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Marrow Adipocytes: Origin, Structure, and Function

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Abstract

The skeleton harbors an array of lineage cells that have an essential role in whole body homeostasis. Adipocytes start the colonization of marrow space early in postnatal life, expanding progressively and influencing other components of the bone marrow through paracrine signaling. In this unique, closed, and hypoxic environment close to the endosteal surface and adjacent to the microvascular space the marrow adipocyte can store or provide energy, secrete adipokines, and target neighboring bone cells. Adipocyte progenitors can also migrate from the bone marrow to populate white adipose tissue, a process that accelerates during weight gain. The marrow adipocyte also has an endocrine role in whole body homeostasis through its varied secretome that targets distant adipose depots, skeletal muscle, and the nervous system. Further insights into the biology of this unique and versatile cell will undoubtedly lead to novel therapeutic approaches to metabolic and age-related disorders such as osteoporosis and diabetes mellitus.

1. INTRODUCTION

Bone marrow adipocytes exist in a unique environment, in an organ that integrates metabolic homeostasis simultaneously with the turnover of millions of cells essential for life (1). Adipocytes are the sole constituent of what is now referred to as bone marrow adipose tissue (MAT). However, the structure and function of the bone marrow adipocyte are not well defined in part because this depot is relatively isolated, hypoxic, and immune protected (2). The site of origin of these adipocytes likely distinguishes marrow adipocytes from subcutaneous or visceral adipocytes, but delineating their progenitors has been difficult (3). In fact, isolating these adipocytes is daunting, let alone trying to define their function. Notwithstanding, major advances in our understanding of the bone marrow adipocytes have occurred over the past half-decade.

Developmentally, MAT is virtually absent at birth, expands during growth and development of the skeleton, and accelerates its expansion during aging and menopause in the long bones and less dramatically in the vertebrae of mammals (4). Historically, the marrow adipocyte was considered inactive, filling the bone marrow cavity when bone mass was low or hematopoiesis impaired. This belief was based on observations more than a century ago that, in states of arsenic poisoning or malignant infiltration of the marrow, there were unilocular cells thought to be adipocytes that filled a major vacuum left by the absence of bone or blood components in the marrow space (5, 6). Further studies in the late twentieth century linked osteoporosis to increased marrow fat infiltration, suggesting an inverse relationship between bone and fat (7, 8). But animal and human studies during the past two decades have shown that the relationship of MAT to bone and to the hematopoietic system is far more complicated, despite many observations that the increase of marrow adipocytes in conditions such as osteoporosis, aging, cancer and, paradoxically, anorexia nervosa is associated with low bone mass (8–10). Notwithstanding that complexity, fracture risk is increased among individuals with high vertebral MAT in many conditions, although this association may not be causal (11). Similarly, the functional association between MAT and hematopoietic diseases such as multiple myeloma and myeloid leukemia has recently been recognized (12, 13). Some investigators have even considered the marrow adipocyte as pathogenic in tumor progression (14). Taken together, these putative associations suggest that the bone marrow adipocyte could be more than a passive bystander within the marrow niche. Indeed, there is evidence these adipocytes actively participate in whole body homeostasis through their secretory nature. Further validation for that concept comes from the study of genetically engineered mouse models that have illustrated a surprising impact from gain or loss of MAT on other adipose tissue depots and whole-body energy metabolism (3, 15). This review focuses on what is known about the origin, structure, and function of the marrow adipocyte and how these determinants relate to normal physiology and the pathophysiology of several diseases. Clinical implications from high or low MAT are also discussed within the context of this unique adipocyte.

2. STRUCTURE AND FUNCTION OF WHITE, BROWN, AND BEIGE ADIPOCYTES

It is now widely accepted there are at least three types of adipocytes—white, brown, and beige—based not only on their appearance but also on their function and site of origin (16). Importantly, this classification has relevance for understanding the marrow adipocyte and determining whether this marrow cell may represent a fourth type of adipocyte (17). White adipocytes are the classic large, lipid droplet–rich cells, storing and releasing fatty acids in a classical manner, particularly with an array of lipases that control lipolysis, enzymes that regulate lipid storage and respiration (16). White adipocytes compose 99% of subcutaneous and visceral adipose tissue (VAT) depots. They are defined by surface markers such as CD24 and expression of perilipin 1, which regulates

lipid droplet formation, as well as peroxisome proliferator–activated receptor gamma (PPARγ) and CCAAT/enhancer binding protein alpha or beta (C/EBPα or β), essential transcription factors for the adipogenic differentiation process (16, 18). White adipocytes principally use oxidative phosphorylation to generate ATP, and the production of reactive oxygen species (ROS) is essential for full adipocyte differentiation. Recent work suggests that the degree of triglyceride storage in white fat cells impacts insulin resistance and sensitivity. Hence, too few adipocytes (lipodystrophy) leads to an excess of circulating fatty acids that contributes to insulin resistance (19). Similarly, excess fat intake can overwhelm the storage capacity of the adipocyte and lead to ectopic fat storage in liver, muscle, and bone, which in turn can also cause insulin resistance. Obesity can also drive an inflammatory program with macrophage infiltration and ultimately fibrosis of adipocytes.

Brown adipocytes are small, multilocular, mitochondria-rich cells, capable of uncoupling energy by burning fatty acids and releasing heat in response to sympathetic tone activation. The cell morphology of these brown adipocytes distinguishes them from white adipocytes. As noted, brown adipose tissue was long considered to exist only in the neonatal period, but it is now clear that substantial brown adipose tissue can persist into adulthood in the interscapular regions of humans and rodents. Beige adipocytes in the subcutaneous and inguinal depots intermingle with classic white adipocytes and share some of the functional properties of brown cells, particularly in terms of their thermogenic capacity via uncoupling protein 1 (Ucp1) and their morphology (i.e., multilocular small fat cells) (18). However, these cells differ somewhat in their transcriptional program. Four major transcription factors are shared by both beige and brown adipocytes: PPARγ, C/EBPβ, PGC1α, and PRDM16. In addition, TLE3, KLF11, IRF4, IRX3, IRX5, LXR, and SRC2 also are shared by both cell types (20). On the other hand, brown adipocytes arise from cells expressing Myf5^{+}, a muscle transcription factor that is not expressed in beige adipocytes (18, 21). Controversy still exists as to whether the beige adipocytes in the subcutaneous depot arise de novo or occur through transdifferentiation of classic white adipocytes (18, 22). Thus, certain stimuli, such as cold exposure via adrenergic signaling or β3 agonists, induce beige adipocytes that at some time point could have been typical white fat cells (18). These cells have significant thermogenic properties in mice, but in humans it is unclear whether beige adipocytes modulate body temperature. All adipocytes, in addition to storing or burning of fatty acids, secrete mediators and hormones collectively called adipokines. The classic adipokines are leptin and adiponectin, which both regulate systemic energy metabolism, but many more adipokines have been identified, and their function is linked to several homeostatic processes (23).

