Curr Osteoporos Rep (2014) 12:235–242
DOI 10.1007/s11914-014-0199-y

HOT TOPIC

Fat and Bone Interactions

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Published online: 7 March 2014

Abstract Fat and bone have a complicated relationship. Although obesity has been associated with low fracture risk, there is increasing evidence that some of the factors that are released by peripheral fat into the circulation may also have a deleterious effect on bone mass, thus, predisposing to fractures. More importantly, the local interaction between fat and bone within the bone marrow seems to play a significant role in the pathogenesis of age-related bone loss and osteoporosis. This "local interaction" occurs inside the bone marrow and is associated with the autocrine and paracrine release of fatty acids and adipokines, which affect the cells in their vicinity including the osteoblasts, reducing their function and survival. In this review, we explore the particularities of the fat and bone cell interactions within the bone marrow, their significance in the pathogenesis of osteoporosis, and the potential therapeutic applications that regulating marrow fat may have in the near future as a novel pharmacologic treatment for osteoporosis.

Keywords Osteoporosis · Fat · Bone · Adipocytes · Osteoblasts · Osteoclasts · Osteocytes · Mesenchymal stem cells · Lamin A · Aging · Fractures · Osteoporosis · RUNX2 · PPARγ · BMP · SMADs · β-catenin

Introduction

Osteoporosis is a major public health problem that affects nearly 75 million people around the world and causes more than 2 million fractures annually [1]. This creates a major health burden by costing billions annually and causing significant morbidity and mortality within the older population.

The pathophysiology of osteoporosis has been associated with a misbalance between bone formation and resorption [2]. During the menopause, bone resorption by the osteoclasts is increased, thus inducing a significant bone loss [3], whereas with aging there is a significant reduction in bone formation due to low number and function of the bone forming osteoblasts [4]. More recently, a third pathophysiological mechanism for osteoporosis has been proposed involving the increasing presence of fat within the bone marrow, which is known to affect osteoblast differentiation and function, while increasing osteoclastic activity and also affecting mineralization [5, 6].

In fact, the relationship between fat and bone is complex. Several studies have differentiated this relationship into either systemic (endocrine) or local (auto and paracrine) [7, 8•]. The systemic interaction between fat and bone refers to those factors that are released by peripheral fat (subcutaneous, visceral, etc) and affect bone metabolism either in a negative or positive manner [4, 9]. In contrast, the local relationship refers to the activity of fat within the bone marrow milieu and its interaction with other bone cells [7].

Interest in the systemic effect of fat on bone has increased in recent years mostly due to the obesity epidemic. Some studies have reported that obesity reduces the risk for osteoporosis and that low body weight is a major risk for fractures [9, 10]. However, more recent evidence indicates that obesity could be detrimental to bone and that there is an inverse relationship between body mass index, bone mineral density and bone formation [11•, 12, 13]. Although this negative systemic effect of fat on bone metabolism has been associated with circulating adipokines, the mechanism of this deleterious effect remains unclear.

In contrast, the local relationship between fat and bone has been better understood and extensively explored in the last

