Bone Marrow Adiposity: Basic and Clinical Implications

Zachary L. Sebo,[1,2] Elizabeth Rendina-Ruedy,[3] Gene P. Ables,[4] Dieter M. Lindskog,[5] Matthew S. Rodeheffer,[1,2] Pouneh K. Fazeli,[6] and Mark C. Horowitz[5]

[1]Department of Comparative Medicine, Yale University School of Medicine, New Haven, Connecticut 06510; [2]Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, Connecticut 06510; [3]Maine Medical Center Research Institute, Scarborough, Maine 04074; [4]Orentreich Foundation for the Advancement of Science, Cold Spring, New York 10516; [5]Department of Orthopaedics and Rehabilitation, Yale University School of Medicine, New Haven, Connecticut 06510; and [6]Neuroendocrine Unit, Massachusetts General Hospital, Boston, Massachusetts 02114

ORCiD numbers: 0000-0002-0726-045X (M. C. Horowitz).

ABSTRACT The presence of adipocytes in mammalian bone marrow (BM) has been recognized histologically for decades, yet, until recently, these cells have received little attention from the research community. Advancements in mouse transgenics and imaging methods, particularly in the last 10 years, have permitted more detailed examinations of marrow adipocytes than ever before and yielded data that show these cells are critical regulators of the BM microenvironment and whole-body metabolism. Indeed, marrow adipocytes are anatomically and functionally separate from brown, beige, and classic white adipocytes. Thus, areas of BM space populated by adipocytes can be considered distinct fat depots and are collectively referred to as marrow adipose tissue (MAT) in this review. In the proceeding text, we focus on the developmental origin and physiologic functions of MAT. We also discuss the signals that cause the accumulation and loss of marrow adipocytes and the ability of these cells to regulate other cell lineages in the BM. Last, we consider roles for MAT in human physiology and disease. ***(Endocrine Reviews 40: 1187 – 1206, 2019)***

Mammalian bone marrow (BM) is a heterogeneous tissue, located in the medullary canal of the tibia, femur, and humerus, as well as in flat bones such as the sternum and iliac crest. Cell types that constitute BM are thought to be predominantly mesodermal in origin (1, 2). These include myeloid, lymphoid, and erythroid cells, which make up the hematopoietic lineage, as well as adipocytes, osteoblasts, chondrocytes, and marrow stromal cells (stroma). Nerve fibers and vasculature that invade the marrow space compose the remainder of BM cells (1, 2).

Marrow adipose tissue (MAT) has been recognized histologically for more than a century (3). In humans, marrow adipocytes rapidly increase with age and become a prominent component of BM. However, their ability to regulate other cells, either by direct interaction or through the secretion of soluble factors (adipokines), has been largely unexplored. Because MAT resides in bone, it is much more difficult to study than the adipose tissue outside bone. This difficulty contributes to the paucity of data and has prompted the development of new tools to study MAT.

Because of this new effort, it is now recognized that, in addition to age, a variety of conditions [*e.g.*, high-fat diet feeding, peroxisome proliferator–activated receptor (PPAR)γ agonist treatment, and irradiation] can be used to induce BM adipogenesis in mice. These methods, coupled with new transgenic reporter mouse strains and the ability to quantify MAT *in vivo*, have made MAT amenable to experimental analyses that historically have been exceedingly labor intensive or simply not possible. Owing to these advances, the functional distinctions between marrow adipocytes and their white, brown, and beige counterparts are beginning to be understood. It is also clear that MAT is an important endocrine organ that regulates systemic metabolism (4). Indeed, excessive MAT is associated with several conditions marked by metabolic derangement, including obesity, diabetes and, paradoxically, anorexia nervosa (5). Moreover, it is known that in many model

ISSN Print: 0163-769X
ISSN Online: 1945-7189
Printed: in USA

Received: 15 May 2018
Accepted: 3 April 2019
First Published Online: 25 April 2019

ESSENTIAL POINTS

- Marrow adipocytes increase with age and other physiologic cues, representing a major population of bone marrow cells, especially in adult humans
- Bone marrow is unique in that it is the only tissue where adipocytes and bone cells are in close juxtaposition
- Although marrow adipocytes arise from a mesenchymal stem cell, there appears to be a small number of downstream progenitor cells with the capacity to differentiate into mature adipocytes
- Although marrow adipocytes possess some characteristics of white adipocytes, they appear to be a distinct (*i.e.*, fourth) population of fat cells, different from white, brown, and beige adipocytes; thus, bone marrow is a previously unrecognized fat depot
- Marrow adipose tissue is an important endocrine organ that can regulate systemic metabolism
- Marrow adipocytes can have both positive and negative effects on bone density and hematopoiesis, depending on the specific model, by mechanisms that are only partially understood

systems, increased MAT is associated with decreased bone mass and osteoporosis (5–7). A role for the marrow adipocyte lineage in hematopoiesis is emerging as well and may have implications for understanding age-related deficits in bone health and fracture repair (5, 8).

Although largely intractable for many decades, MAT is finally giving up some of its long-held secrets. In this review, we critically evaluate our current understanding of MAT biology, as well as discuss areas of speculation where future work is required. Our working definition of MAT is detailed in the following section.

Marrow adipose tissue

Marrow adipocytes arise from mesenchymal lineage cells within the BM. Mature marrow adipocytes are unilocular and can be found, for the most part, in bones that contain BM. In long bones, marrow adipocytes can be seen just below the growth plate and can extend into the metaphysis and diaphysis. MAT in mice and humans increases with age, and in mice, cell number can be increased by exposure to a group of "stressors" (*e.g.*, x-irradiation, thiazolidinediones, high-fat diet). Increased MAT is also associated with a number of pathophysiologic conditions. Marrow adipocytes secrete adipokines that can function locally, affecting other BM cells, and at distant sites functioning in an endocrine manner (*e.g.*, adiponectin). Marrow adipocytes express numerous characteristics that distinguish them from white, brown, and beige adipocytes.

White, Brown, Beige, and Marrow Adipose Tissues

White adipose tissue

White adipose tissue (WAT) is indispensable for maintaining proper metabolic homeostasis. It is the major hub for lipid stockpiling and distribution, and functions as an endocrine organ to regulate systemic insulin sensitivity (9–13), as well as feeding behavior (14, 15) (Fig. 1). In mice, white adipocytes do not fill with lipid until after birth and are derived predominantly from progenitors that originate in the somites or lateral plate mesoderm (16). White adipocytes contain a single unilocular lipid droplet (17, 18). In humans, the final morphological and functional characteristics of white adipocytes are similar to rodents. However, WAT emerges in the second trimester of gestation rather than postnatally (19). In the adult body WAT is anatomically divided into various spatially distinct depots that are broadly classified as either subcutaneous (just beneath the skin) or visceral (within the abdominal cavity). In women, WAT mass is preferentially distributed subcutaneously around the hips and thighs, whereas men have comparatively reduced subcutaneous WAT and a proportionally similar amount of visceral WAT (VWAT) (20).

Unsurprisingly, sex hormones play a significant role in body fat distribution; indeed, visceral adiposity and subcutaneous adiposity are sometimes referred to as android and gynoid, respectively. Consistent with this terminology, elevated free testosterone is associated with an increased waist-to-hip ratio (a metric for visceral adiposity) in women with obesity (21), and postmenopausal women have an increased proportion of VWAT relative to premenopausal women as measured by dual-energy x-ray absorptiometry (22). Moreover, ovariectomized mice fed a high-fat diet display a male-like pattern of adipogenesis, and male mice treated with estradiol have increased adipogenesis in subcutaneous WAT relative to male animals without estradiol treatment (23). Interestingly, WAT distribution in obesity is a predictor of the metabolic syndrome independent of sex (24, 25), with excessive visceral adiposity more strongly associated with cardiometabolic disease risk than excessive subcutaneous adiposity (26, 27). Thus, WAT distribution

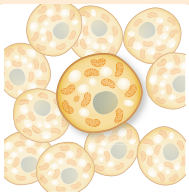
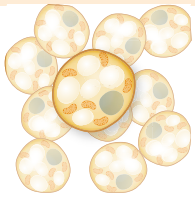
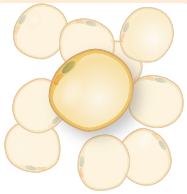
	White adipose	Brown adipose	Beige adipose	Marrow adipose
				
Anatomical				
Location	Subcutaneous: below the skin Visceral: in the abdominal cavity	In mice: intrascapular In human: neck and supraclavicular	In white adipose tissue (IWAT > VWAT)	In bone marrow
Morphology	Unilocular	Multilocular	Multilocular	Unilocular
Lipid storage	+++	+	+	++
Mitochondria	+	+++	+++	++
Fatty acid oxidation	+	+++	+++	+
Expression profile				
UCP1	0	At baseline +++	Activated +++	–
Pgc1α	+	+++	+++	?
Lineage tracing				
Adiponectin	+	+	+	+
PdgfR-α	+	–	+	50%-60%
Myf5+	+ and –	+	?	–
Prx1	asWAT +/– psWAT +++ vWAT –	most –	+++	long bones +++
Modulation				
Aging	↑	↓	↓	↑
High fat diet	↑	↑	↑	↑
Methionine restriction	↓	↓	↑	↑
Cold/β-adrenergic	↓	↑	↑	↓

Figure 1. Phenotypes of white, brown, beige, and marrow adipocytes. A plus sign indicates that the gene/protein is expressed; a minus sign indicates that the gene/protein is not expressed; a plus/minus sign indicates a mixed population of cells; a question mark indicates that expression is unknown; an up arrow indicates increased numbers of cells; and a down arrow indicates decreased numbers of cells. Note that the VWAT includes perigonadal, retroperitoneal, and mesenteric WAT. asWAT, anterior subcutaneous WAT; psWAT, posterior subcutaneous WAT; vWAT, visceral WAT.

is markedly sex-specific, and distinct functional and pathophysiological roles exist for WAT depots.

Brown and beige adipose tissue

Unlike WAT, in mice, brown adipose tissue (BAT) is formed prior to birth and is mainly located in one well-defined depot between the shoulder blades (intrascapular). Brown adipocytes, in contrast to white adipocytes, are multilocular, having multiple small lipid droplets within the cytoplasm. Brown adipocytes have large numbers of mitochondria, facilitating one of their major functions, which is to actively metabolize fatty acids to generate heat (28) (Fig. 1). In humans, BAT is present embryonically and was thought to be lost after childhood and absent in adults. However, within the last few years, BAT has been confirmed to exist in adult humans through modern imaging methods, being located in the supraclavicular region and in the neck, and it can be increased with cold exposure (29, 30). Interscapular BAT is derived from $Myf5^+$ progenitor cells, and brown adipogenesis requires the activity of Ebf2, Prdm16, and BMP7 (18, 30, 31).

Beige adipocytes are similar to brown adipocytes in that they are multilocular, are rich in mitochondria, and metabolize fatty acids (Fig. 1). Unlike BAT, beige adipocyte progenitors are found interspersed within WAT depots, with more in the inguinal WAT (IWAT)

depot than in the visceral depots (32, 33). Although beige progenitor cells are Myf5$^-$ in inguinal fat, similar to brown adipocytes, they can be induced to differentiate *in vivo* by cold exposure or treatment with β-adrenergic agents and feeding mice a methionine-restricted diet (33, 34). Because beige adipocyte precursors can be stimulated to differentiate *in vivo* to mature fatty acid–metabolizing cells, much interest has been focused on this process as a potential therapeutic target to increase energy expenditure and combat obesity. However, beigeing can be detrimental in hypermetabolic states, such as cancer and burns, leading to cachexia [(35–38); for review see (39)].

Marrow adipose tissue

Anatomic distribution and bone and inductive marrow adipogenesis

In the young, BM is rich with hematopoietic, osteogenic, and erythroid cells and is red in appearance. One of the most prominent characteristics of MAT is that it increases with age in humans and mice, resulting in the development of yellow fatty marrow (40, 41). In adult C57BL/6 (B6) mice, a small number of marrow adipocytes can be seen histologically distributed throughout the medullary canal between the tibia–fibular junction and the growth plate. These cells cannot be detected by osmium staining (see "Measurement of mouse MAT *in vivo*" below). In contrast, a small number of marrow adipocytes can be imaged by osmium staining just below the growth plate in 8-week-old mice (42). MAT can also be detected above the growth plate, in the secondary center of ossification at 4 weeks of age.

