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Defining Osteoblast and Adipocyte Lineages in the Bone Marrow

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Abstract

Bone is a complex endocrine organ that facilitates structural support, protection to vital organs, sites for hematopoiesis, and calcium homeostasis. The bone marrow microenvironment is a heterogeneous niche consisting of multipotent musculoskeletal and hematopoietic progenitors and their derivative terminal cell types. Amongst these progenitors, bone marrow mesenchymal stem/stromal cells (BMSCs) may differentiate into osteogenic, adipogenic, myogenic, and chondrogenic lineages to support musculoskeletal development as well as tissue homeostasis, regeneration and repair during adulthood. With age, the commitment of BMSCs to osteogenesis slows, bone formation decreases, fracture risk rises, and marrow adiposity increases. An unresolved question is whether osteogenesis and adipogenesis are co-regulated in the bone marrow. Osteogenesis and adipogenesis are controlled by specific signaling mechanisms, circulating cytokines, and transcription factors such as Runx2 and Pparγ, respectively. One hypothesis is that adipogenesis is the default pathway if osteogenic stimuli are absent. However, recent work revealed that Runx2 and Osx1-expressing preosteoblasts form lipid droplets under pathological and aging conditions. Histone deacetylase 3 (Hdac3) and other epigenetic regulators suppress lipid storage in preosteoblasts and/or control marrow adiposity. Establishing a better understanding of fat storage in bone marrow cells, as well as the osteoblast-adipocyte relationship within the bone marrow niche is necessary to understand the mechanisms underlying disease- and aging-related marrow fat storage and may lead to the development of new therapeutic targets for “fatty bone” and osteoporosis.

Keywords

Bone marrow mesenchymal/stromal cell; osteogenesis; osteoblast; adipogenesis; adipocyte; marrow fat; aging

INTRODUCTION

The Bone Marrow Microenvironment is Heterogeneous

Bone is a metabolically active tissue with complex physiological roles. Bone marrow is a semifluid component of bone that occupies the endosteal space. It consists of a diverse population of cell types including ones involved in bone development, hematopoiesis, tissue remodeling, and endocrine regulation. Many of the mature cell types present in bone marrow are derived from common progenitors. One multipotent progenitor, the bone marrow mesenchymal stem/stromal cell (BMSC), comprises a heterogeneous population of stem cells that can mature into cells of the chondrogenic, myogenic, osteogenic, and adipogenic lineages upon receiving the proper signals (1). BMSC retain their stem-like nature throughout development until triggered to differentiate by stressors and physiological needs. As BMSCs undergo differentiation, changes occur in their transcriptional profile, cellular metabolism, and morphology that are consistent with the respective lineage of commitment. During aging and in pathological conditions, the normal balance between osteogenic and adipogenic cell populations can shift toward the latter, thereby threatening bone health and integrity (2). Here we review the osteoblast-adipocyte relationship within the bone marrow and provide insights into how these two cell types can be better defined. Progress in this area will lead to new insights for preventing age- and pathology-related fractures and metabolic syndromes.

Commitment of BMSCs to Osteoblast or Adipocyte Differentiation

BMSCs are the multipotent progenitors responsible for maintaining the non-hematopoietic cell populations of the bone and bone marrow. The commitment of BMSC to osteogenesis is a tightly regulated process that is dependent on differing signaling mechanisms throughout development and during adulthood (Figure 1). Early regulators of osteogenesis in the BMSC population include Wnt/β-catenin signaling, bone morphogenetic proteins (BMPs), hedgehog proteins (i.e., sonic hedgehog, Indian hedgehog), endocrine hormones such as parathyroid hormone (PTH), epigenetic regulators, and various growth factors (3–5). The master osteoblastic transcription factors runt-related transcription factor 2 (Runx2) and Osterix 1 (Osx1/Sp7) are key and sequential players in the induction of osteogenic differentiation and shift the gene expression profile of BMSC to osteogenic genes that control type I collagen-based extracellular matrix (ECM) deposition (6). BMSC committed to osteogenesis continue to develop the genetic profile and morphology of the osteoblast, expressing genes such as alkaline phosphatase, osteoprotegerin, type I collagen, and later osteocalcin as these cells shift toward a terminal osteocyte morphology (7). In adults, the primary role of BMSC-derived osteoblasts is to produce and secrete osteoid and mineralization factors in a coupled fashion alongside the activity of osteoclasts (which resorb the bone matrix) to remodel the bone as the metabolic and structural needs of the body change. In cases where BMSC are not triggered to commit to osteogenesis, bone formation may become uncoupled from resorption and subsequent declines in bone mass and tissue integrity ensue (8).

