PERSPECTIVES

Osteoporosis as a Lipotoxic Disease

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Abstract

The skeletal system, and adipose tissue, are two body systems that have seen major paradigm shifts in the understanding of their biology in recent years. Going beyond their respective traditional functions of locomotion and energy storage, a complex relationship between the two systems is being unraveled. In this review, we start first by describing current knowledge on the "systemic" interaction between fat and bone. However, the local interaction within the bone marrow milieu seems to play a more important role in the pathogenesis of osteoporosis. Increasing levels of mesenchymal stem cell differentiation into fat may have a lipotoxic effect on osteoblast function and survival while simultaneously stimulating osteoclastic activity. The end result will be increasing levels of bone resorption and decreasing levels of bone formation, the typical features of osteoporosis. Finally, the potential diagnostic and therapeutic implications of marrow fat infiltration will be discussed. *IBMS BoneKEy*. 2010 March;7(3):108-123.
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Introduction

Increasing marrow fat infiltration is one of the constant features of aging bone (1;2). However, the role of marrow fat in bone function and cellularity remains poorly understood (1;3). In contrast with the proposed positive relationship between other types of fat and bone structure, recent evidence has suggested that marrow fat could have a negative effect on bone structure and metabolism (4-6). This negative relationship could be explained by the induction of a predominantly adipocytic differentiation of mesenchymal stem cells (MSCs) and the combination of lipotoxicity (fatty acid release) and adipotoxicity (release of adipokines), which affect bone formation and could induce increasing levels of bone resorption (4).

In this review we will compare the characteristics of the "systemic" relationship between fat and bone (4) versus the recently described "local" relationship occurring within the bone marrow (7). Furthermore, the mechanisms that explain the predominant differentiation of MSCs into fat in aging bone, and the consequences of increasing levels of marrow adiposity, will also be reviewed.

The Fat and Bone Connection Outside the Bone Marrow

High body weight is protective for the skeleton. Indeed, body weight is one of the strongest predictors of bone mass (8). It has also been shown in several studies that weight loss is often accompanied by bone loss, especially in the elderly (9). Fracture risk at the hip and spine is also inversely proportional to body weight (10). In fact, it is possible to use body mass index (BMI) instead of bone mineral density (BMD) in the FRAX® calculator to estimate fracture risk (11). This is likely because a higher body weight confers a greater loading force to stimulate skeletal adaptation in a beneficial manner. Body weight includes 3 major components: fat mass, lean mass and bone. Several groups had previously attempted to determine the relative contributions of fat vs. lean mass on bone density as assessed by DXA studies. It was found that in both pre- and post-menopausal women, fat mass was

the predominant determinant of BMD (12;13) while in men, both fat mass and lean mass were unrelated to BMD after adjusting for the effect of skeletal size (13). However, others have reported that lean mass appears to have a significant effect on BMD (14;15). Investigators have attempted to address this conflicting data in the literature and have found that the relationship between body composition and bone mass/density is critically dependent on the specific parameter that is chosen as the dependent variable in the analysis. Using a multivariate analysis, the authors found that both fat mass and lean mass were significant independent predictors of total body bone mineral content (BMC) in women after accounting for the effects of age and height (16). Importantly, these studies, as well as subsequent ones, did not examine the relationship between fat mass and bone independently of the effects of weight. It has been pointed out (17) that most epidemiologic studies that had small sample sizes could not explore this because of the strong co-linearity between fat mass and body weight. In a large cross-sectional study involving close to 14,000 Chinese subjects, the authors found that a higher percentage of body fat was associated with a higher risk of osteoporosis, osteopenia and non-spine fractures (17). Subsequently, a study involving a large population of Chinese and Caucasian subjects also showed an inverse relationship between fat mass and bone mass after adjusting for the mechanical loading effects of body weight (18). It was also shown that fat mass had a negative correlation, or no correlation, to CT and DXA bone parameters after adjusting for lean mass and trunk height/leg length in adolescents and young adults aged 13-21 years (16). This notion that obesity may not be beneficial to skeletal health is also echoed in the pediatric literature. Studies have shown that children and young adults with distal forearm fractures were more likely to have a higher degree of adiposity (19;20).

