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BONE MARROW AND ADIPOSE TISSUE (G DUQUE AND B LECKA-CZERNIK, SECTION EDITORS)

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 PPARγ2 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 · PPARγ2 · 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 PPARγ2 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_3 (cholecalciferol) is a cholesterol derivative produced in the skin in response to exposure to sunlight or artificial ultraviolet light. Alternatively, cholecalciferol and vitamin D_2 (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_3 is biologically inert and is converted first to 25-hydroxy-D_3 (calcidiol) and then to 1,25-dihdroxy-D_3 (calcitriol) [35, 38] (Fig. 1). While calcitriol is the active vitamin D_3 metabolite, calcidiol is the major circulating vitamin D_3 metabolite, which is measured in serum to determine vitamin D_3 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 PPARγ2".

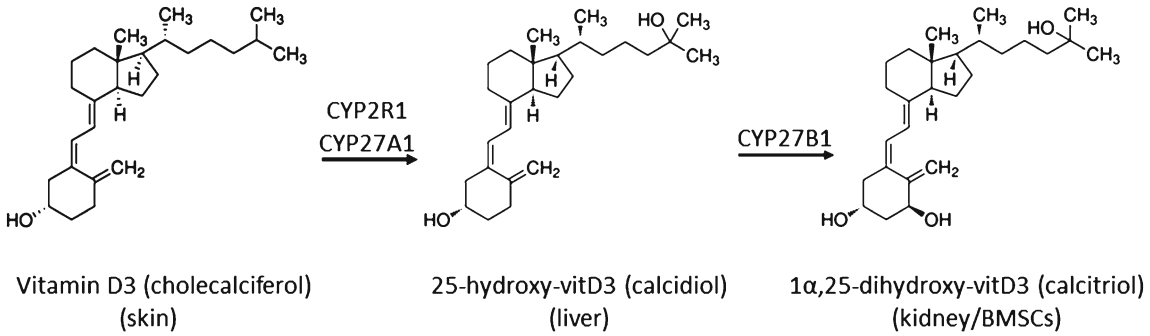

Fig. 1 The conversion of vitamin D3 to its active metabolite, calcitriol. Vitamin D_3 is transported to the liver, for hydroxylation by the cytochrome P450 enzymes CYP2R1 and CYP27A1 to form 25-hydroxy-D3 (calcidiol), and then to the kidney for another hydroxylation step by CYP27B1 (1α-hydroxylase), to form 1α,25-dihydroxy-D_3 (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_3 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 (1α-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_3 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 PPARγ2

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 PPARγ, the master transcriptional regulator of the adipogenic gene programme (reviewed in [14]). The PPARγ agonist troglitazone reversed the inhibition of adipogenesis by calcitriol, and conversely, calcitriol antagonized the transactivation capacity of PPARγ in 3T3-L1 cells [50]. VDR overexpression experiments showed that even unliganded VDR could inhibit PPARγ 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 PPARγ2 in bone marrow and BMSCs. Calcitriol downregulated PPARγ2 expression in human BMSCs [54], and conversely, the PPARγ 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, PPARγ 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•].

PPARγ2 and the Wnt pathway reciprocally inhibit each other to regulate the selection between osteoblastogenesis and adipogenesis (reviewed in [13]), and therefore the inhibition of PPARγ2 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 1α-hydroxylase in the kidney and BMSCs [45, 59]. Conversely, clinical studies have shown that vitamin D_3 supplementation can raise circulating IGF-1 levels [60], and treatment of cultured BMSCs with calcidiol 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 calcidiol 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_3, 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.

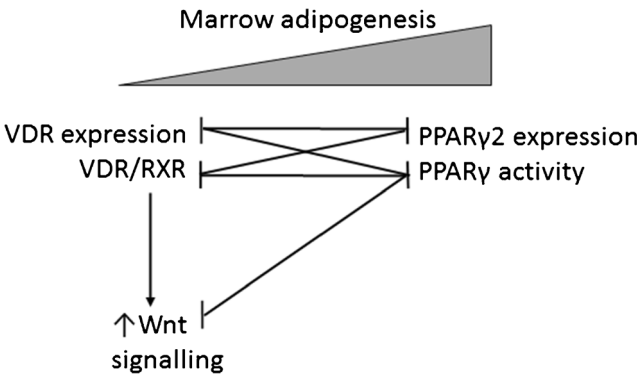

Fig. 2 The interplay between VDR and PPARγ2. Vitamin D, through the VDR, inhibits PPARγ2 expression [54], and conversely, PPARγ2 inhibits VDR expression [55]. In addition, increased VDR expression can suppress the transcriptional activity of PPARγ2 by competing for RXR [42, 50]. Vitamin D also stimulates Wnt signalling by inhibiting the expression of Wnt inhibitors [56], but the Wnt pathway and PPARγ2 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 PPARγ2 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 PPARγ2 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 PPARγ2 (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_3 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 PPARγ2 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 PPARγ2 expression in bone tissue [78] and in cultured BMSCs [80], resulting in increased marrow adipogenesis and decreased

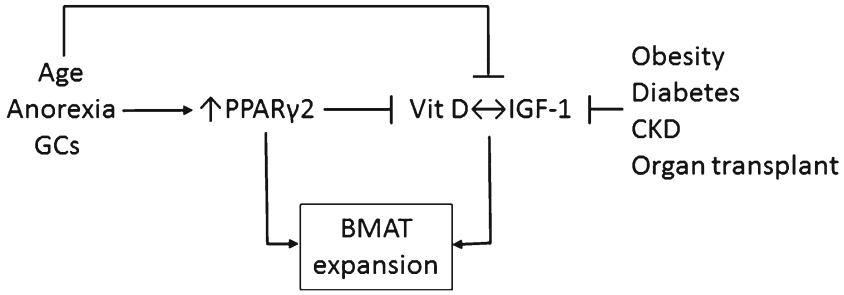

Fig. 3 The effect of various conditions and disease states on vitamin D/IGF-1 status and BMAT, with PPARγ2 as a central regulator. Vitamin D and IGF-1 reinforce each other, both systemically and locally in bone and bone marrow [45, 59, 60]. Through the upregulation of PPARγ2 expression and activity, age, anorexia, and GCs induce a pro-adipogenic shift in the differentiation of BMSCs ([13,14]. PPARγ2 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_3 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 PPARγ2 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 PPARγ2 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 PPARγ2 described in Sections "The Interplay Between VDR and PPARγ2" 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_3 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 1α-hydroxylation of calcidiol to calcitriol, mediated by increased circulating levels of the pro-inflammatory cytokine TNFα [102]. TNFα 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 disoproxyl 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 PPARγ2 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 TNFα [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 TNFα [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.

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