3. THE BONE MARROW ADIPOCYTE: STRUCTURE AND FUNCTION

The presence of adipocytes in the bone marrow was long recognized, but these adipocytes were often considered passive fillers in the absence of bone and hematopoietic cells. Interest in marrow adiposity arose in the 1970s when the dynamic interactions with hematopoiesis became evident after studies of bone marrow transplantation showed a temporal relationship between marrow adiposity and hematopoietic reconstitution (24, 25). Indeed, during chemotherapy and radiation for transplants, there is a very temporal specific pattern of induced marrow adiposity preceding stem cell honing and reconstitution of the hematopoietic marrow. Many studies investigated the association between MAT and clinical conditions such as osteoporosis, anorexia, and obesity (15, 26, 27). Because of the location of those adipocytes in the bone marrow, it has been more difficult to study their characteristics directly. Therefore, studies in animal models, especially in mice, have been key to understanding the origin and function of the marrow adipocytes.

The bone marrow adipocyte is characterized by a unilocular lipid droplet within a cytoplasm that is surrounded by a lipid membrane and an adjacent single nucleus. Although it is often argued

that the marrow adipocyte has beige characteristics because of modest *Ucp1* gene expression in some animal models, no investigator has definitively shown thermogenic capability in this depot, nor significant protein expression of UCP1. However, lineage tracing studies have demonstrated the presence of perilipin+ staining and adiponectin positivity, proving that these droplets are not ectopic lipid deposited in other cell types (e.g., macrophages) but are rather part of the adipocyte lineage. To further assess the structure and function, induction of MAT in experimental mouse models has been illuminating. In the late 1990s, Gimble and colleagues (28) reported an increase in MAT in mice treated with thiazolidinediones. Others showed that estrogen deprivation induced marrow adiposity (29). Subsequent studies by Jilka's and Lecka-Czernik's groups (30–32) described the cellular and molecular signatures by gene expression of marrow adipocytes. More recently, investigators have focused on lineage tracing (see below) using inbred and genetically engineered mutant mice (33–35). These studies have suggested there exists a significant degree of heterogeneity with the MAT compartment as well as among the progenitors. In addition, imaging using osmium micro-CT can determine the quantity and location of MAT within the femur or tibia (36–38). Drugs (e.g., the thiazolidinediones), hormonal determinants, environmental factors (e.g., radiation), and nutritional elements (e.g., high-fat diet or calorie restriction) can induce premature marrow adiposity in the long bones of mice (7, 39–42). This induction may be region (proximal versus distal) specific and possibly cell specific. Inducible marrow adiposity in the proximal region of the tibia has been termed regulated MAT (rMAT) in contrast to its distal site that is referred to as constitutive (cMAT) because of their presence immediately after birth (43). These distinctions were made principally through the use of osmium micro-CT imaging. Functionally, it has been difficult to ascertain whether this distinction has implications. However, in animal models, regulated marrow adipocytes are more responsive to adrenergic drive than constitutive marrow adipocytes (43, 44). However, even this classification has come under scrutiny because it is clear that cMAT can be remodeled by major alterations in metabolic homeostasis such as vertical sleeve gastrectomy or cold exposure (45, 46). In inbred mouse models, rMAT appears to be more dynamic and reversible in contrast to cMAT, which is less so (see **Figure 1**). This is apparent from aging, exercise, and ovariectomy experiments in C57BL6J mice. Whether cMAT and rMAT exist in humans is also not clear, particularly because this classification cannot be employed in the vertebrae where MRI studies can quantitate the volume of MAT (47). But marrow adiposity is also inducible in rodents and humans by states such as estrogen withdrawal, unloading, space flight, or change in body temperature (48–50). However, even in those inducible states, expression profiling and immunohistochemistry have failed to show evidence of enhanced genes related to beiging or thermogenesis (e.g., *Ucp1*, *DiO2*, *Prdm16*) (3).

Despite cell fate mapping studies that support the tenet that all marrow lipid–laden cells arise from the adipocyte lineage, several investigators have suggested that the marrow adipocyte may actually be an osteoblast with a large lipid droplet. Osteoblasts, like all cells, contain lipid droplets, particularly during early differentiation (51). However, these droplets are small and rapidly lipolyzed during energy demands. On the other hand, Westendorf and colleagues (52) showed that in a conditionally deleted mouse model of Hdac3 (i.e., OsterixCre Hdac3$^{-/-}$), approximately 10–15% of the osteoblasts stained positive for both perilipin and runt-related transcription factor 2 (Runx2). Lineage tracing studies also noted that these adipocytes have markers characteristic of both osteoblasts and adipocytes (52). Compounding the difficulty in defining the nature of these particular cells, it remains nearly impossible to sort by flow the mature adipocytes from the marrow owing to their fragility. Hence, current protocols characterizing the size and shape of the marrow adipocyte in vivo rely on histomorphometry of ghost adipocytes that are noted after decalcification of the bone or in frozen sections. Technological improvements beyond ex vivo osmium micro-CT for in vivo imaging are likely to shed more light on the structure of

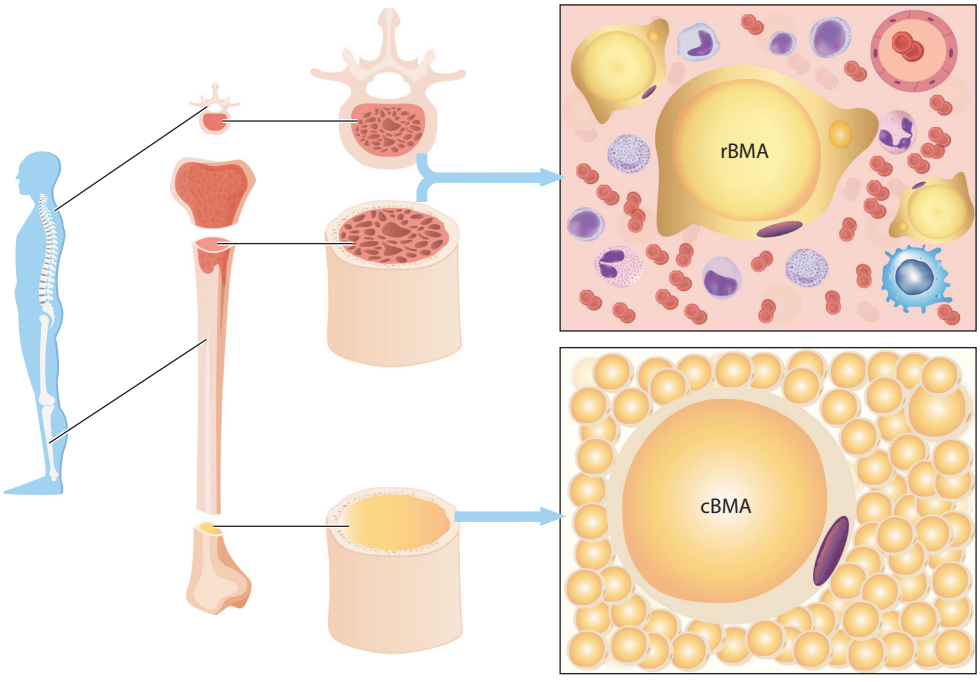

Figure 1

Marrow adipose tissue (MAT) heterogeneity. Residing in different niches of the skeleton, regulated MAT (rMAT) and constitutive MAT (cMAT) show morphological and physiological specificities. Regulated bone marrow adipocytes (rBMAs) are smaller than constitutive BMAs (cBMAs). rMAT colonizes the proximal region of the tibia and vertebrae and is interspaced with hematopoietic lineage cells. rMAT responds quickly to physiological and environmental stimuli. The distal regions of the tibia house cMAT, which adopts an analogous disposition to white adipose tissue, forming dense agglomerates of cells. cMAT is a more stable type of MAT, seldom participating in physiological adaptations.

these adipocytes (36). In the meantime, lineage tracing remains the best hope for understanding the origin and regulation of these cells.

