Clinical Trial

25-Hydroxyvitamin D Threshold for the Effects of Vitamin D Supplements on Bone Density

Secondary Analysis of a Randomized Controlled Trial†

Helen M Macdonald[1], Ian R Reid[2,3], Gregory D Gamble[2], William D Fraser[4], Jonathan C Tang[4], Adrian D Wood[1]

1 School of Medicine & Dentistry, University of Aberdeen, Aberdeen, UK
2 Department of Medicine, University of Auckland, New Zealand
3 Department of Endocrinology, Auckland District Health Board, New Zealand
4 Norwich Medical School, University of East Anglia, Norwich, UK

Correspondence to:
Professor Ian Reid
Faculty of Medical and Health Sciences
University of Auckland
Private Bag 92019
Auckland, New Zealand
Tel: (+64 9) 923 6259
Fax: (+64 9) 308 2308
email: i.reid@auckland.ac.nz
Ian Reid ORCID ID: 0000-0001-6021-5458

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Disclosures

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Abstract

Most trials of vitamin D supplementation have shown no benefits on bone density (BMD), though severe vitamin D deficiency causes osteomalacia which is associated with profound BMD deficits. Recently, the ViDA-BMD study from New Zealand demonstrated a threshold of baseline 25-hydroxyvitamin D (30 nmol/L) below which vitamin D supplementation did benefit BMD. We have now re-examined data from a similar trial in Aberdeen to determine whether a baseline 25-hydroxyvitamin D threshold of 30 nmol/L is also observed in that database. The Aberdeen study recruited 305 postmenopausal women in late winter and randomized them to receive placebo, vitamin D 400 IU/day or vitamin D 1000 IU/day over one year. As previously reported, BMD loss at the hip was reduced by vitamin D 1000 IU/day only, and there was no significant treatment effect of either dose at the lumbar spine. In the present analysis, when the trial participants were grouped according to whether their baseline 25-hydroxyvitamin D was ≤30 nmol/L or above this threshold, significant treatment effects were apparent at both the spine and hip in those with baseline 25-hydroxyvitamin D ≤30 nmol/L, but no significant effects were apparent in those with baseline 25-hydroxyvitamin D above this level. There was evidence of a similar threshold for effects on parathyroid hormone, but no groups showed changes in bone turnover markers during the study.

It is concluded that vitamin D supplements only increase bone density in adults with nadir 25-hydroxyvitamin D ≤30 nmol/L. This moves us further towards a trial-based definition of vitamin D deficiency in adults with adequate calcium intakes, and suggests that supplement use should be targeted accordingly. Future trials of vitamin D supplementation should focus on individuals with 25-hydroxyvitamin D concentrations in this range.

Key Words: vitamin D, vitamin D metabolites, 24,25-dihydroxyvitamin D, osteoporosis, DXA, nutrition, biochemical markers of bone turnover, PTH

Introduction

Vitamin D supplementation is widely recommended and used in the prevention and treatment of osteoporosis.[1] However, meta-analyses have failed to demonstrate effects of these supplements used alone on bone mineral density (BMD) or fracture.[2-5] In contrast, vitamin D treatment of patients with severe vitamin D deficiency resulting in osteomalacia, produces increases in absolute BMD of as much as 50% in 12 months.[6] This suggests that trials to-date have not been carried out in sufficiently D-deficient individuals. This possibility is supported by the finding that when BMD trials are categorized according to baseline 25-hydroxyvitamin D (25OHD) concentrations above or below 50 nmol/L, a significant treatment effect is found in studies below this threshold, but not in the equal number of studies above this level.[2] That division of studies at 50 nmol/L in that analysis was determined *a priori*, but a p*ost hoc* examination of the trial results in that meta-analysis indicated that benefit was only found in trials with baseline 25OHD <40 nmol/L.[2]

The possibility that baseline 25OHD influences treatment response to vitamin D has recently been assessed in detail in the bone density sub-study of the Vitamin D Assessment (ViDA) Study.[7] In that Auckland, New Zealand study, 452 older adults with a mean baseline 25OHD concentration of 56 nmol/L were randomized to vitamin D or placebo for 2 years. In the whole cohort, there was no significant treatment effect in the lumbar spine or total body, but BMD loss at both hip sites was attenuated by ~0.5% over 2 years. There was a significant interaction between baseline 25OHD and treatment effect. With baseline 25OHD ≤30 nmol/L (n=46), there were between-groups BMD changes at the spine and femoral sites of ~2%, significant in the spine and femoral neck. When baseline 25OHD was >30 nmol/L,

differences were ~0.5% and significant only at the total hip. When the cohort was divided at 40 or 50 nmol/L, the contrast in treatment effects above and below the thresholds was less marked, with between-groups differences for change in BMD being very similar at >30, >40 or >50 nmol/L.