In summary, obesity has been associated with high levels of BMD and viewed as a protective factor for fractures. This protective effect seems to be strongly dependent on the effect of mechanical loading by body weight on bone. However, recent evidence suggests that increasing adiposity (independently of weight) might be detrimental to bone.

Systemic Fat Products and Bone Mass

Adipose tissue is associated with both protective as well as negative factors. A comprehensive review of the role of systemic factors secreted by adipose tissue and bone has been published recently (21). Here, we will focus only on factors that may have a detrimental impact on bone health.

Adipokines

Bone and energy are functionally related through a complex neuroendocrine circuit that features leptin. Leptin regulates appetite and energy use by binding to a receptor in the hypothalamus. Through the sympathetic nervous system, leptin activates β-2 adrenergic receptors on osteoblasts, decreasing osteoblast activity while increasing bone resorption via RANKL (22). On the other hand, peripheral leptin signaling has been reported to increase cortical bone growth and bone MSC differentiation into osteoblasts rather than adipocytes (23;24). However, the exact relationship between serum leptin and BMD remains unclear, with studies reporting both positive and negative associations, particularly after body composition adjustments (4).

Lipids/oxidative stress and bone

Hyperlipidemia has been shown to increase osteoclastic bone resorption (25-28). Systemic oxidative stress that is associated with visceral obesity (29) has also been shown to increase bone resorption and impair bone formation (30). Lipid oxidation products can inhibit the differentiation of pre-osteoblasts into osteoblasts (29;30) and activate bone-resorbing osteoclasts by increasing receptor activator of NF-κB ligand (RANKL) (31); they also increase peroxisome proliferator-activated receptor-γ (PPARγ) expression, and diminish Wnt signaling, causing progenitor MSCs to undergo adipogenic instead of osteogenic differentiation (32).

Chronic low grade inflammation

Obesity is associated with chronic low-grade inflammation (33). The visceral adipose tissue depot releases adipocytokines that stimulate a greater hepatic release of acute phase response proteins such as C-reactive protein (CRP) (34) and this is associated with macrophages, which secrete inflammatory cytokines (TNF-α, IL-6, MCP-1, PAI-1) (35). IL-6 can stimulate osteoclasts to increase the rate of bone resorption (36) while higher circulating hsCRP levels are associated with higher serum NTX, a marker of bone resorption (37), and lower bone mass (38).

Vitamin D

While vitamin D is not produced by adipose tissue, it is stored there. Obesity has been associated with vitamin D deficiency (39-42) and cross-sectional studies have reported that serum 25-OHD levels are inversely correlated with total body fat (43-45). It has been suggested that obesity-associated vitamin D insufficiency may be due to the decreased bioavailability of vitamin D_3 from cutaneous and dietary sources because of its deposition in body fat compartments (46;47).

In summary, despite a direct relationship between BMI and BMD, there is evidence suggesting that high levels of fat, and more importantly high levels of fat's secreted factors, may have a deleterious effect on bone mass. This negative effect of fat on bone could be significantly higher within the bone marrow milieu, which experiences increasing fat infiltration with aging and where fat and bone cells share the same microenvironment.

The Bone Marrow Milieu

The bone marrow milieu is a complex combination of millions of specialized cells, proteins and lipids sharing a limited amount of space and in constant movement due to the effect of active blood flow. For the purposes of this review we will focus on the interaction between bone and fat cells within the bone marrow milieu.

At the same time that bone marrow is infiltrated by fat, several changes occur in other cells within the marrow milieu. Hematopoietic cells occupy most of the bone marrow space at birth (3;48). With aging, hematopoietic tissue is replaced by fatty bone marrow. This switch from a hematopoietic bone marrow into a fatty one seems to begin early in life across most species (48).

In addition, osteoblast number and function are decreased with aging (49;50), probably as a consequence of increasing levels of marrow fat. The potential mechanisms of this effect are reviewed in a later section of this article. In contrast, the number of osteoclasts increases with aging (51;52). Although this increase in osteoclast number has been associated with low levels of estrogens (53), the fact that it also happens in men along with new evidence showing that marrow fat could induce an increase in osteoclast number and activity suggest that factors other than hormones could explain this phenomenon.