4. THE BONE MARROW ADIPOCYTE: REGULATION

Marrow adipocytes arise from a multipotent mesenchymal stromal cell, often termed a skeletal stem cell, although investigators use different classifications for the early progenitor in the bone marrow. Skeletal stem cells give rise to either osteoblasts or chondrocytes, the cartilage forming cells, and are likely somewhat more differentiated than the more primitive progenitor cell that can differentiate into adipocytes or muscle cells. However, adipocyte and osteoblast differentiation are closely related, and both types of cells share some common steps during their differentiation (**Figure 2**). Several transcription factors are upregulated during early preosteoblast differentiation specific for the bone lineage: Runx2 and osterix (Sp7). Preadipocyte differentiation requires platelet-derived growth factor receptor β (PDGFRβ), C/EBPα, zinc finger proteins (Zfp) 423, 467, and 521, and PPARγ. Prrx1 is essential for osteoblast differentiation but is also expressed in inguinal depots and the neural crest (53). For a particular window of time, plasticity might exist, and adipocyte and osteoblast progenitor cells may interconvert within the context of an environmental, hormonal, or nutritional cue. Terminal differentiation is regulated by phenotype-specific

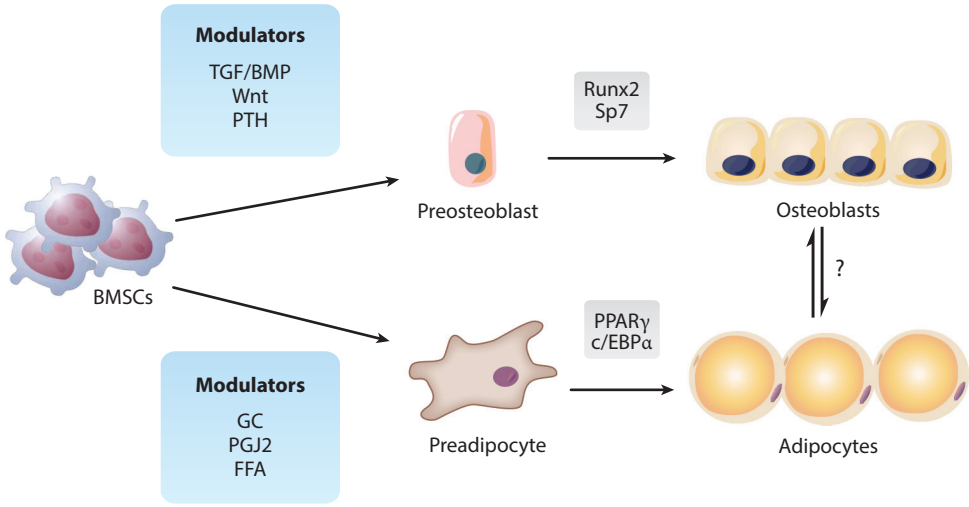

Figure 2

Skeletal stem cells are the remote link between osteoblast and marrow adipocytes. The finely tuned regulation in osteoblast and adipocyte differentiation seems to determine the slow cycles of bone mass development, maintenance, and degeneration during life. Disruption in this programmed process, favoring marrow adipose tissue expansion, leads to bone loss, as occurs under caloric restriction and hypercortisolism. Abbreviations: BMP, bone morphogenetic protein; c/EBPα, CCAAT/enhancer-binding protein alpha; FFA, free fatty acid; GC, glucocorticoid; PGJ2, prostaglandin J2; PPARγ, peroxisome proliferator–activated receptor gamma; Runx2, runt-related transcription factor 2; Sp7, osterix; TGF, transforming growth factor.

transcription factors in the case of osteoblast collagen type 1 (col1a1) and osteocalcin (ocn). And in respect to adipocytes, PPARγ, fatty acid–binding protein 4 (FABP4), perilipin 2 (Plin2), and fatty acid synthase (FASN) are all expressed sequentially during differentiation. Transdifferentiation at a later developmental stage has also been proposed but has not been proven definitively. Finally, osteoblasts terminally differentiate into osteocytes after the cells are surrounded by matrix, and these cells secrete sclerostin (SOST) and fibroblast growth factor (FGF)23.

The direction of skeletal stem cell differentiation toward osteoblasts or adipocytes can be strongly affected by endocrine molecules, particularly estrogen. Natural (menopause) or induced (experimental ovariectomy) hypoestrogenism promotes MAT expansion that can be efficiently reversed by estrogen therapy (29, 49). Moreover, marrow adipocyte proliferation may occur when estrogen receptor alpha (ERα) is silenced, whereas it is not affected in *Erβ* knockout mice. Therefore, ERα is the estrogen receptor directly involved on the allocation of skeletal stem cells toward osteoblasts or adipocytes (54, 55). Evidence also suggests that estrogen acts in progenitor cells before the expression of adiponectin, a marker of late adipocyte differentiation that may also be transiently expressed early in progenitors. The deletion of *Erα* in mature adipocytes does not affect MAT. Indeed, in vitro studies suggest that estrogen has dual effects, promoting osteoblast differentiation and, in parallel, suppressing adipocyte differentiation (56).

Premenopausal women who are estrogen deficient also have high marrow adiposity as measured by MRI, and estrogen supplementation reduces adipocyte number and size. Interestingly, selective estrogen receptor modulators may increase marrow adiposity, potentially by blocking ERα in vivo (57).

Decreasing estrogen concentrations coincide with increasing follicle-stimulating hormone (FSH) concentrations due to the endocrine feedback of the gonadal-pituitary axis. A recent study

showed that blocking the binding of the FSH ligand to its receptor with a polyclonal antibody prevented the increase in MAT after ovariectomy (58). Furthermore, treatment with this antibody in sham-operated mice also decreased MAT volume. The authors showed that both adipocytes and mesenchymal stromal cells express the FSH receptor and that FSH receptor–deficient mice have increased mesenchymal stromal cell adipogenesis, suggesting that postmenopausal-elevated FSH contributes to the increased adipogenic differentiation of the skeletal stem cell. Most recently, work from the AGES cohort in Iceland revealed a strong positive relationship between circulating FSH and MAT volume. Similar findings were noted in a large Chinese cohort (Z. Cheng, personal communication). Interestingly, the relationship between adiposity and FSH in humans may be depot specific, as subcutaneous fat measurements aligned nicely with those from MAT versus FSH, whereas visceral fat depot volume was inversely related to circulation (59).

Parathyroid hormone has a crucial role on the regulation of the circulatory levels of calcium, promoting bone resorption during times of calcium deprivation. On the other hand, intermittent pulses of parathyroid hormone (PTH) can induce bone formation, promoting both skeletal stem cell differentiation to osteoblasts and recruiting osteoblast progenitors from bone lining cells and perivascular cells (60, 61). In the latter scenario, PTH also inhibits marrow adipogenesis, as was observed in rats (62) and mice submitted to caloric restriction (63). Experimental models of deactivation or constitutive activation of the PTH receptor, respectively, promote expansion (64) or suppression of MAT (65, 66). In support, in vitro studies also show the suppressive effect of PTH on skeletal stem cell differentiation toward adipocytes (67, 68).