Macdonald et al have carried out a similar trial over one year in 305 postmenopausal women in Aberdeen.[8] Like the ViDA-BMD study, all participants were enrolled at the end of winter and 25OHD was measured using liquid chromatography–tandem mass spectrometry. In the Aberdeen study, baseline 25OHD concentrations were lower, at 34 (SD 15) nmol/L. Mean BMD loss at the hip was reduced by vitamin D 1000 IU/day only. There was no significant treatment effect of either dose at the lumbar spine. No analysis of the effect of baseline 25OHD on treatment response was carried out.

The present report presents a re-analysis of the Aberdeen trial to determine whether the 30 nmol/L threshold of baseline 25OHD for a vitamin D treatment effect on BMD found in the ViDA-BMD study, can be confirmed in this independent trial cohort which shared important design elements. In other words, we are using the Aberdeen trial as a validation cohort for the results found in the ViDA study.

Methods

The details of the Aberdeen study have already been described.[8] In brief, this was a 1-year, double-blind, placebo-controlled trial to determine the effects of daily oral vitamin D_3 in doses of 400 IU or 1000 IU, compared with placebo, on BMD in 305 non-smoking women from the northeast of Scotland, aged 60 – 70 years.[8] All participants started the trial between January and March 2009. BMD was measured using a Lunar iDXA, (GE Medical, Madison, WI), and 25OHD by tandem mass spectrometry using the US National Institute of Standards and Technology standard,[9] in a laboratory which takes part in the DEQAS quality-control scheme for vitamin D and has full certification via this scheme. Inter-assay coefficients of variation were <10% for both $25OHD_2$ and $25OHD_3$, and the sum of these analytes is reported here. The 25OHD metabolite, 24,25-dihydroxyvitamin D was also measured by tandem mass spectrometry using a prior de-lipidation procedure to maximise recovery.[10] Overnight fasted blood samples collected at each visit were stored at -80°C, and each participant's complete set batched together before analysis. Details of analysis techniques have already been reported.[8]

Since this was a reanalysis of an existing study no power analyses were performed. The primary comparison of the effects of treatment on BMD in those with baseline 25OHD levels above and below 30 nmol/L was pre-specified. Time-course data were analyzed using a mixed models approach to repeated measures with an unstructured covariance matrix. Significant main and or interaction effects were further explored using the method of Tukey.

Changes in BMD across treatment groups have been assessed separately for those with baseline 25OHD concentrations above or below 30 nmol/L by two-way analysis of covariance with baseline BMD included as covariate, using the programs of SAS (version 9.4 SAS Institute Inc., Cary NC). Analyses are by intention-to-treat. Since all comparisons were pre-planned no adjustment to the overall critical significance level ($P<0.05$) was employed.

Results

Baseline characteristics of the study participants, divided according to baseline 25OHD are shown in Table 1. BMI and PTH were higher in those with 25OHD ≤ 30 nmol/L, and 24,25-dihydroxyvitamin D was lower.

Biochemistry

Changes in 25OHD according to its baseline levels are shown in Figure 1. The placebo group showed the expected seasonal variation in 25OHD, with a peak-to nadir difference of about 20 nmol/L in those starting at ≤ 30 nmol/L but of about half this magnitude in those whose 25OHD was > 30 nmol/L at baseline. In the ≤ 30 nmol/L participants, the effects of the two vitamin D doses on 25OHD were almost comparable, whereas in participants with baseline 25OHD > 30 nmol/L a dose-response was apparent.