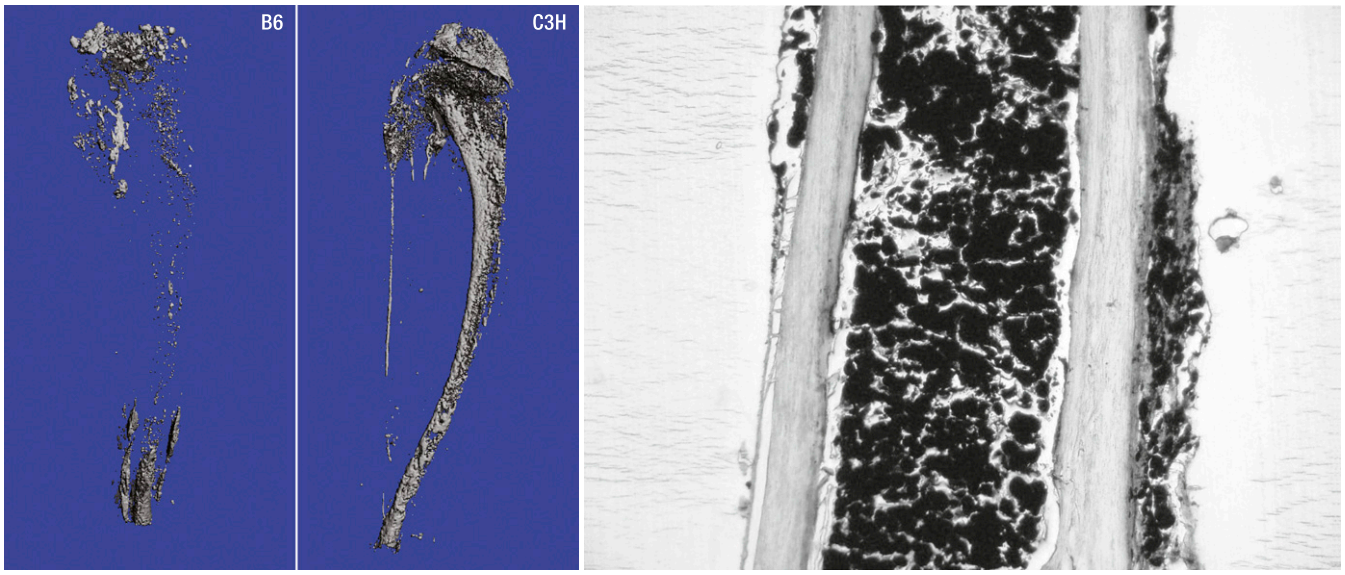
Although MAT does not have the same spatially distinct depots as WAT, marrow adipocytes appear in the distal tibia developmentally well in advance of large numbers of adipocytes in the proximal tibia (42). MAT can be seen in caudal (tail) vertebrae, but not in thoracic or lumbar vertebrae (6). The reason for this uneven distribution in vertebrae is unclear but may relate to temperature differences in the tissue (43). The increase in marrow adipocytes with age correlates with age-related bone loss. This correlation is often cited to indicate that marrow adipocytes are a negative regulator of bone mass. However, this correlation does not uniformly hold. B6 mice have one of the lowest trabecular and cortical bone densities of any mouse strain and very low numbers of marrow adipocytes in their long bones. In contrast, C3H/HeJ (C3H) mice (a distinct strain) have one of the highest bone densities and higher numbers of marrow adipocytes than do B6 mice (44). Interestingly, marrow adipocytes appear in the distal tibia (distal to the tibia–fibular junction extending to the end of the tibia) as early as 4 weeks of age in both B6 and C3H mice and fill the marrow space (42). Whereas few marrow adipocytes can be detected in the proximal tibia of B6 mice, C3H mice have measurable marrow adipocytes in their proximal tibia. The mechanism by which these marrow adipocytes arise is unknown, and a comprehensive analysis of marrow adipocyte distribution by mouse strain remains to be done. Nonetheless, these observations suggest that differentiation of marrow adipocytes is regulated, at least in part, genetically. Therefore, an analysis of MAT using Collaborative Cross mice or Diversity Outbred mice, which are the most genetically diverse mouse resources available, would provide valuable data about the genetics of MAT (45, 46).

Independent of mouse strain, however, marrow adipocytes can be induced in the proximal tibia and femur by a variety of methods. For example, placing mice on 6 weeks of a rosiglitazone (PPARγ agonist)-containing diet potently induces marrow adipogenesis in these bones. Marrow adipocytes extend from below the growth plate, through the metaphysis, and into the diaphysis as a result of this treatment. Increased marrow adipocytes can also be observed above the growth plate in the secondary center of ossification. Eight weeks of a methionine-restricted diet or whole-body x-irradiation are also potent inducers of MAT (8, 47). In contrast to methionine restriction inducing MAT, it causes a marked reduction in IWAT and VWAT mass due to an impaired lipid cycle. Interestingly, although a methionine-restricted diet induces beige adipogenesis with increased mitochondrial biogenesis and amplified expression of uncoupling protein 1 as well, methionine restriction–induced marrow adipocytes do not express uncoupling protein 1, indicating that these cells are not thermogenic in this context (48–51). However, it has been reported recently that treatment of B6 or C3H mice with CL316,243, a β-adrenergic receptor agonist, caused the formation of a small number of multilocular cells in the distal tibia. Nonetheless, these cells did not resemble classic beige adipocytes (52). Further work is required to identify whether marrow adipocytes can take on thermogenic properties *in vivo*.

Measurement of mouse MAT in vivo

Because of the inability to accurately quantitate marrow adiposity in mice, we developed a method to measure MAT *in vivo* (53). In brief, mouse tibiae or femurs are isolated and fixed in formalin overnight. The bones are gently decalcified in EDTA for 20 days, washed, and then stained with osmium tetroxide. Osmium stains lipid in the adipocytes, and because it is a heavy metal, it can be imaged by micro-CT (Fig. 2). Histologic sectioning of osmium-stained bones shows that >95% of the stain is present in marrow adipocytes. Moreover, the amount and position of the marrow adipocytes measured by this method correspond directly with the histology (Fig. 2). This method provides a reproducible, volumetric (three-dimensional, adipocyte volume/total volume) measurement of MAT, including in the distal

Figure 2. (Left) The tibia from B6 or C3H mice. The tibia was isolated, fixed in 10% formalin, and decalcified for 20 d in 4% EDTA. The bones were washed and stained with osmium tetroxide for 48 h, washed, and MAT was imaged by micro-CT. The tibia from B6 mice had low MAT in the proximal tibia with significant MAT visible in the distal tibia and above the growth plate. In contrast, the tibia from C3H mice had extensive MAT throughout the medullary canal. Even the fibula can be imaged in the C3H mice because of MAT filling the marrow space. (Right) A 5-μm thin histologic section of osmium-stained distal tibia (below the tibia–fibular junction) is shown. Marrow adipocytes fill the BM space from endosteum to endosteum.



tibia. Measurements can be obtained above the growth plate in the secondary center of ossification, but because of the variability of structure at this site, comparisons between bones can be difficult.

The BM Adipocyte Lineage

The Cre/Lox system

Modern lineage-tracing experiments invariably depend on the Cre/Lox system (54). It therefore merits a brief description and a word of caution relating to its effective use in defining bona fide cell lineage relationships. In the conventional Cre/Lox system, Cre recombinase is expressed under the control of a tissue-specific promoter to permanently activate a reporter gene that functions to mark the original Cre-expressing cell population and all daughter cells thereof. To accurately interpret any Cre/Lox-based lineage tracing experiment, one must have a thorough understanding of the Cre's spatiotemporal expression domain. Otherwise, erroneous conclusions regarding the tissue of origin may be put forward. Equally important is the nature of the reporter gene used. For example, adipocytes possess little cytoplasm relative to other cell types, and therefore cytoplasmic reporters such as LacZ are suboptimal in this context. Instead, membrane-targeted reporters such as membrane-targeted dTomato (mT)/membrane-targeted eGFP (mG) are preferred (55, 56). For extensive discussions on this topic, see Jeffery *et al.* (57) and Sanchez-Gurmaches *et al.* (58).

Several groups have performed lineage tracing *in vivo* using the fluorescent *mT/mG* reporter mouse in concert with various mouse models driving cre-recombinase from lineage-specific promoters. The expression of cre-recombinase induces the permanent excision of the upstream cassette encoding an mT reporter protein to allow expression of a downstream cassette encoding an mG reporter protein. In this system, cells expressing cre-recombinase "flip" from being dTomato$^+$ to being eGFP$^+$ (Fig. 3). Importantly, expression of eGFP reflects the expression of the gene of interest during the life of the cell and will be maintained in daughter cells (Fig. 3). Therefore, this system allows us to infer progenitor cell lineage (59). In our experiments, BM adipogenesis was induced by either whole-body x-irradiation or feeding mice a rosiglitazone-containing diet. BM plugs were collected from freshly isolated femurs by removing the femoral head, and sliding a 20-gauge needle through the marrow canal from the proximal to the distal end of the femur, and the cellular fluorescence was analyzed by confocal microscopy within hours of isolating the BM. Thus marrow adipogenesis can be analyzed *in vivo*.

BM adipocyte precursor identity

It is generally thought that adipocytes, regardless of type, arise from precursors within their specific depot. In an effort to identify the adult marrow adipocyte

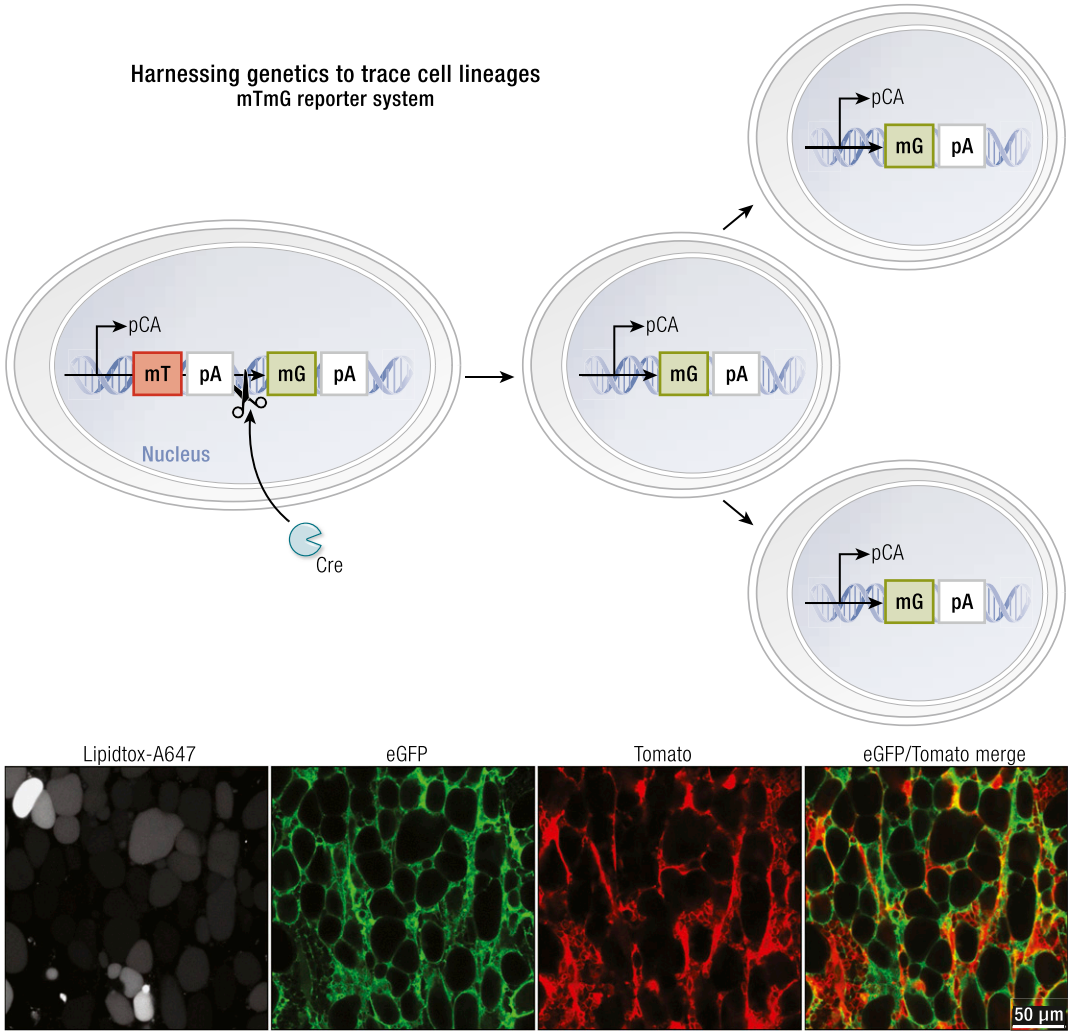


Figure 3. (Top) Schematic of the mT/mG double reporter system. (Bottom) Adiponectin-cre:mT/mG double reporter mice were fed a rosiglitazone-containing diet for 6 wk. The femur was isolated and the femoral head was removed. A 20-gauge needle was slipped down the medullary canal and punched out through the distal epiphysis. The needle was attached to a 5-cc syringe, and the BM was extruded onto a microscope slide. The BM was stained with LipidTox (a lipophilic dye), washed, immersed in Fluoromount, and coverslipped, and fluorescence was viewed by confocal microscopy. LipidTox (left) was used to identify marrow adipocytes. All of the marrow adipocytes were traced with eGFP, (center left), whereas none was with dTomato$^+$ (center right). These data indicate that all marrow adipocytes and their progenitors express adiponectin.

precursor, B6 or *mT/mG* mice were lethally irradiated and reconstituted with either *mT/mG* or B6 BM respectively. Marrow adipocytes in B6 mice reconstituted with *mT/mG* BM showed no fluorescence, whereas marrow adipocytes from irradiated *mT/mG* mice reconstituted with B6 BM were uniformly dTomato$^+$. Marrow adipogenesis could be observed as early as 5 days after irradiation. Importantly, in a separate set of experiments, mice were sub-lethally irradiated and were not reconstituted with BM cells. Tracing of marrow adipocytes was similar to the reconstituted mice. These data suggest that marrow adipocytes arise from a radio-resistant progenitor cell population that is present in adult BM (47).

