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Vitamin D supplementation suppresses age-induced bone turnover in older women who are vitamin D deficient☆,☆☆

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ABSTRACT

There is a lack of evidence that improving vitamin D status, without changing calcium intake, has a positive effect on bone turnover as indicated by bone marker changes. The objective was to measure the effect of vitamin D supplementation, in vitamin D deficient women (25(OH)D concentration <50 nmol/L), on osteocalcin (OC) and C-telopeptide (CTX). The study design was a randomised controlled intervention administering 4000 IU vitamin D_3 or placebo daily for 6 months to South Asian women, aged >20 years. Subjects were stratified by age and menopausal status. Median (25th, 75th percentile) serum 25(OH)D increased significantly from 21 (11, 40) to 75 (55, 84) nmol/L with supplementation. In women >49 years or postmenopausal ($n = 26$), who were not supplemented ($n = 13$), CTX and OC levels increased ($P = 0.001$, $P = 0.004$ respectively), indicating an increased rate of bone turnover. With supplementation CTX decreased ($P = 0.012$) and there was no significant change in OC. In women who were under 49 years and premenopausal ($n = 55$; 29 supplemented), there was no significant response to supplementation in either CTX or OC. We conclude that correcting vitamin D deficiency in older women suppresses the age-induced increase in bone turnover and reduces bone resorption which would normally be exacerbated in conditions of low serum 25(OH)D.

1. Introduction

The role of vitamin D in the control of calcium homeostasis and bone metabolism is well known. Calcium absorption is reduced in a state of vitamin D deficiency, and is believed to plateau when circulating 25(OH)D reaches a concentration of 80 nmol/L [1–4]. When dietary calcium intake is low calcitriol (1,25(OH)2D3) acts in concert with PTH to activate osteoclastogenesis, but it also appears to have a regulatory effect, influencing the mature osteoblasts to inhibit bone resorption via a change in the ratio between osteoprotegerin (OPG) and receptor activator nuclear factor-κB ligand (RANKL) [5,6].

Biochemical markers of bone resorption and formation are measurable in the blood or urine, and provide an indication of the rate of bone turnover [7]. Increased bone turnover is associated with poor bone mineral density (BMD) and increased fragility [8], and is associated with vitamin D levels <50 nmol/L [9–13]. Despite this, to date there has been no conclusive evidence that an improvement in vitamin D status brings about a measurable change in overall bone turnover, an increase in bone formation or decrease in bone resorption as indicated by changes in bone markers.

Of the limited number of intervention studies which measured bone markers, only one has supplemented with vitamin D_3 alone. This study, in adolescent girls (11.4 ± 0.4 years) with adequate calcium intake, showed a dose-dependent increase in bone mineral content (BMC) over 12 months, but no significant differences in bone markers between doses of 5 μg (200 IU), 10 μg (400 IU) vitamin D_3 and placebo [14]. Two other reported studies which examined changes in bone markers supplemented with high doses of calcium (1200 and 1500 mg) in addition to vitamin D_3. Hitz et al. [15] observed an increase in bone mineral density (BMD), concurrent with decreased PTH and bone turnover in older people under 70 years and reduced loss in those over 70 years. However there was no change in healthy students aged 18–27 years [16].

The study reported here is a secondary outcome from the Surya Study, a trial investigating vitamin D supplementation and insulin resistance [17]. The primary aim of the Surya Study was to investigate the effect of improved vitamin D status on insulin resistance in women of South Asian origin who were insulin resistant and vitamin D insufficient or deficient (25(OH)D < 50 nmol/L). However, because the relationship between vitamin D and bone is so important, we took the opportunity to measure the effect of supplementation on markers of bone turnover. We have previously

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Table 1
Correlations between serum 25(OH)D, parathyroid hormone (PTH) and calcium at baseline and endpoint, stratified by groups.

	Correlation at baseline (r)	P value	Correlation at 6 months (r)	P value
Serum 25(OH)D and PTH				
Group 1	−0.399	0.003	−0.465	<0.001
Group 2	−0.502	0.009	−0.511	0.008
Serum 25(OH)D and calcium				
Group 1	0.298	0.027	−0.100	0.468
Group 2	0.455	0.022	−0.240	0.238
Serum PTH and calcium				
Group 1	−0.352	0.008	0.013	0.923
Group 2	−0.235	0.259	0.211	0.301

Group 1: premenopausal and <49 years. Group 2: ≥49 years and/or postmenopausal.

reported low vitamin D status in this population [18]. There is evidence of South Asian women having compromised bone health, possibly due to poor vitamin D status [19–21].