Baseline concentrations of the 25OHD metabolite, 24,25-dihydroxyvitamin D, were ~50% lower in those with 25OHD ≤ 30 nmol/L compared with those with 25OHD > 30 nmol/L, but after supplementation similar concentrations of this metabolite were reached in participants starting either above or below this 25OHD cut-point. Ratios of 25OHD to 24,25-dihydroxyvitamin D were comparable in the low and high 25OHD groups at baseline (Table 1), and declined following supplementation, slightly more so in those given 1000 IU/day (ratios at 12 months in those with low baseline 25OHD: placebo 12.0 [95%CI 10.84,13.22], 400 IU/day 11.1 [10.1,12.2], 1000 IU/day 10.2 [9.1,11.2]; ratios at 12 months in those with high baseline 25OHD: placebo 13.1 [11.8,14.3], 400 IU/day 10.5 [9.3,11.6], 1000 IU/day 10.0 [9.4,10.6]).

Baseline concentrations of 1,25-dihydroxyvitamin D were comparable in those with 25OHD below or above 30 nmol/L (Table 1) and there were no changes in this metabolite with treatment, irrespective of baseline 25OHD status (change from baseline at 12 months in those with low baseline 25OHD: placebo -2 [-17, 13] pmol/L; 400 IU/day 1 [-11, 14] pmol/L; 1000 IU/day 4 [-9, 18] pmol/L; change from baseline at 12 months in those with high baseline 25OHD placebo -8 [-18, 2] pmol/L; 400 IU/day 0 [-10, 10] pmol/L; 1000 IU/day -2 [-13, 9] pmol/L; P > 0.4).

At baseline, mean PTH was 5.5 (SD 1.3) pmol/L in those with 25OHD ≤ 30 nmol/L, and 4.8 (1.2) pmol/L in those with 25OHD > 30 nmol/L (P = <0.0001, Table 1). Changes in PTH during the study are shown in Figure 2. PTH concentrations decreased in all groups during the first 6 months associated with the transition from winter to summer. In participants with baseline 25OHD ≤ 30 nmol/L, PTH was decreased at 12 months by both doses of vitamin D (ΔPTH: -0.2, -0.7, and -1.0 pmol/L in placebo, 400 IU and 1000 IU groups, respectively, P = 0.0003). In those with baseline 25OHD >30 nmol/L, PTH was only decreased at 12 months by vitamin D 1000 IU/day and the changes were smaller (ΔPTH: -0.2, -0.3, -0.6 pmol/L, respectively, P = 0.05).

Baseline concentrations of C-telopeptide (CTX) were comparable in those with 25OHD below or above 30 nmol/L (381 ± 160 ng/L [mean ± SD] and 389 ± 163 ng/L, respectively, P = 0.70) and there were no changes in this marker after supplementation (change from baseline at 12 months in those with low baseline 25OHD: placebo -5 [-38, 27] ng/L, 400 IU/day -4 [-39, 31] ng/L, 1000 IU/day 18 [-13, 49] ng/L; change from baseline at 12 months in those with high baseline 25OHD

placebo 11 [-26, 47] ng/L, 400 IU/day 12 [-23, 47] ng/L, 1000 IU/day -1 [-27, 26] ng/L; P > 0.5).

Baseline concentrations of the N-terminal propeptide of type I procollagen (PINP) were comparable in those with 25OHD below or above 30 nmol/L (44.4 ± 20.7 µg/L and 45.2 ± 20.7 µg/L, respectively, P = 0.75) and there were no changes in this marker after supplementation (change from baseline at 12 months in those with low baseline 25OHD: placebo 0.4 (-4.9, 5.7) µg/L, 400 IU/day -1.2 (-4.7, 2.3) µg/L, 1000 IU/day 0.6 (-2.3, 3.5) µg/L; change from baseline at 12 months in those with high baseline 25OHD placebo 1.1 (-2.6, 4.9) µg/L, 400 IU/day 0.1 (-3.6, 3.8) µg/L, 1000 IU/day -3.2 (-6.5, 0.06) µg/L; P > 0.2).

There were no changes in serum concentrations of calcium or phosphate between baseline and 12 months in any participant groups.