PTH1R expression in marrow adipocytes is significantly higher than it is in inguinal or gonadal depots (64). That might have profound implications for understanding PTH actions in the marrow niche. But more importantly, it highlights the unique nature of the marrow adipocyte. It has been shown that the marrow fat cell makes RANKL, and this may be part of the mechanism surrounding the inverse relationship between bone and marrow fat content (64). As such, at least two genes with osteogenic capacity are highly expressed in the marrow adipocyte, *PTH1R* and *RANKL*. In addition, *SP7*-positive cells, another osteogenic transcription factor, are also reported in the marrow by lineage tracing (69). Thus, evidence that the marrow adipocyte could be a hybrid osteoblast/adipocyte has not been proven but remains a possibility.

The presence of the PTH1R in marrow adipocytes raises another question: Is there clinical relevance to this finding? Previously, Spiegelman's group (70) showed that PTHrp, which binds the same receptor as PTH, drives cachexia through the beiging of inguinal depots in patients with chronic renal disease and cancer. Maridas et al. (63) recently showed that PTH could induce a lipolytic cascade in marrow adipocytes cultured in vitro. Similarly, in vivo they demonstrated that PTH administered intermittently reduces not only the number of adipocytes but also their size, suggesting a marrow lipolytic effect. The transfer of fatty acids from neighboring adipocytes to osteoblasts via lipolysis to enhance energy utilization and mitochondrial respiration during states of increased bone formation is likely to be an important mechanism during pubertal growth and with anabolic therapy (63). Similarly, evidence suggests that osteoblasts may also respond to PTH by regulating lipid metabolism via their lipid droplets. It should be noted that the bioenergetics of the marrow adipocyte is not well defined due to the difficulty in isolating these adipocytes in situ. However, when marrow skeletal stem cells are cultured in adipogenic media and then treated with PTH, there is a reduction in intracellular lipid droplets and an increase in lipolytic enzyme expression. Furthermore, oxidative phosphorylation is enhanced after PTH treatment of these osteoblast progenitors, further supporting the concept that the marrow adipocyte exhibits characteristics of both.

Hypophosphatemia is a classic cause of rickets, a disorder characterized by a defect on bone mineralization installed during skeletal development. Experimental studies of hypophosphatemic

rickets in mice have shown that, in addition to the defect in bone mineralization, there is a decrease in osteoblasts and an expansion of MAT (71). In vitro, the hypophosphatemic environment was not sufficient to alter skeletal stem cell differentiation, meaning that other endocrine and paracrine disturbances exist in vivo. Although the mechanistic determinant of marrow adipogenesis in this condition is yet to be determined, FGF23 seems not to be involved in this process.

Leptin is a multifunctional peptide acting on the modulation of appetite, reproduction, and energy and bone metabolism (72). In addition, leptin participates in the regulation of hematopoiesis (73). Leptin has dual effects on bone; the central pathway activates the sympathetic nervous system, determining impairment in bone formation, whereas direct action stimulates osteoblast activity (74, 75). There are also studies showing that subcutaneous or hypothalamic administration of leptin decreases obesity-induced marrow adiposity (76, 77). Adipocytes in the marrow can secrete leptin, and the leptin receptor (LepR) is an essential characteristic of the adult osteoblast, further supporting the tenet that paracrine and autocrine hormonal regulation occurs between marrow adipocytes and osteoblasts (78).

The Wnt signaling pathway regulates osteogenesis by stimulating β-catenin activation in the nucleus. Ligand binding to the Lrp5 receptor blocks adipogenesis in the marrow and potently enhances osteogenesis (79). Targeted overexpression of Wnt10b using the osteocalcin promoter blocks calorie restriction–induced marrow adipogenesis (15). Sclerostin is a potent osteocyte-generated protein that antagonizes the Wnt signaling pathway. It blocks bone formation and increases bone resorption (80). Sclerostin has recently been shown to induce adipogenesis in vitro, and in vivo treatment with antibodies to sclerostin also leads to reduced MAT in rats (M.R. Reagan, unpublished observation).

Romosozumab is an antisclerostin antibody recently approved by the US Food and Drug Administration (FDA) that enhances bone mass and also suppresses MAT volume in mice and rats (81). Taken together, it is clear that the marrow adipocyte is regulated by a host of hormonal factors that likely also play a role in age-related marrow adiposity (47, 75). In addition to the factors noted above, nutritional stimuli have a major impact on the fate of the marrow adipocyte. For example, calorie restriction in mice induces a significant degree of MAT, particularly in the regulated regions of the long bones (15, 41). In humans, anorexia nervosa is strongly associated with high MAT in the vertebrae. On the other hand, high-fat diets in mice and rats induce an increase in MAT, although the magnitude of this increase is not as great as calorie restriction and can vary by the type of diet and the duration (37, 40, 82). Obese individuals in some studies but not others have increased marrow adiposity (27, 83, 84). Ten days of a high-fat diet fed to healthy volunteers in a supervised environment increased vertebral marrow adiposity more than calorie restriction to the same individuals (P. Fazeli, personal communication).

5. TECHNIQUES TO IDENTIFY THE MARROW ADIPOCYTE PROGENITOR

There are a number of ways to trace a cell and determine its origin. However, it is somewhat trickier to do it in the marrow owing to its location and because decalcification is necessary to identify critical cell components. Lineage tracing is not a new technique; it was originally developed using a vital dye to assess cell fate in *Xenopus* embryos in the late 1920s. Weissman and colleagues (85), however, made the most important breakthrough in the field by tracing hematopoietic stem cells after transplantation using specific antibodies to cell surfaces. This was followed by isolation of cell surface markers on skeletal stem cells that could ultimately be used to trace progenitors in the marrow (86, 87). By the early twenty-first century, Cre-lox technology became widespread for

conditional deletions of specific genes, and over time this technique has been applied to tracing skeletal stem cells in the marrow (88).

Expression of Cre recombinase can be engineered for a specific cell type or tissue (87, 89, 90). That line is then crossed to a reporter line carrying enzymes or fluorescent proteins inserted into ubiquitously expressed loci such as Rosa26. The reporter expression is prevented by a stop cassette flanked by two loxP sites. Upon Cre activation in a particular tissue that expresses the promoter, the reporter will express genes in a cell-specific manner. It should be noted that removal of the stop cassette is permanent; hence, all Cre-activated cells will be expressed during the lifetime of the individual, leading to lifelong deletion of the target reporter gene. The shortcoming to that approach is the long-term nature of the deletion; therefore, inducible Cre reporters were developed to conditionally label cells at a specific time point using CreER, in which Cre recombinase is fused with mutated forms of the estrogen receptor that binds just tamoxifen, which can then be administered at any time point in the life of the mouse. Doxycycline-inducible Cre lines were also developed and are widely used. Generally, the most reliable system for lineage tracing in the skeletal stem cell is a two-color system whereby mTomato red represents all cells being labeled, and green fluorescent protein (GFP) is inducible when the Cre driver is expressed in that cell as the stop cassette is removed and the promoter drives GFP.

