



# The Regulation of Marrow Fat by Vitamin D: Molecular Mechanisms and Clinical Implications

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## Abstract

**Purpose of Review** To review the available literature regarding a possible relationship between vitamin D and bone marrow adipose tissue (BMAT), and to identify future avenues of research that warrant attention.

**Recent Findings** Results from in vivo animal and human studies all support the hypothesis that vitamin D can suppress BMAT expansion. This is achieved by antagonizing adipogenesis in bone marrow stromal cells, through inhibition of PPAR $\gamma$ 2 activity and stimulation of pro-osteogenic Wnt signalling. However, our understanding of the functions of BMAT is still evolving, and studies on the role of vitamin D in modulating BMAT function are lacking. In addition, many diseases and chronic conditions are associated with low vitamin D status and low bone mineral density (BMD), but BMAT expansion has not been studied in these patient populations.

**Summary** Vitamin D suppresses BMAT expansion, but its role in modulating BMAT function is poorly understood.

**Keywords** Vitamin D · Bone marrow fat · Bone mineral density · PPAR $\gamma$ 2 · Marrow fat unsaturation

## Introduction

Bone marrow adipose tissue (BMAT) is virtually absent in neonates, but develops through normal physiological processes post-natally, so that BMAT occupies approximately 70% of the bone marrow space in healthy adults (reviewed in [1••]). The presence of such a considerable quantity of adipocytes in the bone marrow strongly indicates that BMAT must perform physiologically essential functions, and indeed, a wealth of literature on the functions of bone marrow adipocytes (BMAs) and BMAT has become available in recent years. It is not within the scope of this review to provide an exhaustive description of these functions, or a comprehensive bibliography of the literature on this topic, but the reader is referred to

several excellent reviews on this topic published recently [1••, 2, 3••, 4–11]. BMAs are developmentally, anatomically, and functionally distinct from extramedullary white and brown adipocytes, but exhibit genetic and metabolic features of both (reviewed in [1••, 2, 3••, 4, 5]). Consequently, BMAT is now recognized as a separate fat depot [1••]. In addition, two different subtypes of BMAs, namely constitutive and regulated BMAs (cBMAs and rBMAs, respectively), have recently been characterized in animal models [3••]. BMAs may serve as an energy source during bone formation and remodelling [4, 6], although the response of BMAs to classic lipolytic signals differs from that of white adipocytes [7]. BMAs also have complex endocrine functions within the bone marrow, secreting RANK ligand, fatty acids, inflammatory cytokines, and adipokines that have both positive and negative effects on osteoblast differentiation and function [2, 5, 8, 9]. Moreover, BMAs modulate hematopoiesis through complex mechanisms [10], and may play a role in tumor development and metastasis [6, 11].

Bone marrow mesenchymal stem cells (BMSCs) serve as common progenitors for both BMAs and osteoblasts, with competitive lineage selection resulting in the well-described inverse reciprocal (“see-saw”) relationship between adipogenesis and osteoblastogenesis [12]. Molecular mechanisms

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involved in governing this relationship have been discussed in detail in recent publications [2, 13, 14, 15•]. In essence, any signalling factor, physiological change, or disease state that suppresses osteoblastogenesis has the potential to stimulate marrow adipogenesis, and vice versa. However, two molecular mechanisms that have emerged as strong regulators of this selection process are the master pro-adipogenic transcription factor PPAR $\gamma$ 2 and Wnt signalling [13, 14, 15•]. The competition between adipogenesis and osteoblastogenesis in BMSCs is reflected in the often-observed negative correlation between bone mineral density (BMD) and BMAT volume [16–20], although it is recognized that BMAT can also drive bone loss through its paracrine functions post-differentiation (reviewed in [1•, 8, 9]). Indeed, many physiological and pathophysiological conditions result in pathological BMAT expansion, together with loss of bone mass and increased fragility, as will be discussed in the present review, but conversely, factors that can manipulate the adipogenesis/osteoblastogenesis ratio in BMSCs may be applied to mitigate pathological increases in BMAT volume.

Vitamin D insufficiency is a predictor of low BMD and fracture risk in children and adults [21–23], both in health and in a variety of disease states and chronic conditions, as will be discussed below. Vitamin D exerts osteoanabolic effects through several different mechanisms, which will be discussed in more detail below. However, circulating vitamin D levels are negatively correlated with body fat [24, 25], suggesting that vitamin D status may regulate whole-body adiposity, or alternatively, that vitamin D status may be regulated by whole-body adiposity. The question then becomes: does vitamin D also regulate marrow fat? This review will explore the relationship between vitamin D status and BMAT volume, and will examine a few of the mechanisms possibly underpinning this relationship. In addition, the clinical impact of this relationship in a variety of chronic conditions and diseases will be discussed, while unanswered questions and future research avenues will be reflected upon in the final section.

## BMAT: When, Where, What Type, and How Much?

Throughout life, the red hematopoietic marrow in the bone cavities is gradually replaced with yellow fatty marrow in a site- and gender-specific manner, from the appendicular to the axial skeleton, and from the distal to the proximal ends of the long bones. Consequently, age is one of the major determinants of BMAT volume at any given skeletal site, independent of other physiological and pathophysiological factors [2, 4, 6]. However, it has recently emerged that, in addition to BMAT volume per se, the lipid composition of BMAT also has pathological implications, with lower degrees of marrow fat unsaturation having a stronger correlation with bone fragility

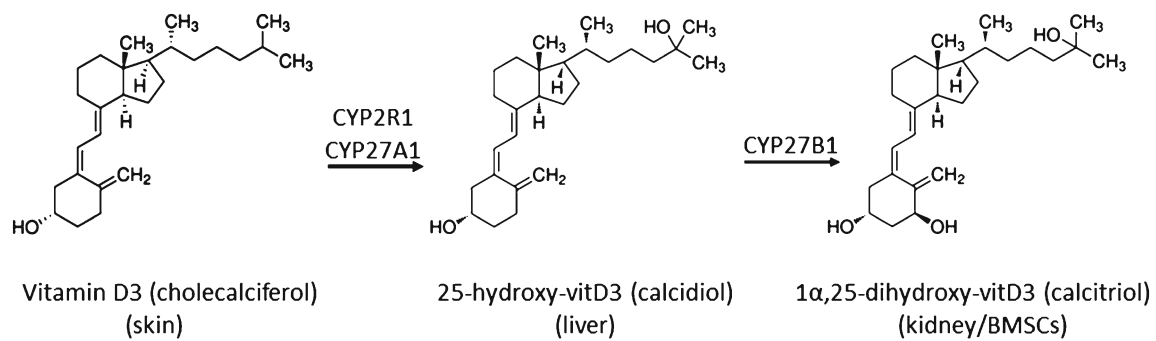
for a given BMAT volume (reviewed in [26•]), due to the lipotoxic effects of saturated fatty acids on osteoblasts (reviewed in [1•]).

Due to its sequestered nature within the marrow cavity, BMAT can only be quantified using highly specialized and specifically targeted techniques, and cannot be evaluated through X-rays or dual energy X-ray absorptiometry (DEXA) used for BMD determination. In the clinical setting, invasive methods of BMAT quantification, such as iliac crest biopsies [6], have largely been replaced with non-invasive methods that are based on either magnetic resonance imaging/magnetic resonance spectroscopy (MRI/MRS) or computed tomography (CT) [27•, 28], which can also quantify differences in marrow fat unsaturation and lipid composition [27•, 28–31]. BMAT studies in animal models often involve sacrificing of the animal and histological analyses of isolated bones [32, 33], although non-invasive imaging techniques are also used in animal studies [32], and allow for longitudinal studies with repeated measurements.

## Vitamin D—Meet the Family

Vitamin D<sub>3</sub> (cholecalciferol) is a cholesterol derivative produced in the skin in response to exposure to sunlight or artificial ultraviolet light. Alternatively, cholecalciferol and vitamin D<sub>2</sub> (ergocalciferol) can be obtained from supplements or from dietary sources, although non-fortified foods are generally bereft of vitamin D content [34, 35]. Ergocalciferol has been shown to be less potent than cholecalciferol at maintaining adequate vitamin D status [36], and has lower bioactivity and vitamin D receptor (VDR) activation potential than cholecalciferol [37]. Vitamin D<sub>3</sub> is biologically inert and is converted first to 25-hydroxy-D<sub>3</sub> (calcidiol) and then to 1,25-dihydroxy-D<sub>3</sub> (calcitriol) [35, 38] (Fig. 1). While calcitriol is the active vitamin D<sub>3</sub> metabolite, calcidiol is the major circulating vitamin D<sub>3</sub> metabolite, which is measured in serum to determine vitamin D<sub>3</sub> status (reviewed in [34, 38]).