Bone Mineral Density

Changes in BMD at the hip and spine by treatment group and by baseline 25OHD level are shown in the Figure 3. When baseline 25OHD was ≤30 nmol/L, the placebo group showed significant bone loss at both sites, and this loss was prevented by vitamin D 1000 IU/day. The results with vitamin D 400 IU/day are comparable to the higher dose at the spine but not at the total hip. In those starting with 25OHD >30 nmol/L, there was no loss of spine BMD in the placebo group and no significant treatment effect at either site.

While this study set out to validate the 30 nmol/L threshold for 25OHD identified in the ViDA study, exploratory analyses of other thresholds have also been undertaken. Dividing the cohort at 25 nmol/L tended to produce even more marked contrasts: a >2% treatment effect at the spine (1000 IU/day compared with placebo, P = 0.002) and ~1% at the hip, in those with 25OHD ≤ 25 nmol/L, compared with no treatment effects in those starting above this level (P 0.14 – 0.44). The distribution of 25OHD concentrations in this cohort made it difficult to rigorously assess 50 nmol/L and 75 nmol/L thresholds, since numbers per treatment group above these levels were 9-17 and 0-3, respectively. No treatment effects were seen at the hip above these higher thresholds. In the spine in subjects >50 nmol/L, the 400 IU vitamin D dose tended to reduce BMD and the 1000 IU dose to increase it; with only 9 in each treatment group, these findings are not reliable.

An exploratory analysis was conducted to determine whether the baseline ratio of 25OHD: 24,25-dihydroxyvitamin D predicted the BMD response to vitamin D supplementation. Grouping participants by quartiles of this ratio showed no interaction with BMD response in either the spine (P = 0.51) or hip (P = 0.40).

Discussion

The present analysis confirms that a 25OHD concentration of 30 nmol/L represents a threshold for the beneficial effects of vitamin D supplements on BMD. Supplementation of individuals below this level results in gains in BMD, but above this level there is no significant change. This finding relates to late winter levels of 25OHD using an appropriately calibrated assay in a fair-skinned population, and should be applied to other contexts with these caveats in mind. The mechanism of this effect is likely to be the correction of secondary hyperparathyroidism, since the present analyses also show differential changes in PTH according to baseline 25OHD status. This agrees with previous studies suggesting a threshold for PTH suppression by vitamin D in the region of 40 – 50 nmol/L.[11,12] However, this does not prove that the change in PTH causes the changes in BMD. In individuals with the lowest 25OHD concentrations, healing of osteomalacia may also be a mechanism contributing to the increase in BMD.

Interestingly, there was no evidence of changes in markers of bone turnover in the D-deficient participants in the present study, nor of suppression of markers following vitamin D supplementation. We have previously observed reductions in PINP in individuals with 25OHD < 30 nmol/L given a 500,000 IU bolus of vitamin D,[11] but daily dosing in deficient individuals seems to produce no change in alkaline phosphatase [13] nor in osteocalcin, pyridinoline or deoxypyridinoline,[14] the latter two markers actually appearing to rise in some groups. These mixed results might reflect the complexity of vitamin D action on bone: the correction of secondary hyperparathyroidism tends to reduce turnover, but direct effects of vitamin D on bone cells can have the opposite effect, as seen is osteomalacia treatment where marker

levels rise substantially. BMD changes following vitamin D supplementation might also result from changes in bone mineralization occurring independently of bone turnover. There is evidence that 1,25-dihydroxyvitamin D directly regulates pyrophosphate concentrations in bone thus influencing mineralization, which might mediate such an effect.[15]

Several factors are likely to have contributed to the congruence between the ViDA-BMD and the Aberdeen results. Both studies recruited participants in late winter or early spring, when 25OHD levels are at their nadir. In most other trials of vitamin D supplementation, participants have been recruited over the whole year so changing seasons will have added to variability of 25OHD concentrations by 20 nmol/L or more.[16,17] Thus, an individual whose 25OHD is 30 nmol/L at its nadir, may be 50 nmol/L or more following the summer peak, as seen in the placebo group in Figure 1. In trials with this extent of variation in baseline 25OHD, identifying a threshold for effect is much more difficult.