5.1. Marrow Adipocyte Lineage Tracing

Seminal work from several labs has shown that mTm:Gfp mice crossed with the adiponectin Cre defines adult cells in the femoral marrow as being true adipocytes. These mice also show labeling in all of the peripheral adipocyte depots. Similarly, treating B6 mice with rosiglitazone, a PPARγ agonist, reveals that all the inducible marrow adipocytes stain green. However, when using the Pdgrβ Cre mice only about half of the marrow adipocytes are traced, whereas all of the peripheral depots are green. This implies that the marrow fat cell may differ in its origin from other adipocytes or that there is significant heterogeneity within the marrow compartment.

Further support for that comes from studies with the Prrx1-Cre, which labels early mesenchymal progenitors and is often used to conditionally delete genes related to osteoblast differentiation (69). As expected, osteoblast-lining cells are traced with Prrx1, as are chondrocytes in the growth plate. Similarly, all of the marrow adipocytes are traced, although in the periphery, only adipocytes in the inguinal depot trace to Prrx1 (91). Myf5, a transcription factor for brown adipocytes, shows absolutely no tracing in the marrow depot, nor did Vav, a marker of the macrophage-osteoclast lineage. Surprisingly, Sp7-Cre also traces all marrow adipocytes but very few peripheral fat cells, again reinforcing the bone-fat nature of these progenitors (3, 92).

Morrison and colleagues (93) have definitively shown that the expression of the LepR is a very important trait in mesenchymal stromal cells. They found from the whole population of cells within bone marrow that 0.3% express LepR, 10% of which can differentiate into osteoblasts, chondrocytes, and adipocytes in culture and in vivo. $LepR^+$ cells generate most of the osteoblasts and adipocytes in adult bone marrow, showing high capacity for proliferation when bone regeneration is required (93). In addition, Nestin positivity in early stem cells of the marrow suggests there may be cross talk between those neural crest type cells and $LepR^+$ cells. Other investigators have identified α smooth muscle actin+ cells as another early marker of the skeletal stem cell (94). Using lineage tracing, Bianco et al. (95) convincingly demonstrated that the pericyte, a cell found in microvascular and periendothelial locations along the vascular basement membrane, was a source of stem cells that labeled for CD146 in humans. Most investigators now believe that the site of origin of the marrow adipocyte is in or around the vascular network in the stromal regions.

5.2. Marrow Adipocyte Progenitors by Cell Sorting

Robey et al. (96) used another approach to identify the marrow adipocyte progenitor where surface antibodies were sorted by flow cytometry. Schulz and colleagues (97) have done the most work with flow cytometry to assess progenitor identification, using the technique to delineate the heterogeneous population of mesenchymal lineage cells from bone marrow. Combinations of specific markers were used to define diverse populations; for instance, non-hematopoietic clusters of differentiated ($CD45^-$) and non-endothelial ($CD31^-$) cells that can be further subdivided in those expressing and those not expressing the stem cell antigen (Sca1). In vitro assay shows that cells expressing $CD45^-$ $CD31^-Sca1^+$ have great adipogenic and limited osteochondrogenic capacities, the opposite of the profile exhibited by those expressing $CD45^-$ $CD31^-Sca1^-$. These investigators went on to also show that the cells were Zfp423 positive, a transcriptional factor that promotes white but not beige adipocyte development. In addition, they found that these adipocyte populations expressed high levels of dipeptidyl peptidase-4 (Dpp4), a protease that is targeted by inhibitors to reduce hyperglycemia in diabetics. Taken together, there is consistent evidence that despite the degree of heterogeneity in marrow progenitors, subpopulations destined to become adipocytes can now be recognized. Altering their cell fate will be a challenge but may be important therapeutically.

6. LIPID STORAGE AND THE ENDOCRINE/PARACRINE ROLE OF MARROW ADIPOCYTES

Energy availability is an essential requirement for the existence and maintenance of life. As such, the capacity to store energy within an appropriate structure provides autonomy and versatility, enabling survival in hostile environments. Moreover, it circumvents ectopic deposition of lipids, avoiding oxidative stress, functional abnormalities, and inflammation. In common to all types of adipocytes, no matter whether they are white, brown, or marrow, all have the capacity to store triglycerides. Intriguingly, there is a major difference between adipocytes from white adipose tissue (WAT) and MAT in their responses to variations in nutritional exposure. Under caloric restriction, MAT expansion represents a remarkable difference in comparison to the expected shrinkage that happens with WAT. MAT may also secrete adipokines that further regulate other adipose depots and whole body homeostasis.

6.1. Marrow Adipose Tissue and the Renewal of White Adipocytes

The great efficiency of adipose tissue for energy storage depends on two processes that allow expansion: cell hyperplasia and hypertrophy. Age determines which process prevails over the other. Hyperplastic adipose tissue is characterized by a high number of small adipocytes, whereas hypertrophic adipose tissue has fewer but larger cells.

Adipocyte renewal is a very dynamic process akin to their unique flexibility to contract or expand in size and number. Cell replacement rate of adipocytes is projected to be on the order of 10% each year and in part occurs via bone marrow progenitors (98). Evidently, such operational replacement requires a continuous supply of progenitor cells. It was hypothesized that WAT adipocytes originated from local progenitors only. Recently, it has been suggested that bone marrow could be a source of progenitor cells that colonize WAT and evolve into mature white adipocytes (**Figure 3**) (99). These new findings are rapidly transforming previous conceptions about bone marrow's role in the whole-body energy homeostasis, including its metabolic influence in obesity.

MAT is naturally programmed to emerge during the perinatal period and predetermined to progressively increase throughout life. Thus, MAT expansion evolves during times of both bone anabolism and bone catabolism. Most likely, during periods of bone acquisition, MAT is a source

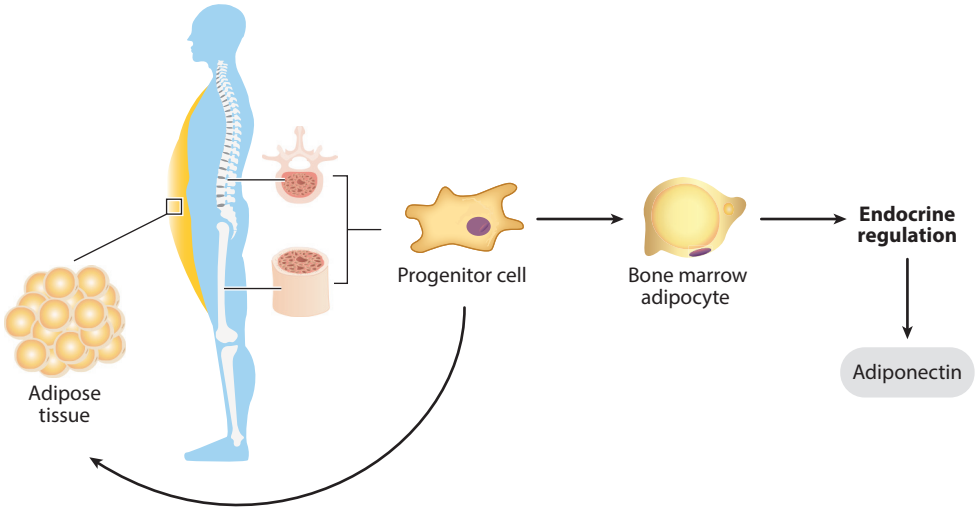

Figure 3

Marrow adipose tissue (MAT) provides multipotent mesenchymal stem cells exhibiting higher differentiation capacity than adipose tissue-resident preadipocytes (98). MAT contributes to metabolic adjustments in energy metabolism; adipocytes from this niche are a reliable source of adiponectin in times of famine.

