**Cut-points for associations between vitamin D status and multiple musculoskeletal outcomes in middle-aged women**

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**ABSTRACT**

*Purpose*This study aimed to determine whether cut-points exist for associations between serum 25-hydroxyvitamin D (25OHD) and musculoskeletal health outcomes in middle-aged women, below which greater 25OHD levels are associated with musculoskeletal health benefits and above which no such associations exist.

*Methods* Cross-sectional study of 344 women aged 36-57 years. Cut-points for associations of serum 25OHD with lumbar spine (LS) and femoral neck (FN) bone mineral density (BMD), lower limb muscle strength (LMS), timed up and go test (TUG), functional reach test (FRT), lateral reach test (LRT) and step test (ST) were explored using locally weighted regression smoothing and nonlinear least-squares estimation, and associations above and below the identified cut-points estimated using segmented regression.

*Results* The prevalence of low 25OHD was 28% (<50 nmol/L). Significant cut-points (nmol/L) were identified for FN BMD 31 (95% confidence interval (CI): 18, 43), LS BMD 31 (17, 45), TUG 30 (24, 36), ST 33 (24, 31), FRT 31 (18, 43) and LMS 29 (8, 49) but not LRT (42 (-8, 93). Below these cut-points, there were beneficial associations between higher 25OHD level and each outcome while above the cut-points there were no beneficial associations.

*Conclusions* In middle-aged women, there are thresholds for associations between serum 25OHD concentrations and bone density and most balance measures, suggesting that a 25OHD levels of at least 29 to 33 nmol/L are required for optimal musculoskeletal health in this population. The current cut-off of 50 nmol/L may be higher than needed for some outcomes but appears warranted overall.

***Keywords:*** 25-hydroxyvitamin D; muscle strength; balance; bone density; middle-aged women

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**Mini abstract**

This was the first study examining optimal vitamin D status for musculoskeletal health in middle-aged women. A 25-hydroxyvitamin D level of at least 29 to 33 nmol/L appears required for optimal musculoskeletal health, but the current cut-off of 50 nmol/L may be warranted.

**Introduction**

Low bone mass in the premenopausal period is as important as fast rate of bone loss for the risk of fracture in later life([1](#_ENREF_1)). Falls are another important contributor to fracture risk. Impaired balance and mobility increases risk of falls([2](#_ENREF_2)), with 4-39% of falls in people older than 65 years accounted for by gait/balance disorders([3](#_ENREF_3)). Muscle weakness is an important contributor to decreased balance and functional limitations in older people ([4](#_ENREF_4)). Middle-aged women may have an accelerating decline in muscle mass, strength([5](#_ENREF_5)), bone mineral density (BMD) and bone strength([6](#_ENREF_6)). Balance also begins attenuating after 45-55 years of age([7](#_ENREF_7)), particularly in women. Therefore, it is critical to maintain or even improve BMD, muscle strength and balance in middle-aged people in the effort to reduce the risk of functional limitations, falls and fractures in older age.

Sufficient vitamin D is critical for bone health and potentially the prevention of muscle weakness, impaired balance and falls([8](#_ENREF_8)). However, there is controversy concerning the optimal vitamin D status for musculoskeletal health([9](#_ENREF_9)). The level of serum 25-hydroxyvitamin D (25OHD) which maximally suppresses serum PTH has been most commonly used to define the optimal vitamin D status, but these estimates have a wide range from 25 to 122 nmol/L([10](#_ENREF_10),[11](#_ENREF_11)). This may be due to the high fluctuation of PTH levels, varying with diet, physical activity, and time of day([12](#_ENREF_12)). Consequently, other musculoskeletal outcomes have been used to estimate the optimal vitamin D level, such as BMD, grip strength, falls, physical performance and fractures. However, evidence is primarily based on older individuals, and the results have been inconclusive and differ by different outcomes; cut-points were either not identified([13](#_ENREF_13),[14](#_ENREF_14)) or estimated to vary largely across studies (50 to 120 nmol/L)([15-18](#_ENREF_15)). In contrast, only one study examined threshold for 25OHD levels in younger US adults([19](#_ENREF_19)), but this was only based on the association with total hip BMD and no threshold was found. To our knowledge, cut-points for associations of vitamin D with muscle strength and balance, important factors for falls, have not been investigated in this age group.