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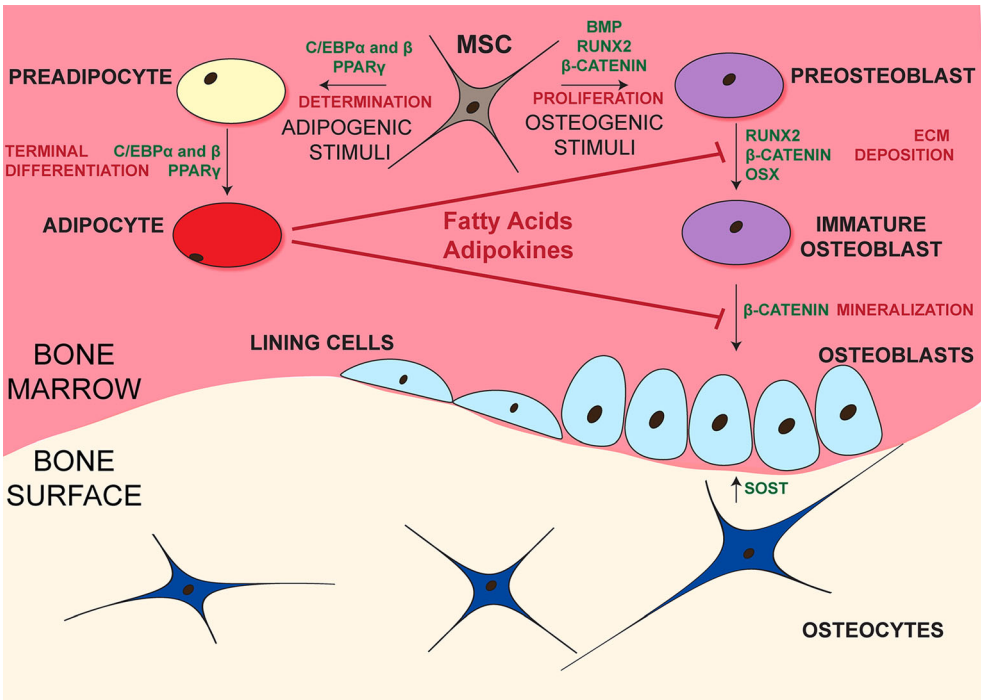

Fig. 1 Fat and bone relationship within the bone marrow milieu. MSCs differentiate toward osteogenic and adipogenic lineage. MSCs are stimulated by either osteogenic or adipogenic factors at each stage of differentiation. Furthermore, fatty acids and adipokines released by adipocytes are toxic and block the osteoblast differentiation pathway. *BMP* Bone morphogenic protein, *C/EBPαβ* CCAAT/enhancer binding protein alpha and beta, *ECM* extracellular matrix, *OSX* Osterix

years. In this review, we summarize the current understanding on the role of marrow fat in bone metabolism, its interaction with other cells in the bone marrow milieu and the potential therapeutic applications that regulating marrow fat volume and activity would have on bone formation (Fig. 1).

The Multicellular Bone Marrow Milieu: Cell Differentiation and Their Interactions

The bone marrow is a complex environment, in which a variety of cell types share a common space locally releasing cytokines and growth factors that could affect the cells in their vicinity. Major cellular groups within the bone marrow include blood cells, bone mass-regulating cells (osteoblasts and osteoclasts), and marrow adipocytes [14••].

In terms of their origin, mesenchymal precursors give rise to osteoblast, adipocytes and chondrocytes whereas blood cells are derived from hematopoietic stem cells. On the other hand, osteoclasts are derived from hematopoietic monocytes or macrophages [15]. Although both hematopoietic and mesenchymal precursors are present during the embryonic and early stages of life, marrow fat (yellow fat) only acquire significant levels during the second decade of life [16] in a process of progressive infiltration of the bone marrow space, which finally occupies a significant proportion of the bone marrow [17, 18•]. In addition, this increase in adipocyte number and volume is associated with reduced hematopoietic function and decreased osteogenesis [19].

The first local linkage between bone and fat relies on their cellular origin. Osteoblasts and marrow adipocytes derive from bone marrow mesenchymal stem cells (MSCs), which must not only differentiate but also proliferate in order to reach the appropriate cell numbers required for tissue regeneration, growing, and repair. Human MSCs fulfill the following characteristics: (1) have a specific antigenic profile that includes CD9+, CD54+, CD73+, CD90+, CD105+, CD166+, CD29+, CD44+, CD14-, CD19-, CD31-, CD34-, CD45-, HLA-DR-, and Nestin+ [20•]; (2) show a fibroblast-like morphology in culture with significant adherence to tissue culture plastic; (3) are isolated from specific niches in postnatal tissues, principally from bone marrow; (4) can remain undifferentiated and have the multipotent in vitro capacity to differentiate into mesenchymal lineage such as osteoblasts, adipocytes, chondrocytes and myoblasts [21–24, 25••]. Finally, MSCs show low immunogenicity and have pro-angiogenic and anti-inflammatory properties, which made them attractive not only for preventing the graft-versus-host disease and modulating the immune system after transplantation [26], but also to be used in regenerative medicine [21] and antitumor therapy [27].

