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Review

Vitamin D receptor overexpression in osteoblasts and osteocytes prevents bone loss during vitamin D-deficiency

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ABSTRACT

There are several lines of evidence that demonstrate the ability of 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$), acting via the vitamin D receptor (VDR) to mediate negative or positive effects in bone. Transgenic over-expression of VDR in osteoblasts and osteocytes in a mouse model (OSVDR) has been previously shown to inhibit processes of bone resorption and enhance bone formation, under conditions of adequate calcium intake. While these findings suggest that vitamin D signalling in osteoblasts and osteocytes promotes bone mineral accrual, the vitamin D requirement for this action is not well understood. In this study, 4 week old female OSVDR and wild-type (WT) mice were fed either a vitamin D-replete (1000 IU/kg diet, D+) or vitamin D-deficient (D−) diet for 4 months to observe changes to bone mineral homeostasis. Tibial bone mineral volume was analysed by micro-CT and changes to bone cell activities were measured using standard dynamic histomorphometric techniques. While vitamin D-deplete WT mice demonstrated a reduction in periosteal bone accrual and overall bone mineral volume, OSVDR mice, however, displayed increased cortical and cancellous bone volume in mice which remained higher during vitamin D-depletion due to a reduced osteoclast number and increased bone formation rate. These data suggest that increased VDR-mediated activity in osteoblast and osteocytes prevents bone loss due to vitamin D-deficiency.

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Contents

1. Introduction

Adequate vitamin D levels, as defined by high levels of serum 25-hydroxyvitamin D_3 (25D), can reduce the risk of fracture in the elderly [1,2]. Levels of serum 25D above 20 nmol/L which normalise intestinal calcium absorption may also be

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insufficient to normalise bone mineral volume [3–5]. We have previously demonstrated positive effects of local synthesis of 1,25D in osteoblasts and osteocytes in stimulating cell differentiation and mineralisation [6–11]. However, other lines of evidence demonstrate alternative effects of 1,25D with regards to mineralisation and bone resorption [11–17], which suggest the actions of 1,25D appears to depend on the physiological context and is closely associated with the fundamental role for vitamin D on the maintenance of plasma calcium homeostasis.

With respect to osteoblasts, VDR-mediated activities have been shown to regulate processes of bone resorption and bone formation [18–20]. One of the first in vivo accounts for the direct action of vitamin D on osteoblasts was established using the transgenic mouse model of osteoblast and osteocyte VDR overexpression (OSVDR) using an osteocalcin promoter driven expression construct [21]. OSVDR demonstrate increased bone volume and strength due to increased mineral apposition as well as decreased osteoclast formation [21,22]. While these findings suggest that vitamin D signalling in osteoblasts and osteocytes promotes bone mineral accrual, the physiological circumstances in which this occurs are not well understood.

The role of vitamin D activity within osteoblasts and osteocytes during vitamin D deficiency is unclear thus in this study we employed the OSVDR transgenic mouse as model to investigate the role of enhanced vitamin D activity in osteoblasts during circumstances where vitamin D supply is limited.

2. Materials and methods

4-week old FVB/N wild-type and OSVDR female mice (OSV3 line) ($n=6$) were fed either vitamin D-replete (1000 IU D_3/kg) or vitamin D-deficient diet containing 1% Ca and 0.625% phosphorus and based on the recommended semi-synthetic diet for rodents (AIN-93-VX, ICN, CA, USA) for 16 weeks. At the end of the dietary study the animals were killed, fasting bloods were taken for biochemistry and the left tibiae were collected for bone structural and histomorphometry analyses. All animal procedures were approved by the Institute of Medical and Veterinary Science Animal Ethics Committee.

2.1. Histological analyses

The micro-architecture of the tibia and vertebra were evaluated using a high resolution micro-CT system (Skyscan 1174, Brussels, Belgium), as previously described [23]. Prior to death, mice were injected with the fluorescent tetracycline compounds calcein and demeclocycline (Sigma Chemical Company, St. Louis, MO), each at 10 mg/kg, 6 days and 2 days prior to collection [5]. Tibia were prepared for histomorphometric analyses as previously described [5]. Analyses of bone formation and bone resorption were performed as previously described [5].

2.2. Serum biochemistry

16 h fasting blood samples were collected at time of death for analyses. Serum calcium and phosphate were measured using a chemistry analyser (Cobas Bio, Roche, IN, USA). Serum 1,25D and 25D were measured by a ^{125}I radioimmunoassay (RIA) (Immunodiagnostic Systems Ltd., Bolden, UK). Serum FGF23 was measured using an ELISA-kit method (Kainos, Japan). Serum PTH was measured using a rat-specific, two-site immunoradiometric assay (IRMA) (Immutopics, CA, USA).