Therefore, this cross-sectional study using follow-up data of a population-based randomised controlled trial aimed to: a) determine whether there are identifiable cut-points of serum 25OHD for associations between serum 25OHD and multiple musculoskeletal outcomes in middle-aged women; and if so, b) whether below those identified cut-points greater 25OHD concentrations have beneficial associations with these outcomes and if above them no such beneficial associations exist.

**Materials and Methods**

**Study sample**

Participants were from a 10-years additional follow-up of 2-year randomised controlled trial (RCT) conducted in 2000 in Southern Tasmania, Australia, details of which have been reported elsewhere([20](#_ENREF_20)). At baseline, women aged 25-44 years were randomly selected from the 2000 Tasmanian Electoral Roll. Women were excluded if they had previous measurement of bone density; had medical conditions affecting BMD (thyroid disease, renal failure, malignancy, or rheumatoid arthritis); a history of hysterectomy or were taking hormone replacement therapies; or who were pregnant, planning pregnancy within 2 years of study entry, or lactating. At baseline, 470 women were randomly assigned to one of two osteoporosis educational interventions: group education using the Osteoporosis Prevention and Self-management course (OPSMC) or an information leaflet. Participants had their BMD measured at the spine and hip at baseline, 2 years and 12. At baseline, those with a mean spine and hip T-score <0 were informed that they were at a higher risk in later life whereas those with a mean T-score of 0 or greater were informed that they were not at higher risk. The primary outcomes of the RCT were changes in femoral neck and lumbar spine BMD, calcium intake, physical activity, fitness and lower limb muscle strength. At two years, feedback of high compared to normal fracture risk had beneficial effects on change in femoral neck BMD, calcium supplement use and self-reported physical activity, but not on lower limb muscle strength. Group education had no benefits for any outcome over provision of a leaflet([20](#_ENREF_20)).

The present study is a cross-sectional analysis of the 344 women retained in the study and who had serum 25OHD levels measured (mean age of 50.0 years, 36.2 to 56.8 years of age) at 12 years. Ethics approval was obtained from the Tasmania Health and Medical Human Research Ethics Committee and all participants gave written informed consent.

**Serum 25OHD levels**

Venous blood samples were taken. Serum 25OHD was assayed by liquid chromatography tandem mass spectrometry (LC-MS/MS). The assay measures 25OHD2 and 25OHD3 separately with a CV 3-6%, using an internal standard.

**BMD**

BMD was measured at the lumbar spine (LS) and femoral neck (FN) by dual-energy X-ray absorptiometry (DXA) using fan beam setting on an in-house Hologic Delphi bone densitometer (Hologic QDR2000, Waltham, MA), calibrated daily with coefficient of variation (CV) 1%.

**Balance measurements**

Balance was assessed using 4 clinical balance tests - the timed up and go test (TUG), the step test (ST), the functional reach test (FRT) and the lateral reach test (LRT). All have been validated in older women and have normative values determined in women of the age in our study([21](#_ENREF_21)).

The TUG requires participants to sit in a normal armchair (45 cm high) with their back against the chair. They were timed when standing up, walking a distance of 3 m, turning around, walking back and sitting down with back against chair again. The average time of two trials was used for analysis. The test is reliable (intraclass correlation coefficient (ICC) = 0.99 for both interrater and retest reliability)([21](#_ENREF_21)).

The ST measures speed of performing a dynamic standing task. Participants stood on one leg 5 cm from an 8.5-cm-high block positioned against a wall and placed the whole foot of another leg onto the block and then returned it to the floor repeatedly as fast as possible for 15 seconds. The number of steps was recorded. Both sides were tested, and the mean number of steps for each side was calculated for analysis. The ST has a high reliability (ICCs > 0.90 in healthy older people)([22](#_ENREF_22)).

The FRT measures ability to reach forward with each arm from a bilateral stance position([23](#_ENREF_23)). Participants stood with feet a comfortable distance apart behind a line perpendicular and adjacent to a wall. The arm closest to the wall was raised to shoulder height and the position of the knuckle of the middle finger measured ([23](#_ENREF_23)). Keeping the feet flat they leaned forward as far as possible and the position of the knuckle was recorded at the point of furthest reach. FRT was the difference between the two measures. The mean score of three trials for each side was calculated for analysis. This test has a high interrater reliability (ICC = 0.98)([23](#_ENREF_23)).