Indeed, the main and most studied source of MSCs is still the bone marrow [28] with other diverse sources such as peripheral blood and subcutaneous fat being tested as alternative sources, which would facilitate their collection and culture in a less invasive way. However, the plasticity of MSCs from those extra medullar sources compared as bone marrow MSCs is limited [29••]. In vitro, non-bone marrow MSCs have a reduced capacity to proliferate and differentiate and also show variable characteristics changing according to the culture conditions [20•, 30].

Although present in a very low numbers under physiological conditions, minimal quantities of MSCs are required to support bone formation during development and adulthood. However, their numbers normally decrease after estrogen withdraw and during ageing [22, 31]. These changes in MSC number within the bone marrow environment would contribute to a shifting of MSC differentiation into an adipogenic lineage instead of an osteogenic one, which has been a common finding in in vivo and ex vivo studies [22, 31].

In contrast, in vitro studies have been less successful in mimicking the fat and bone interaction. This is due to the fact that in vitro models, although allowing the control of several factors such as the cell number and conditioning media, critically impede to simulate the marrow microenvironment where cells and their products interact in a reciprocal crosstalk. With regard to these limitations, biomedical engineers have developed 3D systems of co- and triculture with artificial mineral-coated scaffolds, thus, mimicking the mineralized extracellular matrix and allowing a paracrine multiple-way cellular interaction [32, 33]. Others have replaced the usual conditioning media with supernatants of other bone marrow cells in culture, thus, exposing MSCs to factors present in the bone marrow milieu [32]. Our group has used a different approach by testing the interaction between fat and bone cells in vitro separated by a porous membrane, which allows growth factors and cytokines to unidirectionally flow into the other cell group [34].

Indeed, there is enough evidence to suggest that, in the process of osteogenic differentiation of MSCs, an inverse relationship is established, in which mechanisms that promote osteoblastogenesis prevent adipogenesis and vice versa [35, 36]. Certain states such as ageing [7], metabolic diseases such as diabetes mellitus [37], estrogen withdrawal [38], immobilization [39], and glucocorticoid treatment [40] favor fat accumulation at the expense of bone formation. Overall, these risk factors induce the MSC to switch into its default lineage, which is considered to be adipocytic. In addition to high levels of adipogenesis observed in these conditions, it has been demonstrated that adipocytes products (fatty acids and adipokines) potentiate this negative scenario in a vicious circle, which has been termed as lipotoxicity [8•]. Finally, other cellular and molecular changes associated with ageing such as bigger MSC size, diminished differentiation potential, proliferation and growth rate as well as shortened telomeres could affect the capacity of MSCs to differentiate into osteoblasts while increase their adipocytic differentiation [22].

Molecular Mechanisms of Adipogenesis vs Osteoblastogenesis

The ultimate major controllers of the switching of a MSC toward either osteogenic or adipogenic lineages are runt-related transcription factor 2 (RUNX2) [41] for osteogenesis and the peroxisome proliferator-activated receptor-γ (PPARγ) [42] for adipogenesis, with many regulating routes and epigenetic factors being directly or indirectly involved [43•]. To reach the nucleus and to form their DNA-binding complex, RUNX2 and PPARγ should interact with a set of elements of osteogenic and adipogenic pathways, respectively. The most important pathway involved in these 2 differentiation processes is known as canonical Wnt/β-Catenin pathway (Fig. 1). Pro-osteogenic Wnts such as Wnt10b, Wnt1, Wnt6, Wnt7a, and Wnt10a are soluble proteins that prevent β-Catenin degradation and ultimately allow its nuclear translocation to form transcriptional complexes along with Tcf/Lef (T-cell factor/lymphoid enhancer-binding factor) and RUNX2, thus, stimulating osteogenic commitment of MSCs [44]. This process stimulates mineralization and alkaline phosphatase (ALP) activity in pre-osteoblasts [45] while inducing osteoprotegerin (OPG) expression, thus, indirectly downregulating bone resorption.