BMSCs may alternatively be directed to adipogenesis under certain conditions. In fact, some consider the differentiation of BMSC to the adipocyte lineage to be a “default” pathway (9).

The differentiation of the BMSC into a bone marrow adipocyte involves expression of key transcription factors: peroxisome proliferator-activated receptor γ (Pparγ) and CCAAT/enhancer-binding protein α (c/EBPα) (10). Bone marrow adipocytes characteristically store cytoplasmic lipid droplets and express a number of genes related to lipid storage (i.e., Cidec, Plin1), fatty acid metabolism (i.e., Fasn, lipases like ATGL and HSL), and adipocyte function (i.e., adiponectin, lepR) (11). While the roles of bone marrow adipocytes are still in question, there is evidence that these cells may serve as energy reservoirs for the bone (12). Additionally, marrow adipocytes interact with other bone cells in distinct niches where they may regulate the bone remodeling, repair and endocrine regulatory processes (13). As such, these lipid-storing cells are an important component to skeletal biology and may provide novel insights into the activity of osteoblasts and other BMSC-derived cell types.

Cellular Energetics of BMSC-Derived Osteoblasts and Adipocytes

Much like the varying energy needs of different tissues based on their physiological functions, BMSC undergo a shift in their cellular energetics profiles during the commitment to terminal differentiation (Figure 2). The mesenchymal progenitors primarily rely on glycolysis for energy metabolism, whereas the energy needs of these cells increase dramatically during osteogenesis (14). Importantly, increased glucose metabolism within BMSCs—which is facilitated by upregulated *Glut1* transporter expression that precedes the expression of Runx2 in differentiating preosteoblasts—provides the energy necessary to produce the type 1 collagen that comprises the ECM (15). Mature osteoblasts also require a high yield of ATP from fuel sources in order to produce and deposit significant amounts of osteoid and, consequently, utilize fatty acid oxidation in addition to glucose metabolism to meet those energy needs (16). While the energetics of late-stage osteogenic cells are sufficient for their functions, the use of oxidative phosphorylation for a high energy yield can contribute to the production of excess reactive oxygen species that may cause cellular damage over time.

Bone marrow adipocytes tend to have different metabolic needs from osteogenic cell types, and this is also reflected in their cellular energetics. Rather than utilizing glucose metabolism for energy, these lipid-storing cell types are characterized by fatty acid metabolism and rely heavily on the lipolysis of their intracellular lipid stores to provide free fatty acids (FFA) for metabolism through oxidative phosphorylation (17). As a result of harnessing fatty acids as intracellular lipid droplets, marrow adipocytes may serve as important reservoirs for energy when other sources (i.e., glucose) are depleted. However, an excess of FFA produced by the metabolism of lipid droplets can have lipotoxic effects in BMSCs and osteoblasts by interrupting cellular functions and inducing cell death (18). The detrimental effects of this fatty acid metabolism on osteoblasts can be prevented with the inhibition of fatty acid synthesis (19). The intriguing relationship between fat-storing and bone-forming cells involves crosstalk at the metabolic level. The delicate balance between glycolysis and oxidative phosphorylation—particularly of lipid metabolites—in osteogenic and adipogenic cell types is an active area of interest that may provide novel insights into the maintenance of skeletal health.

Lipid Storage in Non-Adipogenic Skeletal Cells

While it is clear that both osteoblast progenitors and adipocytes reside in the bone marrow, their identities can be difficult to discern because lipid accumulation is often used a defining characteristic of adipocytes instead of lineage-defining transcription factors. A challenge in histology-based examination of bone marrow adiposity is that the lipid droplets in bone marrow cells consume much of the cellular volume, leaving little room to study other organelles, including the nucleus where lineage-directing transcription factors typically reside. However, the biochemical and metabolic pathways that create and dissolve lipid droplets are common to all cells and are not unique to adipocytes. The storage of intracellular lipid droplets in non-adipogenic lineage cells is not a novel concept—for example, lipid storage has been widely studied in hepatocytes of the liver as a consequence of increased alcohol consumption, but intracellular lipid storage in osteoblast-lineage cells is not currently recognized widely by the bone field despite growing evidence of this mechanism's existence. Osteocytes have increased intracellular lipid deposition as a result of chronic alcohol consumption, which often precedes osteocyte apoptosis and osteonecrosis due to the disease (20). Chondrocytes, as well, were shown to contain intracellular lipids, the abundance of which increases with aging (21).