The LRT measures ability to reach to the side in bilateral stance([24](#_ENREF_24)). Participants stood with their backs near but not touching a wall with the heels 10 cm apart. Participants raised both arms to shoulder height and maintained equal weight bearing while the position of the third finger’s tip on the side being measured was marked on the wall. Participants then lowered the arm not being measured and reach sideways as far as possible with the arm being measured. The position of furthest reach was marked and the difference between the two marks calculated. The mean of the three trials on each side was calculated for analysis. LRT has a high retest reliability (ICC > 0.94) in healthy older women([24](#_ENREF_24)).

**Lower limb muscle strength (LMS)**

LMS was measured to the nearest kilogram using a dynamometer (TTM Muscular Meter, Tokyo, Japan) ([25](#_ENREF_25)). This test examines isometric strength, predominantly of the quadriceps and hip extensors. The examiner demonstrated the correct technique to the participant before testing. Participants stood on the back of the dynamometer platform, with back straight against a wall and knees flexed to an angle of 115°. They held a bar, connected to the dynamometer by a chain, and lifted the bar using maximum force using their legs, with the back and neck straight. Two readings were made, and the mean calculated for analysis. The intraclass correlation coefficient for LMS was 0.94 (95%CI, 0.93, 0.95) in this study.

**Dietary intake**

Usual food intake was estimated using a food frequency questionnaire (Anti-Cancer Council of Victoria), which has been validated against 7-day food diaries with energy-adjusted correlation coefficients for nutrient intakes ranging from 0.28 for vitamin A to 0.78 for carbohydrate([26](#_ENREF_26)). Intakes of calcium, energy, fat, protein, carbohydrate, cholesterol, iron, magnesium, phosphorus, sodium, vitamin C, vitamin E, and zinc were calculated using NUTTAB 2010. The content of calcium in various food categories was determined by Australian food composition tables([27](#_ENREF_27)). Participants were also asked to recall if they had regularly used calcium and vitamin D supplements during the last year, where regular use means taking supplements at least 5 times per week for more than 9 months of the year.

**Physical activity**

We measured physical activity using a validated questionnaire([28](#_ENREF_28)), which was modified for Tasmanian conditions and used previously in women of this age. This questionnaire assessed strenuous and light physical activity levels by asking participants how many days in the last 2 weeks they reported performing at least 20 minutes of strenuous exercise and light exercise, represented by five categories (1 = 0 days, 2 = 1-2 days, 3 = 3-5 days, 4 = 6-8 days, 5 = 9 or more days).

**Anthropometry and other factors**

Height was measured by stadiometer (The Leicester height measure, Invicta Plastics Ltd, Oadby, England), weight by a single set of calibrated scales (Heine, Dover NH USA) and body mass index (BMI) (calculated weight (kg)/height (m)2). Questionnaire assessment was made of smoking history (current/former/never), breastfeeding history, number of children, family history of osteoporosis and/or fracture, and fracture history in the subject, education level, employment status of main financial provider in the household, menopausal status, and marital status. Prescription medication use was assessed by asking participants to report all medication, prescribed by a doctor that they had taken in the last 2 weeks. We also asked the history and total years of oral contraceptive pill use.

**Statistical analysis**

To adjust for the seasonal variation of 25-hydroxyvitamin D, deseasonalised vitamin D levels were calculated by regressing the measured 25OHD level on the sinusoidal function: sin(2π[day of year drawn/365]) + cos(2π[day of year drawn/365])([29](#_ENREF_29)), and then adding the residuals to the predicted mean value of 25OHD concentration from the model (mean = 63.1 nmol/L, occurred in 25th May i.e. late Autumn) to create a deseasonalised vitamin D level for each individual. The deseasonalisation provided us estimated 25OHD values for all participants as if they were measured at the same time of year, thus reducing the influences of the time when blood sample was taken.

Participants’ characteristics were presented using mean (SD) or number (%) as appropriate. Difference in characteristics between women with deseasonalised 25OHD level below and above 30 nmol/L, and between interventions were tested using Student’s t-test or Kruskal-Wallis or Chi-square test as appropriate.