Considering that this is a highly regulated pathway, the exact mechanism by which the Wnt ligands and β-Catenin regulate MSCs differentiation within the bone marrow remains partially understood. Mutations in several up or down stream components and controllers of the Wnt/β-Catenin signaling pathway have been associated with bone-related diseases either by up- or downregulating osteogenic genes, thus, causing sclerosteosis or osteoporosis [46–49]. During osteogenesis, several pro-osteogenic Wnt proteins crosstalk and then bind to low-density lipoprotein (LDL) receptor–related proteins 5 and 6 (LRP5/6). This communication, which is followed by β-Catenin translocation, promotes osteoblastogenesis while blocks adipogenesis [50•]. In contrast, the presence of other group of Wnt proteins such as Wnt4, Wnt5a, and Wnt5b blocks these interactions, facilitate the degradation of β-Catenin and induce adipogenesis [51].

In addition, there are also secreted antagonists of the Wnt/β-Catenin pathway that could affect osteoblast differentiation and function. Sclerostin, which is a protein encoded by the SOST gene and is exclusively produced by the osteocytes, binds to the Wnt co-receptors LRP5/6 in osteoblasts to block the Wnt/β-Catenin signaling pathway while increases osteoblast apoptosis [52]. In contrast, mechanical loading sensed by osteocytes, and parathyroid hormone (PTH) diminish sclerostin production and favor osteoblastogenesis [53•, 54]. Furthermore, other antagonist of the Wnt/ β-Catenin pathway known as Dickkopf-related protein 1 (DKK-1) play an important role in skeletal formation since rare polymorphisms are present in patients with juvenile osteoporosis [55]. Also, the animal transgenic model Dkk-$1^{+/-}$ has increased osteoblast number and bone formation rate [56]. Current clinical trials are employing antibodies to neutralize these antagonists of bone formation and to increase bone formation in patients with osteoporosis [57, 58, 59••, 60].

In addition to the soluble Wnt proteins, other growth factors are involved in osteoblastogenesis. Members of the transforming growth factor-beta (TGF-β)/bone morphogenic protein (BMP) signaling pathway crosstalk along with multiple enhancers such as PTH, fibroblast growth factor (FGF), Wnts, Hedgehog, and others, integrate and amplify their signals and ultimately activate RUNX2 in an either SMAD-dependent or independent manner [61••]. Overall, most of the members of these pathways are crucial for bone formation as it is demonstrated by the critical bone impairment in genetic models lacking any of their multiple components [61••].

Additionally, vitamin D_3 has been proved to be stronger than BMP-2 inducing all stages of osteogenesis in a dexamethasone-dependent manner both in vitro [62] and in vivo [63•]. Indirectly, vitamin D enhances calcium and phosphate re-absorption in kidney and intestine, which improves bone mineralization. Finally, vitamin D inhibits marrow adipogenesis by inhibition of PPARγ in vivo [63•].

Interestingly, the downstream response that determines osteoblast differentiation is dependent on the translocation of several transcription factors from the cytoplasm to the nucleus. This has allowed to the discovery that osteogenic/adipogenic transcription factors could only reach the nucleus by means of the mechanical coupling and physical interaction between these activated factors and the proteins of the inner nuclear envelope [64]. Amongst these proteins, lamin A has acquired special relevance due to its regulatory role in the nuclear translocation and DNA-binding of essential transcription factors [65, 66]. In the case of osteoblastogenesis, it has been demonstrated that lamin A integrity and function are crucial to allow its progression [67, 68], otherwise, in the presence of low levels of lamin A expression, adipogenesis would take place [69, 70].

Furthermore, the molecular mechanism linking the Wnt pathway with proteins of the nuclear envelope relay on how β-Catenin translocates into the nucleus and also on the prevention of its degradation while in the cytoplasm. Initially β-Catenin is phosphorylated [71], then it can diffuse or migrate freely through the nuclear pore complexes [72] or can interact with other proteins of the nuclear envelope while facilitates their interaction with the RUNX-related osteogenic complex [73].