of energy substrate for bone modeling. Its paracrine/endocrine function also provides a positive environment for osteoblastogenesis, while at same time constraining osteoclastogenesis (100). Later, as part of the senescence process, the relationship between MAT and bone is reprogrammed, leading to a negative imbalance in bone remodeling (101). The mechanisms determining both these positive and negative influences of MAT in bone are as yet unknown but likely include a secretome and lipidome. As a rule, abnormal increases in MAT have a negative relationship to bone mass, independent of age (e.g., young women with anorexia nervosa, postmenopausal women, and individuals using glucocorticoids or thiazolidinediones all exhibit both bone loss and MAT expansion) (7, 26, 102, 103).

Although WAT expansion is directly dependent on the balance of caloric intake and disposal, MAT evolves in a predetermined program of bone marrow filling as the requirement of hematopoiesis decreases. A recent well-designed study collected important evidence showing that marrow adipocytes provide substrates for osteoblast disposal (63). The authors take advantage of previous data showing the following: first, that calorie restriction leads to bone catabolism and stimulates marrow adipogenesis and, second, that intermittent administration of PTH has bone-anabolic action and decreases bone adipogenesis. The authors showed that, in mice subjected to a calorie-restricted diet, intermittent administration of PTH reduces bone marrow adipocyte number and size and increases osteoblast quantity. In vitro, it was observed that PTH increased lipolysis in adipocytes originated from skeletal stem cells. An additional observation of this study was obtained in cocultures of bone marrow adipocyte and osteoblast progenitors. PTH stimulated the transfer of fatty acids from adipocytes to osteoblasts (63). Taken together, these concrete pieces of evidence endorse the previous hypothesis that MAT actively responds to physiological and hormonal stimuli and provides substrates to osteoblast disposal.

Although MAT has a prominent capacity to store fat, its relationship with caloric availability has a distinct pattern in comparison to WAT, particularly in humans. MAT does not appear to be the destiny for the storage of extra lipids under conditions of energy surplus. Currently, several groups are using magnetic resonance to estimate MAT quantity and quality in clinical and experimental

investigation (104–109). ^{1}H spectroscopy applied to magnetic resonance allows for the accurate and reproducible assessment of MAT in vivo (110). There is some controversy about whether obese individuals have similar or greater amounts of MAT compared to control lean subjects. Findings in several studies show no positive association between MAT and VAT (27, 83, 107, 111). Furthermore, a recent study showed that, while the deposition of fat into the liver occurs in a spectrum of variation in lean control, obese, and type 2 diabetes mellitus subjects, there is no difference between the amounts of MAT in the same groups (83). These results reinforce the hypothesis that MAT is not used for accommodation of the overspill of lipids.

There is another physiological distinction between MAT and VAT, as the former has no association with insulin resistance (27, 83, 107). During expansion of peripheral adipose depots there is marked progression of macrophage infiltration and fibrosis. This does not occur in the marrow compartment. And the passive behavior of MAT, observed in conditions of energy surplus, disappears when it is exposed to caloric restriction. Curiously, instead of shrinking, MAT expands under this unfavorable scenario, in contrast to the typical reduction in WAT. Also, there is no evidence of a fibrotic or inflammatory response to MAT expansion. Experimental studies in mice and rats on caloric restriction, as well as clinical investigations evaluating women diagnosed with anorexia nervosa, show significantly increased MAT, mostly in the proximal region (15), but it may not be restricted to rMAT (112). Moreover, on a long-term basis (i.e., one year) (8), MAT decreased in women after recovery from anorexia nervosa (113). Other studies show that nutritional therapy limits MAT expansion in clinical models of malnutrition. Individuals diagnosed with short bowel syndrome receiving parenteral nutrition show similar amounts of MAT compared to a control group (111). Importantly, in both women after recovery from anorexia nervosa and individuals with short bowel syndrome receiving nutritional therapy, the relationship between bone mass and MAT is positive. Therefore, it seems that under extreme conditions of calorie scarcity the access to nutrients not only reduces the expansion of MAT but also changes the association between it and bone remodeling. The hormonal background of caloric restriction includes increased circulatory levels of cortisol (114), whereas leptin serum levels decrease. Either hypercortisolism or hypoleptinemia (76) may theoretically be noted as the cause of MAT expansion. However, in a recent study Cawthorn et al. (112) collected data suggesting that cortisol might be more involved in this process rather than leptin. On the other hand, insulin sensitivity is enhanced during caloric restriction in rodents and humans, and at least one group has linked this sensitivity to enhanced adiponectin secretion (see below).

It has been hypothesized that MAT might function as a strategic last reserve of the body of energy to be used in conditions of famine. Also, an additional contribution of MAT to metabolic adjustment in this scenario would be the production of adiponectin, as observed by Cawthorn et al. (112). Alternatively, bone formation is a highly costly process. In circumstances of restricted nutrients, metabolic adaptations target saving energy for essential activities necessary to guarantee survival. For instance, in previous studies, Gat-Yablonski & Phillip (115) highlighted that growth capability in humans occurs in plenitude only in circumstances of appropriate good nutrition. In all probability, bone mass development and maintenance are not a priority under feeding deprivation; paradoxically, in this case, marrow obesity seems to be the most cost-effective option to save energy. Further investigation will be necessary to unveil whether adipocyte transdifferentiation to an osteoblast is a natural alternative to restore bone mass after refeeding.

7. THE ENDOCRINE ROLE OF MARROW ADIPOSE TISSUE

Adults harbor a significant amount of MAT, comprising approximately 10% of the total body fat. This sole fact indicates its metabolic relevance. It has been recognized that the marrow adipocyte

is a target of endocrine molecules (e.g., insulin, growth and thyroid hormones), but it also has relevant endocrine/paracrine functions secreting peptides such as leptin and adiponectin and cytokines [e.g., interleukin (IL)-6, IL-1β, and tumor necrosis factor alpha (TNF-α)] (116). Only in the beginning of this century did the interaction between bone and adipose tissues start to receive proper attention. Certainly, two aspects contributed to the additional delay in the interest to investigate the interaction of MAT with bone and its contributions to systemic homeostasis. First, body weight has a positive correlation with bone mass, and it was reasoned that obesity was protective for fracture (117). Second, as mentioned above, MAT was anticipated as a passive, space-filling component of bone marrow. In 2010—unexpectedly at that time—prospective clinical investigation and experimental studies reevaluated this subject. A study performed in the United Kingdom reported that obesity in postmenopausal women was not protective of fracture risk (118). Similar results were also obtained in older men in the United States (119). Experimental studies revealed a complex network linking WAT to the modulation of bone remodeling (74). Leptin and adiponectin are two of the most relevant peptides secreted in adipose tissue; they have sufficient effects on the whole body economy, which encouraged investigation into their effects in different systems. Both are expressed in marrow adipocytes, and their local actions have been recognized (120). More recently, the endocrine contribution of marrow adipocytes to the regulation of energy homeostasis has started to be documented (**Figure 3**).