Hematopoietic stem cells (HSCs) reside in BM, and it is possible that marrow adipocytes arise from these cells, although HSCs are uniquely radiosensitive due to their rapid proliferation. Regardless, it has been reported that WAT can develop from HSCs (60). However, lineage tracing using *Vav1-cre:mT/mG* mice, which traces more than 95% of HSCs and their progeny, failed to mark white adipocytes residing in inguinal subcutaneous WAT (59). Marrow adipocytes induced by a rosiglitazone diet were also not traced using this Vav1-Cre tracing paradigm, indicating that marrow adipocytes do not arise from hematopoietic cells (47).

In mice, brown adipocytes are traced in the *Myf5-cre:mT/mG* system, indicating that brown adipocytes arise from Myf5$^+$ progenitors. In contrast, marrow adipocytes are untraced in the same *Myf5-cre:mT/mG* mice (47). In addition to this apparent developmental difference, mature marrow adipocytes are unilocular whereas brown adipocytes are multilocular. In our experience of examining numerous histologic sections of mouse long bones (tibias, femurs, and humeri) from different mouse models, in which MAT was induced or not induced, we have never observed a multilocular mature marrow adipocyte. Taken together, these data suggest that marrow adipocytes are developmentally and morphologically distinct from brown and beige adipocytes.

Compared with WAT and BAT, less is known about the embryonic origin of MAT. Regardless, it would be surprising if the marrow adipocyte lineages did not follow known developmental trajectories for other BM cell types, at least until the final stages of differentiation. As mentioned previously, marrow adipocytes are closely related to osteoblasts ontogenetically. To explore this relationship, we performed lineage tracing of marrow adipocytes using two mouse reporter stains, which label mesenchymal/osteogenic cells. Paired related homeobox 1 is a protein that in humans is encoded by the *PRRX1* (Prx1) gene. Prx1 expression is restricted to limb bud mesenchyme during development, and Prx1 and Prx2 are expressed in mesenchymal tissue in adult mice (61). In *Prx1-cre:mT/mG* mice that were either irradiated or fed a rosiglitazone diet, all marrow adipocytes were $eGFP^+$, indicating that Prx1 traced all of the marrow adipocytes and that these cells arise from lateral plate mesoderm (similar to most other BM cells) (51). Prx1 has a variable expression pattern in anterior subcutaneous WAT, whereas 96% of adipocytes in posterior WAT, including beige adipocytes, are traced by Prx1. In contrast, Prx1 does not trace most BAT or any VWAT (retroperitoneal WAT, mesenteric WAT, or perigonadal WAT) (62). These data indicate that Prx1 is an early marker of marrow adipocytes. Consistent with the expression of Prx1 in limb bud mesenchyme, cells on bone surfaces, osteocytes embedded deep in bone, and articular cartilage were also traced with Prx1 (data not shown). Because Prx1 expression is more restricted, in whole-body adipose tissue, than many other Cre drivers (*e.g.*, adiponectin-cre), it is useful in adipocyte lineage tracing and gene deletion. Additionally, it has been shown that Osx^+ cells give rise to the osteolineage in developing (embryonic day 13.5) fetal BM (63). Osx can also be detected in osteocalcin (osteoblast marker)-negative BM stromal cells, suggesting that Osx marks nonosteolineage cells as well. In adult BM Osx continues to mark osteolineage cells, but the Osx population of stromal cells is lost. A separate marker, Nestin, labels mesenchymal progenitor cells that are present in developing mouse BM. $Nestin^+$ cells appear to overlap with $CD45^-CD90^-CD51^+CD105^+$ and $Prx1^{lin+}PDGFR\alpha^+Sca1^+$ cell populations (64, 65). Notably, most (85%) of the Osx^+ BM cells are also $nestin^+$ and highly enriched in colony forming units–fibroblasts. This population of cells is able to differentiate to yield osteoblasts and chondrocytes. Importantly, irradiation-induced adipocytes are Osx^+ (63).

In a separate set of experiments, cells expressing the leptin receptor ($LepR^+$) accounted for most colony forming units–fibroblasts (94%). A LepR-Cre traced most marrow adipocytes and osteoblasts in adult BM but not in developing BM (8). Interestingly, the $LepR^{lin+}$ BM population highly overlaps with the Nestin-marked BM population (66). These data suggest that $Nestin^+/LepR^+$ progenitor cells can give rise to marrow adipocytes in adult BM. In addition to these reports, it has been shown that Gremlin1-expressing BM cells, which are capable of self-renewal, can also give rise to osteoblasts, chondrocytes, and marrow stromal cells, but not adipocytes (67). This suggests $Gremlin1^+$ precursors represent a more lineage-restricted cell population than $Nestin^+/LepR^+$ precursors and may be useful for distinguishing adipogenesis-competent cells from incompetent cells in the BM.

Intermittent PTH administration stimulates new bone formation. Although PTH increases the activity of mature osteoblasts, it may also recruit osteoblast–marrow adipocyte progenitor cells into the osteoblast lineage. To evaluate the role of PTH, or its related protein PTHrP, on mesenchymal stem cell fate, *Prx1-cre:PTH1R*$^{fl/fl}$ mice were generated to delete the PTH/PTHrP receptor from $Prx1^+$ mesenchymal progenitor cells (68). These mice had a bone phenotype characterized by increased bone resorption, low bone mass, severely deformed tibias, increased numbers of tartrate-resistant acid phosphatase–positive ($TRAP^+$ osteoclasts) cells, and increased MAT. RT-PCR analysis showed that the marrow adipocytes from the Cre^+ cells but not the Cre^- cells expressed RANKL and are likely responsible for the increased numbers of osteoclasts and bone loss. Some of the $RANKL^+$ cells were also preadipocyte factor-1 $(Pref\text{-}1)^+$. Additionally, PTH treatment reduced marrow adipocyte numbers in humans by 27% after 18 months (68). These data show that the anabolic effect of PTH on bone can be attributed, at least in part, to regulation of osteoblast/adipocyte lineage allocation. In a separate set of experiments, mice were placed on a calorie-restricted or control diet and treated with PTH or vehicle. A calorie-restricted diet induces bone loss and increases MAT in mice and humans (69, 70). In contrast, PTH treatment significantly increased bone mass with increased numbers of osteoblasts in calorie-restricted and control groups and reduced marrow adipocyte number (71). These data are consistent with the experiments deleting the PTH/PTHrP receptor, in that PTH signaling can suppress the development of marrow adipocytes through a mechanism that likely alters lineage allocation away from adipogenesis and toward osteoblastogensis.

PPARγ coactivator 1α (PGC-1α) regulates cell fate allocation and is expressed preferentially in BAT compared with WAT. PGC-1α is highly expressed in mesenchymal stem cells, and its expression decreases with age in mice and humans (72, 73). Pgc1α global knockout mice have decreased femoral bone mineral density (BMD) with an associated loss of osteoblast number and bone formation rate. The bone loss was associated with a marked increase in marrow adipogenesis (74). To determine the role of Pgc-1α on the mesenchymal lineage, *Prx1-cre:Pgc1*$\alpha^{fl/fl}$ mice were used to delete PGC-1α from cells expressing Prx1.

In ovariectomized *Prx1-cre:Pgc-1α*$^{fl/fl}$ mice, BMD and bone volume were decreased relative to sham-operated controls. In the proximal tibia of the *Prx1:Pgc-1α*$^{fl/fl}$ mice, marrow adipocytes extend from just below the growth plate through the metaphysis into the diaphysis as measured by osmium staining. These data suggest that the loss of PGC-1α in mesenchymal precursor cells results in increased bone loss and marrow adipogenesis during ovariectomy-induced osteoporosis. Interestingly, *LepR-cre:Pgc-1α*$^{fl/fl}$ mice had a bone and marrow fat phenotype similar to the *Prx1-cre:Pgc-1α*$^{fl/fl}$ mice. This is important because LepR is expressed on the mesenchymal progenitor cells that give rise to marrow adipocytes and osteoblasts in adult mice (8). Thus, expression of Pgc1α functions to regulate the balance between marrow adipocytes and osteoblast lineage allocation (75).

With menopause there is a decrease in bone mass and a concomitant increase in subcutaneous, visceral, and BM adiposity. Treating these conditions, especially together, has been difficult. During this time FSH levels rise, whereas estrogen levels are relatively stable (76). A polyclonal antibody directed at the β-subunit of Fsh was used *in vivo* to determine the role of FSH in regulating obesity and bone mass. Injection of the anti-FSH antibody in wild-type male and female mice that were on a high-fat diet showed a significant reduction in visceral and subcutaneous adiposity with an increase in lean mass compared with an isotype (IgG) control antibody (77). Antibody-treated mice also had a significant increase in BMD. Using FSH receptor–deficient mice (Fshr$^{+/-}$), the lack of actions of the antibody were observed, confirming the effect of the antibody in Fshr-replete mice. Ovariectomized and sham-operated control mice treated with antibody also showed a reduction in fat mass and an increase in lean mass. Importantly, ovariectomized mice treated with antibody showed a significant decrease in MAT (77). Interestingly, mice placed on a high-fat diet and treated with antibody showed increased numbers of multilocular, Ucp1^{+} staining adipocytes in IWAT, consistent with increased beige adipogenesis. These data provide evidence that interfering with FSH signaling *in vivo* can reduce whole-body WAT, with an increase in metabolically active beige adipocytes, while increasing bone mass. Thus, suppressing FSH may provide a new approach to treat a variety of pathologic conditions associated with high fat.

Distinguishing cells according to cell surface marker profile has also proven to be an effective strategy for identifying adipogenesis-competent BM-derived cells. CD45^{-}CD31^{-}PdgfRα^{+}Sca1^{+} BM cells have been shown to be highly adipogenic but have limited osteochondrogenic potential *in vitro*. In contrast, CD45^{-}CD31^{-}PdgfRα^{+}Sca1^{-} cells do not differentiate into adipocytes *in vitro* but have a strong osteochondrogenic potential. The CD45^{-}CD31^{-}PdgfRα^{+}Sca1^{+} population can be further separated based on CD24 expression. The CD45^{-}CD31^{-}PdgfRα^{+}Sca1^{+}CD24^{+} population is able to differentiate into osteoblasts, chondrocytes, and adipocytes, whereas the CD45^{-}CD31^{-}PdgfRα^{+}Sca1^{+}CD24^{-} population gives rise only to adipocytes (7).