2. Method

The study protocol is described in greater detail elsewhere [17]. The study design was a randomised, placebo-controlled, double-blind trial with 4000 IU vitamin D_3 (4 capsules) or 4 capsules of placebo per day for 6 months. Volunteers were screened for hypovitaminosis D (<50 nmol/L) plus insulin resistance (HOMA-IR ≥ 1.93) and/or triglyceride/HDL-C ratio ≥3.0. Exclusion criteria included fasting serum glucose ≥7.2 mmol/L, medication for diabetes and vitamin D supplementation ≥1000 IU per day. Ethical approval was granted by the Massey University Human Ethics Committee (Southern A), Reference No. 06/67 and the subjects gave written informed consent for participation in the study.

Subjects were matched into pairs by age and BMI. Randomisation of the vitamin D/placebo capsules and allocation to pairs was performed by Blackmores Ltd using nQuery Advisor®, version 6.0 (Statistical Solutions, Cork, Ireland). Randomisation and allocation were fully concealed from the researchers until after statistical analysis of the data.

Fasting blood samples and anthropometric measurements were obtained at baseline and the end of the study. The intervention in the original cohort commenced in July 2007 which is mid-winter in New Zealand, and a second small cohort (n = 7) commenced in October 2007. Subjects were recalled for their final blood test 6 months later i.e. January 2008 (mid-summer) and April 2008. There is considerable circadian variability in bone markers; osteocalcin (OC) levels are increased by 20% at peak (very early morning) and C-terminal cross-linked telopeptide of type 1 collagen (CTX) levels at the nadir in the early afternoon may be half those at the nocturnal peak [7]. Accordingly, all blood samples were obtained within a consistent time period, between 8 am and 10 am.

Methods for the measurements and laboratory analysis of PTH, calcium, and 25(OH)D have been described previously [17]. Osteocalcin and CTX were measured in EDTA plasma samples stored at −80 °C, by Canterbury District Health Board Laboratory (Christchurch, New Zealand), performed on the automated Roche Elecsys 2010 analyser.

Subjects were divided by age and menopausal status – group 1 were premenopausal and <49 years, group 2 were postmenopausal and/or ≥49 years. Four-day food diaries were completed by subjects at baseline and analysed using Foodworks 2007 (Xyris Software, New Zealand Foods Database).

3. Statistical methods

Power calculations were based on the requirements and outcomes of the primary objective of the study. Serum 25(OH)D was not normally distributed and is reported as median (25th, 75th percentiles). Normally distributed data is reported as mean ± standard deviation. Non-parametric tests were used to compare groups (Mann–Whitney U), and to compare baseline and endpoint measures within groups (Wilcoxon). A two-tailed P value of <0.05 was considered as statistically significant. Spearman's correlations were used for correlations involving 25(OH)D status, but where data was normally distributed, Pearson's correlations were used.

4. Results

Two hundred and thirty-five women were recruited and screened for insulin resistance and hypovitaminosis D. One hundred and fourteen qualified for selection, and from those, 106 women volunteered to take part in the intervention trial. Twelve were lost to the study due to becoming pregnant (n = 3), moving overseas (n = 4) perceived side-effects (n = 2) and medical practitioner prescribing vitamin D (n = 3). A further 13 could not be contacted/traced at the end of the trial. The baseline characteristics of this group of 25 did not differ significantly from those participants who remained in the study. Blood samples were taken at 6 months from 81 women, 42 from the vitamin D group and 39 from placebo group.

The majority of participants (91%) were Indian, with 6% from Sri Lanka and 3% from Pakistan. Seventy-nine percent had been in New Zealand for ≤10 years. Mean dietary calcium intake was 700 ± 300 mg/day. Despite the low baseline serum 25(OH)D concentrations present in all subjects, there was still a significant inverse relationship at baseline between PTH and 25(OH)D in both groups and between PTH and serum calcium in group 1 but not group 2. There was also a significant correlation between baseline 25(OH)D and serum calcium but this relationship was lost at 6 months. Although there was no significant change in serum PTH in response to the supplementation, the relationship between PTH and 25(OH)D was stronger at 6 months in both groups (Table 1).