Vitamin D exerts its action through the VDR, a member of the steroid hormone receptor transcription factor superfamily, binding to vitamin D response elements (VDREs) in the promoter regions of target genes (reviewed in [34]). In addition to these genomic actions, rapid non-genomic responses may also occur via membrane-bound VDRs [39]. VDR expression has been demonstrated in most bone cells, including osteoblasts, osteoclasts, and BMSCs [40, 41]. The molecular mechanisms involved in transcriptional regulation by VDR have been discussed elsewhere [34, 38]; however, pertinent for the present review is the finding that VDR requires the retinoid X receptor (RXR) as a heterodimer partner for transcriptional activity [42], the relevance of which will be discussed in more detail in Section “[The Interplay Between VDR and PPAR \$\gamma\$ 2](#)”.



**Fig. 1** The conversion of vitamin D3 to its active metabolite, calcitriol. Vitamin D<sub>3</sub> is transported to the liver, for hydroxylation by the cytochrome P450 enzymes CYP2R1 and CYP27A1 to form 25-hydroxy-

D3 (calcidiol), and then to the kidney for another hydroxylation step by CYP27B1 (1 $\alpha$ -hydroxylase), to form 1 $\alpha$ ,25-dihydroxy-D<sub>3</sub> (calcitriol) [35, 38]. Calcitriol can also be produced locally by BMSCs [45]

Although a detailed overview of the actions of vitamin D on bone falls outside of the scope of this review, these have been comprehensively discussed elsewhere [34, 35, 38, 43]. Briefly, vitamin D maintains circulating calcium levels by increasing intestinal absorption and renal re-absorption of calcium, in order to support bone mineralization and provide adequate calcium for other cellular processes [34]. Physiological levels of vitamin D suppress bone resorption, maintain bone mass, and complement anti-resorptive treatments, while supraphysiological levels stimulate osteoclastic differentiation and bone resorption through increasing RANK ligand production by osteoblasts [43]. Numerous studies have confirmed a direct role for vitamin D and VDR in osteoblast differentiation and function, which are discussed in section “The Effects of Vitamin D<sub>3</sub> on Osteoblast and Adipocyte Differentiation: Results from Cell Culture Models”. In addition to the impact of circulating calcitriol on bone, osteoblasts [44] and BMSCs [45] also express CYP27B1 (1 $\alpha$ -hydroxylase) for local conversion of calcidiol to calcitriol, allowing for autocrine/paracrine actions of calcitriol on bone and bone marrow.

### The Effects of Vitamin D<sub>3</sub> on Osteoblast and Adipocyte Differentiation: Results from Cell Culture Models

Studies in cultured BMSCs have demonstrated that calcitriol has weak osteoblastogenic effects, but potentiates the pro-osteogenic effects of low doses of glucocorticoids [46, 47]. These findings confirm a direct pro-osteogenic effect of calcitriol on bone cells, supporting the idea that the bone-sparing effects of vitamin D are not solely due to the regulation of systemic calcium metabolism and osteoclast function, but also involve the direct stimulation of osteoblast differentiation and function [38]. Consistent with these pro-osteogenic effects of calcitriol, and the proposed inverse relationship between osteoblastogenesis and adipogenesis in BMSCs [12], several studies have shown that calcitriol has anti-adipogenic effects

in BMSCs [48, 49] and in 3T3-L1 pre-adipocytes [50], although in other studies, calcitriol had ambiguous [51] or pronounced pro-adipogenic effects [52] in BMSCs. The reason for these differences in the calcitriol response of cultured BMSCs is not clear. However, different vitamin D metabolites at varying dosages elicit different responses in BMSCs and osteoblasts [53], indicating that the *in vitro* response to vitamin D may be sensitive to dose and the differentiation status of the cells used.

## Factors that Mediate the Relationship Between Vitamin D and BMAT

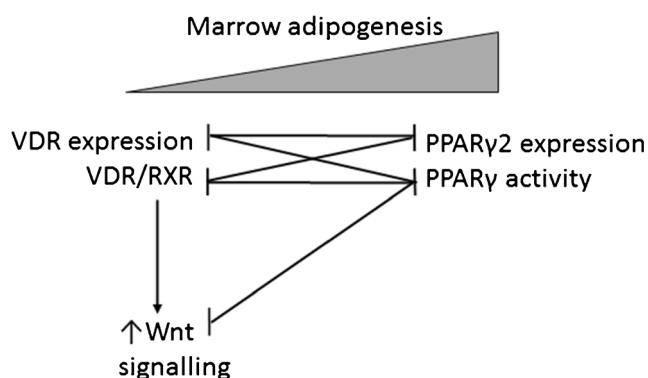
### The Interplay Between VDR and PPAR $\gamma$ 2

Following from their work in 3T3-L1 pre-adipocytes, Kong et al. [50] proposed that the VDR inhibits adipogenesis through direct inhibition of the expression of PPAR $\gamma$ , the master transcriptional regulator of the adipogenic gene programme (reviewed in [14]). The PPAR $\gamma$  agonist troglitazone reversed the inhibition of adipogenesis by calcitriol, and conversely, calcitriol antagonized the transactivation capacity of PPAR $\gamma$  in 3T3-L1 cells [50]. VDR overexpression experiments showed that even unliganded VDR could inhibit PPAR $\gamma$  transactivation activity, by competing for binding to their common heterodimer partner RXR [42, 50]. Consistent with this mechanism, several studies have also shown competitive effects of calcitriol and PPAR $\gamma$ 2 in bone marrow and BMSCs. Calcitriol downregulated PPAR $\gamma$ 2 expression in human BMSCs [54], and conversely, the PPAR $\gamma$  antagonist BADGE upregulated VDR expression in bone, allowing calcitriol to potentiate the anti-adipogenic actions of BADGE in bone marrow [55]. In cultured BMSCs from VDR null mice, PPAR $\gamma$  expression was upregulated, along with increased adipogenesis and higher expression levels of DKK1 and SFRP2, inhibitors of the pro-osteogenic canonical Wnt signalling pathway, and expression of these Wnt inhibitors was downregulated by calcitriol in wild-type BMSCs, in both the absence or presence of adipogenic inducers [56].

PPAR $\gamma$ 2 and the Wnt pathway reciprocally inhibit each other to regulate the selection between osteoblastogenesis and adipogenesis (reviewed in [13]), and therefore the inhibition of PPAR $\gamma$ 2 and the stimulation of Wnt signalling may provide a key mechanism whereby vitamin D could suppress BMAT expansion (Fig. 2).

### Insulin-Like Growth Factor-1 and Its Interaction with Vitamin D and BMAT

Second to the liver, bone is a major source of circulating insulin-like growth factor-1 (IGF-1) [57]. The bone-sparing effects of IGF-1 are well documented (reviewed in [58]), and IGF-1 deficiency has been linked to increased BMAT volume in animals and humans (reviewed in [13]). IGF-1 administration can increase both circulating calcitriol levels and local calcitriol levels in bone by upregulating the expression and activity of 1 $\alpha$ -hydroxylase in the kidney and BMSCs [45, 59]. Conversely, clinical studies have shown that vitamin D<sub>3</sub> supplementation can raise circulating IGF-1 levels [60], and treatment of cultured BMSCs with calcitriol also increased IGF-1 expression [45]. However, Bredella et al. [61] found that while growth hormone treatment in obese women increased both IGF-1 and calcitriol levels as well as bone turnover, BMAT volume was also increased, possibly as a response to the increased energy demands of the remodelling bone tissue. These findings suggest that the systemic interaction between the GH/IGF-1 axis, vitamin D<sub>3</sub>, and BMAT may be complex and may impact other aspects of bone function not directly related to the balance between osteoblastogenesis and adipogenesis in the bone marrow.