To adjust for potential confounders in cut-points estimation, adjusted values were generated for each outcome by regressing each measured outcome on its specific confounding factors, and then adding the residuals to the mean of each measured outcome. Adjusted values for deseasonalised 25OHD levels were also generated in the same way using the same outcome specific covariates. The raw data and adjusted values were used for unadjusted and adjusted analyses, respectively. Cut-points were identified by three steps. Firstly, associations between serum 25OHD concentrations and outcomes were explored visually using locally weighted regression smoothing (LOWESS) plots to determine if the relationships appeared linear or nonlinear and in the latter case, if there were potential cut-points. Secondly, nonlinear least-squares estimation was used to determine the exact values of the cut-points by using the cut-points and starting values (i.e., intercept and slopes) identified from the LOWESS plots as initial values (Table 2). Lastly, segmented regression using adjusted values was utilized to determine associations (beta coefficients) for participants with 25OHD below and above the identified adjusted cut-points (Table 3), where linearity for each segment was verified based on LOWESS plots. A cut-point was confirmed when statistically significant in nonlinear least-squares estimation (Table 2), and below the cut-point the association between serum 25OHD levels and the outcomes were statistically significant and the effect size substantially greater than that above the cut-point (Table 3).

We selected potential confounders based on the biological plausibility of an association of a factor with both the outcome and the exposure of interest. Thus we considered age, menopausal status, weight, height, educational intervention, dietary calcium intake, education level, employment status, marriage status, strenuous physical activity, hours of watching TV, current smoking status, current use of estrogens and oral contraceptive pill, history and total years of taking oral contraceptive, history of fractures, and family history of osteoporosis and fractures as potential confounders. Weight and height were included in all models. Other factors were retained in the final model for each outcome when the estimated coefficient of serum 25OHD for that outcome changed by more than 10%. The type of fracture risk feedback given (i.e. of high or normal risk) was determined by baseline BMD. This meant that it was highly correlated with BMD so we could not adjust BMD models for fracture risk feedback group. At baseline, unsurprisingly, being in the high versus normal risk feedback group i.e. in the lower half of the BMD distribution was strongly associated with weight, height, BMI and lower limb strength. As we already adjusted for BMI in our models, further adjusting for risk feedback group would be an overadjustment. Furthermore, risk feedback group was not associated with change in lower limb muscle strength at 2 years. Thus we did not adjust for fracture risk feedback group.

All analyses were performed in Stata version 12 (Stata Corporation, Texas, USA). A two-tailed p value <0.05 was considered statistically significant.

**Results**

Baseline characteristics of participants who did and did not complete the 12 year follow-up have been previously reported([30](#_ENREF_30)). Briefly, women lost to follow-up (26%) were younger, had lower levels of educational attainment, and were more likely to be current smokers or to have ever smoked, and less likely to be married or in a de facto relationship compared to those who were retained, but other anthropometric and demographic factors were comparable. Table 1 gives characteristics of the study participants. The prevalence of low deseasonalised 25OHD was 6% (<30 nmol/L) and 28% (<50 nmol/L). Season distribution of 25OHD was: 111 (32%) late winter/early spring, 67 (20%) later spring/early summer, 76 (22%) later summer/early autumn, and 90 (26%) later autumn/early winter. All characteristics and outcomes at 12 years were comparable between educational interventions; however, women in the high fracture risk group were taller, heavier and had greater BMI, FRT, LMS and BMD of femoral neck and lumbar spine, compared to those in the normal risk group.

Unadjusted and adjusted LOWESS scatter plots showing exploratory views of non-linear associations of serum 25OHD with multiple musculoskeletal outcomes are given in **Figure 1** and **2**, respectively, with cut-points from nonlinear least squares estimation indicated by vertical lines. Table 2 gives the adjusted and unadjusted cut-points with their 95% confidence intervals. Cut-points were similar and statistically significant for most outcomes in adjusted and unadjusted analyses, except for LRT (33 (1-64) nmol/L unadjusted and 42 (-8, 93) nmol/L adjusted, respectively). Comparisons of characteristics of women with deseasonalised 25OHD <30 nmol/L with those of women with 25OHD of 30 nmol/L or more are given in Table 1. Women with deseasonalised 25OHD < 30nmol/L were less likely to be current smokers and had lower weight and body mass index (BMI) but higher strenuous physical activity levels .

Adjusted analyses for associations between 25OHD level and outcomes in participants with 25OHD level above and below the adjusted cut-points are given in Table 3. Below the cut-points, greater 25OHD levels were associated with increased FN and LS BMD (equivalent to an average improvement of 1% per nmol/L increase in 25OHD concentrations at each site) as well as improved TUG, ST FRT and LMS (equivalent to 1.8, 0.9, 1.1 and 4.3% improvements per nmol/L increase in 25OHD respectively). Above the cut-points, there were no beneficial associations and the only statistically significant association was a deleterious one, being a small increase in TUG.