Table 1
Serum 25D, 1,25D, calcium, phosphate, PTH and body weights of WT and OSVDR animals at 20 weeks of age fed vitamin D+ (1000 IU $VitD_3$/kg) and D− (0 IU $VitD_3$/kg) diet.

Vitamin D (IU/day)	WT		OSVDR	
Dietary Ca (%)	D+	D−	D+	D−
Serum 25D, nmol/L	150.1	26.3*	157.4	29.1*
(SEM)	(9.0)	(1.8)	(5.5)	(2.4)
Serum 1,25D, pmol/L	72.3	55.4	28.3	43.0
(SEM)	(30.3)	(9.0)	(6.5)	(12.5)
Serum Ca, mmol/L	2.31	2.14*	2.39	2.34
(SEM)	(0.02)	(0.03)	(0.02)	(0.04)
Serum Phos, mmol/L	2.13	1.79	1.87	1.96
(SEM)	(0.12)	(0.1)	(0.04)	(0.18)
Serum PTH, pmol/L	24.4	21.2	14.3#	12.7#
(SEM)	(4.5)	(2.7)	(3.6)	(2.8)
Serum FGF23, pg/ml	153.5	104.5*	184.8#	189.3#
(SEM)	(14.9)	(6.3)	(18.1)	(36.2)

Values are mean ± SEM, $n=6$.
* $P<0.05$ vs. D+ mice.
\# $P<0.05$ vs. WT mice.

2.3. Statistical analysis

The effects of dietary vitamin D and transgene were statistically analysed using a 2-way ANOVA and Tukey's posthoc test analysis. Significance was set at $P \leq 0.05$.

3. Results

3.1. Serum biochemistry

Mice fed the vitamin D-deficient diet demonstrated low serum 25D levels (Table 1). Serum 1,25D levels were unchanged between groups suggesting that serum 25D levels were low but adequate for renal production of 1,25D in these 1% Ca fed mice. Serum calcium levels were marginally lower in WT mice fed the vitamin D-deficient diet, despite no observable changes in serum PTH or 1,25D levels due to vitamin D depletion. However, serum FGF23 levels were lower in vitamin D-deplete WT mice. OSVDR mice demonstrated reduced serum PTH when compared to WT mice, regardless of vitamin D status and raised serum FGF23 levels, particularly in vitamin D deficient OSVDR mice when compared WT counterparts.

3.2. Histological analyses

In WT mice, tibial cortical bone volume and width were reduced in vitamin D-depleted WT mice ($P<0.01$), due largely to a reduction in periosteal circumference ($P<0.01$) (Fig. 1A and B). In OSVDR mice, tibial periosteal circumference ($P<0.05$) and cortical bone volume ($P<0.01$) were increased compared to WT mice regardless of vitamin D status (Fig. 1A and B). Unlike WT mice, no change in cortical bone volume was observed in vitamin D-deplete OSVDR mice. In WT mice, as a result of vitamin D depletion, tibial Tb.Th were decreased ($P<0.05$) (Fig. 2B) which was associated with a moderate decline in BFR ($P<0.05$), rather than a change in Oc.Sur (Fig. 3A and B). The decline in BFR and Tb.Th were consistent with a trend towards decreased BV/TV% ($P=0.11$). In OSVDR mice, increased tibial BV/TV% compared to WT mice was associated with increased Tb.N ($P<0.01$) (Fig. 2A and B), BFR ($P<0.01$), and decreased Oc.Sur% ($P<0.01$) (Fig. 3A and B). Unlike in WT mice, vitamin D depletion did not significantly reduce BFR, Tb.Th or BV/TV% in OSVDR animals.