**Fig. 2** The interplay between VDR and PPAR $\gamma$ 2. Vitamin D, through the VDR, inhibits PPAR $\gamma$ 2 expression [54], and conversely, PPAR $\gamma$ 2 inhibits VDR expression [55]. In addition, increased VDR expression can suppress the transcriptional activity of PPAR $\gamma$ 2 by competing for RXR [42, 50]. Vitamin D also stimulates Wnt signalling by inhibiting the expression of Wnt inhibitors [56], but the Wnt pathway and PPAR $\gamma$ 2 are subject to reciprocal inhibition [13]. Consequently, the extent of adipogenesis in the bone marrow is determined by the balance between vitamin D/VDR activity and Wnt signalling, on the one hand, and PPAR $\gamma$ 2 expression and activity on the other

## Conditions and Disease States Where a Relationship Between Vitamin D and BMAT Has Been Demonstrated

### Aging

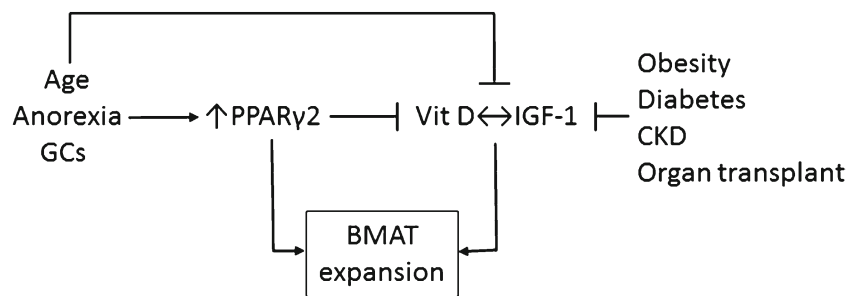
BMAT expansion occurs with increasing age in humans [16] and in animal models of aging [62–64]. This phenomenon has been attributed to a fundamental shift in the differentiation potential of BMSCs, favoring adipogenesis at the expense of osteoblastogenesis due to increased PPAR $\gamma$ 2 expression and activity (reviewed in [14, 64]). In addition, IGF-1 levels in serum and expression in bone decrease with age (reviewed in [13, 58]), which may also be mediated via the age-associated upregulation of PPAR $\gamma$ 2 (reviewed in [13]), and may contribute to BMAT expansion during aging (Fig. 3).

As discussed in Section “[Insulin-Like Growth Factor-1 and Its Interaction with Vitamin D and BMAT](#)”, vitamin D<sub>3</sub> supplementation can raise circulating IGF-1 levels [60], which may assist in counteracting the age-associated loss of IGF-1 and the concomitant increase in BMAT. Consistent with this idea, Lai et al. [76] found that in aged ovariectomized rats, vitamin D supplementation resulted in increased circulating IGF-1 levels, increased bone mineral content, and reduced numbers of adipocytes in the bone marrow. Similarly, in senescence-accelerated mice (SAM-P/6), a model of senile osteoporosis, calcitriol infusions reversed the aging-associated increase in marrow adipogenesis, by downregulating the expression of PPAR $\gamma$ 2 and other pro-adipogenic genes in bone marrow and BMSCs, and coordinately increasing the expression of pro-osteogenic genes [62, 77].

However, aging also has a negative impact on vitamin D status and signalling itself, through decreased availability of vitamin D precursors in the skin [65] and downregulation of VDR and CYP27B1 expression and activity [66, 67]. Taken together, these findings show that calcitriol may reduce BMAT volume through direct regulation of gene expression in vivo, but impaired calcitriol production and responsiveness in aging may be a driving factor in age-related BMAT accumulation (Fig. 3).

### Glucocorticoid-Induced Osteoporosis

Excess glucocorticoids (GCs), whether due to endogenous hypercortisolemia (Cushing’s disease) or pharmacological treatment, result in BMAT expansion [78, 79]. Raised levels of circulating GCs are associated with low vitamin D status [68] and IGF-1 deficiency [69], of which the effect on BMAT was described in Section “[Insulin-Like Growth Factor-1 and Its Interaction with Vitamin D and BMAT](#)” (Fig. 3). Moreover, on a cellular level, it was shown that high levels of GCs upregulate PPAR $\gamma$ 2 expression in bone tissue [78] and in cultured BMSCs [80], resulting in increased marrow adipogenesis and decreased



**Fig. 3** The effect of various conditions and disease states on vitamin D/IGF-1 status and BMAT, with PPAR $\gamma$ 2 as a central regulator. Vitamin D and IGF-1 reinforce each other, both systemically and locally in bone and bone marrow [45, 59, 60]. Through the upregulation of PPAR $\gamma$ 2 expression and activity, age, anorexia, and GCs induce a pro-adipogenic shift in the differentiation of BMSCs ([13, 14]. PPAR $\gamma$ 2 activity also suppresses

VDR expression and transcriptional activity [54, 55]. Calcitriol production is also impaired during aging [65–67]. In addition, anorexia, GC use, obesity, diabetes, and organ transplant are accompanied by a high prevalence of vitamin D and IGF-1 deficiency [14, 68–75], resulting in an inability to suppress BMAT expansion

osteoblastogenesis. Vitamin D<sub>3</sub> supplementation forms a standard component of the treatment guidelines for glucocorticoid-induced osteoporosis (GIO), in order to minimize bone loss through resorption [81], but through the restoration of IGF-1 status and the suppression of PPAR $\gamma$ 2 expression (Figs. 2 and 3), vitamin D may also directly reduce GC-induced marrow adipogenesis and promote osteoblastogenesis.

### Anorexia Nervosa

Patients with anorexia nervosa (AN) exhibit increased BMAT volume that persists even after weight recovery [82]. AN is characterized by skeletal unloading, various endocrine disturbances including hypercortisolemia, and dysregulation of the growth hormone/IGF-1 axis, as well as increased PPAR $\gamma$ 2 transcriptional activity, all culminating in a pro-adipogenic shift in BMSC differentiation potential (reviewed in [14, 70]). Low vitamin D status is also prevalent in AN [71]. Given the interactions between vitamin D, IGF-1, GCs, and PPAR $\gamma$ 2 described in Sections “The Interplay Between VDR and PPAR $\gamma$ 2” and “Insulin-Like Growth Factor-1 and Its Interaction with Vitamin D and BMAT”, as well as in Figs. 2 and 3, it is possible that vitamin D<sub>3</sub> supplementation may reduce BMAT expansion during AN, although studies specifically addressing this question appear to be lacking.

### Obesity and Diabetes

Paradoxically, at the other end of the body weight spectrum, obesity is also associated with low vitamin D status [24, 25, 72], which is likely due to the sequestering of the fat-soluble vitamin D metabolites in excess adipose tissue, thereby decreasing their bioavailability, but may also be exacerbated by decreased physical activity and sunlight exposure (reviewed in [73]). In humans, total body fat, subcutaneous fat, and visceral fat have all been positively correlated with BMAT content in some studies [83–85], although other studies are in disagreement with these findings [19, 86]. On a cellular level, it was

shown that obesity induced a pro-adipogenic shift in the differentiation potential of BMSCs [87, 88], and vitamin D deficiency in obese humans may aggravate this shift, via the mechanisms shown in Figs. 2 and 3. Unfortunately, gastric bypass surgery, in an effort to alleviate morbid obesity, can also predispose patients to vitamin D deficiency due to intestinal malabsorption [89]. While increased BMAT volume and decreased BMD have been demonstrated in some subjects after gastric bypass surgery [90, 91], the correlation between vitamin D status and BMAT volume in these subjects was not examined.

While obesity is a risk factor for developing type 2 diabetes mellitus (T2DM), vitamin D deficiency is also a risk factor for developing metabolic syndrome and T2DM, which is only partially mediated via the effects of obesity on vitamin D status (reviewed in [73]). Plasma IGF-1 levels have also been found to be reduced in T2DM patients, as well in T1DM patients [74], which may be a risk factor for increased BMAT, as discussed in Section “Insulin-Like Growth Factor-1 and Its Interaction with Vitamin D and BMAT”. Correspondingly, several clinical studies have demonstrated increased BMAT in T2DM, which was correlated with indicators of diabetic status, such as homeostatic model assessment of insulin resistance (HOMA-IR) and hemoglobin A1c [20, 85, 86]. In addition, some studies found that diabetes was not correlated with increased BMAT volume per se, but with a lower BMAT unsaturation index [84, 92], an indicator of lipotoxicity in the bone marrow [1]. However, direct correlations between vitamin D status and BMAT volume and composition were not established.