A second factor influencing the apparent 25OHD threshold for the effect of vitamin D supplements is the calibration of the 25OHD assay. The fact that both the ViDA and Aberdeen studies used state-of-the-art tandem mass spectrometry assays is likely to have contributed to the congruence of the findings between the studies, since variations in calibration between different 25OHD assays of up to 80% have been reported in the past.[18] It is of interest to note that the Fraser laboratory, which carried out the present 25OHD measurements, has now added a delipidation step to their assay, with a resultant increase in measured values of approximately 10 nmol/L, both versions of this assay still sitting within acceptable DEQAS limits. (The

data used here did not use the delipidation step). Similar variations will exist between laboratories even if they are involved in external quality control programs. Allowance for this variability is necessary when setting recommended target levels for 25OHD.

The present study is unusual in having measurements of 24,25-dihydroxyvitamin D during vitamin D supplementation. These parallel those of 25OHD, reflecting the fact that 24,25-dihydroxyvitamin D is a metabolite of 25OHD. While it has been speculated that this metabolite, or the ratio of 25OHD to it, might be more useful in prediction of BMD responses to vitamin D supplementation, the present analyses offer no support for this possibility. It is of interest that there is no change in 1,25-dihydroxyvitamin D as a result of supplementation, yet changes in BMD are present in the D-deficient group. This highlights that moderate deficiency of vitamin D does not impact on total concentrations of 1,25-dihydroxyvitamin D, though it might reduce free 1,25-dihydroxyvitamin D since there is less 25OHD to displace it from their common carrier protein. Those cells that produce 1,25-dihydroxyvitamin D as an autocrine factor are likely to produce less when 25OHD is low.

In conclusion, the present analysis suggests that beneficial effects of vitamin D supplements on BMD are only evident in adults whose nadir 25OHD concentrations are below 30 nmol/L. This moves us further towards a trial-based definition of vitamin D deficiency in adults with adequate calcium intakes, and suggests that supplement use should be targeted to those with nadir 25OHD levels of < 30 nmol/L. An important corollary of this is that any future studies of the benefits of vitamin D on bone should focus on individuals with 25OHD concentrations in this range, since

there is virtually no trial evidence of benefit to BMD from supplementation given to individuals starting above this level.

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Authors' Roles

HMM, IRR and GDG had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. HMM was involved in the original study design and study management, and the writing of the manuscript. IRR proposed the present re-analysis and wrote first draft of the manuscript. GDG undertook the analyses and produced the figures. WDF and JCT offered analysis and interpretation of biochemical measurements. ADW was responsible for the day-to-day running of the original study. All authors critically appraised the manuscript.

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Figure Legends

Figure 1

Serum levels of 25OHD throughout the study period by treatment group and by baseline 25OHD level. Data are mean with 95% confidence intervals.

Figure 2

Changes in serum levels of parathyroid hormone (ΔPTH) throughout the study period by treatment group and by baseline 25OHD level. Data are mean with 95% confidence intervals.

Figure 3

Changes in BMD at the spine and hip by treatment group and by baseline 25OHD level. P_1 values are for a treatment effect across the 3 groups at each site. P_2 values are for the comparison of the placebo and 1000 IU/day groups only. Data are mean with 95% confidence intervals.

Table 1: Baseline Characteristics of Study Participants

Characteristic	Baseline 25OHD		P
	≤ 30 nmol/L	>30 nmol/L	
N	126	137	
Age (y)	64.6 (2.1)	64.5 (2.2)	0.61
BMI (kg/m^2)	27.3 (4.6)	26.2 (3.6)	0.034
Physical activity (MET h/week)	73.4 (35.1)	74.1 (32.1)	0.88
Sunlight exposure (SED/week)	0.46 (0.24, 1.04)	0.52 (0.24, 1.21)	0.40
Calcium intake (mg/d)	1265 (511)	1298 (518)	0.61
Dietary vitamin D (ug/d)	4.9 (2.7)	5.4 (3.0)	0.20
Energy intake (MJ/d)	9.2 (3.0)	9.3 (2.8)	0.71
25OHD (nmol/L)	22.7 (5.4)	44.7 (12.1)	<0.0001
1,25-dihydroxyvitamin D (pmol/L)	138 (45)	142 (40)	0.4
24,25-dihydroxyvitamin D (nmol/L)	1.9 (0.8)	3.9 (1.7)	<0.0001
25OHD:24,25-dihydroxyvitamin D ratio	13.2 (5.0)	12.8 (5.9)	0.61
PTH (pmol/L)	5.5 (1.3)	4.8 (1.2)	<0.0001
C-telopeptide (ng/L)	381 (160)	389 (163)	0.7
PINP (µg/L)	44.4 (20.7)	45.2 (20.7)	0.8
Bone mineral density (g/cm^2)			
Lumbar spine	1.08 (0.16)	1.12 (0.16)	0.058
Total hip	0.91 (0.11)	0.93 (0.13)	0.33