Adjusting for educational intervention, estrogen use, oral contraceptive use or its duration resulted in similar results for all outcomes, therefore they were not included in the final models.

**Discussion**

To our knowledge, this is the first study to assess the optimal level of serum 25OHD for musculoskeletal health using multiple clinically important endpoints and the first examining associations with LMS and balance in middle-aged women from a follow-up of a population-based RCT. Cut-points for associations between serum 25OHD level and the majority of outcomes were identified ranging from 29 to 33 nmol/L. Below these greater 25OHD level is associated with increased FN and LS BMD, LMS and better performance on balance tests (an average improvement of 0.9% to 4.3% per nmol/L increase in 25OHD concentrations, while above them no beneficial associations were observed, suggesting these are minimum levels required for optimal musculoskeletal health. These effect sizes compare favourably to an average annualized loss of 1.0% and 1.4% for total hip BMD in the late perimenopausal period ([31](#_ENREF_31)) and annualized decline of 2.2% and 2.5% for grip strength, 0.3% and 1.6% for the standard Romberg test, and 0.19% and 0.25%% for gait velocity observed in women aged 50 and 60 years at baseline, respectively([32](#_ENREF_32)).

Previous estimates of the optimal serum 25OHD level have mainly been based on data from older populations([13](#_ENREF_13),[14](#_ENREF_14),[16](#_ENREF_16),[17](#_ENREF_17)) and results have been inconsistent with some failing to identify cut-points([13](#_ENREF_13),[14](#_ENREF_14)) and the others supporting a level of serum 25OHD greater than 50 nmol/L and up to 120 nmol/L([15-18](#_ENREF_15)). This may be explained by the methodological differences, for example differences in endpoints, study design and population, statistical methods and serum 25OHD assay methods. Accordingly, the choice of optimal 25OHD level for skeletal health remains controversial, with a range from 50 to 100 nmol/L supported by some but not all experts, though there is an agreement that a level of less than 50 nmol/L is suboptimal for skeletal health([9](#_ENREF_9)). The cut-points we identified should not be interpreted as values at which a sharp transition in slopes occurs, but rather a region of transition between strong and weak association as seen in the figures and as indicated by the wide 95% confidence intervals (CI) of the cut-points. The lower 95% CIs were less than 25 nmol/L for all outcomes and the upper 95% CIs ranging from 36 to 49 nmol/L. Therefore, even though the cut-points of 29 to 33 nmol/L we identified are somewhat lower for most outcomes than the currently accepted cut-off of 50 nmol/L, this higher level may still be warranted for optimal musculoskeletal health. When assessing serum 25OHD status of individuals compared to the current definition of deficiency of 50 nmol/L, it has been suggested that the season of measure needs to be considered in judging the clinical importance of the degree of deficiency. The allowance made for seasonal differences will differ according to location, particularly latitude([33](#_ENREF_33)). For example, in current Australian guidelines suggest that a target level of 60 nmol/L be applied in summer([34](#_ENREF_34)). By deseasonalising, we were able to remove the component of variation in serum 25OHD between individuals that was due to the season in which serum 25OHD was measured. However, similar clinical judgement would still need to be applied in interpreting the deseasonalised cut-points for individual patients.

The 1% greater BMD per 1 nmol/L higher serum 25OHD is a large effect size. In elderly women, it has been estimated that for each 5% loss in FN BMD there is a 40% and 90% increase in all fractures and hip fracture risk, respectively([35](#_ENREF_35)). This may also apply in younger populations because BMD tracks throughout lifetime([36](#_ENREF_36)), i.e., people with lower BMD during midlife remain on a trajectory for having lower BMD than others into old age. If raising serum 25OHD in deficient women by 5 nmol/L could increase BMD by 5%, this would be a major and clinically important effect but ideally a RCT is required to confirm whether this can be obtained. A RCT in younger postmenopausal women (aged 50-65 years) has shown beneficial effects of daily 1000 IU vitamin D supplementation on incidence of falls, postural balance, muscle strength and loss of lean mass([37](#_ENREF_37),[38](#_ENREF_38)), but such a trial of correcting vitamin D deficiency in middle-aged women with bone density outcomes is lacking and should be a high research priority.