In contrast to the role of RUNX2 in osteoblastogenesis, PPARγ is the master transcription factor regulating adipogenesis that affects bone mass not only by blocking RUNX2 activity and thus, bone formation but also stimulating osteoclastogenesis [74]. Activation of PPARγ has a strong adipogenic effect that is reached through the inhibition of osteogenic Wnts, degradation of beta β-Catenin and low levels of lamin A expression. Based on this antagonistic effect between RUNX2 and PPARγ activity, it is proposed that mechanisms blocking marrow adipogenesis through PPARγ inhibition would facilitate RUNX2-related response and would improve osteoblastogenesis and bone formation. This therapeutic effect was obtained by using either pharmacologic antagonists or molecular inhibitors of PPARγ in vivo [63•, 75, 76].

In addition to local release of osteogenic proteins and growth factors, systemic adipokines could also play a role in the regulation of bone marrow MSCs. Leptin, an adipocyte-specific adipokine, centrally inhibits bone formation by preventing serotonin release from the hypothalamus [77•]. In addition, absence of serotonin receptors and tryptophan hydroxylase (Tph2) deletion cause osteoporosis and anorexia [78]. On the other hand, gut-derived serotonin peripherally contributes to prevent bone formation through LRP regulation [79], thus, inhibitors of gut-serotonin have shown to be effective in rescuing osteoporosis in an animal model of bone loss [80]. In addition, Confavreux (2011) reported the relationship between energy metabolism and bone remodeling via the neuronal regulation of leptin (neuropeptide Y: NPY, neuromedin: NMU, serotonin and β2-adrenergic receptor mediated) and osteocalcin (adiponectin and insulin mediated) [81]. Finally, insulin inhibits OPG production in osteoblasts, thus, the increased OPG/RANKL ratio favors osteocalcin activation and release from the ECM toward the stimulation of insulin in the pancreas [82].

Fat-Induced Osteoporosis

Both human and animal studies have demonstrated that marrow fat volumes are inversely related to bone mineral density and to bone integrity [83, 84•]. An in vitro study by Verma et al has proved that there is a clonal switch between adipocytic and osteoblastic lineages in subjects with osteoporosis [84•]. This increase in bone marrow adipogenesis could be the result from a reduction in osteoblast formation by stromal cells due to ageing or apoptosis of bone cells [85]. In addition, bone marrow adiposity may influence bone remodeling in 3 ways: (1) secretion of cytokines; (2) production of adipokines; and (3) paracrine influences on adjacent bone cells that decrease osteoblast number and increase adipocyte number while stimulating osteoclastic activity [18•].

Several lines of evidence have revealed that there is a correlation between fractures and fat marrow adipogenesis. Meunier et al studied iliac crest biopsies and found out that high bone marrow adipocytes in osteoporotic samples compares with healthy samples [86]. Wehrli et al reported MRI assessment of bone marrow in old women compared with their bone mass. They reported that increased fracture risk is associated with increased marrow fat together with lower bone mass [87].

In addition, studies have reported that lipotoxicity was detected after osteoblasts were exposed to adipocyte-secreted factors in vitro [34, 88]. This is supported by

experiments using co-cultures of adipocytes and osteoblasts, which revealed that adipocytes inhibit osteoblast proliferation and function through the secretion of adipokines and fatty acids [34, 89]. When the adipocytes were treated with cerulenin (inhibitor of fatty acid synthase) osteoblasts survived longer and mineralized better than their untreated controls suggesting that the secretion of fatty acids by bone marrow adipocytes could have a lipotoxic effect on osteoblasts that can be prevented through the inhibition of fatty acid synthase [34].

Among the adipocyte-secreted factors, palmitic acid was found to be highly prevalent and very toxic to osteoblasts [5, 34]. A recent study has revealed the mechanism of lipotoxicity induced by palmitic acid on osteogenesis by demonstrating that this fatty acid affects Wnt signaling and BMP2/RUNX2 / SMADs pathways as well as mineralization [5]. Furthermore, proteomic analysis investigating changes in adipocytes has shown there is shift in ageing bone marrow in mice from pro-osteogenic, anti-adipogenic and anti-apoptotic phenotype to a toxic and pro-adipogenic one in old mice, which could be associated with adipokines production [90]. Taken together, new evidence on lipotoxicity in bone allows suggesting that yellow fat plays a toxic role within the bone marrow milieu and that inhibition of this toxicity could alleviate this negative effect on bone formation.