Serum 25(OH)D concentrations increased significantly in response to supplementation, from 21 (11, 40) nmol/L at baseline to 75 (55, 85) nmol/L at 6 months in the vitamin D group. There was a much smaller, but significant increase in serum 25(OH)D in the placebo group overall, from 22 (15, 32) nmol/L to 32 (24, 46) nmol/L. Baseline serum 25(OH)D concentration in placebo group 1 was significantly lower than in group 2 (P = 0.001), and the increase from baseline to end in the placebo group was only significant in group 1 (Table 2). Within the two groups, baseline values between the vitamin D and placebo arms were not significantly different in any of the variables reported. There were no significant differences in baseline values between group 1 and group 2 in PTH, OC or CTX, however the premenopausal group (group 1) had significantly lower baseline 25(OH)D concentrations than group 2: 19 (11, 29) nmol/L vs 32 (22, 53) nmol/L (P < 0.001).

Table 2
Change in serum 25(OH)D, PTH, bone markers from baseline to endpoint.

	Serum 25(OH)D (nmol/L)		*P* value between groups	Parathyroid hormone (pmol/L)		*P* value between groups
	Vitamin D	Placebo		Vitamin D	Placebo	
Group 1	*n* = 29	*n* = 26		*n* = 29	*n* = 26	
Baseline	20 (11, 39)	18 (12, 27)		5.44 ± 2.84	5.65 ± 1.90	
End	75 (55, 83)	30 (23, 42)		4.77 ± 1.74	5.40 ± 1.69	
P value within group	<0.001	0.001		0.09	0.46	
Change: end – baseline	48 (21, 69)	11 (0, 19)	<0.001	−0.672 ± 2.072	−0.246 ± 1.673	0.41
Group 2	*n* = 13	*n* = 13		*n* = 13	*n* = 13	
Baseline	31 (17, 57)	32 (23, 40)		6.05 ± 2.91	4.52 ± 1.40	
End	74 (56, 99)	40 (27, 53)		6.23 ± 4.47	5.54 ± 1.53	
P value within group	0.002	0.17		0.8	0.008	
Change: end – baseline	49 (23, 57)	7 (−4, 9)	<0.001	0.18 ± 2.21	1.02 ± 1.15	0.23
	Osteocalcin (μg/L)		*P* value between groups	C-telopeptide (μg/L)		*P* value between groups
	Vitamin D	Placebo		Vitamin D	Placebo	
Group 1	*n* = 29	*n* = 26		*n* = 29	*n* = 26	
Baseline	19.88 ± 7.45	20.04 ± 5.44		0.30 ± 0.15	0.31 ± 0.15	
End	19.55 ± 7.12	22.01 ± 7.27		0.29 ± 0.14	0.32 ± 0.16	
P value within group	0.60	0.022		0.574	0.906	
Change: end – baseline	−0.036 ± 3.35	1.96 ± 4.03	0.026	−0.011 ± 0.108	0.002 ± 0.103	0.636
Group 2	*n* = 13	*n* = 13		*n* = 13	*n* = 13	
Baseline	21.38 ± 4.99	20.0 ± 6.56		0.39 ± 0.15	0.317 ± 0.18	
End	21.15 ± 4.04	23.38 ± 8.03		0.36 ± 0.17	0.372 ± 0.19	
P value within group	0.8	0.004		0.012	0.001	
Change: end – baseline	−0.231 ± 3.876	3.385 ± 3.500	0.02	−0.030 ± 0.037	0.055 ± 0.046	<0.001

Group 1 is women who are premenopausal and <49 years; group 2 is women who are postmenopausal and/or ≥49 years. Serum 25(OH)D is not normally distributed and is expressed as mean (25th, 75th percentiles). All other values expressed as mean ± standard deviation. *P* value between groups is the difference in change from baseline to end between Vitamin D and placebo groups.

