference in the risk of osteoporotic fracture and a 50% difference in the risk of hip fracture (29), therefore an increase of 0.02 g/cm^2 total hip BMD (2.5%) from the lower to the middle range of 25OHD as shown in Figure 1 would be of clinical implication. Meta-analysis of randomized controlled trials (RCTs) that showed no significant effects of vitamin D supplementation on BMD do not undermine this conclusion given that few RCTs have used a threshold of 25OHD <50 nmol/L as an inclusion criteria (30, 31). Two recent Mendelian randomization studies did not find a causal positive association between variation in serum 25OHD and BMD (32, 33) or fracture risk (33). However, these Mendelian randomization studies only evaluated linear relationships, which may miss any threshold or "non-linear" effects of vitamin D insufficiency or deficiency, like those examined in our study.

With regard to fracture outcomes, the high risk of fracture in this ambulant population and thus increase statistical power may have contributed towards the relationship observed with 25OHD. The penalized spline curve identified a decline in hip fracture risk up to 65 nmol/L, and a decline in any fracture-related hospitalization up to 75 nmol/L. Nonetheless, we acknowledge that the clinical importance of a reduction in any fracture at 25OHD levels from 50 to 75 nmol/L in the penalized spline is uncertain, given the similarity in actual fracture rates in the intermediate (26.3%) and higher 25OHD (25.2%) groups. In the categorical analysis, women with intermediate (50-75 nmol/L) and higher

(≥75 nmol/L) 25OHD both had a significant and similar lower risk for any fracture and hip fracture-related hospitalization compared with individuals with lower 25OHD (<50 nmol/L).

The threshold level we identified for hip fracture and any fracture-related hospitalization is similar to the level reported in previous studies with follow-up time of 5-13 years (6, 34-36), however another study reported a level of 30 nmol/L (37), whereas a study in 773 Japanese community-dwelling women aged 69 years and older reported a serum 25OHD ≥71 nmol/L as best discriminating those with a lower risk for lower limb and vertebral fracture risk over 6 years follow-up (38). An early meta-analysis showed that by quartiles of actual vitamin D intake, reduction in the risk of hip and any non-vertebral fracture was observed only at the highest intake level with a median of 800 IU/day (39). Recent meta-analysis of RCTs reported that in the intention to treat analyses vitamin D supplementation was ineffective for fracture prevention in community dwelling older adults (31, 40). However, both trials using daily dose and intermittent high dose (such as 300,000IU (41) or 500,000IU annually (42)) were included in these meta-analyses, and there is now strong evidence of toxicity of vitamin D 60,000 IU monthly on increased falls risk (43). In addition both men and women were included in the same analysis but there are large differences in their fracture epidemiology such that men have very different thresholds for fracture risk and thus are more resistant to lower levels of 25OHD. Finally most RCTs did not select the study populations for low vitamin D status, while studies that did and conducted in high risk population (44) were not included in the meta-analysis (31, 40). The US Preventive Services Task Force (USPSTF) concludes that the current evidence is insufficient to assess the balance of benefits and harms of screening for vitamin D deficiency in asymptomatic adults (45). In community-dwelling, postmenopausal women who are not vitamin D deficient, the

USPSTF recommended against daily supplementation with 400 IU or less of vitamin D for the primary prevention of fractures. The USPTF also found inadequate evidence to estimate the benefits of doses greater than 400 IU of vitamin D to prevent fractures (46). Our data suggest that 25OHD levels below 50 nmol/L is related to increased risk for negative bone health outcomes such as impaired bone structure and increased fracture risk. Consequently, women with 25OHD below this level should be the target population of vitamin D intervention.

Unlike the all fracture outcome, the association of vitamin D status with hip fracture-related hospitalization remained after accounting for femoral neck BMD and TBS, possibly suggesting a non-bone structural benefit of 25OHD on reducing hip fracture risk (such as possible reduction in falling propensity) or skeletal benefits that cannot be detected by DXA-based techniques. Finally, TBS data presented here contradicts previous work suggesting that 25OHD is associated with cortical but not trabecular volumetric BMD (47).