Whereas MAT can be induced by external stressors, it is regulated by the expression of cell-intrinsic molecules. Thy-1 (CD90) is a glycosylphosphatidyl-anchored cell surface protein belonging to the immunoglobulin superfamily and is expressed on mesenchymal stem cells (78, 79). Thy-1 regulates a variety of cell functions, including the balance between proliferation and apoptosis (80). Mesenchymal stem cells from Thy-1–deficient (knockout) mice cultured *in vitro* had decreased osteoblast differentiation with a concomitant increase in marrow adipogenic differentiation compared with wild-type control mice (81). Thy-1$^{-/-}$ mice exhibited decreased bone volume, bone formation rate, and increased cortical porosity, resulting in biomechanically weaker bones. Interestingly, subcutaneous WAT (SWAT), GWAT, and MAT were increased in the Thy-1$^{-/-}$ mice compared with controls. These data suggest that similar to expression of Pgc1α, Thy1 expression can regulate lineage allocation between marrow adipocytes and osteoblasts.

When marrow adipogenesis is stimulated in long bones (*e.g.*, by rosiglitazone feeding), marrow adipocytes form just below the growth plate through the metaphysis and into the diaphysis. Whether marrow adipocyte precursors are concentrated in the primary spongiosa or distributed throughout the BM is unclear. It has been reported that marrow adipocyte precursors are not located on the endosteum but rather in the BM close to the bone surface and perivascularly, being evenly distributed over the metaphysis and diaphysis (7). However, as previously discussed, the identity of these cells remains vaguely defined. In contrast, osteogenic progenitors were found on the endosteum with more in the metaphysis than in the diaphysis (7). This raises the interesting possibility that distinct marrow adipocyte precursor niches exist in bone. In keeping with this idea, mature marrow adipocytes can directly interact by cell-to-cell contact with other BM cells and with the sympathetic nervous system, which may provide another pathway to regulate marrow adipocytes (82).

Taken together, it is apparent that marrow adipocytes are derived from BM-resident mesenchymal progenitor cells. However, a precise lineage hierarchy within BM for marrow adipocytes and the molecular mechanisms by which they differentiate remain unclear. Further investigation is required to determine whether marrow adipocytes arise from a single population or from a small number of different progenitors in the BM. Additionally, the lineage relationships between marrow adipocytes and white, brown, and beige adipocytes has yet to be elucidated.

BM adipocyte metabolism

The intracellular metabolism of marrow adipocytes is largely unknown due to technical challenges associated with their isolation. Nonetheless, the defining feature of these cells is their ability to store lipids as large, unilocular lipid droplets, sometimes referred to as lipid bodies or adiposomes. The presence of these lipid droplets suggests that marrow adipocytes experience similar cellular processes responsible for the storage, mobilization, and metabolism of fatty acids as in other metabolically active adipose depots. Given this defining organelle, any gaps in knowledge relative to marrow adipocyte metabolism can be, to a certain extent, extrapolated from other classic fat depots. This section therefore aims to review these processes and how they likely contribute to BM adipocyte metabolism at a cellular level.

Fatty acid uptake and lipid storage

MAT lipid droplets are composed of a core of neutral lipids, including triacylglycerol (TAG) and cholesteryl esters, surrounded by a phospholipid monolayer that includes embedded proteins (83–85). As such, marrow adipocytes must first accumulate fatty acid substrates that can then be esterified and stored. Similar to other adipose depots, these fatty acids are thought to be acquired either through uptake from the circulation or through *de novo* synthesis from glucose substrates.

Upon entry into the cell, fatty acids destined for storage must be esterified to glycerol or cholesterol via a two-step, ATP-dependent reaction catalyzed by acyl–coenzyme A synthetase (ACS) that also involves ACSL1, ACSL3, and ACSL4 (85). Once activated, these fatty acids can begin to be incorporated into the lipid droplet core. Although the relative amounts of TAGs and cholesteryl esters can vary, TAGs are by far the most predominant lipid found in MAT (83–85). As the neutral lipid core continues to expand, the lipid droplet membrane must be synthesized as well. Aside from the phospholipids, lipid droplet membranes may contain lipid droplet–associated proteins, including the PAT proteins [perilipin A, adipose differentiation-related protein (ADRP), and tail-interacting protein of 47 kDa (TIP47)]. The PAT family of proteins or perilipins consists of PLIN1, PLIN2, PLIN3, PLIN4, and PLIN5 (86). PLIN1 has been identified as a component of marrow adipocytes both through immunohistochemistry and western blot analysis (52). Interestingly, in other adipose depots, it is thought that small lipid droplets are first coated with PLIN2 and PLIN3 (87), and as the droplet enlarges they are replaced by PLIN1 (88); however, other members of the PAT family have yet to be identified on marrow adipocytes. Pertinent to this observation, researchers have often studied MAT by biochemical fractionation owing to their lipid droplet–mediated buoyancy (89). However, it is reasonable to speculate that marrow adipocytes with smaller, denser lipid droplets are not included in this fraction, and therefore are not represented (90). Consequently, this technique is expected to bias data toward large lipid droplets/mature marrow adipocytes, which could confound any interpretation. Therefore, although marrow adipocytes are unique and much remains to be learned about marrow adipocyte lipid droplets, their biogenesis appears to involve common machinery found in classic white adipocytes.

Lipolysis and fatty acid mobilization

Once lipid droplets have accumulated in marrow adipocytes, they are speculated to serve as a source of stored energy or ATP. When cellular energy is in demand, either by the marrow adipocytes themselves or other cells, lipid droplets are presumed to be broken down to free fatty acids via lipolysis. Lipolysis is a generalized term describing the catabolism of lipids via mechanisms involving (i) lipid droplet–associated cytoplasmic lipases, and/or (ii) autophagosome sequestration of neutral lipids and subsequent degradation by the lysosome, termed lipophagy.

The more commonly described lipolysis involves cytoplasmic lipases acting on the lipid droplet in three distinct, sequential steps: (i) adipose triglyceride lipase hydrolyzes TAGs to release a fatty acid, yielding diacylglycerol (DAG) (91); (ii) hormone-sensitive lipase then converts DAG to monoacylglycerol (92), again releasing a fatty acid, while (iii) the last fatty acid is liberated from glycerol via monoglyceride lipase (93). Importantly, note that hormone-sensitive lipase can hydrolyze TAGs as well; however, its activity is markedly higher for DAGs. Until recently, MAT lipolysis has remained underappreciated and rarely discussed. For example, histological analysis of MAT would often shed some light on the size or volume of marrow adipocytes/lipid droplets, but owing to the nature of lipid flux, one could not conclude whether this was due to increased storage of fatty acids or diminished lipolysis. This point is further underscored by the fact that although MAT was recently shown to undergo cytoplasmic lipase-mediated lipolysis, this unique depot appears to be less responsive to β-adrenergic stimulation compared with WAT (52). Therefore, although the process of lipolysis appears to occur in marrow adipocytes similar to other adipose depots, the stimulus and/or responsiveness may be unique to this depot. These data provide an interesting clue about how MAT metabolism is regulated and indicate that continued investigation is required.

Aside from cytoplasmic lipase lipolysis, lipophagy has been shown to be a crucial means by which white adipocytes can mobilize lipids as well. Macroautophagy, referred to hereafter as autophagy, is a multistep cellular process that involves initiation, membrane nucleation, phagophore formation, sequestration, and autophagosome formation, followed by autophagosome–lysosome fusion (94). As such,

"Although the association between MAT and BMD is clear, what is not clear is the mechanism of action."

lipophagy invokes a similar series of steps; however, instead of the indiscriminate engulfment of cytoplasmic organelles, this process selectively targets lipid droplets (95–97). Although the process of lipophagy has been described in white and brown adipocytes, to date, we are unaware of any studies that have investigated lipolysis via lipophagy in MAT; however, this remains an interesting prospect for future studies. Whether cytoplasmic-mediated lipolysis and/or lipophagy are initiated in marrow adipocytes, the end product of free fatty acids and glycerol remains conserved between pathways.

Fatty acid oxidation and ATP generation

Aside from MAT's presumed ability to mobilize fatty acid stores to other cells (*i.e.*, osteoblasts, osteoclasts, and their precursors), marrow adipocytes may also use the fatty acids for their own ATP generation. Similar to the esterification that takes place during lipid droplet biogenesis, fatty acids would first need to be activated in the cytoplasm via ACS, prior to entry into either the mitochondria or peroxisome for β-oxidation (98, 99). More specifically, MAT in both the distal tibia and proximal tibia and femur contains a large proportion of long-chain fatty acids and, therefore, primarily β-oxidation is thought to occur (83, 100). Acetyl–coenzyme A can now enter into the tricarboxylic acid cycle where it undergoes oxidative phosphorylation, generating ATP. This ATP is now capable of suppling the bone with the energy demands required for cellular function and maintenance.

MAT in Humans

Measurement of MAT in humans

Imaging techniques have allowed for the noninvasive quantification of MAT in humans. The imaging methods used to quantify MAT include magnetic resonance spectroscopy (MRS) (101, 102), T1-weighted MRI (103, 104), a modified Dixon method (also known as fat fraction MRI or the water-fat MRI) (105, 106), as well as CT (107, 108). MRS and MRI are the preferred methods for measurement of MAT in humans given that CT imaging involves radiation exposure. These methods all differ slightly in their methods of quantitating MAT. For example, MRS quantification is limited to a specifically demarcated region and may be more technically challenging as compared with T1-weighted MRI, which is a more standard procedure and therefore may be more feasible (105). T1-weighted MRI, alternatively, uses a threshold method to distinguish MAT and therefore is considered a semiquantitative method, as only pixels reaching a given threshold are quantitated as MAT (105). Despite these differences, these methods have been compared with each other as well as to histologic examination of MAT and, importantly, they have been shown to produce highly concordant results. For example, Shen *et al.* (105) compared MAT quantification in the L3 vertebra in postmenopausal women using MRS, T1-weighted MRI, and a modified Dixon method and found strong concordance among the three techniques (0.78 to 0.88). Bredella *et al.* (108) found strong concordance between dual-energy CT and MRS quantification of MAT. Arentsen *et al.* (109) measured vertebral MAT using the Dixon method as well as dual-energy CT in human cadavers (<24 hours postmortem) and found a high concordance between these imaging methods (r = 0.88). They then compared histologic sections taken from the imaged region of interest and found that adipose volume/tissue volume (AV/TV) (the histologic quantification of MAT) was consistent with the imaging methods (r = 0.77 to 0.80) (109). Therefore, all of these methods provide an accurate, noninvasive means of quantifying MAT.

MAT in Healthy Populations

Development

As in mice, at birth, the composition of BM is predominantly red with hematopoietic/osteogenic cells (110). Thereafter, MAT rapidly begins to accumulate in the human skeleton (110), and this accumulation occurs more rapidly in the distal bones as compared with the proximal bones (111). This accumulation occurs during the time of peak bone acquisition and by the age of 25 years, 70% of marrow in humans contains MAT (111). MAT continues to increase with age in both males and females (102, 103, 112, 113), yet after the age of 55 years, although MAT continues to increase steadily in males, it increases dramatically in females, likely due to the estrogen deficiency state characteristic of menopause (114). *In vitro* studies demonstrate that 17β-estradiol decreases PPARγ agonist-induced adipocyte differentiation of human mesenchymal stem cells (115). In murine models, estrogen deficiency, for example through ovariectomy, also enhances adipocyte formation in BM, and estrogen supplementation can prevent increases in marrow adiposity in these estrogen-deficient animals (116). Similarly, in women, marrow AV/TV and marrow adipocyte number increase after menopause, and AV/TV decreases with estrogen replacement (117), thereby demonstrating the effects of estrogen deficiency on MAT accumulation in humans. Levels of endogenously produced estradiol have also been associated with MAT in older adult men, with higher levels of estradiol being associated with lower vertebral MAT (118). Another factor that may influence accumulation of MAT during development is exercise. In young, school-aged children, individuals who participated in 20 minutes of moderate physical activity 3 days per week for 10 weeks had a significant

decrease in femoral MAT as compared with children in the control group whose femoral MAT did not change (119). In mice, exercise reduces not only VWAT but also the accumulation of MAT induced by rosiglitazone feeding and a high-fat diet (120, 121) [for a review, see (122)]. Therefore, systemic factors likely play an important role in the accumulation or loss of MAT throughout human development and the lifespan.