No significant response to supplementation was observed in the premenopausal group in any of the variables (other than 25(OH)D), although there was a decrease of borderline significance in PTH ($P = 0.09$). In group 2, there was a significant decrease in CTX ($P = 0.012$) in response to supplementation, but no change in OC. There were, however, significant increases in both OC ($P = 0.004$) and CTX ($P = 0.001$) in the group 2 women taking the placebo over the course of the study.

5. Discussion

In women who were under 49 years and premenopausal (group 1), there was no significant response to supplementation in either CTX or OC, although OC did increase significantly in the placebo arm ($P = 0.02$). There are no established reference intervals for OC or CTX in South Asian women. However, Glover et al. [22] have published reference intervals for CTX in premenopausal women based on an assessment of Caucasian women from Belgium, France, UK and USA. Their median of 0.299 μg/L (geometric mean 0.317 μg/L) compares with the premenopausal women in this study at 0.310 ± 0.150 μg/L.

During the course of the trial, bone turnover rate continued to increase in the placebo arm of the postmenopausal women, with both OC and CTX increasing significantly. However, supplementation appeared to abrogate this menopause-related increase in bone turnover; CTX decreased ($P = 0.012$) and there was no significant change in OC. OC is primarily a marker of bone formation, but because bone resorption and formation are closely linked, OC is also a reliable marker for bone turnover, and correlates with increased risk of fracture in postmenopausal women [23]. Increased rate of bone turnover is associated with low bone mass [24] and increased risk of fragility and fracture [25]. Higher levels of circulating CTX indicate increased bone resorption and a corresponding elevation in bone fragility [7].

Osteoprotegerin (OPG) is a powerful protector of bone. Secreted by mature osteoblasts, it competes with the receptor nuclear factor-κB (RANK) for binding with RANKL, and in this way it interrupts the RANK–RANKL signalling system and suppresses osteoclast formation and maturation [5,26]. Both calcitriol and oestrogen appear to stimulate mature osteoblasts to produce more OPG and less RANKL, thus slowing bone resorption in preparation for the osteoblasts to commence rebuilding the bone [5,27], and consequently protecting the bone from excessive resorption.

In the perimenopausal period, when oestrogen concentrations are declining, the protective effects of oestrogen on bone are compromised [23], possibly by the reduced secretion of OPG [27]. Markers of bone resorption have been shown to decrease with hormone replacement therapy in postmenopausal women [28]. If, during this period, serum 25(OH)D concentrations are also less than adequate, bone is threatened by increased rates of both resorption and overall turnover. In the absence of sufficient 25(OH)D, absorption of dietary calcium from the intestine will be reduced and PTH levels will increase to stimulate the reabsorption of calcium from the kidney, as well as activating osteoclasts to commence bone resorption and release calcium into circulation [29,30]. At the same time, the regulatory effect of calcitriol on the osteoblasts (via increased OPG secretion) will be impaired due to low 25(OH)D levels and compounded by declining oestrogen concentrations [5,23].

Secretion of PTH is regulated primarily by a drop in serum calcium [31,32] and does not appear to be affected by vitamin D deficiency until serum 25(OH)D concentrations drop below 30–40 nmol/L [16,32,33]. This may explain the lack of PTH response to supplementation in this study. There were small, not significant decreases in PTH levels in both the vitamin D and placebo arms of group 1 at the end of the study. The median baseline 25(OH)D concentration in this group was 19 (11, 31) nmol/L and this had increased significantly in the placebo arm as well as the vitamin D arm after 6 months. A strong inverse correlation between 25(OH)D and PTH which was present at baseline was retained through the study and was actually stronger in both groups at 6 months (Table 1). Meanwhile mean dietary calcium intake across the entire subject group was 700 ± 300 mg/day, which although less than the New Zealand RDI of 1000 mg [34] is similar to the mean

for all New Zealand adult females of 735 mg/day [35], and possibly sufficient to suppress secondary hyperparathyroidism.

We conclude that increasing serum vitamin D levels in older women who are vitamin D deficient suppresses the increase in bone turnover induced by age and decline in oestrogen, and reduces bone resorption which would normally be exacerbated in conditions of low serum 25(OH)D. It is also possible that the dietary calcium intake in this group of South Asian women was at a level which also offered some protection against the stimulation of osteoclast activity by PTH.

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