The storage of lipid droplets within osteoblast progenitors in the bone marrow is not as well-documented as other cell types, but there is recent evidence that BMSC-derived bone-building cells may store intracellular fat when their differentiation is challenged—similar to skeletal muscle (Figure 1) (22). Recent studies on the conditional deletion of the epigenetic regulator histone deacetylase 3 (Hdac3) in osteoblastic or chondroblastic cells (cells targeted by Osx1-Cre, Col2-Cre) revealed increases in the prevalence of lipid-containing, Runx2+ BMSC-derived osteoblasts that are hypothesized to contribute to high marrow fat observed in Hdac3-deficient models (23–25). An important aspect of these studies is that Runx2+ cells expressed lipid droplets when cultured in osteoblastic medium. Of interest, deletion of Hdac3-associated cofactors (e.g., Ezh2 and Zfp521) in osteoprogenitors also increased marrow adiposity (26,27). These data indicate that the Hdac3 cofactor complex controls energy homeostasis in osteoprogenitors like it does in the liver (28–30). Hdac3 levels decline in human bone with age (24), suggesting a novel potential mechanism that could contribute to marrow fat increases with aging and certain pathologies (i.e., aberrant glucocorticoid signaling, excess oxidized amino acid metabolites), as Hdac3 levels can decrease in these conditions as well (24,31–34).

Deletion or overexpression of other important osteoblast proteins in osteoprogenitor cells can also alter marrow adiposity. For example, conditional deletion of Cbfβ, a stabilizing co-factor for Runx transcription factors, in osteoprogenitors (with Osx1-Cre, Prxx1-Cre, or Col2-Cre) increases bone marrow adiposity (35), as does deletion of GNAS (Gsα) with Prxx1-Cre (36,37) or Vegf with Osx1-Cre (38). In contrast, transgenic animals overexpressing osteoanabolic agents such as Wnt10b (driven by the OCN promoter; (39)) or a gain-of-function form of Gsα do not have marrow adiposity (40).

Several groups performing fate-mapping studies with Osterix-Cre mice showed that cells in which the Cre is active contain lipid droplets, as defined by perilipin-positive rings, in vivo (41,42). The conclusions of these studies were that adipocytes express Osterix-Cre.

However, an equally plausible conclusion is that osteoblasts are capable of forming lipid droplets when differentiation is challenged as it may be if one or both copies of Osx is deleted.

While considered potentially deleterious in the aforementioned studies, lipid storage within osteoblasts has also been proposed as a critical step for osteogenesis. Impairment of lipid droplet formation in BMSC with the drug triascin C caused a notable decrease in osteoblastic differentiation as measured by alkaline phosphatase and Von Kossa stain (43). Thus, while fat accumulation in osteoblasts is often associated with pathologies, the physiological roles of these lipid-storing cells still remains unclear and may vary depending on the stage of osteoblastic differentiation.

Despite the potential for lipid droplets to support osteoblast function, it is important to consider that excess marrow fat storage can compromise bone mineral density and tissue health if lipid deposition extends beyond supplying the energy needs of differentiating BMSC. Fatty tissue in the metaphyseal region of the bone marrow cavity tends to occupy space that could otherwise hold trabecular bone, and there is increasing evidence that lipid metabolism within the bone marrow can hinder bone formation (44,45). Lipolysis of adipocyte lipid droplets can release fatty acids such as palmitate, ultimately impairing osteoblast differentiation and function by interrupting β-catenin and Runx2 signaling mechanisms (18,19). Additionally, accumulation of free fatty acids can have lipotoxic effects on osteoblasts by inducing autophagic and apoptotic mechanisms that result in long-term bone loss (46).