Strengths of the current study include the prospective design, long-term follow-up and population-based setting with ascertainment of verified fracture-related hospitalizations including hip fractures independent of self-report. As few people of this age group move interstate, our study is likely to have nearly complete follow-up. Detailed information of potential confounders including previous osteoporotic fracture, BMI, lifestyle factors such as calcium and alcohol intake, smoking and physical activity was available and considered in the analyses. Despite the strengths of our study, limitations need to be acknowledged, which include circulating 25OHD concentration only measured at one time point. However, an American study showed high reproducibility for 25OHD and substantial agreement for the assignment of 25OHD quartile when blood samples were

collected 1-3 years apart matched on calendar date (48). Due to the observational nature of our investigation, causal links cannot be established and the observed associations could still be due to residual or uncontrolled confounding, such as time spent outdoors. Nonetheless, we adjusted for physical activity levels, which are likely to be strongly related to the amount time spent outdoors. The use of fracture-related hospitalization would result in an underestimation of fracture incidence and reflect more serious type of fractures. However, it offers the advantage of allowing ascertainment of fracture-related hospitalization independently of patient report with the associated problems such as loss to follow-up. In addition, fractures requiring hospitalization often have greater social, economic and clinical implications. Furthermore, participants of our study were Caucasian females, with a greater proportion had 25OHD levels above 50 and 75 nmol/L compared with levels reported in the general US population (45), thus our suggested threshold levels could be data dependent and have uncertainties (i.e. estimated 95% CI of the threshold) and may not be applicable to other populations.

In conclusion, in older Caucasian women our findings support 25OHD levels >50 nmol/L as a minimum public health target, whereas no additional reduction in fracture risk was observed with 25OHD levels above 75 nmol/L. These data support the Institute of Medicine recommendations that a circulating 25OHD level of 50 nmol/L would protect 97.5% of the population against adverse skeletal outcomes such as falls and fractures (20).

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Tables

Table 1 Characteristics of study participants according to baseline plasma 25-hydroxyvitamin D level

		< 50 nmol/L (n = 384)	**50-75 nmol/L (n = 491)**	**≥75 nmol/L (n = 473)**
Plasma 25OHD, nmol/L		35.9 ± 10.0[a]	62.3 ± 7.2[b]	96.8 ± 20.8[c]
Season of blood sampling, %				
	Winter	43.0	31.0	33.4
	Spring	46.1	41.5	32.1
	Summer	1.8	3.5	0.6

	Fall	9.1[a]	24.0[b]	33.8[c]
Age, years		75.4 ± 2.7	75.1 ± 2.7	75.1 ± 2.8
Body weight, kg		70.2 ± 13.3[a]	68.3 ± 12.6[a,b]	67.1 ± 11.6[b]
Height, cm		158.6 ± 5.9	158.9 ± 5.9	158.7 ± 6.0
BMI, kg/m^2		27.9 ± 5.0[a]	27.0 ± 4.7[b]	26.7 ± 4.5[b]
Physical activity, kcal/day		90 (0, 190)[a]	115 (37, 203)[b]	118 (48, 215)[b]
Ever smoking, %		39.1	36.9	34.9
Alcohol consumption of ≥ 3 unit/day, %		5.5	2.9	3.2
Socioeconomic status*		5 (3, 6) [a]	4 (3, 6) [b]	5 (3, 6) [a,b]
Timed Up and Go, seconds		9.8 (8.3-11.6)[a]	9.5 (8.2-11.1)[a]	9.2 (8.0-10.8)[b]
Calcium intake, mg/day		965 ± 365	949 ± 333	959 ± 366
Calcium treatment baseline to 5 yr, %		49.7	50.9	49.3
Prevalent fracture, %		26.8	26.3	29.0
Bisphosphonates ever used baseline to 5 yr, %**		8.7	8.2	6.0

Values are mean ± SD, median (inter-quartile range), or %. *Range 1-6, where a score of 1 indicates the top 10% most highly disadvantaged, and a score of 6 indicates the top 10% least disadvantaged; **Percentage based on n=252, 340, 331 for the <50, 50-75 and ≥75 nmol group who had medication data collected baseline to 5 yr. 25OHD, 25-hydroxyvitamin D; BMI, body mass index. Values in a row with different superscript letters are significantly different, $P < 0.05$ (analysis of variance with Tukey's HSD post

hoc test) or P < 0.017 in non-parametric test.