Finally, recent evidence looking at the role of adipocytes within the marrow milieu suggests that rather than being passive cells, adipocytes are able to affect other cells in their vicinity due to their strong secretory activity and also due to their capacity to induce other cells to differentiate into adipocytes (54;55).

In summary, the bone marrow milieu is a microenvironment in which hematopoietic, fat and bone cells interact in a particular manner either by inhibiting or by inducing their activity. In general, aging is the most important factor that determines a change of bone marrow from hematopoietic to fatty, and therefore the understanding of the role of aging in this process is essential.

The Aging Bone Marrow

Apart from the switch from red to yellow marrow, there are other age-induced changes within the bone marrow milieu that may explain the negative relationship between fat and bone. With aging, there is a predominant differentiation of MSCs into adipocytes (3;5;6) (Fig. 1) at the expense of
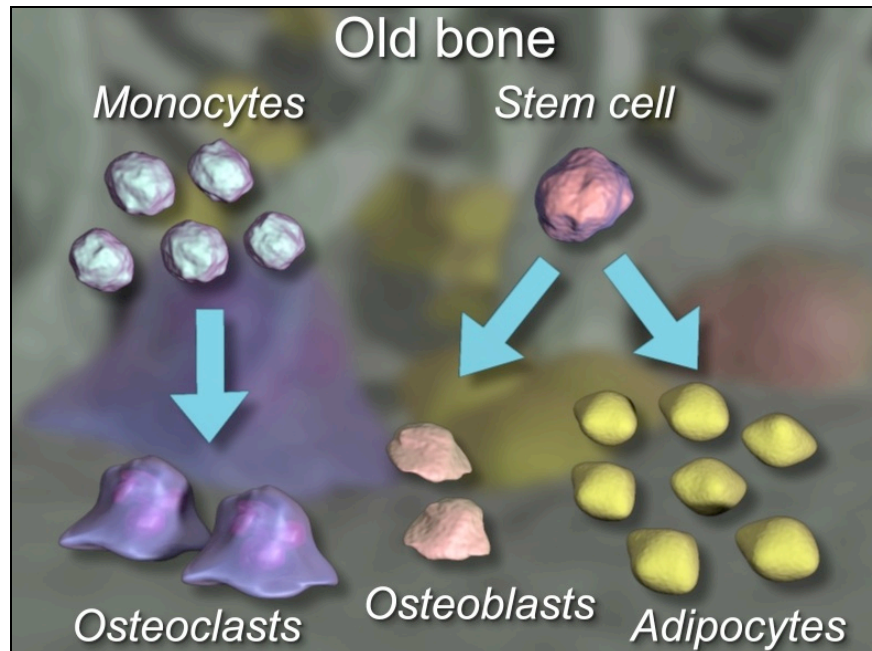
Fig. 1. In old bone, there is a predominant differentiation of MSCs into adipocytes. In addition, high levels of osteoclastogenesis are seen during post-menopause.

osteoblasts. Although the mechanisms that explain this switch remain poorly understood, there are several theories that could be classified as intrinsic (happening within the MSC) or extrinsic (a consequence of external factors).

Among the intrinsic factors that may determine the predominant differentiation of MSCs into adipocytes with aging, there is evidence suggesting that telomerase and lamin A/C are the two most important determinants of this process. Telomerase stabilization in MSCs has been demonstrated to prolong their lifespan and maintain their potential for osteogenic differentiation (56). In addition, human telomerized stromal cell clones exhibited the phenotype of hematopoietic-supporting osteoblastic and myofibroblastic cells after long-term culture (57).

In the case of lamin A/C, this protein of the nuclear envelope has been shown to be essential for osteoblast differentiation (58;59). Low levels of lamin A/C expression, as seen in aging osteoblasts (60), induce a predominant differentiation of MSCs into adipocytes (59;61) whereas osteoblastogenesis is inhibited (61). Furthermore, inducers of lamin A/C activity

such as zoledronic acid in combination with statins have been found to correct the osteoporotic phenotype in lamin A/C-deficient mice (62). This evidence suggests that young MSCs express sufficient levels of lamin A/C to differentiate into osteoblasts but these levels of expression decrease with aging and therefore a predominant differentiation into adipocytes may occur.