The Identity of the Adipocyte-Osteoblast

Intracellular lipid deposition in osteoblasts raises concern regarding their distinction from marrow adipocytes as well as the purpose of lipid storage within bone. Though the molecular identity of lipid-storing, non-adipogenic cells in the bone marrow is still in early stages, there is speculation that these cells may represent an intermediate stage between osteogenic versus adipogenic differentiation endpoints. Because BMSC are the common progenitors for osteoblasts and adipocytes, the overlap in their morphologies suggests potential for transdifferentiation between cell types (rather than distinct terminal differentiation) under certain physiological conditions (47). The phenomenon of transdifferentiation has been observed in various tissues—from regenerative studies in the liver and eye to limb regeneration in amphibians—but a remaining question is whether committed unipotent osteoblasts may differentiate directly into marrow adipocytes (or vice versa) or whether an intermediate dedifferentiation step is required (48,49). There are also questions regarding the triggers for transdifferentiation, as microRNAs, loss of transcription factor signaling, and lineage specific growth conditions have been shown to influence lineage switching in committed cell types (50,51). Runx2 (Cbfa1) deficient calvarial progenitors cannot become osteoblasts but retain potential to become adipocytes and other mesenchymal lineages when cultured in conditions favoring their development (52). Alternatively, an intermediate cell type with both osteogenic and adipogenic properties may be a key contributor to marrow fat storage under pathological conditions. As described earlier, recent work has shown that Runx2-positive BMSC-derived osteoprogenitors can

store lipid droplets and contribute to increased marrow adiposity in models of Hdac3 conditional deletion and aging (24). This phenomenon may be a result of incomplete lineage switching or another method of cell adaptation to changes within the bone marrow niche, but further characterization of BMSC-derived cell morphology and metabolism is needed to determine the identity of these lipid-positive bone cells. Such studies will require the ability to track the fate of single cells using lineage-defining transcription factors and care in accounting for cell culture conditions and media components. Indeed, a limitation of many published studies attempting to track cell fate and transdifferentiation is that heterogeneous cell populations and lineage-driving growth conditions (e.g., adipogenic or osteoblastic culture medium) are used to influence cell fate and conclusions are inferred to entire cell population. In a heterogeneous BMSC pool, there will be cells more inclined to become osteoblasts or adipocytes (or something else), but their potential is only achieved and observed under certain conditions. The observations of a cell population are insufficient to track individual cells or transdifferentiation.

Aging-Associated Marrow Fat

In vivo, marrow adiposity increases with age (45). In older individuals, the increase in marrow fat coincides with bone loss, but the physiological link between the two phenotypes is not easy to study in vivo (53). Bone marrow adipose tissue (MAT) can be classified into two groups—constitutive MAT (cMAT) and regulated MAT (rMAT)—that have unique formation patterns in addition to potentially unique roles (54). The former describes early MAT development in distal regions of the skeleton whereas the latter, rMAT, can form with age and tends to develop in the proximal regions of the bone (i.e., proximal tibia, femur, etc.) as it occupies the marrow distributed throughout the hematopoietic populations (55). The staining of murine tibia MAT with radio-opaque osmium tetroxide followed by micro-computed tomography (micro-CT; μCT) shows the accumulation of marrow fat that follows the formation patterns of cMAT (distal) and rMAT (proximal) that ultimately occupies most of the bone marrow cavity at 22 months of age (Figure 3). At the later stages of marrow fat accumulation, the low trabecular bone mass can put individuals at risk for osteopenia and the related comorbidities of fracture, increased healthcare costs, and even immortality.

There are many factors involved in skeletal aging and downstream effects, making it difficult to define the hormonal, nutritional, epigenetic regulators and signaling mechanisms involved in the osteoblast-adipocyte biology. In general, after skeletal maturity, bone density decreases with age and is met with increased marrow adiposity, and the aged bone is at an increased risk for skeletal fracture (8,56). One major contributor to skeletal aging is elevated glucocorticoid signaling, which promotes lipid accumulation in a number of tissues and may be a key factor in inducing adipogenesis within the aging bone marrow (57–60). Finally, lifestyle—including reduced mechanical loading and diet—can influence the metabolic needs of the bone as well as alter the extracellular signals that dictate BMSC fate during aging. Load-bearing exercises can reduce bone marrow adiposity (61), but both high fat and calorically restricted diets induce bone marrow fat storage and consequent bone loss (11,62). Despite this evidence, whether these cellular responses to lifestyle and diet are efforts to store excess fuel sources in the form of lipid droplets remains unclear. The underlying

mechanisms dictating BMSC differentiation with age are still an active area of research interest.