Table 2 Bone measures at year 1 and 5 according to baseline plasma 25-hydroxyvitamin D level

	25OHD < 50 nmol/L	**25OHD 50-75 nmol/L**	**25OHD ≥75 nmol/L**	**P value**
Year 1 hip DXA, n	265	377	357	
Total hip BMD, g/cm^2				
Unadjusted	0.804 ± 0.120	0.810 ± 0.132	0.815 ± 0.117	0.536
Covariate-adjusted	0.795 ± 0.007 [a]	0.812 ± 0.005 [a,b]	0.821 ± 0.006 [b]	0.010
Femoral neck BMD, g/cm^2				
Unadjusted	0.681 ± 0.099	0.689 ± 0.106	0.696 ± 0.100	0.189
Covariate-adjusted	0.675 ± 0.006 [a]	0.690 ± 0.05 [a,b]	0.701 ± 0.005 [b]	0.002
Year 5 lumbar spine DXA, n	274	351	343	
L1-L4 BMD, g/cm^2				
Unadjusted	0.959 ± 0.182	0.959 ± 0.192	0.944 ± 0.176	0.502

Covariate-adjusted	0.947 ± 0.011	0.960 ± 0.009	0.955 ± 0.010	0.660
L1-L4 TBS				
Unadjusted	1.202 ± 0.117 [a]	1.226 ± 0.105 [b]	1.236 ± 0.106 [b]	<0.001
Covariate-adjusted	1.208 ± 0.007 [a]	1.227 ± 0.006 [a,b]	1.232 ± 0.006 [b]	0.020

Values are mean ± SD for the unadjusted analysis, and mean ± SE for covariate-adjusted analysis. Values in a row with different superscript letters are significantly different, $P < 0.05$ (ANOVA with Tukey's HSD test for unadjusted analysis, ANCOVA with Bonferroni's test for covariate-adjusted analysis adjusted for season of blood sampling, baseline age, body mass index, physical activity level, calcium intake, smoking and alcohol consumption, socioeconomic status and calcium treatment group during the intervention phase).

Table 3 Adjusted hazard ratio between plasma 25-hydroxyvitamin D as continuous or categorical variables in relation to fracture-related hospitalization over the 14.5 years follow-up

	All	**Lower**	**Medium**	**Higher**
	Per SD 28 nmol/L change	< 50 nmol/L	50-75 nmol/L	≥75 nmol/L
	n = 1438	n = 384	n = 491	n = 473
	HR (95% CI)	Reference	HR (95% CI)	HR (95% CI)
Any fracture-related hospitalization				

n (%)	372 (27.6%)	124 (32.3)	129 (26.3)	119 (25.2)
Model 1	**0.87 (0.78, 0.97)**	1.00	**0.75 (0.58, 0.97)**	**0.69 (0.53, 0.90)**
Model 2	**0.88 (0.79, 0.98)**	1.00	**0.77 (0.59, 0.99)**	**0.70 (0.54, 0.91)**
Model 3	0.90 (0.79, 1.02)	1.00	0.84 (0.62, 1.14)	0.79 (0.58, 1.09)
Model 4	0.92 (0.81, 1.04)	1.00	0.75 (0.56, 1.02)	0.76 (0.56, 1.04)
Hip fracture-related hospitalization				
n (%)	143 (10.6%)	54 (14.1)	44 (9.0)	45 (9.5)
Model 1	**0.82 (0.68, 0.98)**	1.00	**0.60 (0.40, 0.91)**	**0.61 (0.41, 0.92)**
Model 2	**0.82 (0.68, 0.98)**	1.00	**0.60 (0.40, 0.91)**	**0.61 (0.40, 0.92)**
Model 3	0.83 (0.67, 1.03)	1.00	**0.59 (0.37, 0.94)**	**0.58 (0.36, 0.95)**
Model 4	0.86 (0.70, 1.06)	1.00	**0.58 (0.36, 0.93)**	**0.62 (0.38, 0.99)**

HR, hazard ratio.

Cox proportional hazards regression models adjusted for: Model 1: season of blood sampling, baseline age and body mass index, treatment group during the intervention phase and fracture history; Model 2: Model 1 + timed up and go test, calcium intake, ever smoking, consumption of ≥ 3 unit/day of alcohol, physical activity level and socioeconomic status (n = 1322); Model 3: Model 2 + femoral neck BMD at year 1 (n = 981); Model 4: Model 2 + TBS at year 5 (n = 953). Bold values indicate statistical significance at the $P < 0.05$ level.