Meanwhile, the extrinsic factors that may explain the predominant age-related adipocytic differentiation of MSCs include low levels of bone marrow perfusion with high levels of oxidative stress (63), decreasing levels of estrogens (64), and decreasing levels of osteogenic factors (65). In the case of marrow perfusion, a study using magnetic resonance in men (mean age = 73 years) (66) demonstrated that subjects with osteoporosis have decreased vertebral marrow perfusion and increased marrow fat compared with these parameters in subjects with osteopenia. Similarly, subjects with osteopenia have decreased vertebral marrow perfusion and increased marrow fat compared with these parameters in subjects with normal bone density. *In vitro* studies have also demonstrated a direct relationship between hypoxia and high adipocyte differentiation (67). This evidence

suggests that MSCs exposed to limited oxygenation, as could be the case in the aging bone marrow exposed to low perfusion due to atherosclerotic arteries, would predominantly differentiate into adipocytes.

Furthermore, estrogens have been described recently as inhibitors of adipogenesis (68). A recent study has reported that postmenopausal women show high levels of marrow fat, and this was prevented by estrogen administration (69). We have also demonstrated that old oophorectomized mice receiving estrogen supplementation show significantly lower levels of marrow fat than a young oophorectomized group treated with estrogens, suggesting that old MSCs conserve their capacity to differentiate into osteoblasts in the presence of estrogens (64).

Finally, although aging has been associated with low levels of bone morphogenic proteins and other osteogenic factors (52), few studies have looked at the effect that supplementation of these factors may have on the features of age-related osteoporosis.

Fat's Gain Is Bone's Loss

There is an inverse relationship between fat and bone volume (70). This inverse relationship has been documented in bone obtained from animal models with high levels of marrow fat infiltration such as the 6T (71) and the senescence-accelerated mouse (72). In humans, the reverse relationship between fat and bone has been documented in studies (73) using bone biopsies and in studies using noninvasive techniques such as magnetic resonance (MR) (74). In fact, MR studies have shown that osteoporotic subjects have a higher proportion of marrow fat volume and are at higher risk of fracture when compared to healthy controls (74;75).

Furthermore, this fat infiltration starts much earlier than the third decade of life when bone mass starts its decline. Using CT scan images, a recent study in young individuals showed that marrow fat was already present, and that there was a reciprocal relation between marrow adiposity and the amount of bone in the axial and appendicular skeleton (76).

This evidence suggests that marrow fat infiltration starts before bone mass is affected, suggesting that, contrary to previous theories, marrow fat is not just occupying the space left by bone loss but is in fact inducing bone loss either through the shifting of MSCs into adipocytes at the expense of osteoblasts, or by a lipotoxic effect of fat within the bone marrow microenvironment.

Interestingly, marrow fat behaves differently than other types of fat. Studies in animal models (77;78) and recent studies in patients suffering from anorexia nervosa (79;80) suggest that marrow fat is not involved in energy metabolism under extreme levels of calorie restriction. In addition, high levels of marrow fat have not been associated with high body mass index or with high levels of cholesterol or triglycerides, suggesting that marrow fat has unique characteristics that differentiate it from other types of fat and that make it negligible as a metabolic source.

Mechanisms of Differentiation in Aging Stem Cells

The mechanisms of differentiation of MSCs into either adipocytes or osteoblasts have been extensively reviewed elsewhere (81). Although there are multiple factors involved in this process, for the purposes of this review we will focus on two major transcription factors, namely PPARγ2 for adipogenesis, and runt-related transcription factor 2 (Runx2) for osteoblastogenesis.

The predominant expression of one of these two factors will determine the final differentiation of MSCs into fat or bone cells. With aging, levels of expression of PPARγ2 increase within the bone marrow (82). This increase in PPARγ2 is associated with increasing levels of fatty acids (83) as well as inhibition of osteogenesis via the down-regulation of the expression of cyclooxygenase-2 and inducible nitric oxide (84).

