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Marrow Fat and the Bone Microenvironment: Developmental, Functional, and Pathological Implications

Clifford J. Rosen[1,*], **Cheryl Ackert-Bicknell**[2], **Juan Pablo Rodriguez**[3], and **Ana Maria Pino**[3]

1 *Maine Medical Center Research Institute, Scarborough, ME 04041, USA*

2 *The Jackson Laboratory, Bar Harbor ME 04609*

3 *The University of Chile, Santiago, Chile*

Abstract

Bone marrow adipogenesis is a normal physiologic process in all mammals. However, its function is unknown. The mesenchymal stem cell is the marrow precursor for adipocytes as well as osteoblasts, and PPARγ is an essential differentiation factor for entrance into the fat lineage. Mouse models have provided significant insight into the molecular cues that define stromal cell fate. In humans, accelerated marrow adipogenesis has been associated with aging and several chronic conditions including diabetes mellitus and osteoporosis. Newer imaging techniques have been used to determine the developmental time course of fat generation in bone marrow. However, more studies are needed to understand the interrelationship among hematopoietic, osteoblastic, and adipogenic cells within the marrow niche.

Keywords

adipogenesis; marrow stromal cells; osteoblastogenesis; bone mass; aging

I. INTRODUCTION

Although often overlooked, ignored, or misunderstood, fat is an integral part of the bone marrow microenvironment. The developmental, mechanical, and physiological components of adipogenesis within the trabecular milieu have recently become the focus of investigation for several reasons. First, cells in the bone marrow niche communicate with each other, and are essential for the maturation of mesenchymal and hematopoietic stem cells. Similarly, reticular endothelial cells lining the sinusoids of the marrow can become osteoblasts under the right environmental circumstances. Second, not only can marrow fat can be visualized by CT or MRI, but there is an inverse relationship between the amount of marrow fat, measured by spectroscopy, and bone mineral density by DXA or quantitative CT.[1] Third, commonly used drugs such as the glucocorticoids and the thiazolinediones can enhance marrow fat, at the expense of skeletal mass. Coincident with those findings, there has also been significant progress in understanding the differentiation programs of osteoblasts and adipocytes, as well as their common ancestor, the mesenchymal stem cell.

This review centers on our current knowledge of marrow fat, from its origins to its physiologic role in the hematopoietic niche, and its function in pathologic conditions. We focus on mouse

*Address all correspondence to Clifford J. Rosen, Maine Medical Center Research Institute, 81 Research Drive, Scarborough, ME 04041; E-mail: rofe@aol.com.

and human models, although some of the conclusions may extend to other vertebrates. It will become evident from this review that although advances have been made, much work still needs to be done, particularly in understanding the function of marrow fat and its relevance to other fat depots and tissues such as the skeleton.

II. HISTORICAL VIGNETTE

Large cells in the bone marrow were recognized in pathologic human specimens as early as the 19th century, although their function was unknown. Neumann was the first to recognize from autopsy samples that marrow giant cells replaced red marrow in the peripheral skeleton with age. Indeed, he advanced the theorem that with advancing age, hematopoietic elements within the marrow are replaced by large yellow cells. In 1893, large “giant” cells in the bone marrow of individuals were noted, although their function was not known. Later that decade, a marked increase in marrow giant cells was noted with arsenic toxicity, and these cells were reported to contain fat globules. Subsequently, it was recognized that adipocytes were a constant feature of bone marrow aspirates and biopsies, and numerous studies reported the absence of marrow fat in hematopoietic disorders or enhanced marrow adiposity in aplastic anemia due to chemicals or radiation. In 1971, observations by two independent investigators set the stage for understanding the relationship between marrow fat and bone mass. The former reported an inverse association between marrow fat and bone volume, and the latter noted that osteoporotic elderly women had abundant marrow fat, which was directly related to age, and negatively correlated with trabecular bone volume.[2] Several reports then showed an age-associated increase in marrow adiposity, but with gender, ethnic, and age variation. By the 1990s, MRI emerged as a means to image and quantitate marrow fat by spectroscopy. Phenotyping in mice for marrow adiposity has only recently been achieved using high-field MRI and μCT with osmium staining.

III. DEVELOPMENTAL SEQUENCE OF MARROW ADIPOGENESIS

Generation of fat in the marrow is presumed to be identical to adipogenesis in other tissues. However, since the function of marrow fat is uncertain, the type of fat tissue (i.e., white or brown adipose tissue) in the bone marrow has not been established. Nonetheless, adipogenesis in mice and humans follows a defined pathway that begins with a common mesenchymal stem cell that is pluripotent.[3] Once adipogenesis begins, it is highly regulated and complex. The central components of this network are Cebpβ and PPARG, two critical transcription factors that initiate a cascade of other factors that enhance differentiation of adipocytes. Interestingly, some of these determinants, including Srebp-1c, Cebp/β, and EBF-1, may precede expression of PPARG or conversely may be downstream of PPARG. Moreover, PPARG is not only modulated by these factors, but also is autoregulated, making it difficult to determine purely by transcript number how PPARG is working.[4] Several other factors modulate PPARG function. For example, phosphorylation of PPARG suppresses transcriptional activity, while ligand binding enhances it in coordination with the recruitment of specific coactivators or corepressors. In regard to the former, endogenous ligands, including PGJ2, fatty acids, and HODE, can activate PPARG. Exogenous ligands for PPARG are the thiazolinediones such as rosi- and pioglitazone. Another level of control resides with the recruited coactivators and corepressors that ultimately define the function of PPARG and the metabolic function of the differentiated adipocyte. For example, recruitment of Pgc-1α, Src-1, and Src-2 promotes the progression of adipogenesis toward brown fat, whereas Traf and Tif2 enhance white adipocyte differentiation.[5] Interestingly, the proximity of adipocytes to osteoblasts in the bone marrow, and their common origin from a pluripotent MSC, implies that preadipocytes could become osteoblasts. For example, recruitment of TAZ and HICS to the PPARG complex with RXRα acts as a corepressor to inhibit further adipocyte differentiation and enhance osteoblastogenesis.[6] Moreover, recent work by the Evans group[7] has established that PPARG

may also enhance osteoclast differentiation by upregulating c-Fos. These findings also place PPARG, an essential factor for adipogenesis, at the center of the bone remodeling process.