Relationship with BMD

Although MAT replaces red or hematopoietic/osteogenic marrow in the long bones during peak bone acquisition (40, 41), suggesting a positive association between bone mass and MAT, most studies demonstrate an inverse relationship between MAT and BMD, including in children and young individuals. For example, Shen *et al.* (123) demonstrated a significant inverse association between pelvic MAT and pelvic BMD in children 5 to 17 years of age. Similarly, Di Iorgi *et al.* (107) have demonstrated this inverse association between BMD and MAT in individuals between the ages of 15 and 25 years. This inverse association has also been demonstrated in many groups of healthy adults, including white women (18 to 88 years of age) (103) and middle-aged African American and white women and men (38 to 52 years of age) (104). Therefore, the preponderance of data in humans demonstrate that across the age spectrum, in healthy populations, there is a strong inverse association between BMD and MAT. Because MAT has also been associated with measures of decreased bone integrity, including vertebral compression fractures (113), the inverse association between BMD and MAT may have important structural consequences and, importantly, marrow adiposity has been associated with fractures. In postmenopausal women with osteoporosis, vertebral fractures were associated with higher MAT volume and larger adipocytes independent of BMD (124).

The association between MAT and BMD is further strengthened by longitudinal studies demonstrating changes in both BMD and MAT in response to treatments for low bone mass. Treatment of postmenopausal women with teriparatide (recombinant human PTH) for 1 year resulted in decreased vertebral MAT concomitant with increases in lumbar spine BMD (125). Similarly, in postmenopausal women treated with estrogen replacement (see "Development" above), a decrease in AV/TV was observed after 12 months of treatment (117). In contrast, although an *in vivo* rodent model demonstrated a significant decrease in MAT in ovariectomized rats treated with raloxifene (a selective estrogen receptor modulator used in the treatment of osteoporosis), postmenopausal women treated with raloxifene for 2 years did not demonstrate significant changes in marrow adipocyte volume or number (124, 126). Whether the differences observed with raloxifene (125) vs estrogen replacement (117) are due to the time course of treatment (*i.e.*, perhaps changes would have been observed after 12 months of treatment with raloxifene but not at the 2-year time point) or due to the fact that estrogen replacement has been shown to have greater effects on BMD as compared with raloxifene after 12 months of treatment (127), and therefore may have greater effects on MAT, is not known. Pioglitazone, an insulin sensitizer that activates PPARγ, has also been shown to influence levels of MAT (128). In a 12-month study in subjects with obesity with metabolic syndrome, subjects randomized to pioglitazone had a 4.1% increase in femoral MAT and a 1.4% decrease in total hip BMD (128). Importantly, in this study there was a statistically significant correlation between the change in femoral MAT and decrease in hip BMD, strengthening the association between BMD and MAT in humans (128).

Although the association between MAT and BMD is clear, what is not clear is the mechanism of action. One likely possibility is that marrow adipocytes inhibit osteoblast differentiation through effects on lineage allocation. Alternatively, marrow adipocytes could affect mature osteoblast and or osteoclast function. It is also likely that other molecules, yet to be discovered, will have significant effects on the relationship of marrow adipocytes and bone.

"...women and adolescents with anorexia nervosa have more MAT compared with normal-weight controls...."

Composition of MAT

The degree of saturated fatty acid composition (saturation) of MAT has also been examined in a number of studies. In healthy populations, the degree of unsaturated fatty acid composition (unsaturation) has been shown to increase with age (129, 130) and is higher in women as compared with men (129). Importantly, the composition of MAT has been associated with BMD. In MAT at the lumbar spine and iliac crest, lower amounts of unsaturation have been associated with osteopenia and osteoporosis in postmenopausal women (131, 132). Some (133), but not all (132), studies have demonstrated that individuals with fractures have lower proportions of unsaturated lipids as compared with those without fractures. Similarly, in women with anorexia nervosa, a psychiatric disease characterized by low body weight due to self-induced starvation (see "MAT in anorexia nervosa" below), higher estimates of saturation in MAT at the femoral diaphysis are associated with lower BMD (134), suggesting that the composition of MAT may be an important factor contributing to the association between MAT and BMD.

In considering the degree of unsaturation in MAT in humans, it is important to consider the location of MAT measurement. We have shown that in humans, MAT in the distal tibia has an increased degree of unsaturation relative to MAT in the more proximally

located femur (42). This difference in degree of unsaturation at different MAT sites may explain the differences observed in studies looking at the association between MAT composition and metabolic parameters. For example, one study in humans found that a higher degree of unsaturation in MAT from the tibia was associated with increasing insulin resistance (135), whereas other studies examining MAT in the lumbar spine and femur found that those with type 2 diabetes mellitus (DM) had a significantly lower proportion of unsaturated lipids compared with nondiabetics (136, 137). Therefore, in humans MAT may have different metabolic functions depending on its site of accumulation.

MAT in hematopoiesis

Marrow adipocytes are generally considered to be negative regulators of hematopoiesis (6, 7). However, marrow adipocytes have been shown to promote hematopoietic stem cell survival (138) and support the regeneration of hematopoietic stem cells following irradiation or 5-fluorouracil treatment by the secretion of stem cell factor (139). Acute myeloid leukemia is characterized by uncontrolled proliferation of myeloid progenitor cells, resulting in fatal infections due to suppressed differentiation of myeloid and erythroid cells. MAT has been shown to promote the proliferation of tumor cells in acute myeloid leukemia (140), and as tumor cells increase in the BM there is a correlated loss of marrow adipocytes, whereas osteoblast numbers are unaffected (141). This suggests that the effect of tumor is marrow adipocyte specific. *In vitro* culture experiments demonstrate that marrow adipocytes support myeloerythroid differentiation, and administration of PPARγ agonists increased marrow adipogenesis and reduced leukemia progenitor pools.

Marrow adipocytes may also play a role in the progression of other human tumors. Postmenopausal women with breast cancer have more MAT than do age- and body mass index–matched controls, with significant positive associations observed between MAT and tumor size and histologic grade (142). *In vitro* studies have also demonstrated preferential migration of breast cancer cells toward adipocytes isolated from marrow (143). Similarly, in prostate cancer, marrow adipocytes have been shown to promote the invasiveness of prostate cancer cells in bone, in part by driving increased expression of fatty-acid binding protein 4 on tumor cells (144). Therefore, MAT may play a critical role in the development or progression of skeletal metastases in human malignancy.

MAT and Metabolic Disease

MAT in obesity

In women who are overweight/obese, MAT is positively correlated with VWAT, such that individuals with higher VWAT have higher MAT (145). This is in direct contrast to what is observed in women who are underweight, in which VWAT and MAT are not correlated (70) (see "MAT in anorexia nervosa" below). This positive correlation between VWAT and MAT in individuals who are overweight/obese but not in individuals who are lean suggests that MAT may serve different functions depending on an individual's nutritional status. As in healthy individuals, the inverse association between MAT and BMD is also observed in women who are overweight/obese (134, 145). Similarly, in men who are obese, an inverse association between MAT and parameters of bone microarchitecture has been reported (146). Similar to humans, mice fed a high-fat diet develop increased VWAT and SWAT with associated insulin resistance and metabolic syndrome. The mice also develop increased MAT, although the time of onset (12 to 17 weeks) is variable for reasons that are unclear (147, 148).

MAT in diabetes mellitus

Individuals with type 1 DM have a significantly increased risk of low bone mass, impaired bone microarchitecture, and an increased risk of fractures (149, 150). Mouse models of insulin deficiency, including the spontaneous diabetic NOD mouse, and streptozotocin-induced B6 mice (151, 152) demonstrate increased expression of proadipogenic genes, including PPARγ and aP2 (fatty-acid binding protein 4), in long bones concomitant with decreased osteocalcin expression (151, 152). Whereas treatment with a PPARγ antagonist (bisphenol A diglycidyl ether) decreased MAT in the streptozotocin-induced diabetic mice, it did not reverse loss of bone mass (153). In humans, individuals with type 1 DM have comparable amounts of MAT as compared with nondiabetic controls, although MAT is inversely associated with BMD in this population (154). Although MAT did not correlate with glycemic control, as estimated by hemoglobin (HbA1c), in type 1 DM, a significant correlation was observed with serum lipid levels (155). In postmenopausal women with type 2 DM and in women who are morbidly obese with type 2 DM, MAT is also inversely associated with BMD, but in this group, HbA1c levels are positively associated with MAT (136, 137). Therefore, in both type 1 DM and type 2 DM, there are significant associations between MAT and metabolic risk markers, providing evidence of a functional role for MAT in systemic metabolism.

Similarly, in a pediatric population, MAT at the lumbar spine has been positively associated with hepatic fat content in adolescent boys with nonalcoholic fatty liver disease (155), an entity associated with increased metabolic risk. However, in young, healthy individuals between 16 and 25 years of age, an association between femoral MAT and metabolic risk markers, including serum lipids, measures of insulin

resistance, carotid intima-media thickness, waist-to-hip ratio, and blood pressure, was not found (156). In contrast to what has been observed in women who are overweight and obese (145), in this population of young, healthy individuals with a mean body mass index of <24 kg/m^2, an association between VWAT and MAT was not observed (156), suggesting that MAT may be a more important factor in systemic metabolism in individuals who are overweight/obese and/or those with metabolic disease as compared with young, healthy individuals.

In individuals who are morbidly obese, changes in MAT after the significant weight loss associated with Roux-en-Y gastric bypass (RYGB) appear to depend on the presence or absence of type 2 DM, again arguing for a role for MAT in metabolic disease and health. Whereas women with type 2 DM lose MAT after RYGB, in those without type 2 DM, levels of MAT remained stable (157). In individuals who are morbidly obese undergoing sleeve gastrectomy, levels of MAT increased 12 months postsurgery, and changes in MAT and VWAT were positively correlated as compared with individuals undergoing RYGB in whom MAT did not change, despite that both groups lost comparable amounts of weight (158). As the mechanism of weight loss differs in these two surgical procedures, with malabsorption being a component of RYGB, the divergence in changes in MAT postsurgery suggests that factors other than weight loss alone may play an important role in MAT homeostasis.

MAT in anorexia nervosa

On the other end of the weight spectrum, anorexia nervosa is a psychiatric disorder predominantly affecting females characterized by self-induced starvation and low body weight. Individuals with anorexia nervosa have low SWAT and VWAT stores (70). Despite having low SWAT and VWAT compared with normal-weight controls, women and adolescents with anorexia nervosa have more MAT compared with normal-weight controls, as measured by MRI and MRS (70, 159, 160). The findings of these noninvasive studies are substantiated by a morphometric study of BM aspirate and biopsy specimens in 44 individuals with anorexia nervosa (161). Thirty-nine percent of the subjects had hypoplastic or aplastic marrow with an increase in the fat fraction and nearly 50% of subjects had an increase in the proportion of adipocytes (161). Importantly, after recovery from anorexia nervosa, levels of MAT normalize. Abella *et al.* (161) found normalization of the marrow in a subset of subjects who had repeat biopsies after treatment, and using a noninvasive imaging modality (MRS), we have shown that women with a history of anorexia nervosa who have recovered (defined as normal weight and achievement of eumenorrhea) have levels of vertebral MAT that are comparable to normal-weight controls and significantly lower than levels in women with active anorexia nervosa (162).

Potential hormonal determinants of MAT in anorexia nervosa

Estrogen

A number of hormonal adaptations occur in anorexia nervosa to minimize energy expenditure in this state of chronic starvation. Functional hypothalamic amenorrhea, a result of disruption of GnRH secretion (163, 164), is one of those adaptations that allows for a decrease in energy expenditure on the costly process of reproduction during periods of nutritional stress. In postmenopausal women, marrow AV/TV and marrow adipocyte number increase after menopause, and AV/TV decreases with estrogen replacement (117) (see "Development" above). Whether estrogen is also a mediator of elevated MAT in premenopausal populations is not known but is an area of active research.

Leptin

Girls and women with anorexia nervosa have low levels of leptin, an adipokine secreted primarily by SWAT (165, 166). In animal models, subcutaneous or intracerebroventricular administration of leptin decreases marrow adiposity (167–169). In women with anorexia nervosa, leptin levels are inversely associated with vertebral MAT (170), but longitudinal studies are necessary to definitively determine whether low leptin levels are a determinant of MAT in anorexia nervosa.

"BM should be considered a new fat depot and MAT the fourth type of fat."

Cortisol

Hypothalamic–pituitary–adrenal axis activation in states of physiologic stress results in the release of cortisol, and women with anorexia nervosa have higher levels of cortisol than do normal-weight controls (171, 172). *In vitro* studies demonstrate that cortisol may be necessary for marrow adipocyte differentiation (173). The effects of cortisol as a potential determinant of MAT have been evaluated in a study comparing women with anorexia nervosa to women with Cushing syndrome who have frankly elevated cortisol levels. Using quantitative CT, women with anorexia nervosa were found to have higher levels of vertebral adipose tissue compared with healthy controls, whereas the patients with Cushing syndrome had similar levels of marrow adiposity compared with healthy controls (174). In contrast, in a more recent study, patients with Cushing syndrome had higher levels of MAT at the vertebra and femur as compared with healthy controls (175). Therefore, further studies are necessary to evaluate the possible role of cortisol as a determinant of MAT in humans.