In the case of Runx2, the effect of aging in Runx2 expression and activity remains poorly understood. A 2008 study (85) in aging group IVA phospholipase A2 (iPLA2beta)-null mice showed that undifferentiated bone marrow stromal cells (BMSCs) from KO mice express higher levels of PPARγ and lower levels of Runx2 mRNA, and this correlates with increased adipogenesis and decreased osteogenesis in BMSCs from these mice. Although the complexity of this mouse model prevents it from being considered as an aging mouse model, the fact that this model shows the particular features of senile osteoporosis suggests that Runx2 levels could also be decreased during normal aging. Nevertheless, further studies looking at age-related changes in Runx2 expression in MSCs and their significance in low levels of osteoblastogenesis are still required.

In summary, aging MSCs show high levels of PPARγ2 expression that induce the release of pro-adipogenic and toxic factors within the bone marrow. The mechanisms that explain this predominant expression of PPARγ2 by MSCs in aging bone marrow are unclear but could be dependent on age-related factors such as low lamin A/C expression or oxidative stress. Although there is evidence that low lamin A/C expression is associated with low Runx2 activity (59), the mechanisms that explain low levels of Runx2 expression in aging bone remain unknown.

Bone Lipotoxicity

Fat is usually accumulated in adipose tissue. However, aging is associated with ectopic lipid deposition in non-adipose tissues, such as the liver, skeletal muscle, the pancreas and bone (55). Ectopic lipid deposition is associated with the local release of adipokines, which have been associated with multiple pathological conditions that include the metabolic syndrome (86), type II diabetes mellitus (87), and chronic inflammation with the infiltration of macrophages (88). In general, the toxic effect of ectopic fat is a constant in all infiltrated tissues, therefore, bone would not be an exception.

The term "lipotoxicity" involves a variety of effects due to the presence of ectopic fat. These effects include the release of adipokines, fatty acids and other metabolites that may affect the function and survival of other cells in their vicinity (55) (Fig. 2). The pancreas is probably the best-studied example of lipotoxicity. In the pancreas, progressive fat infiltration induces activation of apoptotic pathways in β cells, inducing cell death and affecting the total population of β cells and inducing pancreatic failure (55;89). This effect, known as lipoapoptosis, is one of the multiple consequences of the presence of ectopic fat and has been explained by the secretion of fatty acids and the activation of deleterious pathways such as ceramide production (90).

In fact, the effect of fat infiltration in bone is still unclear, however, there is enough evidence to suggest that fat and bone cells sustain a negative relationship when sharing the same environment. The first evidence of this negative relationship came from experiments using a system of cocultures that demonstrated that the presence of adipocytes inhibits proliferation of human osteoblasts. (91) In a further study (92), it was reported that polyunsaturated fatty acids were responsible for this inhibition. We further assessed this interaction using the same model to identify the mechanisms that explain this inhibitory effect. Our study demonstrated that adipocytes not only inhibit osteoblast proliferation but also affect levels of Runx2 expression and induced osteoblast apoptosis (93). Furthermore, analysis of the potential factors involved in this effect showed that two fatty acids, stearate and palmitate, were responsible for this lipotoxicity. Finally, treatment of adipocytes with cerulenin, an inhibitor of fatty acid synthase, prevented the negative effect of fatty acids on osteoblast function and survival in our model.