The associations between 25OHD and other outcomes may also be clinically relevant. For example, in the case of muscle strength, a 25-yr prospective study of initially healthy middle-aged men (45-68 years old) showed that compared to those in the highest tertile of baseline grip strength, those in the lowest and middle tertiles were at greater risk of developing functional limitations and disabilities in old age (ORs ranging from 1.07 to 2.80)([39](#_ENREF_39)). Similar long-term data in women are lacking. Interpreting effect sizes of balance tests is challenging in our setting because of a lack of studies. However, balance tests have been shown to accurately predict falls risk in older adults([23](#_ENREF_23)). In addition, Zhu et al. showed that TUG test was a risk factor for incident nonvertebral fracture in elderly women (hazard ratio = 1.54 (95% CI: 1.15-2.07) for <10.2 vs. >10.2 seconds), independent of BMD and other risk factors([40](#_ENREF_40)). However, it should be noted that direct evidence that deficits in balance in middle-age have effects in older adult life is lacking. Given the lengthy period of follow-up required to assess such associations, this is likely to remain problematic.

Previous studies in this age group are limited. A previous large cross-sectional study by Bischoff-Ferrari et al. did not identify a cut-point for the relationship between serum 25OHD levels and hip BMD([19](#_ENREF_19)). Higher serum 25OHD levels were associated with greater BMD in the hip throughout a reference range of 22.5 to 94 nmol/L, though most benefit was seen with a 25OHD level below ~50 in younger women (aged 20 to 49 years) ([19](#_ENREF_19)). This is broadly consistent with our findings, though in our study there was an identifiable cut-point of 31 nmol/L and benefit of greater 25OHD concentrations on FN and LS BMD only existed in those with 25OHD level below this. One potential explanation for the discrepancy is the relatively higher calcium intake of 1186 mg/day in our study compared to 881 mg/d in the study by Bischoff-Ferrari et al.([19](#_ENREF_19)). In elderly people with low calcium intake a higher serum 25OHD concentration (up to 120 nmol/L) is needed to keep PTH within the normal range([15](#_ENREF_15)). Also, compared to the nonlinear least-squares estimation utilized in our study, the LOWESS used by Bischoff-Ferrari et al.([19](#_ENREF_19)) is an exploratory approach, which does not allow for an accurate determination of the cut-point.

In addition to the effect of vitamin D on calcium homeostasis and BMD, its protective effect on fractures may be mediated by improving lower-extremity function, thus reducing falls risk([41](#_ENREF_41)). Significant cut-points were identified for associations between serum 25OHD, LMS and most balance tests in our study, ranging from 29 to 33 nmol/L. Similar results were reported in a cross-sectional study in US older adults (aged ≥60 years), indicating that lower-extremity function (i.e. sit-to-stand test and 8-foot walk test) increased continuously with greater serum 25OHD level throughout a reference range from 22.5 to 94 nmol/L, with most of the improvement occurred in 25OHD level below around 40 nmol/L([41](#_ENREF_41)). This is important because balance begins attenuating in midlife([7](#_ENREF_7)), and it has been suggested that the prevention of functional limitations in older age should begin in midlife([42](#_ENREF_42)). Thus the potential for correcting vitamin D deficiency and maintaining ongoing adequate levels to improve muscle strength and balance in middle aged women also appears worthy of exploration by randomised controlled trials.

 There was an association with poor performance on TUG above the cut-point of 30 nmol/L. The effect size was small (0.004 sec/nmol/L increase in 25(OH)D, less than 0.1% of the mean TUG). The reasons for a deleterious association being present above a cut-point as low as 30 nmol/L are unclear, as other studies report that only very high 25OHD levels may be detrimental for lower extremity functiont([41](#_ENREF_41),[43](#_ENREF_43)). However, given the small effect size, this is unlikely to have clinically important effect even at very high levels of vitamin D (above 120 nmol/L).