Leptin is a highly conserved nonglycosylated polypeptide of 16 kDa, containing 146 amino acids encoded by the leptin gene (121). Leptin is mainly produced in WAT and is increased with adipocyte expansion. Investigation in human cell cultures indicated that bone marrow adipocytes express and secrete leptin at high levels (120). In animals sensitive to its action, leptin works by limiting lipid storage via a central effect of appetite inhibition, decreasing sensitivity to insulin but also directly expediting glycerol release and inhibiting fatty acid synthesis (122). Hamrick and colleagues (76) observed that *ob/ob* mice have increased MAT volume in the femur but not in vertebrae that is responsive to leptin treatment. The significance of these findings is not yet fully understood, but it is in line with structural bone analysis showing increased and decreased bone volume/total volume, respectively, in trabecular bone of the vertebrae and femur. Contradictory results also exist concerning the effect of leptin on marrow adipogenesis. A recent study was designed to evaluate the effects of leptin on skeletal stem cells. The authors conditionally deleted Lepr from limb bone marrow stromal cells, but not from the axial skeleton or hypothalamic neurons, using Prx1-Cre. These mice exhibited a normal bone phenotype except limb bones had increased osteogenesis, decreased adipogenesis, and accelerated fracture healing. In addition, *Prx1-Cre;Lepr*$^{fl/fl}$ mice do not show increased adipogenesis and reduced osteogenesis in the limb bones, as observed in wild-type mice (123). Studies also show that within the bone marrow, hematopoietic cells exert a paracrine modulation of leptin secretion. It was also observed that proinflammatory cytokines inhibited leptin secretion in bone marrow adipocytes (124). At this point, our knowledge is insufficient to determine how endocrine-/paracrine-secreted leptin from MAT affects bone remodeling in normal weight and obese individuals.

Adiponectin is a 30-kDa adipokine encoded by the *AdipoQ* gene, mainly synthesized and secreted by adipocytes. Circulatory levels of adiponectin reflect the abundance of the production of this peptide. Adiponectin can improve insulin sensitivity (125) and decrease inflammatory cytokines (126). Adiponectin participates in the modulation of energy homeostasis through its hypothalamic action, increasing energy expenditure. Although adipocytes are the main source of adiponectin, paradoxically there is a negative association between adiponectin and body weight, suggesting that massive increases in adipose tissue downregulate adiponectin. On the other hand, adiponectin secretion increases with body weight loss when there is a progressive contraction of adipose tissue. In obese subjects, the metabolic benefits of weight loss are at least in part attributed

to increased serum levels of adiponectin. There is no explanation for this occurrence, as there is no evidence of impairment in adiponectin clearance under caloric restriction (127). Also, most but not all (127) findings indicate that there is no increase in adiponectin expression in WAT (128). MAT is the only known adipose tissue that expands under the stimulus of caloric restriction. Cawthorn et al. (15) in 2014 collected convergent results using control mice, genetically modified mice (Ocn-Wnt10b mice), rabbits, and humans, demonstrating that MAT acts as an endocrine organ and contributes significantly to the circulatory levels of adiponectin under differing conditions (**Figure 3**). The same group has further contributed to this line of investigation. They observed that unlike mice, rabbits under caloric restriction do not show MAT expansion or increased serum levels of adiponectin, but akin to mice they exhibit bone loss, decreased WAT, and low serum levels of leptin (112). These data reinforce the hypothesis that the elevation of adiponectin in caloric restriction may require MAT expansion and that decreased levels of leptin are not sufficient to drive MAT expansion. Moreover, based on the findings of rabbits studied under caloric restriction, the authors called attention to the fact that MAT expansion is not an absolute requirement for bone loss (112).

The magnitude of the endocrine role of adipocytes is as important as, or exceeds, its particular capacity to store lipids. It has been suggested that adipocytes synthesize approximately 700 different types of adipokines (23). Leptin and adiponectin are two of the most well-known adipokines; however, investigations into their effects on bone have only just started. The complex action of these two peptides encourages more investigation of their effects on the skeleton and how MAT influences this process. Incongruities in the current data available have opened the door to better scrutinize the effects of adipokines on bone and MAT. The role of MAT as an organ to store and supply energy and simultaneously participate in the regulation of bone remodeling, hematopoiesis, and energy needs to be fully understood.

8. MARROW ADIPOSE TISSUE IN DISEASE STATES

8.1. Primary Osteoporosis

Primary osteoporosis is a degenerative disorder that occurs early or late in life, depending on three aspects: the peak of bone mass attained in the phase of bone acquisition, the subsequent rate of bone loss, and life expectancy. After birth, MAT imprints a continuous process of expansion, signaling that positive osteogenesis and adipogenesis are not mutually exclusive processes. Notwithstanding, the timing of major alterations in bone mass maintenance (i.e., menopause and aging) also leads to acceleration in marrow adiposity and a coincidental loss of bone. Because marrow adipocytes and osteoblasts derive from the same skeletal stem cell, shifting the differentiation from one cell lineage to the other may occur at the expense of the other. However, it should be cautioned that mutual exclusivity between adipogenesis and osteogenesis in the marrow has not been proven.

Estrogens exert tonic inhibition in paracrine secretion of RANKL by osteoblasts and possibly marrow adipocytes, inactivating a central mechanism in osteoclast differentiation and action. The repertory of estrogen actions in bone also includes a direct influence in the differentiation of bipotential progenitor cells into osteoblasts instead of adipocytes. Evidence was obtained in vitro using a mouse skeletal stem cell line, ST-2, modified to overexpress either human ERα or ERβ. The isolated cell treatment with bone morphogenetic protein-2 increased alkaline phosphatase activity and the number of oil red O-positive adipocytes in ERα and ERβ cell cultures. The addition of estradiol to the media of both cell cultures enhanced alkaline phosphatase expression and suppressed lipid accumulation. Moreover, an ER antagonist, ICI182780, reversed these effects (129). Similar results reinforce that estrogens moderate adipogenesis and stimulate osteogenesis

(130), which is in line with clinical observations obtained in a classical model of estrogen deficiency in humans, the menopause of women. Assessment of MAT through bone biopsies and ^{1}H spectroscopy applied to magnetic resonance showed that postmenopausal women diagnosed with osteoporosis harbor increased MAT. Moreover, estrogen therapy reverted bone loss and decreased adipocytes within bone in the postmenopausal women subjected to bone biopsy (49).

In 1971, Meunier and colleagues (26) performed iliac crest bone biopsy in individuals between 20 and 90 years of age. The comparison of bone parameters in young and old individuals showed that with the involutional bone loss of aging there is a concomitant increase in MAT. PPARγ is a master intracellular regulator of adipogenesis (31). Thiazolidinediones are exogenous stimulators of PPARγ, which are efficient controllers of hyperglycemia in type 2 diabetes, but they stimulate adipogenesis, including the bone marrow progenitor. Prospective studies evaluating the antidiabetic effects of rosiglitazone revealed that rosiglitazone, a typical thiazolidinedione, induced bone loss and greater fracture risk (131–133). Rosiglitazone is used in culture media to drive the differentiation of progenitors of mesenchymal cells into adipocytes (12). PPARγ expression increases in bone marrow during aging. Indeed, studies suggest that the increase in MAT and the decrease in osteoblast number that occur during aging are due to increased PPARγ expression (30, 32). It has also been suggested that rosiglitazone therapy intensifies an otherwise normal physiological bone marrow response that occurs late in life (7). Efforts to posttranslationally modify the PPARγ molecule have resulted in experimental evidence in mice that bone loss can be modified while glucose sensitivity is maintained (134).