Summary and Future Directions

The diversity of the bone marrow mesenchymal stem cell population and its derivative cell types highlights the complexities of skeletal tissue. The heterogeneity of the bone marrow microenvironment supports the function of osteogenic, adipogenic, and chondrogenic cells, and BMSCs replenish these populations as the structural and metabolic needs of bone change.

BMSC commitment to osteogenesis is an important pathway during embryogenesis, skeletal growth, and bone remodeling to ensure proper tissue function. The generation of osteoblasts from BMSCs is dependent on tightly regulated signaling mechanisms (e.g., Wnt/β-catenin signaling), hormonal factors (e.g., PTH, glucocorticoids), epigenetic regulators (e.g., Hdac3), and the activity of specific transcription factors such as Runx2 and Osx1. BMSC-derived osteoblasts express genes and proteins related to producing osteoid (which transitions to calcified bone matrix), and this energy-demanding process tends to shift the energetic profile of these cells from predominantly glycolytic to a more complex metabolic profile that incorporates oxidative phosphorylation. The effects of aging on BMSC and osteoblastic bioenergetic profiles are not yet clear, but may shed light on the biological processes that contribute to increased MAT and decreased bone mass with age. Interestingly, BMSC-derived marrow adipocytes tend to utilize fatty acid oxidation but may impair osteoblast function and bone formation by doing so. These Pparγ+ cells characteristically store lipid droplets that may be used to meet energy demands, but recent evidence suggests that excess adipogenesis occurs at the expense of bone volume and that metabolism of adipocyte lipid droplets may hinder the function and survival of neighboring osteoblasts. Moreover, the fatty acids and adipokines (e.g., adiponectin) secreted by marrow adipocytes may drive further adipocyte differentiation (through Pparγ activation) and fatty acid metabolism in a feed-forward fashion. While marrow adipocytes have implications in bone endocrine functions and cellular signaling with osteogenic cell types within the bone, the identity of these cells (which is suggested to be unique from other adipose tissues) and the potential crosstalk with osteoblasts during aging is still in question.

There is increasing evidence that BMSC differentiation into distinct osteogenic, adipogenic, and chondrogenic populations requires intermediate steps and that transitional cell types may exist. Of interest for this review is the accumulation of lipid droplets in non-adipogenic cell types—a phenomenon that occurs in various soft tissues throughout the body during aging and certain pathologies. One argument for increased bone marrow lipid storage is that the cells positive for osteogenic markers may use lipid droplets as energy stores under energetically challenging conditions. Another plausible argument for osteogenic lipid storage is that adipogenesis is the default commitment pattern of BMSCs—as such, epigenetic regulators and signaling mechanisms dictated by the bone marrow microenvironment may downregulate adipogenesis in favor of bone formation and loss of function of these regulators (due to aging, stress, or pathological conditions, for example) can induce the reversion of osteogenic commitment. This “de-differentiation” step could be

an intermediate step to lineage switching that may favor adipogenesis, but current techniques for lineage tracking of the cells in question limit these research endeavors. Lastly, an emerging school of thought in bone marrow fat research suggests that osteoblasts and adipocytes may undergo the phenomenon known as transdifferentiation, or a switch between the morphologies of these terminal cell types in lieu of an intermediate de-differentiation step. Despite recent evidence, it is still difficult to confirm whether observations of transdifferentiation are real or due to the conditions in which the BMSCs are cultured, as the available substrates and fuel sources in culture media can influence the commitment of these cells to certain lineages.

The dynamic between bone marrow osteoblasts and adipocytes changes throughout life and influences bone density and function with age. Marrow fat accumulation increases with age, contributing to osteopenia by decreasing trabecular bone mass. While there are some therapies—for example, bisphosphonates to slow bone resorption and PTH to stimulate new bone growth—in use to treat osteoporotic bone, the long-term administration of these drugs can cause adverse events (63,64). Moreover, existing measures to prevent and treat osteoporosis do not target the marrow fat that contributes to fracture risk and metabolic dysfunction. As such, a better understanding of the mechanisms influencing lipid storage in BMSC-derived cells and bone marrow adiposity can provide novel insights into preventing the decreased bone mass and increased fat deposition with age. Given the recently reported differences in the bioenergetic phenotypes of osteoblasts as compared to adipocytes (65), it will likely be critical to determine the relative importance of cellular energetic profile as compared to lineage commitment in the process of osteoblast versus adipocyte differentiation, particularly with aging.