Clinical Translation: Fat as a Therapeutic Target for Bone-Related Diseases

From a therapeutic point of view, osteoblast differentiation and RUNX2 activation may be stimulated by using agonists of the Wnt osteogenic pathway, direct stimulation of RUNX2, or inhibition of PPARγ. However, and considering that these pathways also regulate growth and differentiation of other cell types, there is always an inherent risk of increasing the risk of malignancy [91]. For example, although LRP5 and GSK3β could be ideal targets since they have crucial roles in either promoting or blocking osteoblastogenesis, respectively, it is extremely challenging to target their activity in MSCs without affecting other cellular processes [92]. Furthermore, as an additional beneficial effect of inducing MSC differentiation into the osteogenic lineage is that myogenesis could also be facilitated while decreasing high levels of fat infiltration also observed in ageing muscle [93].

As a new therapeutic approach to osteoporosis, in addition to their role in regulating glucose and lipids metabolism, several synthetic [94] and natural PPARγ antagonists [95] are being tested as stimulators of osteoblastogenesis and bone formation. One of them, bisphenol-A-diglycidyl ether (BADGE) is a low affinity PPARγ antagonist that has been shown to have a bone anabolic effect [63•]. This effect that was potentiated when administered in combination with vitamin D in an aged animal model [63•].

As an alternative approach to regulate MSCs differentiation and stimulate osteoblastogenesis, interferon gamma (IFNγ), which we previously reported as increased together with its inducible genes during early osteoblast differentiation in vitro [96] and also to regulate osteoblastogenesis in mice, could rescue oophorectomized mice from their osteoporotic phenotype by increasing osteoblastogenesis and inhibiting adipogenesis [97]. However, the exact mechanism of this effect remains to be elucidated.

Finally, and considering that fatty acids released from adipocytes, are toxic for osteoblast differentiation [5] and that inhibition of fatty acid synthase protects osteoblasts from apoptosis, this new evidence supports the notion that regulating the fatty acid synthase (FAS) pathway could effectively prevent adipogenesis while stimulating osteoblastogenesis [34, 98], which is a hypothesis that requires further exploratory using in vivo studies.

Conclusions

In conclusion, the relationship between fat and bone is complicated, especially within the marrow milieu. The inverse relationship between osteoblastogenesis and adipogenesis, the default differentiation of MSCs into adipocytes, and the age-related changes in the differentiation machinery determine that ageing bone marrow becomes fatty at expense of osteoblastogenesis and bone formation. In addition, the presence of marrow fat is associated with a toxic microenvironment that affects other marrow cells and could induce cell dysfunction and cell death. Considering that the regulatory mechanisms involved in MSCs are well known, these mechanisms could constitute novel therapeutic targets for osteoporosis, especially in older persons in whom bone formation is significantly reduced. With new regulators of adipogenesis and inhibitors of lipotoxicity being assessed in animal models, the potential development of osteoporosis treatment focusing on the fat and bone relationship could constitute the future of the pharmacologic approach to this devastating disease.

Acknowledgement The authors' research cited in this review has been funded by project grants from the National Health and Medical Research Council (NHMRC) of Australia (Grants 632766 and 632767) and the Nepean Medical Research Foundation. The authors would like to thank PR Ebeling of the University of Melbourne and EF Eriksen of Oslo University Hospital for their review of the manuscript.

Compliance with Ethics Guidelines

Conflict of Interest S. Bermeo declares no conflicts of interest.
K. Gunaratnam declares no conflicts of interest.
G. Duque declares no conflicts of interest.

Human and Animal Rights and Informed Consent All studies by the authors involving animal subjects were performed after approval by the appropriate institutional review boards.

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- •• Of major importance

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