IGF-1

Although IGF-1 is secreted by the liver in response to GH, in states of undernutrition, IGF-1 levels are low despite normal or elevated GH levels (176). This state of GH resistance minimizes energy expenditure on growth during periods of undernutrition. In cross-sectional studies, low IGF-1 levels have been associated with elevated levels of vertebral MAT in women with anorexia nervosa (170), and therefore IGF-1 may be a potential determinant of MAT in this population.

Pref-1

Pref-1, a member of the epidermal growth factor family of proteins, is an inhibitor of both adipocyte and osteoblast differentiation (177). Despite that Pref-1 inhibits adipocyte differentiation, circulating levels of Pref-1 have been positively associated with femoral MAT in women with anorexia nervosa and inversely associated with lumbar spine BMD (170). In adolescent girls with anorexia nervosa, Pref-1 levels decrease in response to transdermal estrogen treatment, and this decrease is directly associated with an increase in BMD (178), suggesting that Pref-1 may play a role in mediating changes in BMD observed in response to estrogen replacement. Whether Pref-1 also mediates the changes in MAT observed in response to estrogen therapy in women is unknown.

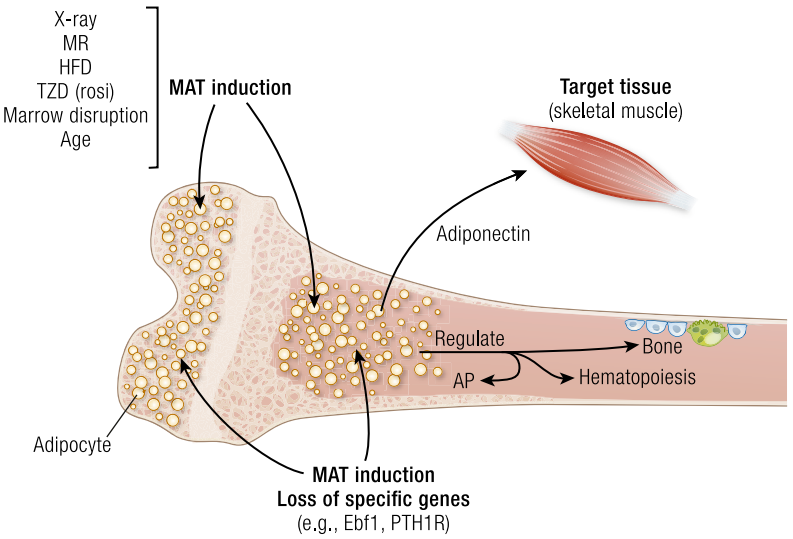


Figure 4. Marrow adipogenesis can be increased in long bones of B6 mice by a variety of external inducers, including x-irradiation, feeding mice a diet containing rosiglitazone, a methionine-restricted diet, or a high-fat diet, or physically disrupting the BM. Alternatively, loss of certain genes [*e.g.*, early B cell factor 1 (Ebf1), PTH/PTHrP receptor (PTH1R)] can also result in increased marrow adipogenesis; however, this occurs in the absence of any exogenous induction. This increased marrow adipogenesis occurs above the growth plate, in the secondary center of ossification, and just below the growth plate, in the intratrabecular spaces. Adipocytes can extend down the medullary canal through the metaphysis and into the diaphysis. Nerves that intercalate the marrow channel can contact these adipocytes directly. Marrow adipocytes may regulate bone cells (osteoblast and osteoclast precursors) as well as hematopoietic cells, either by direct cell contact or the secretion of adipokines in a paracrine manner. It is also possible that mature adipocytes regulate the development of adipocyte precursors in the marrow. Lastly, this ability of marrow adipocytes to regulate other cells is not restricted to BM. Secretion of adipokines, such as adiponectin, can regulate cells outside the BM in an endocrine manner. AP, adipocyte progenitor; HFD, high-fat diet; MR, methionine-restricted diet; rosi, rosiglitazone; TZD, thiazolidinedione; X-ray, x-irradiation.

MAT in lipodystrophy

Lipodystrophy consists of a number of disorders, including both congenital and acquired forms, that are characterized by partial or complete absence or loss of adipose tissue. Congenital generalized lipodystrophy (CGL, also known as Berardinelli–Seip syndrome) is an autosomal recessive form of lipodystrophy with four subtypes. CGL1 is the subtype due to mutations in the gene encoding 1-acyl-gycerol-3-phosphate *O*-acyltransferase 2, an enzyme required for TAG synthesis (179). CGL2 is due to a mutation in the Berardinelli–Seip congenital lipodystrophy 2 gene, whose function remains largely unknown, although it appears to play a role upstream of PPARγ to allow for normal adipogenesis (180). Although there are phenotypic differences between these two subtypes, including the presence of subcutaneous fat in the scalp, retro-orbital space, palms, and soles of the feet in patients with CGL1 but not in patients with CGL2, both subtypes lack MAT (181). Importantly, Simha and Garg (181) suggested that the adipose tissue present in CGL1 patients is "mechanical" (*i.e.*, serves as structural or protective purpose) rather than being metabolically active fat, thus providing support for the concept that MAT is metabolically important in humans. CGL3, due to a mutation in the CAV1 gene, encodes a structural protein present in plasma membrane, is known as caveolin and is characterized by the presence of both MAT and mechanical fat (182). CGL4 is the result of a mutation in polymerase I and transcript release factor, and it is closely associated with CGL3. Because the polymerase I and transcript release factor is important for the formation of caveolae (the invaginations in plasma membrane containing caveolin) (183), it is associated with a much milder metabolic phenotype (183), again suggesting that MAT may be a metabolically important form of adipose tissue.

Patients with HIV often develop a form of acquired lipodystrophy typically associated with their antiretroviral therapy. HIV-associated lipodystrophy is characterized by loss of subcutaneous fat, particularly in the extremities, as well as an increase in VWAT and development of a posterior cervical pad sometimes termed a "buffalo hump" (184, 185). HIV-infected men were found to have significantly lower MAT as compared with noninfected controls, and levels were lowest in those with lipodystrophy (185). Lumbar spine BMD was also significantly lower in HIV-infected men as compared with noninfected controls, suggesting a positive association between BMD and MAT. However, when only subjects in the HIV-infected group were assessed, there was a strong

inverse association between BMD and MAT (186). Therefore, although both BMD and MAT are reduced in HIV-infected males as compared with noninfected controls, the inverse association between BMD and MAT remains intact in this population.

Perspective and Future Directions

Prior to the last 8 to 10 years, the developmental origin and physiologic roles of marrow adipocytes were obscure. However, it is now known that these cells arise from the embryonic mesoderm and, in adult mice, marrow-resident progenitors with proadipogenic properties can be distinguished from other BM cells according to their cell-surface marker profile. Historically, MAT has been observed histologically just below the growth plate in long bones, indicating that progenitor cells reside in these locations. Nonetheless, induction of marrow adipocyte hyperplasia by x-irradiation or rosiglitazone results in MAT formation first in the primary spongiosa, then the metaphysis and, finally, the diaphysis. This suggests that progenitors are located throughout the medullary canal. Alternatively, marrow adipocyte progenitors may only reside in intratrabecular marrow spaces and, upon induction, migrate down the medullary canal before differentiating into mature adipocytes. Moreover, although it is known that at least some progenitors reside in the marrow space, lineage tracing experiments suggest that these cells may also be found on the endosteum. Thus, additional work is required to resolve these points.

It is becoming clear that marrow adipocytes can regulate other cell types (*e.g.*, bone and hematopoietic) in the BM by either direct cell-to-cell contact or the secretion of adipokines (Fig. 4). Whether marrow adipocytes can regulate their own precursors in such ways remains to be shown. Moreover, marrow adipocytes can secrete adipokines that can function on cells and tissues outside the BM in an endocrine manner. Whether this regulation results in a positive or negative effect on the target cells appears to be context-dependent. As an example, in most circumstances, increased MAT correlates with decreased bone mass. Although it is likely that the mechanism involves a shift in lineage allocation away from osteoblast differentiation and toward adipocyte differentiation, it is just as likely that this is not the only mechanism. As such, it is worth a note of caution: consistent with much of the available data, it is difficult to know whether the effects that marrow adipocytes exert on other cells types, particularly in the BM, are caused by marrow adipocytes or are correlated with marrow adipocytes.

It is well documented that MAT forms in response to a variety of external treatments in mice (*i.e.*, x-irradiation, radiomimetic drugs such as 5-fluorouracil, methionine-restricted or high-fat diets) or as a result of aging and disease in humans (*e.g.*, estrogen-deficient osteoporosis and anorexia nervosa). All of these conditions can be viewed as "trauma" to the BM. This suggests that the induction of MAT, in response to these traumas, is part of repairing the damage to the BM. Importantly, the genetic and biochemical pathways leading to increased MAT are unknown. Do these traumas use common pathways, or is the pathway to adipogenesis and repair trauma type–dependent? Certainly, in adult mice, the mechanisms that leads to marrow adipogenesis are suppressed. Loss of specific genes (*e.g.*, Ebf1, Pgc1α, PTH1R, Fsh) and their downstream signaling results in florid marrow adipogenesis. This suggests that marrow adipogenesis is tightly regulated and mediates important functions. This idea is supported by the demonstration that adipogenesis is driven, at least in part, by repression of transcription factors expressed in BM stromal cells (human) that function as brakes on adipogenesis (187). Consequently, the loss of this suppression could account for the age-dependent increase in MAT. Understanding how these conditions induce MAT formation is critical to develop strategies for therapeutically manipulating MAT.

As we have gained a better understanding of MAT, the data show that the origin, development, and function of marrow adipocytes distinguish them from white, brown, and beige adipocytes. Additionally, because of the regulatory activity of these cells, the association with clinically relevant diseases that affect large numbers of people (*i.e.*, osteoporosis and obesity), and their association with bone and BM repair and location within bone, they are unique. As such, BM should be considered a new fat depot and MAT the fourth type of fat.

References and Notes

1. Gregory CA, Prockop DJ, Spees JL. Non-hematopoietic bone marrow stem cells: molecular control of expansion and differentiation. *Exp Cell Res*. 2005;**306**(2):330–335.
2. Bruder SP, Fink DJ, Caplan AI. Mesenchymal stem cells in bone development, bone repair, and skeletal regeneration therapy. *J Cell Biochem*. 1994;**56**(3):283–294.
3. Gimble JM, Robinson CE, Wu X, Kelly KA. The function of adipocytes in the bone marrow stroma: an update. *Bone*. 1996;**19**(5):421–428.
4. Cawthorn WP, Scheller EL, Learman BS, Parlee SD, Simon BR, Mori H, Ning X, Bree AJ, Schell B, Broome DT, Soliman SS, DelProposto JL, Lumeng CN, Mitra A, Pandit SV, Gallagher KA, Miller JD, Krishnan V, Hui SK, Bredella MA, Fazeli PK, Klibanski A, Horowitz MC, Rosen CJ, MacDougald OA. Bone marrow adipose tissue is an endocrine organ that contributes to increased circulating adiponectin during caloric restriction. *Cell Metab*. 2014;**20**(2):368–375.
5. Veldhuis-Vlug AG, Rosen CJ. Clinical implications of bone marrow adiposity. *J Intern Med*. 2018;**283**(2):121–139.