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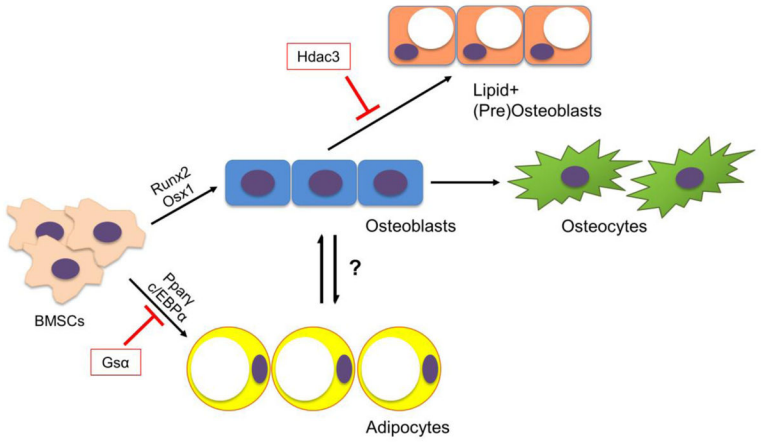

Figure 1.

Differentiation of BMSCs to osteogenic and adipogenic lineages. Bone marrow mesenchymal stem cells (BMSCs) are signaled to differentiate to osteoblasts by transcription factors such Runx2 and Osx1 or to adipocytes by Pparγ and c/EBPα. BMSC-derived osteoblasts can further differentiate into mature osteocytes or may become lipid-storing cell types. This diagram illustrates a potential lineage-switching mechanism between osteoblasts and adipocytes as well as a reduction of lipid storage within osteogenic cells by histone deacetylase 3 (Hdac3) and its associated cofactors.

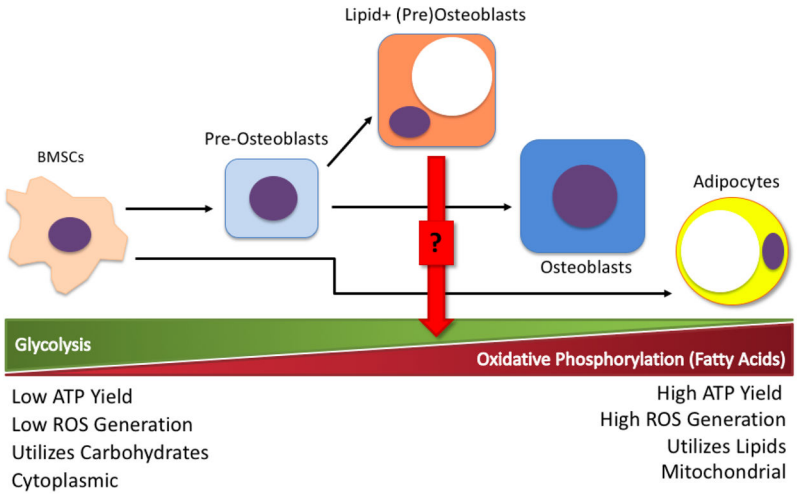

Figure 2.

Shift of cellular energetics profiles through BMSC differentiation. The progenitor BMSC population is highly glycolytic and dependent on glucose for energy metabolism. As BMSCs commit to osteogenesis, the energy demands of osteoid production require metabolic processes that generate more ATP—e.g., the metabolism of fatty acids for oxidative phosphorylation. Mature osteoblasts utilize a combination of glycolysis and oxidative phosphorylation to meet their energy needs, but this energetic balance must be tightly regulated to reduce reactive oxygen species (ROS) generation and subsequent cellular damage. In contrast to BMSCs, mature adipocytes generate much of their ATP from fatty acid oxidation and oxidative phosphorylation. The metabolic profile of the lipid-positive pre-osteoblast is still in question, but the intermediate morphology of this cell type suggests that it may use both energetic processes and may potentially store lipid droplets for fatty acid metabolism under high energy demand.

Figure 3.

Marrow fat accumulates with age in tibiae from female C57BL/6 mice. Osmium tetroxide-stained murine tibias imaged by micro-computed tomography show proximal and distal marrow adipose tissue at **a)** 4 months, **b)** 13 months, and **c)** 22 months of age. Each image is from a different mouse.