Data are mean (SD), except for sunlight exposure which is median (interquartile range)

PINP = N-terminal propeptide of type I procollagen *Wilcoxon test

SED = standard erythemal dose

25OHD = 25-hydroxyvitamin D

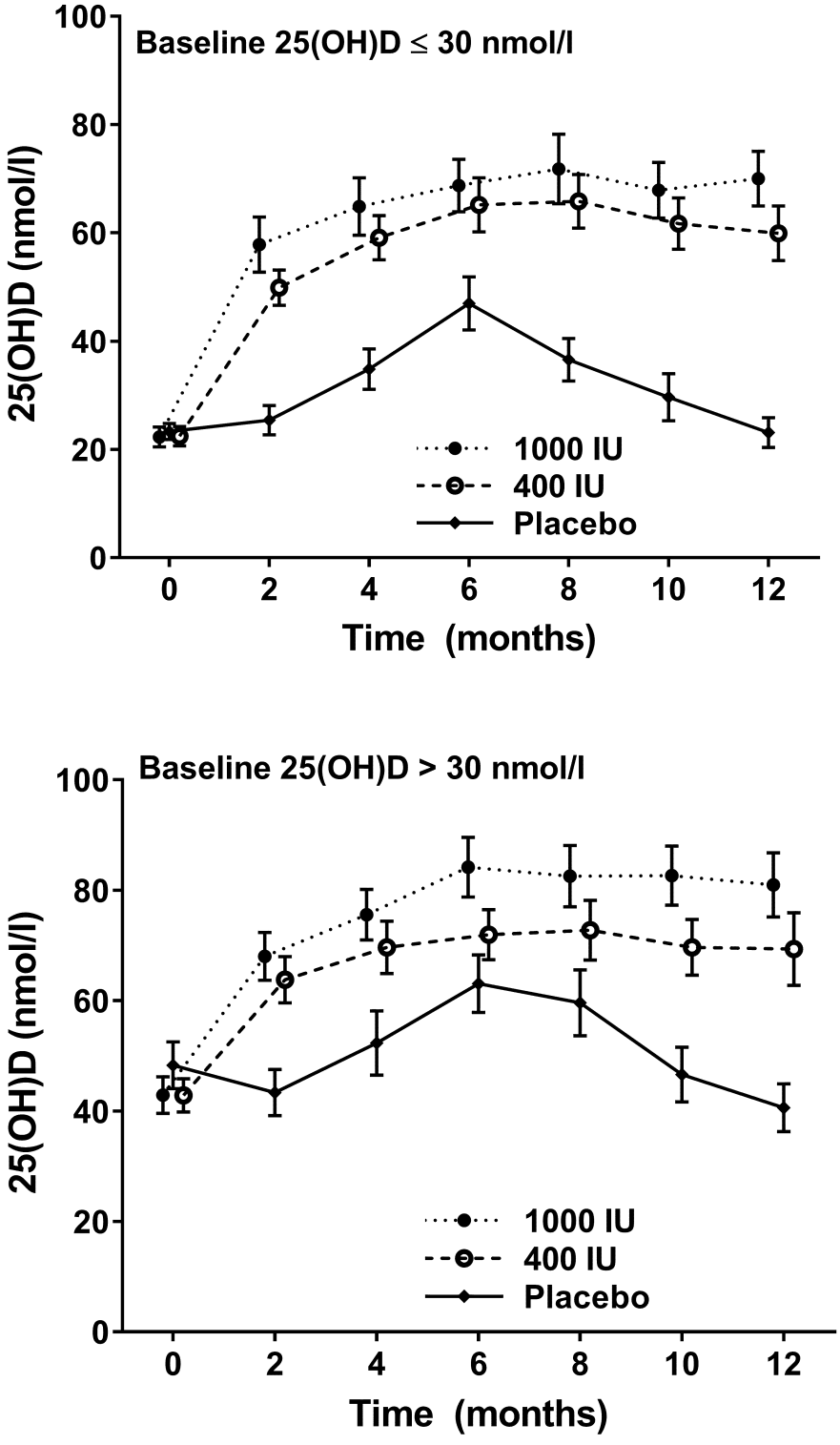