Our study has several limitations. One is the cross-sectional design, which means that causal associations between vitamin D and BMD, muscle strength and balance cannot be demonstrated. RCTs have shown that vitamin D supplementation improved serum 25OHD concentrations and decreased serum PTH levels in premenopausal women([44](#_ENREF_44)) but longitudinal and RCT data in younger women are otherwise lacking. As mentioned, well-designed RCTs are needed to directly confirm a causal relationship and determine the magnitude of any effect of improving vitamin D levels in women with sub-optimal levels. The prevalence of low serum 25OHD was relatively low and this could be explained by the high proportion of women taking vitamin D supplements which may in part be attributed to them taking part in an osteoporosis education intervention trial. Indeed, the National Health Survey component of the Australian Health Survey showed that only 5% Australian adults were taking Vitamin D supplements in 2011-12([45](#_ENREF_45)). As a result, there were relatively few women with 25OHD below the identified cut-points and this lowered the reliability and precision of estimating cut-points and associations below those cut-points, particularly for LMS. Although the original study([20](#_ENREF_20)) had a population-based design, the participants were exposed to an osteoporosis behavioural intervention and there was a dropout rate of 26% by the end of final follow-up. There were some differences in sociodemographic characteristics and smoking behaviour between women retained in the study and those lost to follow-up but the wide spread of education levels at baseline and employment rate at 12 years approximates the overall population figures for these socioeconomic factors and adjustment for potential confounders was performed so our findings are still likely to apply to healthy middle-aged women from a range of sociodemographic backgrounds.

In conclusion, in these middle-aged Australian women cut-points for associations between serum 25OHD level and the majority of outcomes were observed, below which greater 25OHD level is associated with increased BMD and LMS as well as better performance on balance tests, while above which, there are no such associations. A level of 25OHD of at least 29 to 33 nmol/L appears required for optimal musculoskeletal health in this population, but the current cut-off of 50 nmol/L may be warranted. Longitudinal studies are required to further confirm these findings and randomized controlled trials are necessary to accurately assess the effects of correcting vitamin D deficiency on musculoskeletal health in this age group.

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Ethical approval: “All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.”

Informed consent: “Informed consent was obtained from all individual participants included in the study.” **References**

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**Table 1** Characteristics of participants (n=344)

|  |  |  |
| --- | --- | --- |
|  | Total | Deseasonalised 25OHD (nmol/L)† |
| Characteristic | n=344 | <30 (n=20) | ≥30 (n=324) |
| Age (yr.) | 50.0 (5.1) | 50.5 (3.7)  | 50.0 (5.2) |
| Height (cm) | 164.0 (6.2) | 162.9 (4.4) | 164.0 (6.2) |
| Weight (kg) | 73.7 (15.8) | 83.7 (21.7) | 73.1 (15.1)\* |
| Body mass index (kg/m2) | 27.4 (5.8) | 31.5 (8.0) | 27.2 (5.5)\* |
| Currently smoking n (%) | 26 (7) | 6 (30) | 19 (6)\* |
| Strenuous activity level, median (IQR) | 3.0 (1.4) | 1.5 (1-3) | 3 (2-4)\* |
| Deseasonalised 25OHD (nmol/L)† | 63.1 (22.1) | 24.9 (5.3) | 65.5 (20.5)\* |
| Serum 25OHD level (nmol/L) | 63.1 (22.8) | 24.5 (7.7) | 65.5 (21.2)\* |
| Vitamin D supplement use n (%) | 126 (36) | 4 (20) | 121 (37) |
| Calcium supplement use n (%) | 123 (35) | 4 (21) | 118 (36) |
| Dietary calcium intake (mg/d) | 1184 (494) | 1119 (426) | 1188 (499) |
| History of taking contraceptive  |  |  |  |
|  Yes, n (%) | 332 (97) | 20 (100) | 312 (96) |
|  Duration (yr.), median (IQR)# | 5 (4-5) | 5 (4-5) | 5 (4-5) |
| Current use of oral contraceptive n (%)‡ | 37 (11) | 1 (5) | 36 (11) |
| Current use of estrogens n (%)‡ | 24 (7) | 0 (0) | 24 (7) |
| Menopausal status n (%) |  |  |  |
|  Post-menopause | 86 (25) | 6 (30) | 79 (24) |
|  Pre-menopause | 134 (39) | 8 (40) | 123 (38) |
|  Peri-menopause | 102 (29) | 3 (15) | 23 (31) |
|  Status unclear | 26 (7) | 3 (15) | 99 (7) |
| Timed up and go test (seconds) | 5.3 (0.7) | 5.8 (0.8) | 5.3 (0.7) |
| Step test (steps) | 18.6 (4.7) | 16.6 (2.1) | 18.5 (2.6) |
| Functional reach test (cm) | 41.2 (6.3) | 40.6 (6.7) | 41.3 (6.3) |
| Lateral reach test (cm) | 18.7 (3.9) | 18.0 (3.6) | 18.8 (3.9) |
| Lower limbs muscle strength (kg) | 75.8 (25.5) | 72.7 (25.5) | 75.9 (25.5) |
| Femoral neck BMD (g/cm2) | 0.814 (0.125) | 0.800 (0.147) | 0.814 (0.124) |
| Lumbar spine BMD (g/cm2) | 1.035 (0.151) | 1.018 (0.128) | 1.036 (0.152) |

†adjusted for season, see text for details.