### Chronic Kidney Disease and Organ Transplant

Chronic kidney disease (CKD) is associated with increased BMAT, compared to healthy counterparts [93], but kidney transplant is not effective in subsequently normalizing BMAT levels, even when supported with vitamin D supplementation [94]. Osteoporosis and vitamin D deficiency are long-term complications of organ transplantation, irrespective of the organ involved, and result from both the pre-

transplantation disease state and post-transplantation management. Pre-transplantation, vitamin D deficiency may result from limited sunlight exposure and hepatic dysfunction [95], and mechanical unloading due to reduced physical activity may further contribute to loss of BMD (reviewed in [14]). Post-transplantation, immunosuppressive therapy has been related to a higher risk of skin cancer, and consequently these patients are advised to avoid sun exposure, resulting in a high prevalence of vitamin D deficiency [75]. Moreover, GCs form a standard component of immunosuppressive therapies [96], which may independently drive BMAT accumulation, as described in Section “[Glucocorticoid-Induced Osteoporosis](#)”. Combined, these factors place transplant recipients at a high risk of pathological BMAT accumulation.

## Disease States Where BMAT Expansion May Be a Risk Factor

### HIV Infection and Anti-retroviral Treatment

Globally, millions of people live with human immunodeficiency virus (HIV) infection, and approximately 2 million new infections are recorded each year [<http://www.unaids.org/en/resources/documents/2018/unaids-data-2018>]. HIV infection results in multifactorial bone destruction and loss of BMD (reviewed in [97, 98]), mediated in part by the anti-osteogenic effects of HIV proteins [99, 100]. Low vitamin D status is highly prevalent during HIV infection [97] and responds poorly to vitamin D supplementation [101], partly due to impaired renal  $1\alpha$ -hydroxylation of calcidiol to calcitriol, mediated by increased circulating levels of the pro-inflammatory cytokine  $\text{TNF}\alpha$  [102].  $\text{TNF}\alpha$  may also suppress VDR expression and transcriptional activity in BMSCs, as has been shown for other cell systems [103, 104].

The World Health Organization (WHO) recommends that anti-retroviral treatment (ARV) be initiated in all persons with HIV infection, and also as pre-exposure prophylaxis (PrEP) in persons at risk of contracting HIV [<https://www.who.int/hiv/pub/guidelines/earlyrelease-arv/en>]. However, long-term use of ARVs, in particular certain classes of ARVs and individual drugs within classes, may result in an even greater loss of BMD than that observed in untreated HIV infection (reviewed in [98]), and the use of ARVs such as efavirenz and tenofovir disoproxil fumarate (TDF) is a specific risk factor for both vitamin D deficiency and low BMD [105•]. Moreover, various ARVs were found to increase bone marrow adiposity in rats [106••] and stimulate adipogenesis in cultured BMSCs [107].

No clinical data on the prevalence of increased BMAT volume in HIV-positive people appear to have been published, although the abovementioned findings from animal and cell models suggest that these patient groups may be at risk of increased BMAT accumulation. Given the role of VDR

signalling in stimulating the pro-osteogenic Wnt signalling pathway and suppressing PPAR $\gamma$ 2 activity and adipogenesis in the bone marrow, HIV-associated deficiencies in vitamin D metabolism may predispose millions of people globally to pathological BMAT expansion. Although a single unifying mechanism involving HIV- and ARV-associated bone loss, vitamin D deficiency, and BMAT has not been delineated, it is possible that future studies may “connect the dots.” Furthermore, the advent of ARV treatment has transformed HIV from a fatal infection to a life-long chronic disease (<https://www.who.int/hiv/pub/guidelines/earlyrelease-arv/en/>), which increases the probability of developing HIV-induced bone loss and BMAT accumulation over time. Considering the global drive towards increased ARV uptake, in both HIV-positive and -negative persons (<https://www.who.int/hiv/pub/guidelines/earlyrelease-arv/en/>), ARV-induced increases in BMAT in the context of compromised vitamin D status may present a global health challenge in decades to come, and warrant more focused attention.

### Diseases that Are Characterized by Low BMD and Low Vit D Status

Patients suffering from thalassemia major, which results in ineffective erythropoiesis and anemia, also present with decreased BMD, a defective GH/IGF-1 axis and decreased vitamin D levels, due to lack of physical activity and jaundice impairing the production of vitamin D in the skin. However, although these patients have considerable bone marrow abnormalities, BMAT accumulation has not been studied in these subjects [108]. Patients in intensive care have also been found to exhibit vitamin D deficiency that is refractory to supplementation [109]. Given that these patients undergo extended hospitalization and skeletal unloading, BMAT expansion may become a further complication of their disease state, as reviewed in [14]. Similarly, patients suffering from burn injuries also undergo prolonged immobilization and skeletal unloading, coupled with increased GC levels due to the stress response of the burn trauma, as well as an inability to produce vitamin D through the burnt skin [110]. Given the mechanisms described above and shown in Figs. 2 and 3, the combination of all these factors should elevate the risk of increased BMAT accumulation after burn injury, but no studies have been published in this regard.

It has long been recognized that vitamin D has immunomodulatory properties, and may therefore suppress the progression of auto-immune diseases [34, 111]. Vitamin D modulates the activity of T cell populations, favoring a Th2 response over Th1, thereby reducing the production of pro-inflammatory cytokines such as  $\text{TNF}\alpha$  [34, 112] and alleviating disease severity. Conversely, vitamin D deficiency is prevalent in many auto-immune diseases, although the cause-and-effect relationship is not always clear [111, 113]. However, as discussed in Section “[HIV Infection and Anti-retroviral Treatment](#)”, suppression of renal calcitriol conversion [102]

and of VDR transcriptional activity by  $\text{TNF}\alpha$  [103, 104] may contribute to impaired vitamin D status and signalling in inflammation. Several auto-immune diseases are also characterized by low BMD and increased fracture risk, including inflammatory bowel disease, rheumatoid arthritis, and systemic lupus erythematosus [114–116], and are commonly treated with GCs. The combination of vitamin D deficiency and GC excess could predispose these patients to pathological BMAT expansion, but studies examining BMAT in these patient populations are lacking.

## Conclusions and Future Perspectives

The findings from in vivo animal studies and clinical investigations described here largely concur that vitamin D metabolites suppress BMAT expansion by inhibiting adipogenic gene expression and adipocytic differentiation of BMSCs, even though results from in vitro cell culture studies were not always in agreement. However, at this juncture, with the recent heightened interest in BMAT function [1••, 2, 3••, 4–11], several avenues for future research have emerged. Large numbers of studies have measured BMD under various conditions of health and disease, but by comparison, relatively few studies have examined BMAT in vivo in animals and humans, likely due to the specialized nature of the methods and equipment required to quantify BMAT. As described in Sections “[HIV Infection and Anti-retroviral Treatment](#)” and 7.2, many disease states, and treatment for these diseases, are characterized by vitamin D deficiency and other risk factors for pathological BMAT expansion, but studies on BMAT in these patients are lacking. In addition, while the studies cited in this review focused on the relationship between vitamin D and BMAT *volume*, very little is known about the effect of vitamin D on BMAT *function*. However, the observation by Mutt et al. [117] that calcitriol could inhibit the production of inflammatory cytokines by BMSC-derived adipocytes indicates that vitamin D may indeed modulate the impact of the BMAT secretome on surrounding cells. Furthermore, the lipid unsaturation index of BMAT has emerged as a determinant of the pathophysiological impact on bone fragility [1••], but the regulation of BMAT lipid composition by vitamin D has not been studied. It is also not known whether vitamin D may have differential effects on different MSC populations in bone [118••] or on the cMAT and rMAT subpopulations in the marrow [3••]. It also remains to be determined whether vitamin D could regulate BMAT through non-genomic VDR actions [39], along with its recognized genomic actions.

Given the findings in this review, one might envisage a therapeutic application of vitamin D for addressing BMAT dysfunction. Although expanded BMAT volume may result from vitamin D deficiency, and may therefore respond to standard calciferol supplementation, BMAT expansion may also

occur in individuals that do not have gross vitamin D deficiency. Consequently, the calcemic effects of vitamin D metabolites would also have to be considered when developing such therapy. For these purposes, it would be essential to formulate new vitamin D analogs with more targeted effects on bone and bone marrow, to provide optimal regulation of MSC differentiation without concomitant hypercalcemia [34]. Several alternative VDR ligands have been identified and include slightly modified vitamin D metabolites [119, 120] as well as other steroidal [121] and non-steroidal compounds [122], but the effects of these compounds on BMAT have not been studied in detail. Furthermore, certain strains of probiotics have been found to increase vitamin D status and BMD in ovariectomized rats [123•], suggesting that specific probiotics may be used as adjunct therapy to maintain adequate vitamin D levels, especially in the instances listed above where vitamin D status was refractory to supplementation.