IV. THE MOUSE AS A MODEL TO STUDY MARROW FAT

A. Distribution and Developmental Time Course

The laboratory mouse has been a crucial model system for investigating the mechanisms that define skeletal acquisition and bone maintenance. Surprisingly, little attention has been paid to fat in the marrow, and thus it was not previously characterized in mice. In fact, as a phenotype, the amount of marrow fat in the mouse was considered only in terms of its relative proportion to the size of the animal, and its fat content elsewhere. Originally, it was assumed that marrow fat in rodents was important for peripheral heat generation and therefore was restricted to the tail vertebrae and extreme periphery of the skeleton (i.e., tibia and fibula of the distal skeleton).[5] This tenet was supported by the finding that marrow adipocytes expressed UCP-1 (uncoupling protein 1), a mitochondrial protein instrumental in thermogenesis.[5] However, more careful phenotyping revealed that marrow adipocytes are distributed from both the epiphyses to the diaphysis in long bones of mice, including the femur (Fig. 2). Moreover, fat cells can also be found in the lumbar vertebrae, although to a much lesser degree.[8] These findings imply that mitochondrial thermogenesis is not the only function of marrow fat.

The temporal development of marrow fat in the mouse may provide some clues as to its function, in a manner analogous to our knowledge of marrow adiposity in humans. Surprisingly, developmental phenotyping of marrow fat in inbred strains or mutant mice has not been accomplished to date. In part, this has been due to the difficulty of characterizing this phenotype in vivo. The gold standard for quantitating marrow fat has been the enumeration of adipocyte ghosts on routine hematoxylin staining from undecalcified bone samples. Newer imaging techniques with higher resolution have started to define the temporal stages of marrow adiposity. For example, in vivo MRI with a 7 Tesla magnet can be used to perform spectroscopy and quantitate fat and water content in the marrow of mice over several time points. Presently, scanning time, cost, and availability limit widespread use. However, this technique can also be applied ex vivo. Another potential method under development is osmium staining followed by μCT imaging, which may allow for better quantitation and more precise measurements.

B. Function of Marrow Fat

Some studies have suggested that marrow adipocytes, under most physiologically conditions, are metabolically inert.[9] This process is often thought of as a default mechanism whereby mesenchymal stem cells enter the fat lineage because of their inability to differentiate into muscle, bone, or chondrocytes. Hence, fat fills the marrow void vacated by bone. Although this hypothesis can be considered tenable in light of several studies showing an inverse relationship between marrow fat and bone volume, there are mouse models to suggest this may not always be operative. Other studies suggest that marrow adipocytes may be self-promotive such that existing marrow adipocytes can induce differentiation of more MSCs into adipocytes,[9,10] thereby preventing lineage allocation into other cell lines. Finally, we and others have shown that mouse fat from certain depots can suppress osteoblastogenesis when cocultured with mesenchymal stem cells, suggesting that these cells are metabolically active, but self-promotive by suppressing MSC entry into the bone lineage (Rosen, personal communication). In summation, the function of marrow adipocytes is still not well understood in the mouse. In part, this may be a function of insufficient phenotyping and developmental studies. Notwithstanding, much more is known about the molecular mechanisms regulating cell entry into the adipocytic lineage, and its relationship to osteogenesis. It begins with the master differentiation factor, PPARG.

C. Peroxisome Proliferator Activated Receptor Gamma

Peroxisome proliferator activated receptor gamma (PPARG) is a nuclear receptor that heterodimerizes with RXRα and is activated by binding of a ligand to subsequently induce gene transcription.[11–13] There are numerous downstream targets of PPARG activation that are dependent on ligand specificity as well as coactivator and corepressor recruitment. Fatty acid synthetase, UCP-1, lipoprotein lipase, CD36, and aP2 (FABP4) are some of the adipocytic genes activated by PPARG. Ligands for PPARG include the naturally occurring PGJ2 and 9 (*S*)-HODE compounds as well as the thiazolidinedione (TZD) class of synthetic compounds.[4,14] During adipogenesis, CCAATβ-binding protein beta (*Cebpb*) has been shown to induce the expression of PPARG and, in turn, PPARG can then induce the expression of CCAAT/enhancer–binding protein alpha (*Cebpa*) (229). Several transcription factors are downstream of PPARG, but can also induce PPARG expression. These include Cebp-β, Srebp-1c, and Ebf1. Ebf1 (early B-cell factor-1) is a helix-loop-helix DNA-binding protein, and is instrumental for B-cell development.[15] Expression of this nuclear factor has been found in a variety of sites, including white adipose tissue.[15] Very recent studies have shown that Ebf1 binds directly to the PPARG promoter and may act between Cebpβ and Cebpα/PPARG in the adipocyte differentiation cascade.[16]

Elbrecht et al.[17] first showed that PPARG was expressed in bone marrow. A separate group demonstrated that treatment of marrow stromal cells with TZDs resulted in the differentiation of these cells in to adipocytes.[18] UAMS-33 cells are a bipotential cell line that can be induced to either form a mineralized matrix or adopt adipocytelike characteristics. When these cells were transfected with PPARG2 and treated with rosiglitazone, they became adipocytelike cells and were no longer capable of forming a mineralized matrix.[19] These experiments were the first to suggest that activation of PPARG might actually be detrimental to bone by reducing osteoblast differentiation.