‡use in the last two weeks.

#for those who took contraceptive.

25OHD, 25-hydroxyvitamin D; BMD, areal bone mineral density; IQR, inter-quartile range.

Values are Mean (SD) unless otherwise stated;

\*p<0.001 compared to deseasonalised 25OHD <30 nmol/L group.

**Table 2** Unadjusted and adjusted cut-points for associations between deseasonalised serum 25OHD level and multiple musculoskeletal outcomes

|  |  |
| --- | --- |
|  | Cut-points of 25OHD (nmol/L) |
|  | Unadjusted | Adjusted |
| Femoral neck BMD (g/cm2) | **32 (19, 45)** | **31 (18, 43) †** |
| Lumbar spine BMD (g/cm2) | **35 (17, 54)** | **31 (17, 45) †** |
| Timed up and go test (seconds) | **34 (28, 40)** | **30 (24, 36) ‡** |
| Step test (steps) | **36 (29, 43)** | **33 (24, 41) ‡** |
| Functional reach test (cm) | **27 (16, 38)** | **31 (18, 43) ‡** |
| Lateral reach test (cm) | **33 (1, 64)** | 42 (-8, 93) ‡ |
| Lower limb muscle strength (kg) | **31 (19, 44)** | **29 (8, 49) ‡** |

Bold denotes statistical significance, p<0.05, 25OHD, 25-hydroxyvitamin D; BMD, areal bone mineral density;

†Adjusted outcomes and deseasonalised 25OHD level were used, adjusted for weight, height, menopausal status, strenuous physical activity, dietary calcium intake and currently smoking status.

‡Adjusted outcomes and deseasonalised 25OHD level were used, adjusted for age, weight, height, educational level, strenuous physical activity and currently smoking status.

**Table 3** Associations between serum 25OHD level and multiple musculoskeletal outcomes# below and above adjusted cut-points of 25OHD level

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Cut-points |  | Below cut-point |  | Above cut-point |
|  |  | n | β (95% CI) | n | β (95% CI) |
| Femoral neck BMD (g/cm2)† | 31 | 10 | 0.008 (-0.001, 0.017) | 333 | 0.0002 (-0.0003, 0.0008) |
| Lumbar spine BMD (g/cm2)† | 31 | 11 | **0.010 (0.001, 0.018)** | 332 | -0.0005 (-0.0012, 0.0002) |
| Timed up and go test (seconds)‡ | 30 | 9 | **-0.10 (-0.16, -0.04)** | 330 | **0.004 (0.001, 0.007)** |
| Step test (steps)‡ | 33 | 22 | **0.16 (0.02, 0.31)** | 317 | -0.01 (-0.02, 0.003) |
| Functional reach test (cm)‡ | 31 | 10 | 0.44 (-0.21, 1.09) | 329 | -0.01 (-0.04, 0.02) |
| Lower limb muscle strength (kg)‡ | 29 | 5 | **2.64 (0.74, 4.55)** | 334 | -0.04 (-0.17, 0.09) |

Bold denotes statistically significant association within subgroup, p<0.05, 25OHD, 25-hydroxyvitamin D; BMD, areal bone mineral density

#Lateral reach test was not tested as there was not a significant adjusted cut-points (see Table 2).

†Adjusted outcomes and deseasonalised 25OHD level were used, adjusted for weight, height, menopausal status, strenuous physical activity, dietary calcium intake and currently smoking status.

‡Adjusted outcomes and deseasonalised 25OHD level were used, adjusted for age, weight, height, educational level, strenuous physical activity and currently smoking status.

**Figure legends**

**Figure 1, A-G.** Unadjusted scatter plots and Locally weighted regression smoothing (LOWESS) curves for exploratory views of associations of serum 25OHD levels with multiple musculoskeletal outcomes, vertical lines indicate identified unadjusted cut-points (see Table 3) (raw data used).

**Figure 2, A-G.** Adjusted scatter plots and locally weighted regression smoothing (LOWESS) curves for exploratory views of associations of serum 25OHD levels with multiple musculoskeletal outcomes, vertical lines indicate identified adjusted cut-points (see Table 3) (adjusted values for deseasonalised 25OHD level and outcomes used, see text for details).