Recent work has demonstrated that sclerostin, a Wnt inhibitor secreted by bone matrix-embedded osteocytes, can induce adipogenesis in BMSCs [124•]. Given the multifactorial effects of vitamin D on bone, and the direct suppression of other Wnt inhibitors by vitamin D in BMSCs [56], vitamin D may suppress BMAT formation by inhibiting either the production or actions of sclerostin, but more studies are needed to examine these mechanisms. Earlier this year, Fairfield et al. [125••] published the results of their efforts to develop the first three-dimensional in vitro model of BMAT, which may revolutionize the way we study this fascinatingly complex tissue, and may provide answers to at least some of the questions posed here.

## Compliance with Ethical Standards

**Conflict of Interest** Hanel Sadie-Van Gijsen declares no conflict of interest.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

## References

Papers of particular interest, published recently, have been highlighted as:

- Of importance
- Of major importance

1. Hardouin P, Rharass T, Lucas S. Bone marrow adipose tissue: tTo be or not to be a typical adipose tissue? *Front Endocrinol (Lausanne)*. 2016;7:85 **This is the first formal description of bone marrow adipose tissue (BMAT) as a distinct adipose depot.**
2. Ambrosi TH, Schulz TJ. The emerging role of bone marrow adipose tissue in bone health and dysfunction. *J Mol Med (Berl)*. 2017;95:1291–301.

- 3.●● Craft CS, Li Z, MacDougald OA, Scheller EL. Molecular differences between subtypes of bone marrow adipocytes. *Curr Mol Biol Rep*. 2018;4:16–23 **This paper provides a comprehensive description of the two subtypes of bone marrow adipocytes identified in animal models, and highlights future research questions.**
4. Sebo ZL, Rendina-Ruedy E, Ables GP, Lindskog DM, Rodeheffer MS, Fazeli PK, et al. Bone marrow adiposity: basic and clinical implications. *Endocr Rev*. 2019. <https://doi.org/10.1210/er.2018-00138>.
5. Suchacki KJ, Cawthorn WP. Molecular interaction of bone marrow adipose tissue with energy metabolism. *Curr Mol Biol Rep*. 2018;4:41–9.
6. Veldhuis-Vlug AG, Rosen CJ. Clinical implications of bone marrow adiposity. *J Intern Med*. 2018;283:121–39.
7. Scheller EL, Khandaker S, Learman BS, Cawthorn WP, Anderson LM, Pham HA, et al. Bone marrow adipocytes resist lipolysis and remodeling in response to  $\beta$ -adrenergic stimulation. *Bone*. 2019;118:32–41.
8. Horowitz MC, Bery R, Holtrup B, Sebo Z, Nelson T, Fretz JA, et al. Bone marrow adipocytes. *Adipocyte*. 2017;6:193–204.
9. Li Y, Meng Y, Yu X. The unique metabolic characteristics of bone marrow adipose tissue. *Front Endocrinol (Lausanne)*. 2019;10:69.
10. Wang H, Leng Y, Gong Y. Bone marrow fat and hematopoiesis. *Front Endocrinol (Lausanne)*. 2018;9:694.
11. Luo G, He Y, Yu X. Bone marrow adipocyte: an intimate partner with tumor cells in bone metastasis. *Front Endocrinol (Lausanne)*. 2018;9:339.
12. Gimble JM, Zvonic S, Floyd ZE, Kassem M, Nuttall ME. Playing with bone and fat. *J Cell Biochem*. 2006;98:251–66.
13. Sadie-Van Gijzen H, Crowther NJ, Hough FS, Ferris WF. The interrelationship between bone and fat: from cellular see-saw to endocrine reciprocity. *Cell Mol Life Sci*. 2013;70:2331–49.
14. Sadie-Van Gijzen H, Hough FS, Ferris WF. Determinants of bone marrow adiposity: the modulation of peroxisome proliferator-activated receptor- $\gamma$ 2 activity as a central mechanism. *Bone*. 2013;56:255–65.
- 15.● Tencerova M, Kassem M. The bone marrow-derived stromal cells: commitment and regulation of adipogenesis. *Front Endocrinol (Lausanne)*. 2016;7:127 **This review provides an update on the cellular and molecular mechanisms involved in regulating the competitive selection between adipogenesis and osteoblastogenesis in BMSCs.**
16. Justesen J, Stenderup K, Ebbesen EN, Mosekilde L, Steiniche T, Kassem M. Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis. *Biogerontology*. 2001;2:165–71.
17. He J, Fang H, Li X. Vertebral bone marrow fat content in normal adults with varying bone densities at 3T magnetic resonance imaging. *Acta Radiol*. 2019;60:509–15.
18. Cortes ARG, Cohen O, Zhao M, Aoki EM, Ribeiro RA, Abu Nada L, et al. Assessment of alveolar bone marrow fat content using 15 T MRI. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2018;125:244–9.
19. Bani Hassan E, Demontiero O, Vogrin S, Ng A, Duque G. Marrow adipose tissue in older men: association with visceral and subcutaneous fat, bone volume, metabolism, and inflammation. *Calcif Tissue Int*. 2018;103:164–74.
20. Zhu L, Xu Z, Li G, Wang Y, Li X, Shi X, et al. Marrow adiposity as an indicator for insulin resistance in postmenopausal women with newly diagnosed type 2 diabetes—an investigation by chemical shift-encoded water-fat MRI. *Eur J Radiol*. 2019;113:158–64.
21. Lips P, van Schoor NM. The effect of vitamin D on bone and osteoporosis. *Best Pract Res Clin Endocrinol Metab*. 2011;25:585–91.
22. Topor LS, Melvin P, Giancaterino C, Gordon CM. Factors associated with low bone density in patients referred for assessment of bone health. *Int J Pediatr Endocrinol*. 2013;2013(1):4.
23. Swanson CM, Srikanth P, Lee CG, Cummings SR, Jans I, Cauley JA, et al. Associations of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D with bone mineral density, bone mineral density change, and incident nonvertebral fracture. *J Bone Miner Res*. 2015;30:1403–13.
24. Arunabh S, Pollack S, Yeh J, Aloia JF. Body fat content and 25-hydroxyvitamin D levels in healthy women. *J Clin Endocrinol Metab*. 2003;88:157–61.
25. Parikh SJ, Edelman M, Uwaifo GI, Freedman RJ, Semega-Janneh M, Reynolds J, et al. The relationship between obesity and serum 1,25-dihydroxy vitamin D concentrations in healthy adults. *J Clin Endocrinol Metab*. 2004;89:1196–9.
- 26.●● Pino AM, Miranda M, Figueroa C, Rodríguez JP, Rosen CJ. Qualitative aspects of bone marrow adiposity in osteoporosis. *Front Endocrinol (Lausanne)*. 2016;7:139 **This review discusses the emerging concept of the impact of marrow fat lipid composition, rather than marrow fat volume per se, on bone fragility.**
- 27.● Cordes C, Baum T, Dieckmeyer M, Ruschke S, Diefenbach MN, Hauner H, et al. MR-based assessment of bone marrow fat in osteoporosis, diabetes, and obesity. *Front Endocrinol (Lausanne)*. 2016;7:74 **This paper describes how MR-based methods can be applied to quantify BMAT lipid composition.**
28. Singhal V, Bredella MA. Marrow adipose tissue imaging in humans. *Bone*. 2019;118:69–76.
29. Xu K, Sigurdsson S, Gudnason V, Hue T, Schwartz A, Li X. Reliable quantification of marrow fat content and unsaturation level using in vivo MR spectroscopy. *Magn Reson Med*. 2018;79:1722–9.
30. Martel D, Leporq B, Bruno M, Regatte RR, Honig S, Chang G. Chemical shift-encoded MRI for assessment of bone marrow adipose tissue fat composition: pilot study in premenopausal versus postmenopausal women. *Magn Reson Imaging*. 2018;53:148–55.
31. Martel D, Leporq B, Saxena A, Belmont HM, Turyan G, Honig S, et al. 3T chemical shift-encoded MRI: detection of altered proximal femur marrow adipose tissue composition in glucocorticoid users and validation with magnetic resonance spectroscopy. *J Magn Reson Imaging*. 2019;50:490–6.
32. Zhu M, Hao G, Xing J, Hu S, Geng D, Zhang W, et al. Bone marrow adipose amount influences vertebral bone strength. *Exp Ther Med*. 2019;17:689–94.
33. Scheller EL, Troiano N, Vanhoutan JN, Bouxsein MA, Fretz JA, Xi Y, et al. Use of osmium tetroxide staining with microcomputerized tomography to visualize and quantify bone marrow adipose tissue in vivo. *Methods Enzymol*. 2014;537:123–39.
34. DeLuca HF. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr*. 2004;80:1689S–96S.
35. Geng S, Zhou S, Bi Z, Glowacki J. Vitamin D metabolism in human bone marrow stromal (mesenchymal stem) cells. *Metabolism*. 2013;62:768–77.
36. Heaney RP, Recker RR, Grote J, Horst RL, Armas LA. Vitamin D(3) is more potent than vitamin D(2) in humans. *J Clin Endocrinol Metab*. 2011;96:E447–52.
37. Romagnoli E, Pepe J, Piemonte S, Cipriani C, Minisola S. Management of endocrine disease: value and limitations of assessing vitamin D nutritional status and advised levels of vitamin D supplementation. *Eur J Endocrinol*. 2013;169:R59–69.
38. Christakos S, Dhawan P, Liu Y, Peng X, Porta A. New insights into the mechanisms of vitamin D action. *J Cell Biochem*. 2003;88:695–705.
39. Norman AW. Minireview: vitamin D receptor: new assignments for an already busy receptor. *Endocrinology*. 2006;147:5542–8.