The homozygous *PPARG* knockout animals (strain *PPARG*^{tm1Tka}) are embryonic lethal, but the heterozygous mice are viable. Akune et al.[20] showed that male *PPARG* haploinsufficient mice have a pronounced skeletal phenotype of high bone mass and decreased marrow adiposity. Similarly, aP2-Cre-PPARG$^{-/-}$, also called FKOγ mice, are deficient in PPARG during the latter stages of adipocyte differentiation, and are lipodystrophic but have markedly increased bone mass (Lecka-Czernik, personal communication). Tie2-Cre-PPARG$^{-/-}$ mice also have high bone mass; these mice have a significant defect in the hematopoietic precursors, leading to defective osteoclastogenesis.[7] The skeletal phenotype of these mice is osteopetrosis, supporting the notion that PPARG may be very important in regulating not only bone formation, but also bone resorption. Interestingly, the *Ebf1* null mice demonstrate a substantial increase in marrow adiposity that is accompanied by an increase in bone mass and bone formation rate, suggesting this nuclear factor may have a more complex role in bone biology.[21]

Treatment with TZDs strongly affects bone mass and/or marrow adiposity, further substantiating a role for PPARG in marrow adiposity. Tornvig et al.[22] first showed that troglitazone increased marrow adiposity in the *Apoe*$^{-/-}$ strain, but no changes in bone mass were observed in these mice. Darglitazone is 20 times more potent than rosiglitazone and 150 times more potent than pioglitazone.[23] A dose of 10 mg/kg per day in 8-month-old male mice resulted in a profound decrease in both trabecular and cortical bone, but the affect of this TZD on marrow adiposity was not reported.[24] Netoglitazone, a relatively weak TZD, was found to decrease whole-body bone mineral content (BMC), but did not affect trabecular bone volume or whole-body areal (a) BMD in C57BL/6 mice, but this TZD did increase marrow adiposity.[25] Recent studies in four inbred strains have shown a genotype specific response to rosiglitazone for both the skeletal mass and marrow fat (Ackert-Bicknell, personal communication). In vitro, when PPARG2 is transfected into bone marrow stromal cells

(UAMS) and then activated with rosiglitazone, or when this agent is added to primary bone marrow stromal cells, genes in the osteoblast pathway, including Runx2, osterix, Dlx, osteocalcin, and IGF-I are markedly suppressed.[7,18] In addition, there is a profound reduction in key components of the Wnt signaling system (Wnt 10b, β-catenin, TCF/Lef complex), suggesting that a global defect in osteoblastogenesis can occur with TZD treatment.

D. Mutant and Inbred Strains

Genetically engineered and inbred strains of mice that express differences in key cytokines can provide insight into the function of marrow fat. Leptin, a hormone secreted by adipocytes, plays a key role in the regulation of energy intake, appetite, and energy expenditure through its actions in the central nervous system. Numerous studies have also demonstrated a key role for Leptin in the regulation of osteogenesis.[26] The *ob/ob* (obese) strain of mice, first described in 1950 by Ingalls and colleagues,[27,28] carry a spontaneous nonsense mutation at codon 105 of the leptin gene that results in a complete lose of leptin protein. As the name of this strain suggests, these mice are extremely obese: exhibiting profound hyperphagy, glucose intolerance, and hyperinsulinemia.[27,29] In the femur, *ob/ob* mice have increased marrow adipocytes compared to wild-type controls. In contrast, these mice have decreased numbers of marrow adipocytes in the vertebrae.[30] Studies in wild-type mice have shown that osteoblast cells do not appear to express significant quantities of the leptin receptor, whereas the receptor is expressed in preosteoblasts, although its physiologic significance is unclear.[31,32] Experiments in the *ob/ob* mice have shown that peripheral administration of leptin corrects the marrow adiposity phenotype seen in the femur of these mice.[33] In addition, studies in rats have shown that direct stimulation of the leptin receptors of the ventromedial hypothalamus results in apoptosis of marrow adipocytes.[34]

The IRKO-L1, which is an insulin receptor (IR) null mouse, in which expression of IR has been restored in pancreas, liver, and brain, but not muscle or fat, has extremely low numbers of marrow adipocytes, but has normal trabecular BMD.[35] Similarly, we have noted that in a spontaneous mutant, *small*, which has a homozygous recessive mutation in the intact IRS-1 molecule, there is a virtual absence of marrow fat and low bone mass during neonatal and adult life.