Taken together, this evidence suggests that osteoblast function and survival are affected by the presence of marrow adipocytes and their autocrine release of fatty acids, a clear example of lipotoxicity. The implications of these findings may allow us to better understand several mechanisms of osteoporosis that are poorly understood
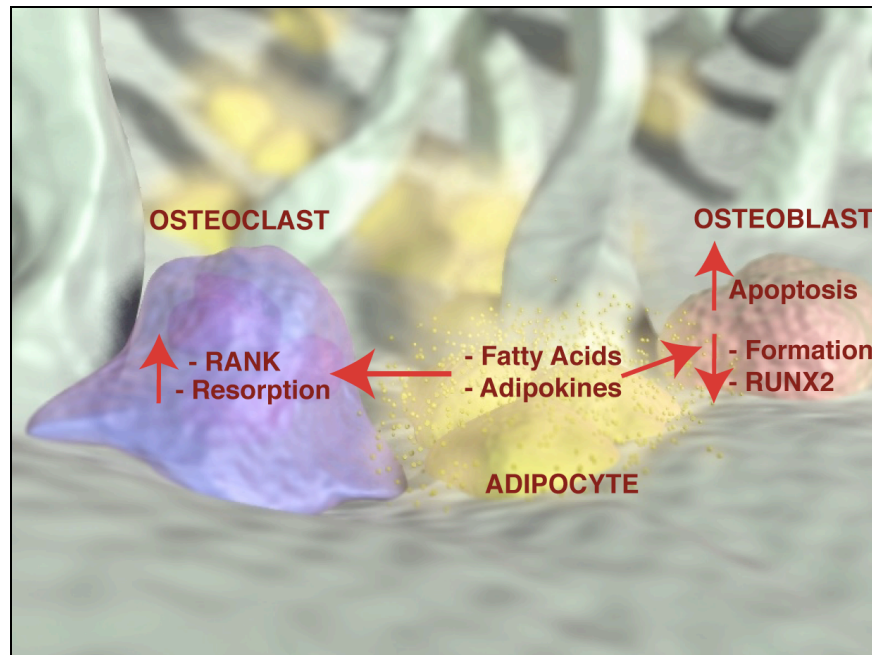
Fig. 2. Increasing levels of marrow fat are associated with the release of adipokines and fatty acids within the bone marrow microenvironment. These factors will have an inhibitory effect on osteoblast function and affect mineralization, whereas they stimulate osteoclastic activity, RANK expression and bone resorption.

such as osteoblast/osteocyte apoptosis (94) and changes in bone structure such as increasing bone porosity with age (95).

In contrast, there is recent evidence suggesting that adipocytes may have a stimulatory effect on osteoclasts. In a recent study, it was shown that bone marrow adipocytes support osteoclast differentiation (96). Furthermore, using a Tie2Cre/flox mouse model in which PPARγ was deleted in osteoclasts but not in osteoblasts, it was shown that PPARγ and its ligands promote osteoclast differentiation and resorption (97).

Furthermore, two recent *in vivo* studies have assessed whether increasing levels of marrow fat also exhibit a "lipotoxic profile." Using young (4-month-old) vs. old (24-month-old) C57BL/6 mice (98) we recently compared the levels of adipokine expression in adipocytes obtained from subcutaneous tissue and bone marrow. Our proteomic analysis showed that, when compared with subcutaneous adipocytes, aging bone marrow adipocytes showed a more pro-adipogenic, anti-osteoblastogenic and pro-apoptotic phenotype. In addition, marrow adipocytes obtained from the old mice showed higher levels of toxic adipokines that

have been associated with the induction of lipoapoptosis in other organs. Another study performed in human subjects of varying bone densities used samples of marrow fat and subcutaneous fat from 126 subjects (98 females, 34 males, mean age 69.7 ± 10.5 years) to analyze for fatty acid composition by gas chromatography. In agreement with our mice data, the authors report that marrow fatty acid composition differs from that of subcutaneous fat and varies between predominantly erythropoietic and fatty marrow sites. The authors found a significant difference in the marrow fat concentration of two fatty acids, cis-7-hexadecenoic acid and docosanoic acid, between normal and osteoporotic subjects (99). These *in vivo* studies constitute the first assessment of the particular components of marrow fat that make it unique and particularly lipotoxic.

In summary, there is increasing evidence suggesting that the pathophysiology of osteoporosis includes a lipotoxic component that deserves further investigation. This new evidence allows us to suggest that with aging, fat infiltrates the marrow space, releases toxic adipokines and fatty acids and expresses high levels of PPARγ, having

as a consequence lower levels of osteoblast function and survival and stimulation of osteoclast differentiation and activity (Fig. 2 and additional information). The final effect of the presence of marrow fat is low bone formation and high levels of resorption, the typical features of osteoporosis in older individuals.