8.2. Secondary Osteoporosis

A long list of disorders is associated with secondary osteoporosis. Osteoporosis is a complex disease characterized by increased bone fragility and altered microarchitecture. Two mechanisms are determinants of bone fragility: bone mass quantity and bone quality. Frequently, both mechanisms are combined, whereas some conditions show a predominant impairment in bone quality, exhibiting normal or increased bone mass (e.g., type 2 diabetes) (83). As part of the heterogeneity of osteoporosis, it has been recently recognized that not all diseases associated with osteoporosis exhibit increased MAT. The detrimental effects that metabolic and other disorders (e.g., obesity, type 2 diabetes, and anorexia nervosa) imprint in bone have unique aspects, only recently recognized.

The magnitude of peak bone mass is a major determinant of the lifetime risk for osteoporosis and fracture. Anorexia nervosa is a psychiatric disease characterized by self-imposed starvation leading to severe reduction in body fat and lean mass as well as hypogonadism. Hypercortisolism, growth hormone resistance, and hypoleptinemia are additional endocrine adaptations to extreme malnutrition (135). Anorexia nervosa affects 0.2–4% of adolescent girls and young women and is a major cause of impairment in bone mass development in this population (136). A decade ago two studies reported the paradoxical increase in MAT of adolescent (mean age $= 16 \pm 1.6$ years) (137) and young women (mean age $= 29.8 \pm 7.6$ years) (102) diagnosed with anorexia nervosa in long bones and vertebrae, respectively. The authors observed that MAT shows a negative correlation with bone mass development and suggested that premature conversion of red to yellow bone marrow may cause osteoporosis. Other studies in these patients reported the following findings: The serum levels of preadipocyte factor 1 (PREF1) increase in women with anorexia nervosa. PREF1 is a member of the epidermal growth factor-like family of proteins and is highly expressed in osteoblastic and adipocytes cell lines, as well as in human skeletal stem cells, working as a negative regulator of adipocyte and osteoblast differentiation. PREF1 was positively correlated with marrow fat content of the proximal femur and negatively with lumbar spine bone mass development.

These results encourage further investigation on the effects of PREF1 on the differentiation of osteoblast and marrow adipocytes.

Obesity and type 2 diabetes mellitus were initially thought to protect against fracture (138). This misconception was, at least in part, due to the increased bone mass observed in both conditions, but it was not supported by studies evaluating prospectively fracture occurrences in large groups of obese and type 2 diabetics (139). Currently, there is a great interest in the study of mechanisms that determine fracture susceptibility and in developing new exams to provide better evaluation of bone weakness in these metabolic disorders. Expectedly, several studies have been developed to evaluate MAT as a link between insulin resistance and bone fragility in these disorders.

There are contradictory results about the impact of high-fat diet on bone mass and MAT in experimental studies and clinical studies targeting the influence of obesity and fat distribution on MAT. In mice, several factors have been suggested as potential determinants of response, including strain (140), gender (104), age (7), composition of lipids in the diet (82), and the experimental duration of diet (141). The microbiome is another component that potentially can have an impact on the ultimate effect of diet on bone and MAT (38). In humans, the association between body weight and MAT has only recently been investigated. In 2015, bone, adipose tissue distribution, and MAT were evaluated in a group of nondiabetic women, showing the following characteristics: age range from 21 to 68 years, weight from 42.8 to 97.8 kg, and BMI from 17.5 to 37.3 kg/m^2. There was no association between body weight and MAT (27). The authors did not observe associations between MAT and parameters strongly related to insulin resistance [e.g., VAT, intrahepatic lipids, and insulin resistance (homeostatic model assessment of insulin resistance, HOMA-IR)] (27). These results were reaffirmed in another study that evaluated normal weight, obese, and type 2 diabetic individuals of both genders (83). Furthermore, both studies called attention to the finding that insulin resistance was not negatively associated with bone mass. The latter results are in line with those obtained in subjects with acquired (142) or severe congenital insulin resistance that exhibit normal or high bone mass (143). However, divergent results have been described in the literature (144, 145). Ermetici et al. (144) reported that, although normal weight and obese premenopausal women show similar amounts of MAT, there is a negative association between MAT and insulin resistance. In a previous study, Russell et al. (146) reported that VAT is an independent inverse determinant of bone mass in obese adolescent girls. More recently, an as yet unpublished study showed that 10 days of a high-fat diet in healthy volunteers resulted in an increase in vertebral MAT. Hence, further work is necessary to define the extent of triglyceride storage in marrow adipocytes.

Few studies have examined MAT in other metabolic bone diseases, but Mendonça et al. (106) reported that in primary hyperparathyroidism, a condition where bone loss occurs in ambient of high bone turnover, there is no increase in MAT. On the other hand, intermittent PTH administration has been associated with a decline in MAT (64). Increased MAT was recently reported in Cushing disease (103). In addition, Bastos et al. (147) described that patients with inactive inflammatory bowel disorders and in long-term withdrawal of glucocorticoids show similar quantities of MAT compared to a normal control group. Two independent studies showed that the amounts of MAT in individuals with type 1 diabetes are similar to those observed in a control group (109, 148). Carvalho et al. (109) highlighted that accompanying the global epidemic of obesity type 1 diabetes is the increasing prevalence of an obese phenotype among these individuals, which has a positive impact on their bone mass.

^{1}H spectroscopy applied to magnetic resonance also allows an analysis of lipid composition within MAT (11, 149). There is a natural difference in the rate of lipid saturation, depending on the bone site; vertebral bodies have a higher rate of lipid saturation than does long bone. Also, as part of the aging process, there is a physiological increase in the amount of saturated lipids in

MAT. Patsch and colleagues (11) reported that both fracture and type 2 diabetes are associated with higher amounts of saturated lipids in the bone marrow.

9. CONCLUSION

In summary, the marrow adipocyte is a unique cell innately programmed for distinct functions that are temporally and spatially specific during the life span of the individual. Inside the skeletal space, marrow adipocyte progenitors in a hypoxic environment are strategically positioned in various phases of differentiation to exert an influence on hematopoiesis and osteogenesis. Mature marrow adipocytes are linked to osteoblasts by sharing the same ancestor, the marrow stem cell. The differentiation of skeletal or mesenchymal stem cells toward marrow adipocytes or osteoblasts results from complex regulation that involves neural and endocrine inputs as well as a rich network of paracrine/autocrine modulatory factors. It is now evident that the balance between osteoblasts and adipocytes defines an individual's peak bone mass. The maintenance of marrow homeostasis is also dependent on that equilibrium and is integrated by overall health, endocrine status, and nutritional well-being. Understanding the origin, structure, and function of the marrow adipocyte will require further systematic and multidisciplinary approaches but is likely to lead to novel therapeutic strategies for several disorders.

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