Bone loss and diabetes have been described in several animal models.[36] Increased marrow adiposity has been reported in both the spontaneous diabetic NOD strain and in mice in which diabetes was induced with streptozotocin.[37,38] In both of these models of type I diabetes, proadipocytic genes such as *PPARG* and *Fabp4* (AP2) were found to be increased in the long bones and this was correlated with decreased expression of osteocalcin.[38] Furthermore, as was noted in *ob/ob* mice, the increase in marrow adipocytes associated with the induction of disease state appears limited to the calvaria and long bones.[35,39]

Growth hormone (GH) induces lipolysis, but in excess promotes insulin resistance. In bone, GH has been shown to have both direct effects on long bone growth and indirect effects via insulinlike growth factor 1 (IGF-I). The *dw/dw* mutation is a spontaneous mutation that arose in the Lewis strain of rat. These rats are dwarfs as a result of GH deficiency, and have a profound increase in both adipocyte number and adipocyte size in the marrow of the long bones, compared to wild-type controls.[40] Treatment with GH results in a decrease in adipocyte number, whereas treatment with IGF-I results in a decrease in adipocyte volume.[41] Recently, Turner and colleagues demonstrated that acute pituitary insufficiency due to hypophysectomy resulted in significant marrow adiposity that can be reversed by GH, but not by IGF-I, estrogen, or cortisol (Turner, personal communication). Interestingly, LID mice, in which hepatic IGF-I is deleted, have very high GH levels and low IGF-I, but do not have marrow adiposity. On the other hand, *little* mice with a spontaneous mutation of the growth hormone releasing

hormone receptor have significant marrow fat. Thus, abnormalities in the GH–IGF-I axis are associated with marrow adipogenesis.

TSH can stimulate osteogenesis, although its role in marrow adipogenesis is unknown. However, mice in which the thyroid receptors (TR)-α1 and -β have been deleted ($TR\alpha1^{-/-}\beta^{-/-}$) exhibit increased marrow adiposity. While TRα1, TRα2, and TRβ1 are known to be expressed in both osteoblasts and in cultured adipocytes, $TR\alpha1^{-/-}\beta^{-/-}$mice also exhibit decreased GH levels. As a result, it cannot be determined if the marrow adiposity phenotype of these mice is a direct result of loss of TR signaling or if this phenotype is secondary to the GH deficiency.[42]

Inbred strains of mice are powerful tools for use in the study of the genetics of complex disease, since each mouse of a given strain represents essentially an identical twin of all other mice of that strain. As a result, phenotypic measures such as bone mineral density (BMD) or serum HDL levels remain relatively constant within a strain, but may vary greatly between strains (see http://www.jax.org/phenome). In a pilot study in which we examined marrow adipocyte numbers in the distal femur (immediately proximal to the growth plate) of four inbred strains of mice, we found that the number of marrow adipocytes per unit area varied greatly between strains (see Table 1).

Interestingly, BV/TV% was only found to be significantly correlated with marrow adipocyte number for the DBA/2J mice ($R^2 = 0.78$, $p = 0.04$). However, vBMD of the femur did not correlate with marrow adipocyte number for any of the strains measured, suggesting that the relationship between bone mass and marrow adiposity is not a simple inverse correlation.

The developmental sequence of marrow adiposity in the inbred mouse is also a relatively new area of investigation. Using high-field MRI of the distal femur in female B6 mice, we recently noted that at 8 weeks of age, female B6 mice have more marrow fat by spectroscopy than at 12, 16, or 32 weeks. This is particularly intriguing since in humans, marrow fat in the long bones converts from red to yellow during the time of peak bone acquisition, which corresponds to 8 weeks in mice. In that same vein, much like in aging humans, B6 mice at 24 months have significant long bone marrow adiposity, and this can be enhanced further by treatment with rosiglitazone. Also, with ovariectomy in both mice and rats, there is a decrease in BV/TV% that is associated with increased marrow adiposity.[43] Hence, there are parallels between rodents and humans relative to the timing and sequence of marrow adipogenesis.

V. MARROW ADIPOGENESIS IN HUMANS

A. Physiology

Marrow fat in humans has often been considered “filler” for the void left by trabecular bone during aging or after radiation.[18,44–46] However, with the acknowledgment that adipocytes are secretory, a revisionist theory has emerged suggesting a role for these cells as an energy source, or as a modulator of adjacent tissue by the production of paracrine, and autocrine factors. In fact, adipokines, steroids, and cytokines[6,47] can exert profound effects on neighboring marrow cells, sustaining or suppressing hematopoietic and osteogenic processes.[48] Thus, the function of bone marrow adipose tissue may be similar to that of extra medullary fat. As such, it has been well established that unbalanced production of signaling products from subcutaneous or visceral fat modulates several human conditions including obesity, lipodystrophy, atherogenesis, diabetes, and inflammation. Whether these factors have a detrimental effect on bone metabolism remains to be determined, but they may, in combination with other aspects of marrow fat, contribute to the regulation of bone remodeling.

B. Developmental Sequence of Marrow Fat in Humans

In newborn mammals there is no marrow fat; however, adipocyte number increases with age in the marrow such that in humans older than 30 years of age, most of the femoral cavity is occupied by adipose tissue.[49] Indeed, recent studies using MRI have shown that in the appendicular skeleton of adults, more than 70% of the marrow space is occupied by fat. Interestingly, this conversion from red to "yellow" marrow occurs around the time of peak bone acquisition, fueling the hypothesis that fat is necessary for osteoblasts that are functioning maximally to produce new bone. By contrast, aging, a state of reduced bone formation, is associated not only with an increase in the number of marrow adipocytes, but also their size.[50] In a cross-sectional study of postmortem iliac crest biopsies, adipose tissue volume increased from 15% to 60% between 20 to 65 years of age, while trabecular bone volume decreased from 26% to 16%.[2] More recently, Justesen et al.[51] found that marrow adipose tissue increased from 40% at age 30 to 68% at 100 years, while bone volume decreased to 12%. Finally, in osteopenic bone it was observed that in addition to the age-related inverse correlation between adipose and bone tissues, hematopoietic tissue was replaced by fat.[52] Other conditions such as osteoporosis, immobilization, microgravity, ovariectomy, diabetes, or glucocorticoid treatment also show an increase in the content of marrow adipose tissue and decreased bone volume.[2,51,53–58]