6. Naveiras O, Nardi V, Wenzel PL, Hauschka PV, Fahey F, Daley GQ. Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment. *Nature*. 2009;**460**(7252):259–263.
7. Ambrosi TH, Scialdone A, Graja A, Gohlke S, Jank AM, Bocian C, Woelk L, Fan H, Logan DW, Schürmann A, Saraiva LR, Schulz TJ. Adipocyte accumulation in the bone marrow during obesity and aging impairs stem cell-based hematopoietic and bone regeneration. *Cell Stem Cell*. 2017;**20**(6):771–784.e6.
8. Zhou BO, Yue R, Murphy MM, Peyer JG, Morrison SJ. Leptin-receptor-expressing mesenchymal stromal cells represent the main source of bone formed by adult bone marrow. *Cell Stem Cell*. 2014;**15**(2):154–168.
9. Frayn KN. Adipose tissue as a buffer for daily lipid flux. *Diabetologia*. 2002;**45**(9):1201–1210.
10. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest*. 1995;**95**(5):2409–2415.
11. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science*. 1993;**259**(5091):87–91.
12. Hotta K, Funahashi T, Bodkin NL, Ortmeyer HK, Arita Y, Hansen BC, Matsuzawa Y. Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes*. 2001;**50**(5):1126–1133.
13. Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med*. 2001;**7**(8):947–953.
14. Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG. Identification of targets of leptin action in rat hypothalamus. *J Clin Invest*. 1996;**98**(5):1101–1106.
15. Pinto S, Roseberry AG, Liu H, Diano S, Shanabrough M, Cai X, Friedman JM, Horvath TL. Rapid rewiring of arcuate nucleus feeding circuits by leptin. *Science*. 2004;**304**(5667):110–115.
16. Sebo ZL, Jeffery E, Holtrup B, Rodeheffer MS. A mesodermal fate map for adipose tissue. *Development*. 2018:**145**(17):dev166801.
17. Han J, Lee JE, Jin J, Lim JS, Oh N, Kim K, Chang SI, Shibuya M, Kim H, Koh GY. The spatiotemporal development of adipose tissue. *Development*. 2011;**138**(22):5027–5037.
18. Sanchez-Gurmaches J, Guertin DA. Adipocytes arise from multiple lineages that are heterogeneously and dynamically distributed. *Nat Commun*. 2014;**5**(1):4099.
19. Poissonnet CM, Burdi AR, Garn SM. The chronology of adipose tissue appearance and distribution in the human fetus. *Early Hum Dev*. 1984;**10**(1–2):1–11.
20. Westerbacka J, Cornér A, Tiikkainen M, Tamminen M, Vehkavaara S, Häkkinen AM, Fredriksson J, Yki-Järvinen H. Women and men have similar amounts of liver and intra-abdominal fat, despite more subcutaneous fat in women: implications for sex differences in markers of cardiovascular risk. *Diabetologia*. 2004;**47**(8):1360–1369.
21. Kissebah AH, Krakower GR. Regional adiposity and morbidity. *Physiol Rev*. 1994;**74**(4):761–811.
22. Ley CJ, Lees B, Stevenson JC. Sex- and menopause-associated changes in body-fat distribution. *Am J Clin Nutr*. 1992;**55**(5):950–954.
23. Jeffery E, Wing A, Holtrup B, Sebo Z, Kaplan JL, Saavedra-Peña R, Church CD, Colman L, Berry R, Rodeheffer MS. The adipose tissue microenvironment regulates depot-specific adipogenesis in obesity. *Cell Metab*. 2016;**24**(1):142–150.
24. Hu G, Qiao Q, Tuomilehto J, Balkau B, Borch-Johnsen K, Pyorala K; DECODE Study Group. Prevalence of the metabolic syndrome and its relation to all-cause and cardiovascular mortality in nondiabetic European men and women. *Arch Intern Med*. 2004;**164**(10):1066–1076.
25. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA*. 2002;**287**(3):356–359.
26. Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu CY, Vasan RS, Murabito JM, Meigs JB, Cupples LA, D'Agostino RB Sr, O'Donnell CJ. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. *Circulation*. 2007;**116**(1):39–48.
27. Porter SA, Massaro JM, Hoffmann U, Vasan RS, O'Donnel CJ, Fox CS. Abdominal subcutaneous adipose tissue: a protective fat depot? *Diabetes Care*. 2009;**32**(6):1068–1075.
28. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. *Physiol Rev*. 2004;**84**(1):277–359.
29. Nedergaard J, Bengtsson T, Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. *Am J Physiol Endocrinol Metab*. 2007;**293**(2):E444–E452.
30. Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scimè A, Devarakonda S, Conroe HM, Erdjument-Bromage H, Tempst P, Rudnicki MA, Beier DR, Spiegelman BM. PRDM16 controls a brown fat/skeletal muscle switch. *Nature*. 2008;**454**(7207):961–967.
31. Rajakumari S, Wu J, Ishibashi J, Lim HW, Giang AH, Won KJ, Reed RR, Seale P. EBF2 determines and maintains brown adipocyte identity. *Cell Metab*. 2013;**17**(4):562–574.
32. Wu J, Boström P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaart G, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerbäck S, Schrauwen P, Spiegelman BM. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell*. 2012;**150**(2):366–376.
33. Guerra C, Koza RA, Yamashita H, Walsh K, Kozak LP. Emergence of brown adipocytes in white fat in mice is under genetic control. Effects on body weight and adiposity. *J Clin Invest*. 1998;**102**(2):412–420.
34. Patil YN, Dille KN, Burk DH, Cortez CC, Gettys TW. Cellular and molecular remodeling of inguinal adipose tissue mitochondria by dietary methionine restriction. *J Nutr Biochem*. 2015;**26**(11):1235–1247.
35. Petruzzelli M, Schweiger M, Schreiber R, Campos-Olivas R, Tsoli M, Allen J, Swarbrick M, Rose-John S, Rincon M, Robertson G, Zechner R, Wagner EF. A switch from white to brown fat increases energy expenditure in cancer-associated cachexia. *Cell Metab*. 2014;**20**(3):433–447.
36. Kir S, White JP, Kleiner S, Kazak L, Cohen P, Baracos VE, Spiegelman BM. Tumour-derived PTH-related protein triggers adipose tissue browning and cancer cachexia. *Nature*. 2014;**513**(7516):100–104.
37. Patsouris D, Qi P, Abdullahi A, Stanojcic M, Chen P, Parousis A, Amini-Nik S, Jeschke MG. Burn induces browning of the subcutaneous white adipose tissue in mice and humans. *Cell Reports*. 2015;**13**(8):1538–1544.
38. Sidossis LS, Porter C, Saraf MK, Børsheim E, Radhakrishnan RS, Chao T, Ali A, Chondronikola M, Mlcak R, Finnerty CC, Hawkins HK, Toliver-Kinsky T, Herndon DN. Browning of subcutaneous white adipose tissue in humans after severe adrenergic stress. *Cell Metab*. 2015;**22**(2):219–227.
39. Abdullahi A, Jeschke MG. Taming the flames: targeting white adipose tissue browning in hypermetabolic conditions. *Endocr Rev*. 2017;**38**(6):538–549.
40. Moerman EJ, Teng K, Lipschitz DA, Lecka-Czernik B. Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stroma/stem cells: the role of PPAR-γ2 transcription factor and TGF-β/BMP signaling pathways. *Aging Cell*. 2004;**3**(6):379–389.
41. Moore SG, Dawson KL. Red and yellow marrow in the femur: age-related changes in appearance at MR imaging. *Radiology*. 1990;**175**(1):219–223.
42. Scheller EL, Doucette CR, Learman BS, Cawthorn WP, Khandaker S, Schell B, Wu B, Ding SY, Bredella MA, Fazeli PK, Khoury B, Jepsen KJ, Pilch PF, Klibanski A, Rosen CJ, MacDougald OA. Region-specific variation in the properties of skeletal adipocytes reveals regulated and constitutive marrow adipose tissues (published correction appears in *Nat Commun*. 2016;**7**:13775). *Nat Commun*. 2015;**6**(1):7808.
43. Tavassoli M, Watson LR, Khademi R. Retention of hemopoiesis in tail vertebrae of newborn rats. *Cell Tissue Res*. 1979;**200**(2):215–222.
44. Beamer WG, Donahue LR, Rosen CJ, Baylink DJ. Genetic variability in adult bone density among inbred strains of mice. *Bone*. 1996;**18**(5):397–403.
45. Chesler EJ, Miller DR, Branstetter LR, Galloway LD, Jackson BL, Philip VM, Voy BH, Culiat CT, Threadgill DW, Williams RW, Churchill GA, Johnson DK, Manly KF. The Collaborative Cross at Oak Ridge National Laboratory: developing a powerful resource for systems genetics. *Mamm Genome*. 2008;**19**(6):382–389.
46. Svenson KL, Gatti DM, Valdar W, Welsh CE, Cheng R, Chesler EJ, Palmer AA, McMillan L, Churchill GA. High-resolution genetic mapping using the Mouse Diversity outbred population. *Genetics*. 2012;**190**(2):437–447.
47. Berry R, Rodeheffer MS, Rosen CJ, Horowitz MC. Adipose tissue-residing progenitors (adipocyte lineage progenitors and adipose-derived stem cells (ADSC)). *Curr Mol Biol Rep*. 2015;**1**(3):101–109.
48. Perrone CE, Mattocks DA, Hristopoulos G, Plummer JD, Krajcik RA, Orentreich N. Methionine restriction effects on 11β-HSD1 activity and lipogenic/lipolytic balance in F344 rat adipose tissue. *J Lipid Res*. 2008;**49**(1):12–23.
49. Perrone CE, Mattocks DA, Jarvis-Morar M, Plummer JD, Orentreich N. Methionine restriction effects on mitochondrial biogenesis and aerobic capacity in white adipose tissue, liver, and skeletal muscle of F344 rats. *Metabolism*. 2010;**59**(7):1000–1011.
50. Hasek BE, Stewart LK, Henagan TM, Boudreau A, Lenard NR, Black C, Shin J, Huypens P, Malloy VL, Plaisance EP, Krajcik RA, Orentreich N, Gettys TW. Dietary methionine restriction enhances metabolic flexibility and increases uncoupled respiration in both fed and fasted states. *Am J Physiol Regul Integr Comp Physiol*. 2010;**299**(3):R728–R739.
51. Horowitz MC, Berry R, Holtrup B, Sebo Z, Nelson T, Fretz JA, Lindskog D, Kaplan JL, Ables G, Rodeheffer MS, Rosen CJ. Bone marrow adipocytes. *Adipocyte*. 2017;**6**(3):193–204.
52. Scheller EL, Khandaker S, Learman BS, Cawthorn WP, Anderson LM, Pham HA, Robles H, Wang Z, Li Z, Parlee SD, Simon BR, Mori H, Bree AJ, Craft CS, MacDougald OA. Bone marrow adipocytes resist lipolysis and remodeling in response to β-adrenergic stimulation. *Bone*. 2019;**118**:32–41.