Figures

Figure 1 Graphic presentation of the dose-response relationship between plasma 25-hydroxyvitamin D (25OHD) and bone mineral density (BMD, g/cm^2) of total hip (A) and femoral neck (B) at year 1 (n = 999), and lumbar spine L1-L4 BMD (g/cm^2) (C) and trabecular bone score (D) at year 5 (n = 968) obtained by generalized additive regression models. Models adjusted for season of blood sampling, baseline age, body mass index, calcium intake, smoking, alcohol intake, physical level and socioeconomic status, and treatment group during the intervention phase as covariates. Dotted lines represent 95% confidence intervals. The reference value for BMD is the value associated with the mean plasma 25OHD for all participants. The rug plot along the bottom of each graph depicts each observation.

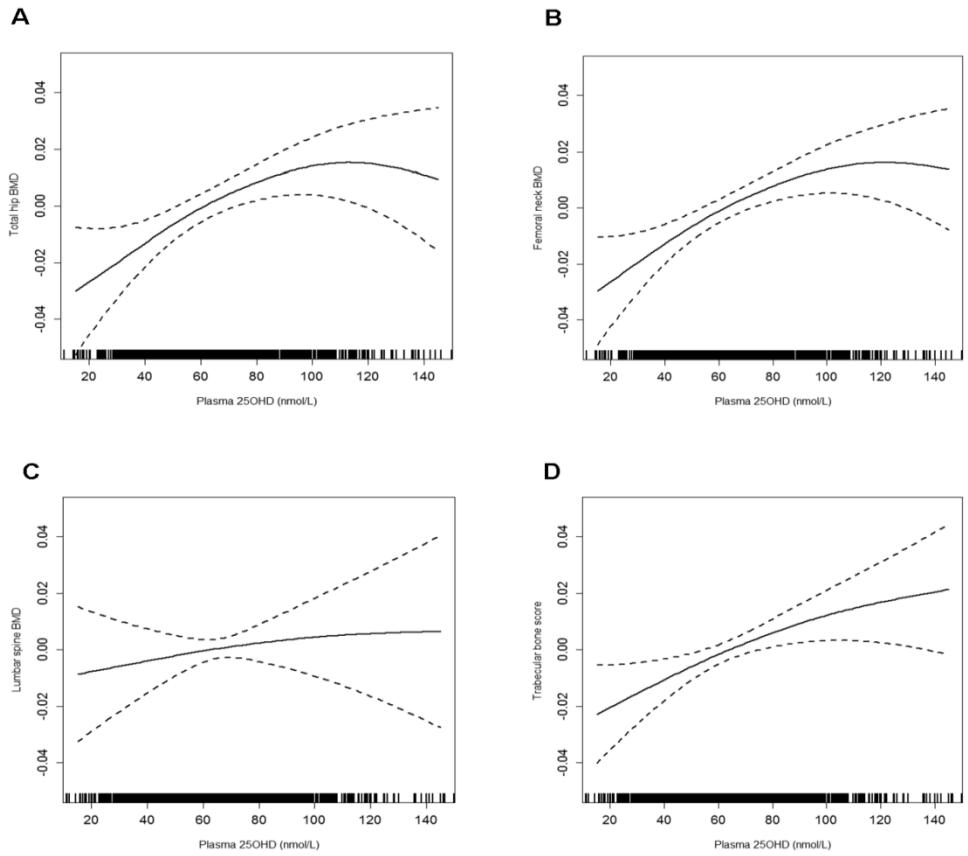

Figure 2 Adjusted hazard ratio (HR) from Model 2, for serum 25-hydroxyvitamin D (25OHD) concentration in relation to risk of hospitalizations related to any fracture (A) and hip fracture (B) based on fitted penalized spline using 75 nmol/L as the reference level, adjusted for season of blood sampling, baseline age and body mass index, fracture history, timed up and go test, calcium intake, ever smoking, consumption of ≥ 3 unit/day of alcohol, physical activity level and socioeconomic status, and treatment group during the intervention phase. Solid line is estimated hazard ratio and dotted lines represent 95% confidence interval.