The juxtaposition of adipose tissue within the bone marrow milieu suggests that its presence may have consequences for the skeleton.[46] Two theories are most prevalent. First, since the total bone marrow cavity is shared among adipose, bone, and hematopoietic tissues, fat overload would displace functional hematopoietic and/or osteogenic cells from the marrow cavity.[50] Second, a balanced bone marrow microenvironment is critical for normal hematopoiesis and osteogenesis, among other processes.[59,60] Increased production of fat-related factors, such as fatty acids, could alter the fat–bone marrow relationship and inhibit hematopoiesis and/or osteogenesis.[6,48,61] Aging results in a preferential shift to adipogenesis among marrow precursor cells. However, there must be a critical limit to adipocyte accumulation, above which excess fat in bone marrow would impede skeletal function and hematopoiesis.[46,61] Since both osteoblasts and adipocytes originate from a common precursor cell, whose lineage fate may be reciprocal, both the precursor cells and their regulatory milieu appear to collaborate to define conditions that result in an unbalanced differentiation scheme. Alternatively, atypical or functionally defective adipocytes could emerge under pathological stimuli, whether from defective precursor cells or from an altered differentiation scheme. For example, adipocytes generated from MSCs derived from osteoporotic donors showed functional differences from those produced by MSCs obtained from control donors.[62–65] This tenet is supported by observations from extramedullary adipocytes where, in humans, subcutaneous and visceral fat are recognized as different not only because of their distribution, but also in respect to metabolic function and their relationship to diseases such as atherosclerosis. Thus, lipolysis rates, the capacity to store triglycerides, and the production and/or response to leptin differ by depot site.[66,67] The cellular structure may also differ by site, such that in obesity, visceral adipocytes develop more profound hyperplasia and become insulin resistant.[68,69] A recent report by Gilsanz and colleagues suggests that in teens and young adults there is absolutely no relationship between the amount of marrow fat and the magnitude of subcutaneous or visceral fat (Gilsanz, personal communication). Moreover, visceral fat, but not marrow fat, was associated with surrogate markers of atherosclerosis.

C. Cellular Aspects of Marrow Adipogenesis in Humans

As noted, bone marrow stroma contain mesenchymal stem cells (MSCs), representing an adult stem cell population capable of extensive self-renewal and plasticity, allowing the formation of differentiated connective tissues.[70] MSCs can enter the osteogenic, chondrocytic or

adipogenic lineages, depending on activation of phenotype-specific transcription factors such as osteoblast-specific Runx2/Cbfa1 and adipocyte-specific PPARG2.[71–74]

PPARG, acting in conjunction with CCAAT/enhancer-binding protein-β (C/EBP-β), is required for entrance of MSCs into the adipocyte lineage.[75,76] PPARG exists in four isoforms, PPARG1–4; however, PPARG2 is fat specific and due to its extra 30 N-terminal amino acids may have enhanced transcriptional activity. Thus, in humans, like in mice, PPARG2 transcripts increase during adipogenic differentiation, while PPARG1 exhibits constitutive expression.[77] The transcriptional activity of PPARG is positively regulated by specific lipophilic ligands and negatively regulated by phosphorylation.[3,78] Phosphorylation-induced inhibition of the transcriptional PPARG activity provides a mechanism to switch off the response to the ligand.[79]

Studies on factors in humans that control MSCs' differentiation along the adipogenic or osteogenic pathways support the proposition that increased adipose tissue in the bone marrow is counterbalanced by decreased production of osteogenic cells. Studies on MSCs' differentiation showed that activation of PPARG2 positively regulates adipocyte differentiation while acting as a dominant negative regulator of osteogenic differentiation, inducing cells to shift to differentiated adipocytes.[18,80,81] Another signal that exerts dual regulatory effects on MSCs' differentiation is the Wnt pathway. Wnt activation controls cell commitment toward osteoblast development by blocking adipogenesis through inhibition of C/EBPβ and PPARG2, as recently demonstrated in human cells.[82] Interestingly, Runx2 expression by mesenchymal cells inhibits differentiation into adipocytes by blocking PPARG2 activity.[83]

Hypothetically, the determination of MSC fate occurs early in the stages of cell differentiation ("commitment"), involving the interplay of intrinsic (genetic) and environmental (local and/or systemic) conditions to ultimately define cell outcome. Although there is much uncertainty about the cellular and molecular events underlying MSCs' fate decisions, it is recognized that the process is tightly and temporally controlled in humans.

The MSCs' niche or microenvironment plays a fundamental role by providing signals from other cell phenotypes, as well as the extracellular matrix and local or systemic factors.[59,84] Taking into consideration only the primary cellular components of the marrow stroma, it is known that a broad range of signals result from the activity of adipocytes, macrophages, fibroblasts, osteoprogenitors, and hematopoietic stem cells and their progeny, as well as from endothelial and reticular cells. At present, most physiologic cellular and molecular interactions of MSCs within their niche are not well defined, in part due to difficulty in extrapolation from in vitro observations and the absence of an appropriate in vivo model for humans. However, disordered stem cell commitment and differentiation are at the origin of several primary diseases of the bone marrow.[85] A clearer understanding of the mechanisms inherited in these disorders is crucial not only for the primary disease process, but also for the secondary skeletal manifestations.