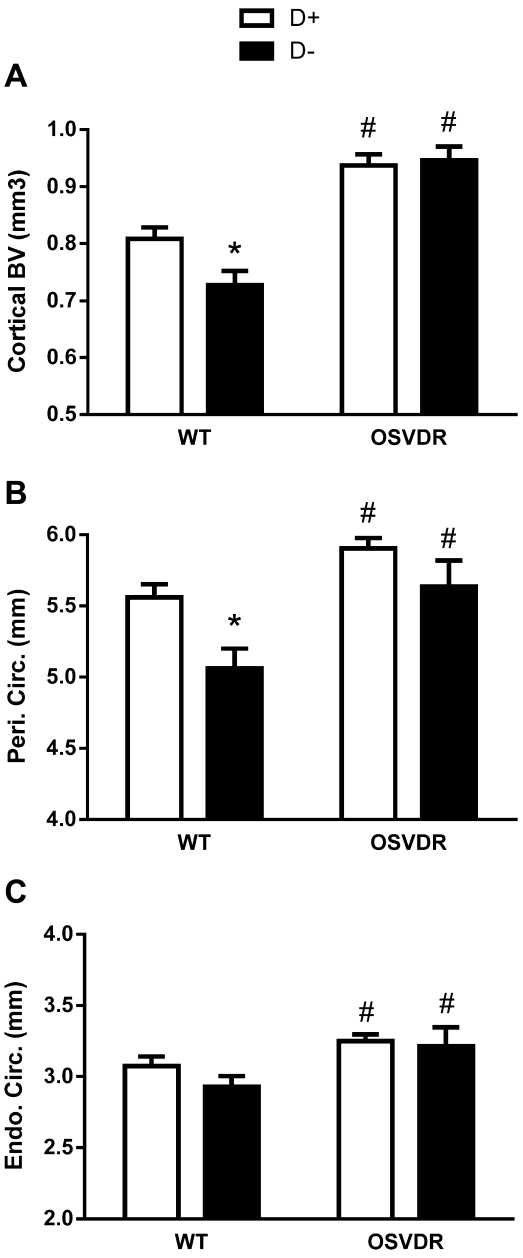

Fig. 1. Tibial cortical bone volume (A), periosteal circumference (B) and endosteal circumference (C) in WT and OSVDR mice fed either vitamin D-replete (D+) or vitamin-deficient (D−) diet. Values are mean ± SEM, $n = 6$/group. $^{*}P < 0.05$ vs. D+ mice. $^{\#}P < 0.05$ vs. WT mice.

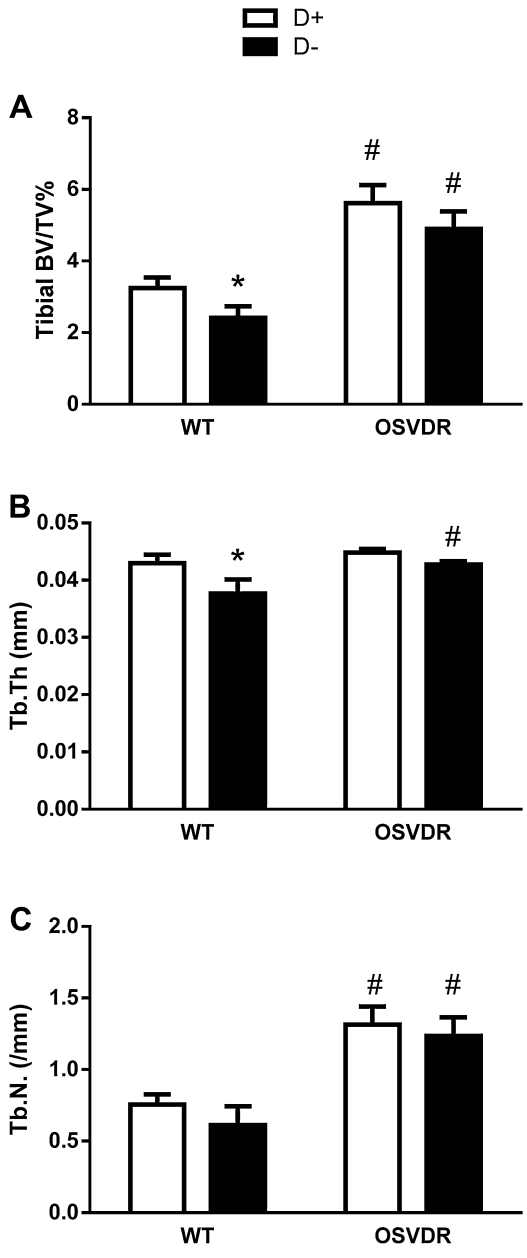

Fig. 2. Tibial trabecular bone volume (A), trabecular thickness (B) and trabecular number (C) in WT and OSVDR mice fed either vitamin D-replete (D+) or vitamin-deficient (D−) diet. Values are mean ± SEM, $n = 6$/group. $^{*}P < 0.05$ vs. D+ mice. $^{\#}P < 0.05$ vs. WT mice.