Potential Therapeutic Applications of Lipotoxicity Prevention in Osteoporosis

The process of marrow fat infiltration could have additional implications in terms of the diagnosis and treatment of osteoporosis. For diagnosis, the fact that fat infiltration starts much earlier than any decline in bone mass and that osteoporotic patients show high levels of marrow fat compared to healthy controls indicates that quantification of marrow fat could become a better predictor of osteoporotic fractures than BMD. In fact, our group (100) and other groups (66) have developed and validated noninvasive methods of marrow fat quantification. However, although there is a strong correlation between bone mass and marrow fat quantified by these methods, they have not been tested in large cohorts in order to determine their predictive value for fractures. In addition, these noninvasive methods have not been tested looking at the effectiveness of current osteoporosis treatments.

Indeed, invasive quantification of changes in marrow fat have provided us with a new understanding of the effect that current osteoporosis treatments may have on bone cells. Although several *in vitro* experiments have shown an inhibitory effect of bisphosphonates (101) and teriparatide (102) on adipocyte differentiation, only two studies have looked at the effect of osteoporosis treatment on marrow fat *in vivo*. One study (69) demonstrated that estrogens decrease marrow fat in postmenopausal women. Furthermore, looking at biopsies obtained from osteoporotic women treated with risedronate for three months, our group has identified a significant reduction in marrow fat and lower levels of PPARγ expression in women treated with risedronate as compared with placebo (103).

These studies suggest that, in addition to their antiresorptive effect, estrogens and bisphosphonates may have a positive effect on bone mass by inhibiting bone marrow adipogenesis. In the case of bisphosphonates, which are known as inhibitors of osteoclastic activity, a reduction in marrow fat would create a friendly environment for secondary mineralization, a well-known effect of bisphosphonates that may explain their effect on bone mass.

In fact, there is evidence suggesting that inhibition of adipogenesis in general or targeting PPARγ in particular could have a positive effect on bone mass (104). Heterozygous PPARγ-deficient mice exhibited high bone mass with increased osteoblastogenesis independently of insulin or leptin (105). In a study treating SAMP6 mice with vitamin D, we showed a significant reduction in fatty bone marrow (106) concomitant with increasing levels of bone formation (107).

A recent study has tested the effect of PPARγ inhibitors on bone mass with negative results (108). These negative results are probably due to the fact that the PPARγ inhibitor was tested in diabetic mice, which show significant differences with a normal aging mouse model. To obviate this limitation, we have treated C57BL/6 mice with an inhibitor of PPARγ. We found that treated mice showed a significant gain in bone mass and showed high levels of osteoblastogenesis (109).

This evidence suggests that quantification of marrow fat and inhibition of marrow adipogenesis may become a novel approach to the diagnosis and treatment of osteoporosis. The inhibition of marrow adipogenesis could have a dual impact on bone cellularity by increasing osteoblastogenesis while decreasing osteoclast differentiation and activity.

Conclusion

In this review, we have summarized the particular features of the fat and bone relationship. Although a systemic relationship between fat and bone has been proposed, the local relationship seems to be

determined by a direct interaction between adipocytes and osteoblasts within the bone marrow milieu.

Increasing levels of MSC differentiation into adipocytes in aging bone could be explained by a combination of age-related mechanisms associated with other factors such as hormones, nutrition, genetics or a miscellany of growth factors. Once the process of fat infiltration starts within the bone marrow, it is associated with the release of adipokines and fatty acids that exert a lipotoxic effect against the cells in their vicinity, including osteoblastic and hematopoietic tissue. In contrast, osteoclastic differentiation and activity is stimulated by the presence of marrow fat. The final consequence of this process is bone loss due to high levels of bone resorption and low levels of bone formation.

Although a significant amount of evidence is still required, the identification of the mechanisms of adipogenesis in bone, the quantification of marrow fat and the potential inhibition of marrow adipogenesis will constitute a new approach to the understanding and treatment of osteoporosis in the near future.

Additional Information

http://www.youtube.com/watch?v=QZ3ac
IPTlbE

Acknowledgement

The authors would like to thank Dr. Jeff Gimble from the Pennington Biomedical Research Center, Louisiana State University System for his careful review of our manuscript.

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