D. Aging and Marrow Fat

With the aging of most mammals, the status of MSCs changes in respect to both their intrinsic differentiation potential and the production of signaling molecules, within a modified marrow microenvironment. For instance, it has been observed in humans that the number of MSCs committed to the adipocytic lineage increases, while the number of those committed to the osteoblastic lineage decreases.[49] Unbalanced, proadipocytic and antiosteoblastic MSC allocation could result from increased activity of PPARG2, or the decreased expression of the TGF-β/BMP, Wnt/β-catenin, and IGF-I signaling pathways.[49] These changes could easily lead to reduced osteoblast differentiation, while the formation of new adipocytes is enhanced.[49]

On the other hand, it should be noted that lineage allocation is not always mutually exclusive; hence, there may be increased marrow adipogenesis but enhanced osteoblast function, as shown recently in several mouse models.

E. Osteoporosis

Osteoporotic individuals, whether of primary or secondary origin, have more fat in the bone marrow than age-matched controls.[2] Studies comparing MSCs from normal and osteoporotic donors and their adipogenic potential have shown alteration in some functional characteristics, either basally or during early cell differentiation.[62,86–88] For instance, the proliferation rate and the mitogenic response to IGF-I are significantly diminished, while the pERK/ERK ratio is increased in osteoporotic MSCs, compared with control MSCs.[62,89] In other cells types, it was shown that activation of the MEK/ERK signaling pathway enhances the activity of adipogenic transcription factors.[90] In contrast, MSCs derived from osteoporotic donors have diminished alkaline phosphatase activity and less calcium deposition under osteogenic differentiation conditions, consistent with their reduced capability to produce mature bone cells. In addition, MSCs derived from osteoporotic women exhibit decreased TGF-β production, as well as decreased capacity to generate and maintain a type I collagen–rich extracellular matrix, conditions that promote cell differentiation into the adipogenic lineage.[63] MSCs isolated from bone marrow of osteoporotic donors also express higher levels of adipogenic differentiation markers than cells obtained from donors with normal bone mass, and these cells show preferential adipogenic differentiation.[63–65,91] PPARG mRNA is higher in osteoporotic than control cells,[65] while the phosphorylated form of this protein (p-PPARG), is similar in both control and osteoporotic MSCs, but higher levels are noted in control MSCs cultured in adipogenic media. These observations are consistent with the hypothesis that activity of the transcription factor PPARG2 is necessary and sufficient for adipogenic differentiation.[3,19]

In addition to the intrinsic characteristic of MSCs involved in cell commitment and differentiation, it is recognized that locally produced factors such as leptin, estrogens, fatty acids, and growth factors may be important in regulating neighboring osteoblasts. Leptin was initially proposed as an adipokine with potentially protective effects on bone tissue, although published data on leptin's direct effects on osteoblast function are contradictory.[61,92–94] For example, in vitro studies confirmed that bone marrow MSCs were responsive to leptin, both through enhanced proliferation and differentiation into the osteoblastic lineage, as well as inhibition of MSCs into adipocytes.[64,95–97] Moreover, leptin-induced activation of the MAPK cascade stimulated osteoblastic differentiation, as well as phosphorylation of PPARG. Leptin may act on early differentiating bone marrow MSCs by increasing aromatase activity, thereby improving estrogen production.[98] However, conditional deletion of the leptin receptor in osteoblasts failed to cause a demonstrable skeletal phenotype in vivo. Other studies have shown that MSCs display a distinct response to leptin during osteogenic and adipogenic differentiation, depending on whether these cells were derived from bone marrow of normal or osteoporotic donors.[24] Leptin significantly inhibited adipocyte differentiation in control cells, but did not affect adipogenic differentiation of osteoporotic cells.[65] These results suggest that in addition to increased PPARG protein level in osteoporotic cells, there may be impairment in the capability for developing a leptin response. Notwithstanding, it should be emphasized that it is now felt that the principal in vivo effect of leptin on bone is indirect and is mediated through a relay in the paraventricular nucleus of the hypothalamus to the sympathetic nervous system and then to bone.[99–103] Several lines of evidence support this premise, and provide a compelling rationale for the role of the central nervous system in controlling bone remodeling.

During menopause, decreasing endogenous estradiol enhances bone turnover. This is accompanied by a shift in the adipocyte-to-osteoblast ratio, favoring fat accumulation in the bone marrow.[51,104] A direct effect of estrogen on the skeleton has been underlined by developmental failure of bone in males with deficient estrogen activity.[105,106] The skeletal response to falling estrogen levels has also been demonstrated in vitro and in vivo. In the former, in human MSCs reciprocal estrogen regulation of osteogenic and adipogenic differentiation has been reported.[98,107] In the latter, bone biopsy samples from postmenopausal women revealed that estrogen replacement was associated with markedly reduced adipocytes compared to women without estrogen replacement.[108] The observation that aromatase (the enzyme responsible of estrogen biosynthesis) and other enzymes implicated in sex metabolism are found in extra gonadal organs, including adipose, and bone tissues, has strengthened the concept that locally generated androgens and estrogens can exert regulatory action on bone marrow cells.[109–112] In fact, aromatase expression was found in MSCs, in osteoblasts and osteoblastlike cells.[107,109,110,113,114] Studies during MSCs' differentiation point to the potential importance of local estrogen production and action for osteogenic and adipogenic commitment and as a negative regulator for adipogenesis.[107,110,115–117] These observations support the hypothesis of a threshold estradiol level for normal skeletal remodeling, which could be attained by both appropriate endogenous aromatase activity and estrogenic precursors.[118,119]