4. Discussion

The effects of vitamin D-depletion on bone volume of WT mice were consistent with our previous report in rats [23]. WT mice with low serum 25D levels exhibited reduced cortical and trabecular bone volume compared to vitamin D-replete mice. Interestingly, this occurred without changes to serum 1,25D or PTH levels, osteoclast recruitment or trabecular numbers. Rather, reduced bone formation rate, periosteal expansion and reduced trabecular thickness were responsible for this bone loss, suggesting that young mice fed a vitamin D deficient diet with high dietary calcium intake fail to accrue normal levels of cortical bone, rather than lose bone mass through bone resorption. Our data indicate that a serum 25D levels greater than 26 nmol/L is required to maintain bone volume in WT mice under these circumstances. We have previously shown that serum 25D levels are an important determinant of trabecular bone mineral volume in rats when fed an adequate level of dietary calcium, and only when serum 25D levels were above 80 nmol/L was peak bone mineral volume achieved [4,5]. More recently, we also showed that in the presence of a low calcium diet and high 1,25D levels, peak bone mineral volume could not be achieved despite high serum 25D levels [24].

In contrast to WT mice, vitamin D-depleted OSVDR animals maintained their increased cortical bone volume over WT mice to levels comparable with vitamin D-replete OSVDR animals at least when dietary calcium levels were adequate. The over-expression of VDR in OSVDR maintained cortical width through maintenance of the periosteal perimeter, suggesting that in addition to benefits of higher 25D levels, the VDR-mediated activity on osteoblasts

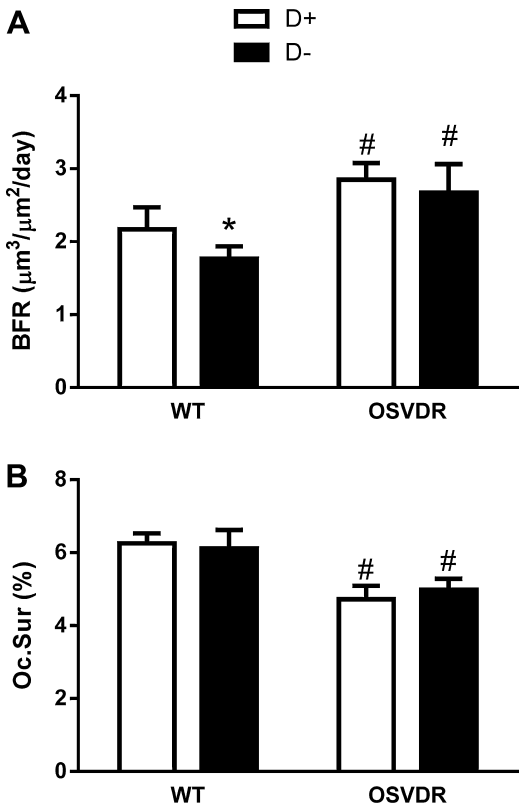

Fig. 3. Bone formation rate (A) and osteoclast number/BS% (B) in WT and OSVDR mice fed either vitamin D-replete (D+) or vitamin-deficient (D−) diet. Values are mean ± SEM, $n = 6$/group. $^{*}P < 0.05$ vs. D+ mice. $^{\#}P < 0.05$ vs. WT mice.

and osteocytes promotes periosteal formation. Over-expression of VDR in osteoblasts and osteocytes has previously been shown to increase tibial cross-sectional cortical area and vertebral trabecular bone in the OSVDR mice model [21,22]. Using more sensitive micro-CT methods, we have extended these findings to demonstrate increased trabecular bone within the proximal tibial metaphysis. OSVDR mice demonstrated increased trabecular thickness and enhanced bone formation rate, in addition to decreased osteoclastic bone resorption, which was unaffected by vitamin D depletion in the presence of a high calcium diet. It is unclear, however, whether the actions of VDR are to solely enhance direct 1,25D activity in osteoblast and osteocytes. Raised serum FGF23 levels in OSVDR mice could play a significant role in feedback on phosphate and calcium homeostasis. Reduced serum PTH levels in OSVDR mice are likely to be important in both in determining bone formation and resorption. Furthermore, enhanced VDR levels in OSVDR mice may play a role in a postulated unliganded VDR-mediated mechanism [25].

Given our previous reports of the relationship between 25D, local vitamin D synthesis and bone formation, the possibility remains that enhanced sensitivity to locally produced 1,25D is responsible for these anabolic activities. Equally, higher VDR levels in OSVDR mice may mediate other indirect feedback signalling involving FGF23 and PTH. These activities warrant further investigations to further understand the role of VDR in osteoblasts and osteocytes. Regardless, our data indicate that prolonged vitamin D deficiency without overt impaired calcium economy causes reduced bone mineralisation, which can be prevented by enhancing VDR-mediated activities in osteoblasts and osteocytes.

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