40. Langub MC, Reinhardt TA, Horst RL, Malluche HH, Koszewski NJ. Characterization of vitamin D receptor immunoreactivity in human bone cells. *Bone*. 2000;27:383–7.
41. Geng S, Zhou S, Glowacki J. Effects of 25-hydroxyvitamin D(3) on proliferation and osteoblast differentiation of human marrow stromal cells require CYP27B1/1 $\alpha$ -hydroxylase. *J Bone Miner Res*. 2011;26:1145–53.
42. Whitfield GK, Hsieh JC, Nakajima S, MacDonald PN, Thompson PD, Jurutka PW, et al. A highly conserved region in the hormone-binding domain of the human vitamin D receptor contains residues vital for heterodimerization with retinoid X receptor and for transcriptional activation. *Mol Endocrinol*. 1995;9:1166–79.
43. Suda T, Ueno Y, Fujii K, Shinki T. Vitamin D and bone. *J Cell Biochem*. 2003;88:259–66.
44. Atkins GJ, Anderson PH, Findlay DM, Welldon KJ, Vincent C, Zannettino AC, et al. Metabolism of vitamin D3 in human osteoblasts: evidence for autocrine and paracrine activities of 1 alpha, 25-dihydroxyvitamin D3. *Bone*. 2007;40:1517–28.
45. Zhou S, LeBoff MS, Glowacki J. Vitamin D metabolism and action in human bone marrow stromal cells. *Endocrinology*. 2010;151:14–22 **This article first demonstrated that BMSCs possess all of the molecular machinery required to produce and respond to calcitriol.**
46. Beresford JN, Joyner CJ, Devlin C, Triffitt JT. The effects of dexamethasone and 1,25-dihydroxyvitamin D3 on osteogenic differentiation of human marrow stromal cells in vitro. *Arch Oral Biol*. 1994;39:941–7.
47. Piek E, Sleumer LS, van Someren EP, Heuver L, de Haan JR, de Grijjs I, et al. Osteo-transcriptomics of human mesenchymal stem cells: accelerated gene expression and osteoblast differentiation induced by vitamin D reveals c-MYC as an enhancer of BMP2-induced osteogenesis. *Bone*. 2010;46:613–27.
48. Kelly KA, Gimble JM. 1,25-Dihydroxy vitamin D3 inhibits adipocyte differentiation and gene expression in murine bone marrow stromal cell clones and primary cultures. *Endocrinology*. 1998;139:2622–8.
49. Ding J, Nagai K, Woo JT. Insulin-dependent adipogenesis in stromal ST2 cells derived from murine bone marrow. *Biosci Biotechnol Biochem*. 2003;67:314–21.
50. Kong J, Li YC. Molecular mechanism of 1,25-dihydroxyvitamin D3 inhibition of adipogenesis in 3T3-L1 cells. *Am J Physiol Endocrinol Metab*. 2006;290:E916–24.
51. Atmani H, Chappard D, Basle MF. Proliferation and differentiation of osteoblasts and adipocytes in rat bone marrow stromal cell cultures: effects of dexamethasone and calcitriol. *J Cell Biochem*. 2003;89:364–72.
52. Mahajan A, Stahl CH. Dihydroxy-cholecalciferol stimulates adipocytic differentiation of porcine mesenchymal stem cells. *J Nutr Biochem*. 2009;20:512–20.
53. Zarei A, Hulley PA, Sabokbar A, Javaid MK, Morovat A. 25-hydroxy- and 1 $\alpha$ ,25-dihydroxycholecalciferol have greater potencies than 25-hydroxy- and 1 $\alpha$ ,25-dihydroxyergocalciferol in modulating cultured human and mouse osteoblast activities. *PLoS One*. 2016;11(11):e0165462.
54. Duque G, Rivas D. Alendronate has an anabolic effect on bone through the differentiation of mesenchymal stem cells. *J Bone Miner Res*. 2007;22:1603–11.
55. Duque G, Li W, Vidal C, Bermeo S, Rivas D, Henderson J. Pharmacological inhibition of PPAR $\gamma$  increases osteoblastogenesis and bone mass in male C57BL/6 mice. *J Bone Miner Res*. 2013;28:639–48.
56. Cianferotti L, Demay MB. VDR-mediated inhibition of DKK1 and SFRP2 suppresses adipogenic differentiation of murine bone marrow stromal cells. *J Cell Biochem*. 2007;101:80–8 **This article identified stimulation of Wnt signalling as a mechanism whereby calcitriol could inhibit marrow adipogenesis.**
57. Nicolas V, Prewett A, Bettica P, Mohan S, Finkelman RD, Baylink DJ, et al. Age-related decreases in insulin-like growth factor-I and transforming growth factor-beta in femoral cortical bone from both men and women: implications for bone loss with aging. *J Clin Endocrinol Metab*. 1994;78:1011–6.
58. Gómez JM. The role of insulin-like growth factor I components in the regulation of vitamin D. *Curr Pharm Biotechnol*. 2006;7:125–32.
59. Wei S, Tanaka H, Seino Y. Local action of exogenous growth hormone and insulin-like growth factor-I on dihydroxyvitamin D production in LLC-PK1 cells. *Eur J Endocrinol*. 1998;139:454–60.
60. Ameri P, Giusti A, Boschetti M, Bovio M, Teti C, Leoncini G, et al. Vitamin D increases circulating IGF1 in adults: potential implication for the treatment of GH deficiency. *Eur J Endocrinol*. 2013;169:767–72.
61. Bredella MA, Gerweck AV, Barber LA, Breggia A, Rosen CJ, Torriani M, et al. Effects of growth hormone administration for 6 months on bone turnover and bone marrow fat in obese premenopausal women. *Bone*. 2014;62:29–35.
62. Duque G, Macoritto M, Kremer R. 1,25(OH)2D3 inhibits bone marrow adipogenesis in senescence accelerated mice (SAM-P/6) by decreasing the expression of peroxisome proliferator-activated receptor gamma 2 (PPARgamma2). *Exp Gerontol*. 2004;39:333–8.
63. Duque G, Rivas D, Li W, Li A, Henderson JE, Ferland G, et al. Age-related bone loss in the LOU/c rat model of healthy ageing. *Exp Gerontol*. 2009;44:183–9.
64. Ambrosi TH, Scialdone A, Graja A, Gohlke S, Jank AM, Bocian C, et al. Adipocyte accumulation in the bone marrow during obesity and aging impairs stem cell-based hematopoietic and bone regeneration. *Cell Stem Cell*. 2017;20:771–84.
65. Holick MF, Smith E, Pincus S. Skin as the site of vitamin D synthesis and target tissue for 1,25-dihydroxyvitamin D3. Use of calcitriol (1,25-dihydroxyvitamin D3) for treatment of psoriasis. *Arch Dermatol*. 1987;123:1677–83a.
66. Montero-Odasso M, Duque G. Vitamin D in the aging musculoskeletal system: an authentic strength preserving hormone. *Mol Asp Med*. 2005;26:203–19.
67. Geng S, Zhou S, Glowacki J. Age-related decline in osteoblastogenesis and 1 $\alpha$ -hydroxylase/CYP27B1 in human mesenchymal stem cells: stimulation by parathyroid hormone. *Aging Cell*. 2011;10:962–71.
68. Skversky AL, Kumar J, Abramowitz MK, Kaskel FJ, Melamed ML. Association of glucocorticoid use and low 25-hydroxyvitamin D levels: results from the National Health and Nutrition Examination Survey (NHANES): 2001–2006. *J Clin Endocrinol Metab*. 2011;96:3838–45.
69. Mazziotti G, Formenti AM, Adler RA, Bilezikian JP, Grossman A, Sbardella E, et al. Glucocorticoid-induced osteoporosis: pathophysiological role of GH/IGF-I and PTH/VITAMIN D axes, treatment options and guidelines. *Endocrine*. 2016;54:603–11.
70. Ghali O, Al Rassy N, Hardouin P, Chauveau C. Increased bone marrow adiposity in a context of energy deficit: the tip of the iceberg? *Front Endocrinol (Lausanne)*. 2016;7:125.
71. Veronese N, Solmi M, Rizza W, Manzano E, Sergi G, Santonastaso P, et al. Vitamin D status in anorexia nervosa: a meta-analysis. *Int J Eat Disord*. 2015;48:803–13.
72. Pereira-Santos M, Costa PR, Assis AM, Santos CA, Santos DB. Obesity and vitamin D deficiency: a systematic review and meta-analysis. *Obes Rev*. 2015;16:341–9.
73. Greco EA, Lenzi A, Migliaccio S. Role of hypovitaminosis D in the pathogenesis of obesity-induced insulin resistance. *Nutrients*. 2019;11(7).
74. Bouillon R, Bex M, Van Herck E, Laureys J, Doms L, Lesaffre E, et al. Influence of age, sex, and insulin on osteoblast function:

- osteoblast dysfunction in diabetes mellitus. *J Clin Endocrinol Metab.* 1995;80:1194–202.
75. Reichrath J. Dermatologic management, sun avoidance and vitamin D status in organ transplant recipients (OTR). *J Photochem Photobiol B.* 2010;101:150–9.
  76. Lai CY, Yang JY, Rayalam S, Della-Fera MA, Ambati S, Lewis RD, et al. Preventing bone loss and weight gain with combinations of vitamin D and phytochemicals. *J Med Food.* 2011;14:1352–62.
  77. Duque G, Macoritto M, Kremer R. Vitamin D treatment of senescence accelerated mice (SAM-P/6) induces several regulators of stromal cell plasticity. *Biogerontology.* 2004;5:421–9 **Combined, references 65 and 66 demonstrate that vitamin D suppress BMAT formation through upregulation of IGF-1 and down-regulation of pro-adipogenic genes, including PPAR $\gamma$ 2.**
  78. Yang YJ, Zhu Z, Wang DT, Zhang XL, Liu YY, Lai WX, et al. Tanshinol alleviates impaired bone formation by inhibiting adipogenesis via KLF15/PPAR $\gamma$ 2 signaling in GIO rats. *Acta Pharmacol Sin.* 2018;39:633–41.
  79. Maurice F, Dutour A, Vincentelli C, Abdesselam I, Bernard M, Dufour H, et al. Active Cushing syndrome patients have increased ectopic fat deposition and bone marrow fat content compared to cured patients and healthy subjects: a pilot IH-MRS study. *Eur J Endocrinol.* 2018;179:307–17.
  80. Pereira RC, Delany AM, Canalis E. Effects of cortisol and bone morphogenetic protein-2 on stromal cell differentiation: correlation with CCAAT-enhancer binding protein expression. *Bone.* 2002;30:685–91.
  81. Compston J. Glucocorticoid-induced osteoporosis: an update. *Endocrine.* 2018;61:7–16.
  82. Badr S, Legroux-Gérot I, Vignau J, Chauveau C, Ruschke S, Karampinos DC, et al. Comparison of regional bone marrow adiposity characteristics at the hip of underweight and weight-recovered women with anorexia nervosa using magnetic resonance spectroscopy. *Bone.* 2019;127:135–45.
  83. Bredella MA, Torriani M, Ghomi RH, Thomas BJ, Brick DJ, Gerweck AV, et al. Vertebral bone marrow fat is positively associated with visceral fat and inversely associated with IGF-1 in obese women. *Obesity (Silver Spring).* 2011;19:49–53.
  84. Baum T, Yap SP, Karampinos DC, Nardo L, Kuo D, Burghardt AJ, et al. Does vertebral bone marrow fat content correlate with abdominal adipose tissue, lumbar spine bone mineral density, and blood biomarkers in women with type 2 diabetes mellitus? *J Magn Reson Imaging.* 2012;35:117–24.
  85. Yu EW, Greenblatt L, Eajazi A, Torriani M, Bredella MA. Marrow adipose tissue composition in adults with morbid obesity. *Bone.* 2017;97:38–42.
  86. De Araújo IM, Salmon CE, Nahas AK, Nogueira-Barbosa MH, Elias J Jr, de Paula FJ. Marrow adipose tissue spectrum in obesity and type 2 diabetes mellitus. *Eur J Endocrinol.* 2017;176:21–30.
  87. Tencerova M, Figeac F, Ditzel N, Taipaleenmäki H, Nielsen TK, Kassem M. High-fat diet-induced obesity promotes expansion of bone marrow adipose tissue and impairs skeletal stem cell functions in mice. *J Bone Miner Res.* 2018;33:1154–65.
  88. Tencerova M, Frost M, Figeac F, Nielsen TK, Ali D, Lauterlein JL, et al. Obesity-associated hypermetabolism and accelerated senescence of bone marrow stromal stem cells suggest a potential mechanism for bone fragility. *Cell Rep.* 2019;27:2050–62.
  89. Mangan A, Le Roux CW, Miller NG, Docherty NG. Iron and vitamin D/calcium deficiency after gastric bypass: mechanisms involved and strategies to improve oral supplement disposition. *Curr Drug Metab.* 2019;20:244–52 **This article provides evidence for increased prevalence of vitamin D deficiency after gastric bypass surgery, which is of clinical concern in an era where such surgery is increasingly used to treat morbid obesity.**
  90. Kim TY, Schwartz AV, Li X, Xu K, Black DM, Petrenko DM, et al. Bone marrow fat changes after gastric bypass surgery are associated with loss of bone mass. *J Bone Miner Res.* 2017;32:2239–47.
  91. Bredella MA, Greenblatt LB, Eajazi A, Torriani M, Yu EW. Effects of Roux-en-Y gastric bypass and sleeve gastrectomy on bone mineral density and marrow adipose tissue. *Bone.* 2017;95:85–90.
  92. Patsch JM, Li X, Baum T, Yap SP, Karampinos DC, Schwartz AV, et al. Bone marrow fat composition as a novel imaging biomarker in postmenopausal women with prevalent fragility fractures. *J Bone Miner Res.* 2013;28:1721–8.
  93. Woods GN, Ewing SK, Sigurdsson S, Kado DM, Ix JH, Hue TF, et al. Chronic kidney disease is associated with greater bone marrow adiposity. *J Bone Miner Res.* 2018;33:2158–64.
  94. Hernandez MJ, Dos Reis LM, Marques ID, Araujo MJ, Truys CAM, Oliveira IB, et al. The effect of vitamin D and zoledronic acid in bone marrow adiposity in kidney transplant patients: a post hoc analysis. *PLoS One.* 2018;13(5):e0197994.
  95. Stein EM, Cohen A, Freeby M, Rogers H, Kokolus S, Scott V, et al. Severe vitamin D deficiency among heart and liver transplant recipients. *Clin Transpl.* 2009;23:861–5.
  96. Anastasilakis AD, Tsourdi E, Makras P, Polyzos SA, Meier C, McCloskey EV, et al. Bone disease following solid organ transplantation: a narrative review and recommendations for management from The European Calcified Tissue Society. *Bone.* 2019;127:401–18.
  97. Kühne CA, Heufelder AE, Hofbauer LC. Bone and mineral metabolism in human immunodeficiency virus infection. *J Bone Miner Res.* 2001;16:2–9.
  98. Ahmad AN, Ahmad SN, Ahmad N. HIV infection and bone abnormalities. *Open Orthop J.* 2017;11:777–84.
  99. Cotter EJ, Ip HS, Powderly WG, Doran PP. Mechanism of HIV protein induced modulation of mesenchymal stem cell osteogenic differentiation. *BMC Musculoskelet Disord.* 2008;9:33.
  100. Butler JS, Dunning EC, Murray DW, Doran PP, O'Byrne JM. HIV-1 protein induced modulation of primary human osteoblast differentiation and function via a Wnt/ $\beta$ -catenin-dependent mechanism. *J Orthop Res.* 2013;31:218–26.
  101. Noe S, Moeckel CI, Schwerdtfeger C, Oldenbuettel C, Jaeger H, Wolf E, et al. Monthly or weekly supplementation with cholecalciferol 20,000 IU in people living with HIV: results from a nested cohort study. *Interdiscip Perspect Infect Dis.* 2018;2018:7502127.
  102. Haug CJ, Aukrust P, Haug E, Mørkrid L, Müller F, Frøland SS. Severe deficiency of 1,25-dihydroxyvitamin D3 in human immunodeficiency virus infection: association with immunological hyperactivity and only minor changes in calcium homeostasis. *J Clin Endocrinol Metab.* 1998;83:3832–8.
  103. Mayur N, Lewis S, Catherwood BD, Nanes MS. Tumor necrosis factor alpha decreases 1,25-dihydroxyvitamin D3 receptors in osteoblastic ROS 17/2.8 cells. *J Bone Miner Res.* 1993;8:997–1003.
  104. Fernandez-Martin JL, Kurian S, Farmer P, Nanes MS. Tumor necrosis factor activates a nuclear inhibitor of vitamin D and retinoid-X receptors. *Mol Cell Endocrinol.* 1998;141:65–72.
  105. Nylén H, Habtewold A, Makonnen E, Yimer G, Bertilsson L, Burhenne J, et al. Prevalence and risk factors for efavirenz-based antiretroviral treatment-associated severe vitamin D deficiency: a prospective cohort study. *Medicine (Baltimore).* 2016;95(34):e4631 **This article discusses the risk of vitamin D deficiency associated with efavirenz and TDF use, which is of clinical concern in a population that already presents with an infection-related burden of vitamin D deficiency.**
  106. Conradi MM, van de Vyver M, Andrag E, Conradi M, Ferris WF. A direct comparison of the effects of the antiretroviral drugs Stavudine, Tenofovir and the combination Lopinavir/Ritonavir on bone metabolism in a rat model. *Calcif Tissue Int.* 2017;101:422–