Fat tissue is an endocrine hormone and it produces multiple other factors that might regulate osteoblast differentiation. For example, we and others have recently shown that a coculture of MSCs with intra-abdominal fat causes marked suppression in osteoblast differentiation, due in part to an increase in programmed cell death of preosteoblasts.[9] This observation would suggest that release of fat-specific products, such as long-chain fatty acids, can have a major impact on skeletal remodeling. Similarly, TNF and IL-6 are cytokines produced by adipocytes that can act locally or systemically to regulate osteoblast differentiation and programmed cell death. Other soluble factors, such as adiponectin, are likely to be a part of this regulatory loop.[120] In summation, these observations point to a critical interaction between adipocytes and osteoblasts within the bone marrow microenvironment. Since aromatase activity is important during the commitment and differentiation of human MSCs,[98,107,110] it can be inferred that high local levels of estrogen affects MSCs' entry into the adipogenic or osteoblastogenic pathways. From these data, it can be inferred that a high local levels of estrogen affect MSCs' commitment, either restraining adipogenic differentiation or facilitating osteogenic differentiation, or both. This, combined with changes in secretory factors from fat cells and endogenous activation of proapoptotic programs in preosteoblasts, must contribute to the marrow fat–bone interface.[98] Notwithstanding, the precise mechanism whereby estrogen suppresses adipocytic differentiation in the marrow has not been defined.

F. Clinical Correlates

Previously, marrow fat was thought to have little or no functional significance; but recent studies have produced a paradigm shift in our understanding of this phenotype and raise an entirely new set of questions. For example, nearly a quarter of a century ago, Klibanski and colleagues noted enhanced marrow fat signals in CT scans from anorexic women who were osteoporotic (Klibanski, personal communication). Abella reported in 2002 that on bone marrow biopsy, anorexia nervosa was associated with significant marrow adiposity.[121] Subsequent studies in rodents undergoing calorie restriction demonstrated a similar phenomenon. Thus, not only does overabundance of fuel increase marrow adiposity, but energy insufficiency may generate signals that enhance marrow fat, even as peripheral fat stores are diminished.

With normal aging, Wehrli and colleagues have noted by MRI that marrow fat in the vertebrae was strongly and inversely associated with bone volume.[122] Griffith et al.[1] reported a similar negative correlation between marrow fat by spectroscopy and areal bone mass. Hence, even in nonpathologic conditions, marrow fat may infiltrate spaces previously occupied by trabecular bone. However, we do not know whether this is a primary or compensatory process, or whether struggling osteoblasts require adjacent adipocytes for energy. Thus, the role of marrow fat in aging, and in pathologic conditions, has still not been clarified.

VI. SUMMARY

Marrow fat is an important phenotypic component of the bone marrow milieu in mice and humans. The intimate relationship among adipocytes, osteoblasts, and hematopoietic stem cells suggest these cell types could work in concert during the course of several homeostatic processes. In contrast, during pathologic conditions, the role of marrow fat may change and function as an antagonist for osteogenesis. There is a need for more studies to understand this basic, yet critically important process. For example, what are the structural consequences of marrow adiposity? Can marrow fat predict fractures in older individuals? And the penultimate question remains unanswered: what is the function of marrow fat? It is found invariably in states of poor osteoblastic function, yet it is also part of the normal process of peak bone acquisition where osteoblasts are working the hardest. Could the infusion of marrow fat be an evolutionary response to the energy needs of the skeleton? Is it purely a default pathway for MSCs that cannot enter other lineages? Answers to these and other questions should permit a much better understanding of the physiological and pathologic role of marrow fat.

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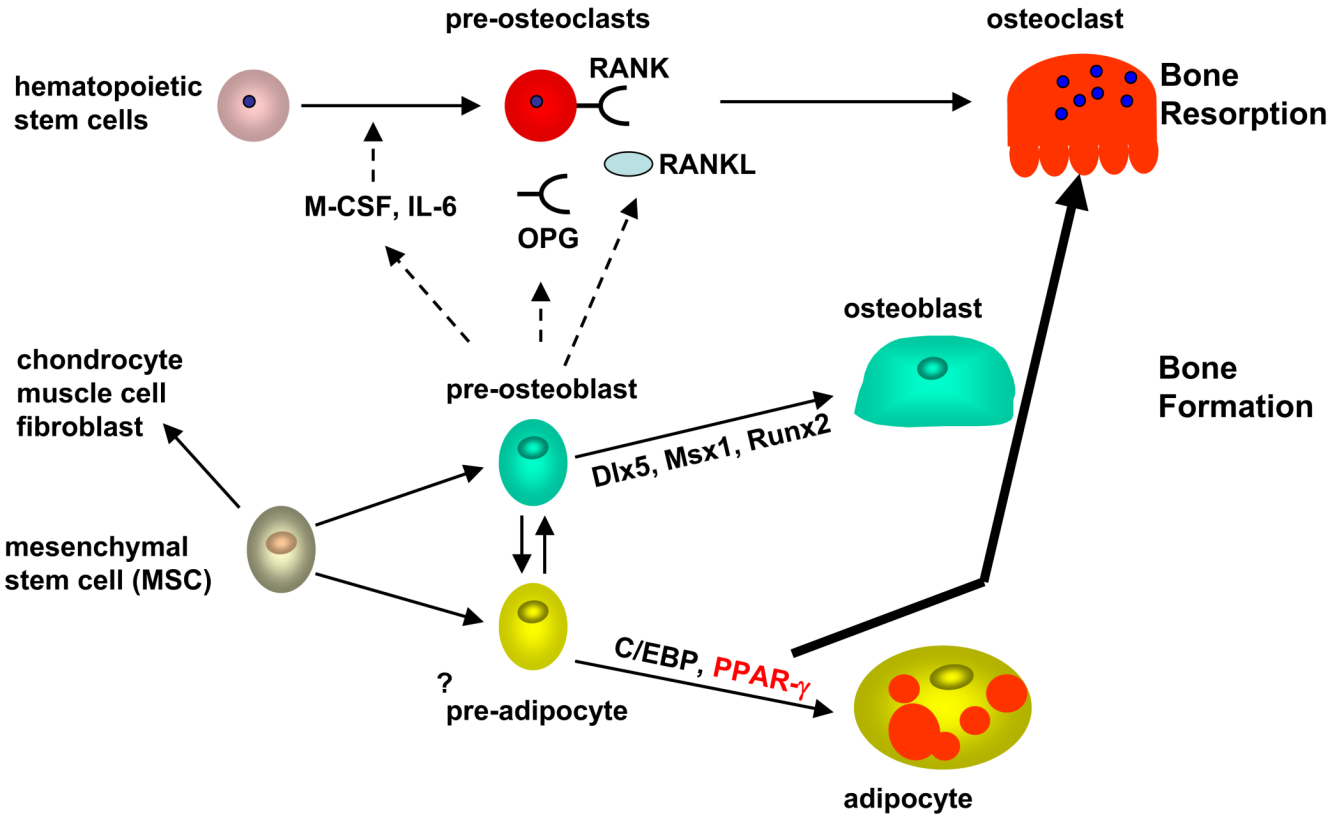