Figure 1

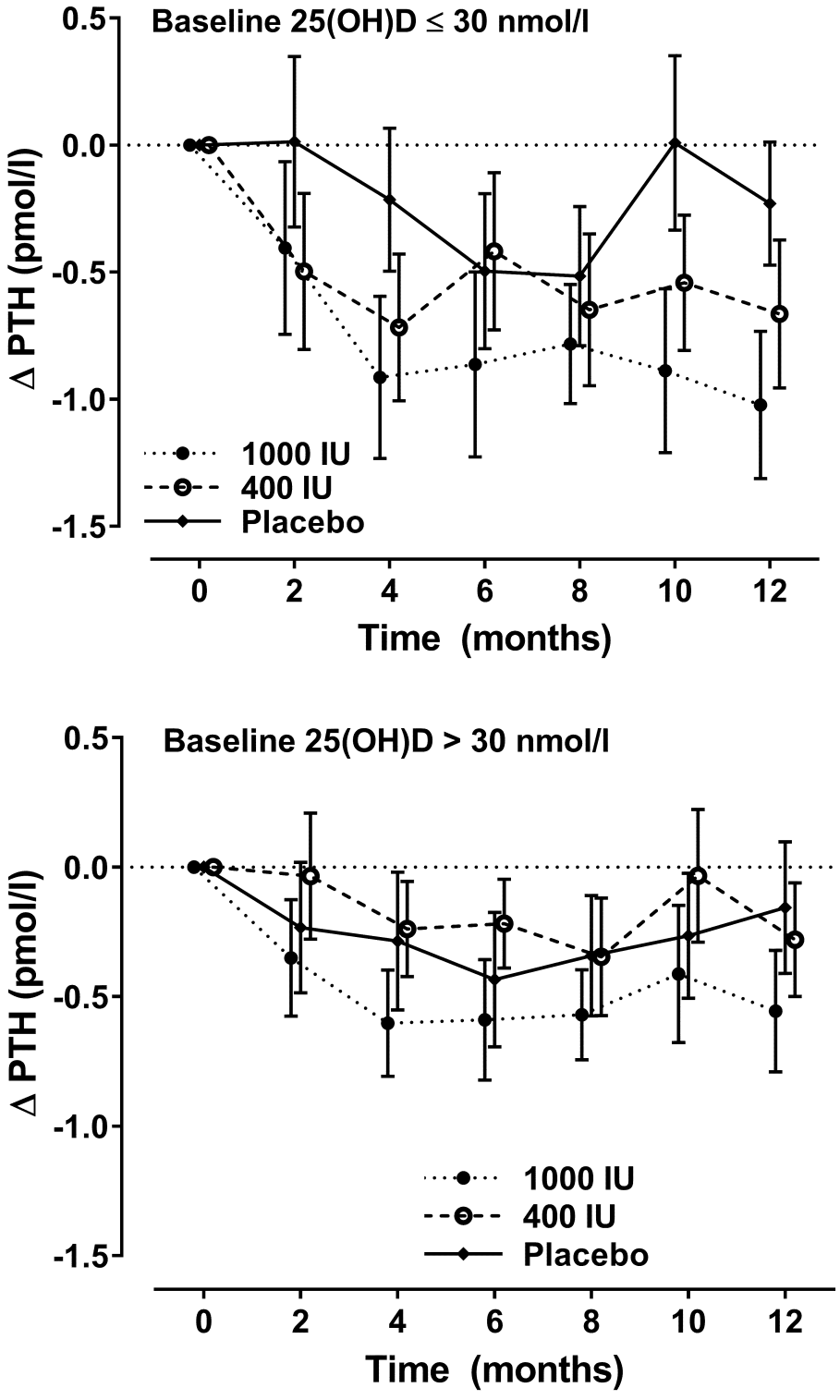

Figure 2

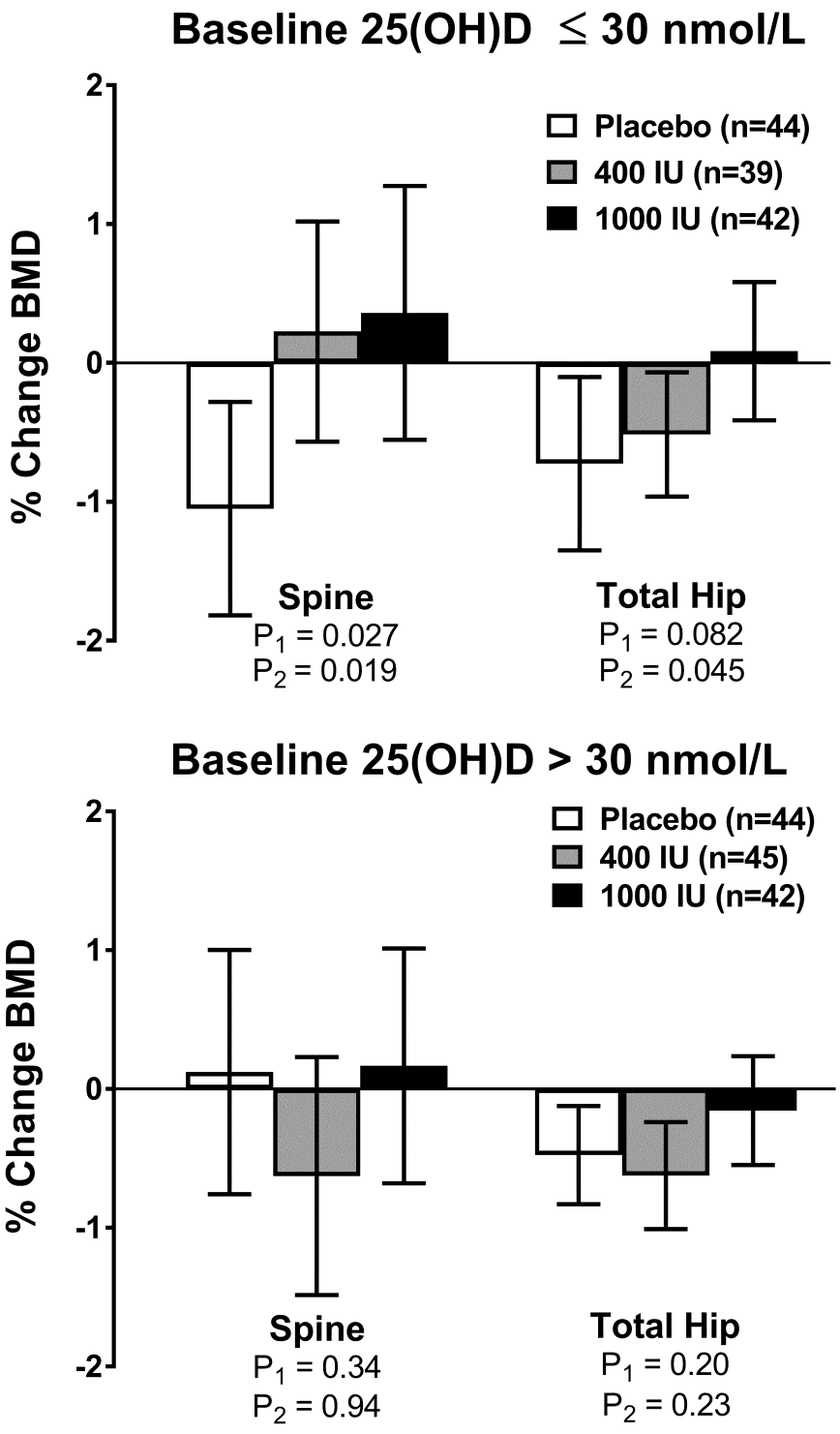

Figure 3