- 32 This article is the only study to date to provide evidence that certain ARVs may cause BMAT expansion in vivo.**
107. Hernandez-Vallejo SJ, Beaupere C, Larghero J, Capeau J, Lagathu C. HIV protease inhibitors induce senescence and alter osteoblastic potential of human bone marrow mesenchymal stem cells: beneficial effect of pravastatin. *Aging Cell*. 2013;12:955–65.
  108. Gaudio A, Morabito N, Catalano A, Rapisarda R, Xourafa A, Lasco A. Pathogenesis of thalassemia major-associated osteoporosis: a review with insights from clinical experience. *J Clin Res Pediatr Endocrinol*. 2019;11:110–7.
  109. Van den Berghe G, Van Roosbroeck D, Vanhove P, Wouters PJ, De Pourcq L, Bouillon R. Bone turnover in prolonged critical illness: effect of vitamin D. *J Clin Endocrinol Metab*. 2003;88:4623–32.
  110. Klein GL. The interaction between burn injury and vitamin D metabolism and consequences for the patient. *Curr Clin Pharmacol*. 2008;3:204–10.
  111. Murdaca G, Tonacci A, Negrini S, Greco M, Borro M, Puppo F, et al. Emerging role of vitamin D in autoimmune diseases: an update on evidence and therapeutic implications. *Autoimmun Rev*. 2019;102350.
  112. Peterson CA, Heffeman ME. Serum tumor necrosis factor- $\alpha$  concentrations are negatively correlated with serum 25(OH)D concentrations in healthy women. *J Inflamm (Lond)*. 2008;5:10.
  113. Fletcher J, Cooper SC, Ghosh S, Hewison M. The role of vitamin D in inflammatory bowel disease: mechanism to management. *Nutrients* 2019;11(5).
  114. Szafors P, Che H, Barnette T, Morel J, Gaujoux-Viala C, Combe B, et al. Risk of fracture and low bone mineral density in adults with inflammatory bowel diseases. A systematic literature review with meta-analysis. *Osteoporos Int*. 2018;29:2389–97.
  115. Choi ST, Kwon SR, Jung JY, Kim HA, Kim SS, Kim SH, Kim JM, Park JH, Suh CH. Prevalence and fracture risk of osteoporosis in patients with rheumatoid arthritis: a multicenter comparative study of the FRAX and WHO criteria. *J Clin Med*. 2018;7(12).
  116. Xia J, Luo R, Guo S, Yang Y, Ge S, Xu G, et al. Prevalence and risk factors of reduced bone mineral density in systemic lupus erythematosus patients: a meta-analysis. *Biomed Res Int*. 2019;2019:3731648.
  117. Mutt SJ, Karhu T, Lehtonen S, Lehenkari P, Carlberg C, Saarnio J, et al. Inhibition of cytokine secretion from adipocytes by 1,25-dihydroxyvitamin D<sub>3</sub> via the NF- $\kappa$ B pathway. *FASEB J*. 2012;26:4400–7.
  - 118.\*\* Jacobs FA, Sadie-Van Gijzen H, van de Vyver M, Ferris WF. Vanadate impedes adipogenesis in mesenchymal stem cells derived from different depots within bone. *Front Endocrinol (Lausanne)*. 2016;7:108 **In this article, we identified two separate MSC populations in the rat femur, with different adipogenic potential, which may have distinct contributions to BMAT expansion.**
  119. Li Y, Bäckerjö CM, Haldosén LA, Lindgren U. Species difference exists in the effects of 1 $\alpha$ ,25(OH)<sub>2</sub>D<sub>3</sub> and its analogue 2-methylene-19-nor-(20S)-1,25-dihydroxyvitamin D<sub>3</sub> (2MD) on osteoblastic cells. *J Steroid Biochem Mol Biol*. 2008;112:110–6.
  120. Wiberg K, Ljunghall S, Binderup L, Ljunggren O. Studies on two new vitamin D analogs, EB 1089 and KH 1060: effects on bone resorption and osteoclast recruitment in vitro. *Bone*. 1995;17:391–5.
  121. Hou QK, Huang YQ, Luo YW, Wang B, Liu YM, Deng RD, et al. (+)-Cholesten-3-one induces osteogenic differentiation of bone marrow mesenchymal stem cells by activating vitamin D receptor. *Exp Ther Med*. 2017;13:1841–9.
  122. Peräkylä M, Malinen M, Herzig KH, Carlberg C. Gene regulatory potential of nonsteroidal vitamin D receptor ligands. *Mol Endocrinol*. 2005;19:2060–73.
  - 123.\* Montazeri-Najafabady N, Ghasemi Y, Dabbaghmanesh MH, Talezadeh P, Koohpeyma F, Gholami A. Supportive role of probiotic strains in protecting rats from ovariectomy-induced cortical bone loss. *Probiotics Antimicrob Proteins*. 2018. <https://doi.org/10.1007/s12602-018-9443-6> **These findings suggest that specific gut microbiota populations may provide support in maintaining or correcting vitamin D status.**
  - 124.\* Fairfield H, Falank C, Harris E, Demambro V, McDonald M, Pettitt JA, et al. The skeletal cell-derived molecule sclerostin drives bone marrow adipogenesis. *J Cell Physiol*. 2018;233:1156–67 **This article identifies sclerostin, another Wnt inhibitor, as a driver of BMAT expansion, similar to the Wnt inhibitors identified in reference 56.**
  - 125.\*\* Fairfield H, Falank C, Farrell M, Vary C, Boucher JM, Driscoll H, et al. Development of a 3D bone marrow adipose tissue model. *Bone*. 2019;118:77–88 **This model may fundamentally change the way that we study BMAT function in vitro.**

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