FIGURE 1.

Bone turnover is a coupled process that involves differentiation of precursor cells from the mesenchymal and hematopoietic lineages. Adipocytes and osteoblasts arise from a common mesenchymal cell, or marrow stromal cell, that is multipotent and can differentiate into chondrocytes or muscle cells. The differentiation of adipocytes requires activation by several transcription factors, including Cebpβ and PPARG2. Co-activators and co-repressors are recruited to the transcription complex with activation of PPARG2 and ultimately determine the type and function of the adipocyte. In some instances, activation of adipogenesis can occur at the expense of osteoblast differentiation, which requires a distinct series of transcription factors (Dlx, Runx2, Msx, Osterix). However, lineage allocation may not be mutually exclusive. Activation of PPARG2 also results in recruitment of hematpoietic cells that can differentiate into osteoclasts under the influence of m-CSF and RANKL, which are produced by preosteoblasts, thereby ensuring a coupled process.

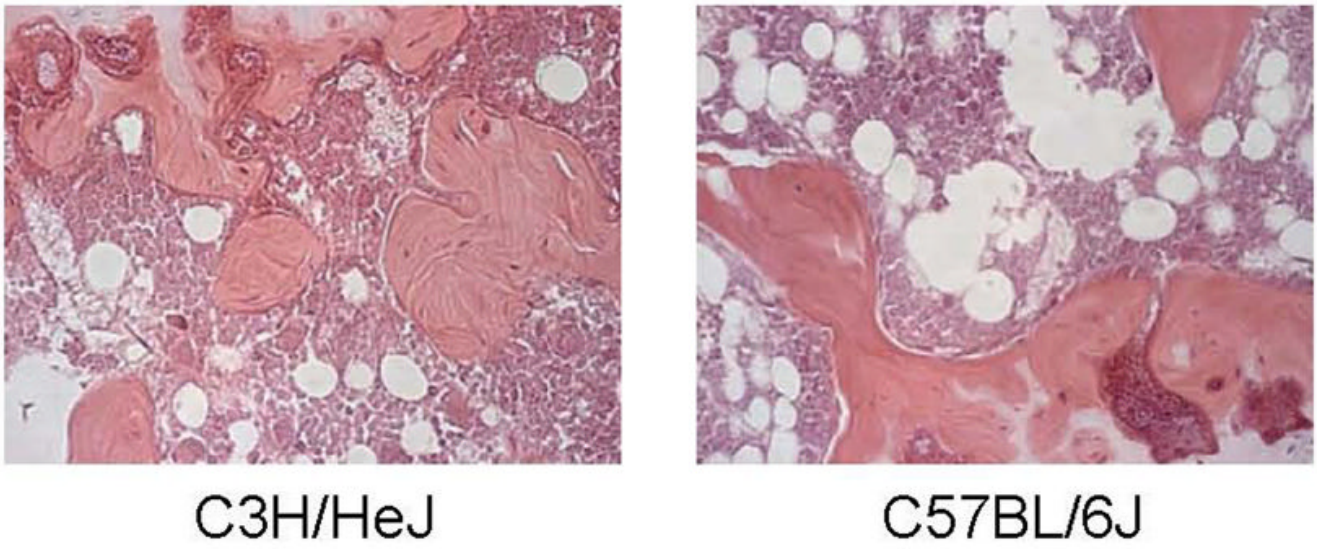

FIGURE 2.
Marrow fat in two inbred strains, C3H/HeJ and C57BL/6J.

TABLE 1

Differences in Adipocyte Number Per High-Power Field by Strain

Strain	# Adipocyte/high-power field
C57BL/6J	31 ± 4.8
C3H/HeJ	17.8 ± 7.2
A/J	14 ± 4.6
DBA/2J	30.6 ± 9.9