53. Scheller EL, Troiano N, Vanhoutan JN, Bouxsein MA, Fretz JA, Xi Y, Nelson T, Katz G, Berry R, Church CD, Doucette CR, Rodeheffer MS, Macdougald OA, Rosen CJ, Horowitz MC. Use of osmium tetroxide staining with microcomputerized tomography to visualize and quantify bone marrow adipose tissue in vivo. *Methods Enzymol*. 2014;**537**:123–139.
54. Kretzschmar K, Watt FM. Lineage tracing. *Cell*. 2012; **148**(1–2):33–45.
55. Soriano P. Generalized *lacZ* expression with the ROSA26 Cre reporter strain. *Nat Genet*. 1999;**21**(1): 70–71.
56. Muzumdar MD, Tasic B, Miyamichi K, Li L, Luo L. A global double-fluorescent Cre reporter mouse. *Genesis*. 2007;**45**(9):593–605.
57. Jeffery E, Berry R, Church CD, Yu S, Shook BA, Horsley V, Rosen ED, Rodeheffer MS. Characterization of Cre recombinase models for the study of adipose tissue. *Adipocyte*. 2014;**3**(3):206–211.
58. Sanchez-Gurmaches J, Hung CM, Guertin DA. Emerging complexities in adipocyte origins and identity. *Trends Cell Biol*. 2016;**26**(5):313–326.
59. Berry R, Rodeheffer MS. Characterization of the adipocyte cellular lineage in vivo. *Nat Cell Biol*. 2013; **15**(3):302–308.
60. Majka SM, Fox KE, Psilas JC, Helm KM, Childs CR, Acosta AS, Janssen RC, Friedman JE, Woessner BT, Shade TR, Varella-Garcia M, Klemm DJ. De novo generation of white adipocytes from the myeloid lineage via mesenchymal intermediates is age, adipose depot, and gender specific. *Proc Natl Acad Sci USA*. 2010;**107**(33):14781–14786.
61. Logan M, Martin JF, Nagy A, Lobe C, Olson EN, Tabin CJ. Expression of Cre recombinase in the developing mouse limb bud driven by a *Prxl* enhancer. *Genesis*. 2002;**33**(2):77–80.
62. Sanchez-Gurmaches J, Hsiao WY, Guertin DA. Highly selective in vivo labeling of subcutaneous white adipocyte precursors with Prx1-Cre. *Stem Cell Reports*. 2015;**4**(4):541–550.
63. Mizoguchi T, Pinho S, Ahmed J, Kunisaki Y, Hanoun M, Mendelson A, Ono N, Kronenberg HM, Frenette PS. Osterix marks distinct waves of primitive and definitive stromal progenitors during bone marrow development. *Dev Cell*. 2014;**29**(3):340–349.
64. Chan CK, Chen CC, Luppen CA, Kim JB, DeBoer AT, Wei K, Helms JA, Kuo CJ, Kraft DL, Weissman IL. Endochondral ossification is required for haematopoietic stem-cell niche formation. *Nature*. 2009; **457**(7228):490–494.
65. Greenbaum A, Hsu YM, Day RB, Schuettpelz LG, Christopher MJ, Borgerding JN, Nagasawa T, Link DC. CXCL12 in early mesenchymal progenitors is required for haematopoietic stem-cell maintenance. *Nature*. 2013;**495**(7440):227–230.
66. Pinho S, Lacombe J, Hanoun M, Mizoguchi T, Bruns I, Kunisaki Y, Frenette PS. PDGFRα and CD51 mark human Nestin^{+} sphere-forming mesenchymal stem cells capable of hematopoietic progenitor cell expansion. *J Exp Med*. 2013;**210**(7):1351–1367.
67. Worthley DL, Churchill M, Compton JT, Tailor Y, Rao M, Si Y, Levin D, Schwartz MG, Uygur A, Hayakawa Y, Gross S, Renz BW, Setlik W, Martinez AN, Chen X, Nizami S, Lee HG, Kang HP, Caldwell JM, Asfaha S, Westphalen CB, Graham T, Jin G, Nagar K, Wang H, Kheirbek MA, Kolhe A, Carpenter J, Glaire M, Nair A, Renders S, Manieri N, Muthupalani S, Fox JG, Reichert M, Giraud AS, Schwabe RF, Pradere JP, Walton K, Prakash A, Gumucio D, Rustgi AK, Stappenbeck TS, Friedman RA, Gershon MD, Sims P, Grikscheit T, Lee FY, Karsenty G, Mukherjee S, Wang TC. Gremlin 1 identifies a skeletal stem cell with bone, cartilage, and reticular stromal potential. *Cell*. 2015;**160**(1–2): 269–284.
68. Fan Y, Hanai JI, Le PT, Bi R, Maridas D, DeMambro V, Figueroa CA, Kir S, Zhou X, Mannstadt M, Baron R, Bronson RT, Horowitz MC, Wu JY, Bilezikian JP, Dempster DW, Rosen CJ, Lanske B. Parathyroid hormone directs bone marrow mesenchymal cell fate. *Cell Metab*. 2017;**25**(3):661–672.
69. Fazeli PK, Klibanski A. Anorexia nervosa and bone metabolism. *Bone*. 2014;**66**:39–45.
70. Bredella MA, Fazeli PK, Miller KK, Misra M, Torriani M, Thomas BJ, Ghomi RH, Rosen CJ, Klibanski A. Increased bone marrow fat in anorexia nervosa. *J Clin Endocrinol Metab*. 2009;**94**(6):2129–2136.
71. Maridas DE, Rendina-Ruedy E, Helderman RC, DeMambro VE, Brooks D, Guntur AR, Lanske B, Bouxsein ML, Rosen CJ. Progenitor recruitment and adipogenic lipolysis contribute to the anabolic actions of parathyroid hormone on the skeleton. *FASEB J*. 2019;**33**(2):2885–2898.
72. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell*. 1998;**92**(6):829–839.
73. Lin J, Handschin C, Spiegelman BM. Metabolic control through the PGC-1 family of transcription coactivators. *Cell Metab*. 2005;**1**(6):361–370.
74. Yu B, Huo L, Liu Y, Deng P, Szymanski J, Li J, Luo X, Hong C, Lin J, Wang CY. PGC-1α controls skeletal stem cell fate and bone-fat balance in osteoporosis and skeletal aging by inducing TAZ (published correction appears in *Cell Stem Cell*. 2018;**23**(4): 615–623). *Cell Stem Cell*. 2018;**23**(2):193–209.e5.
75. Horowitz MC, Tommasini SM. Fat and bone: PGC-1α regulates mesenchymal cell fate during aging and osteoporosis. *Cell Stem Cell*. 2018;**23**(2):151–153.
76. Randolph JF Jr, Crawford S, Dennerstein L, Cain K, Harlow SD, Little R, Mitchell ES, Nan B, Taffe J, Yosef M. The value of follicle-stimulating hormone concentration and clinical findings as markers of the late menopausal transition. *J Clin Endocrinol Metab*. 2006;**91**(8):3034–3040.
77. Liu P, Ji Y, Yuen T, Rendina-Ruedy E, DeMambro VE, Dhawan S, Abu-Amer W, Izadmehr S, Zhou B, Shin AC, Latif R, Thangeswaran P, Gupta A, Li J, Shnayder V, Robinson ST, Yu YE, Zhang X, Yang F, Lu P, Zhou Y, Zhu L-L, Oberlin DJ, Davies TF, Reagan MR, Brown A, Kumar TR, Epstein S, Iqbal J, Avadhani NG, New MI, Molina H, van Klinken JB, Guo EX, Buettner C, Haider S, Bian Z, Sun L, Rosen CJ, Zaidi M. Blocking FSH induces thermogenic adipose tissue and reduces body fat. *Nature*. 2017;**546**(7656):107–112.
78. Craig W, Kay R, Cutler RL, Lansdorp PM. Expression of Thy-1 on human hematopoietic progenitor cells. *J Exp Med*. 1993;**177**(5):1331–1342.
79. Crawford JM, Barton RW. Thy-1 glycoprotein: structure, distribution, and ontogeny. *Lab Invest*. 1986;**54**(2):122–135.
80. Schmidt M, Gutknecht D, Simon JC, Schulz JN, Eckes B, Anderegg U, Saalbach A. Controlling the balance of fibroblast proliferation and differentiation: impact of Thy-1. *J Invest Dermatol*. 2015;**135**(7): 1893–1902.
81. Picke AK, Campbell GM, Vluher M, Krugel U, Schmidt FN, Tsourdi E, Winzer M, Rauner M, Vukicevic V, Busse B, Salbach-Hirsch J, Tuckermann JP, Simon JC, Anderegg U, Hofbauer LC, Saalbach A. Thy-1 (CD90) promotes bone formation and protects against obesity. *Sci Transl Med*. 2018; **10**(453):eaao6806.
82. Robles H, Park S, Joens MS, Fitzpatrick JAJ, Craft CS, Scheller EL. Characterization of the bone marrow adipocyte niche with three-dimensional electron microscopy. *Bone*. 2019;**118**:89–98.
83. Ren J, Dimitrov I, Sherry AD, Malloy CR. Composition of adipose tissue and marrow fat in humans by ^{1}H NMR at 7 Tesla. *J Lipid Res*. 2008;**49**(9): 2055–2062.
84. Miranda M, Pino AM, Fuenzalida K, Rosen CJ, Seitz G, Rodríguez JP. Characterization of fatty acid composition in bone marrow fluid from postmenopausal women: modification after hip fracture. *J Cell Biochem*. 2016;**117**(10):2370–2376.
85. Ellis JM, Frahm JL, Li LO, Coleman RA. Acyl-coenzyme A synthetases in metabolic control. *Curr Opin Lipidol*. 2010;**21**(3):212–217.
86. Itabe H, Yamaguchi T, Nimura S, Sasabe N. Perilipins: a diversity of intracellular lipid droplet proteins. *Lipids Health Dis*. 2017;**16**(1):83.
87. Brasaemle DL, Barber T, Wolins NE, Serrero G, Blanchette-Mackie EJ, Londos C. Adipose differentiation-related protein is an ubiquitously expressed lipid storage droplet-associated protein. *J Lipid Res*. 1997; **38**(11):2249–2263.
88. Blanchette-Mackie EJ, Dwyer NK, Barber T, Coxey RA, Takeda T, Rondinone CM, Theodorakis JL, Greenberg AS, Londos C. Perilipin is located on the surface layer of intracellular lipid droplets in adipocytes. *J Lipid Res*. 1995;**36**(6):1211–1226.
89. Weller PF, Monahan-Earley RA, Dvorak HF, Dvorak AM. Cytoplasmic lipid bodies of human eosinophils. Subcellular isolation and analysis of arachidonate incorporation. *Am J Pathol*. 1991;**138**(1): 141–148.
90. Bartholomew SR, Bell EH, Summerfield T, Newman LC, Miller EL, Patterson B, Niday ZP, Ackerman WE IV, Tansey JT. Distinct cellular pools of perilipin 5 point to roles in lipid trafficking. *Biochim Biophys Acta*. 2012;**1821**(2):268–278.
91. Zimmermann R, Strauss JG, Haemmerle G, Schoiswohl G, Birner-Gruenberger R, Riederer M, Lass A, Neuberger G, Eisenhaber F, Hermetter A, Zechner R. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science*. 2004;**306**(5700): 1383–1386.
92. Kraemer FB, Shen WJ. Hormone-sensitive lipase: control of intracellular tri-(di-)acylglycerol and cholesteryl ester hydrolysis. *J Lipid Res*. 2002;**43**(10): 1585–1594.
93. Fredrikson G, Tornqvist H, Belfrage P. Hormone-sensitive lipase and monoacylglycerol lipase are both required for complete degradation of adipocyte triacylglycerol. *Biochim Biophys Acta*. 1986; **876**(2):288–293.
94. Yang Z, Klionsky DJ. Mammalian autophagy: core molecular machinery and signaling regulation. *Curr Opin Cell Biol*. 2010;**22**(2):124–131.
95. Singh R, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, Tanaka K, Cuervo AM, Czaja MJ. Autophagy regulates lipid metabolism. *Nature*. 2009;**458**(7242):1131–1135.
96. Liu K, Czaja MJ. Regulation of lipid stores and metabolism by lipophagy. *Cell Death Differ*. 2013; **20**(1):3–11.
97. Singh R, Cuervo AM. Lipophagy: connecting autophagy and lipid metabolism. *Int J Cell Biol*. 2012; **2012**:282041.
98. Wakil SJ, Abu-Elheiga LA. Fatty acid metabolism: target for metabolic syndrome. *J Lipid Res*. 2009; **50**(Suppl):S138–S143.
99. Lodhi IJ, Semenkovich CF. Peroxisomes: a nexus for lipid metabolism and cellular signaling. *Cell Metab*. 2014;**19**(3):380–392.
100. Houten SM, Wanders RJ. A general introduction to the biochemistry of mitochondrial fatty acid β--oxidation. *J Inherit Metab Dis*. 2010;**33**(5):469–477.

101. Schick F, Einsele H, Bongers H, Jung WI, Skalej M, Duda S, Ehninger G, Lutz O. Leukemic red bone marrow changes assessed by magnetic resonance imaging and localized ^{1}H spectroscopy. *Ann Hematol*. 1993;**66**(1):3–13.
102. Schellinger D, Lin CS, Fertikh D, Lee JS, Lauerman WC, Henderson F, Davis B. Normal lumbar vertebrae: anatomic, age, and sex variance in subjects at proton MR spectroscopy—initial experience. *Radiology*. 2000;**215**(3):910–916.
103. Shen W, Chen J, Punyanitya M, Shapses S, Heshka S, Heymsfield SB. MRI-measured bone marrow adipose tissue is inversely related to DXA-measured bone mineral in Caucasian women. *Osteoporos Int*. 2007;**18**(5):641–647.
104. Shen W, Scherzer R, Gantz M, Chen J, Punyanitya M, Lewis CE, Grunfeld C. Relationship between MRI-measured bone marrow adipose tissue and hip and spine bone mineral density in African-American and Caucasian participants: the CARDIA study. *J Clin Endocrinol Metab*. 2012;**97**(4):1337–1346.
105. Shen W, Gong X, Weiss J, Jin Y. Comparison among T1-weighted magnetic resonance imaging, modified dixon method, and magnetic resonance spectroscopy in measuring bone marrow fat. *J Obes*. 2013;**2013**:298675.
106. Ojanen X, Borra RJ, Havu M, Cheng SM, Parkkola R, Nuutila P, Alen M, Cheng S. Comparison of vertebral bone marrow fat assessed by ^{1}H MRS and inphase and out-of-phase MRI among family members. *Osteoporos Int*. 2014;**25**(2):653–662.
107. Di Iorgi N, Rosol M, Mittelman SD, Gilsanz V. Reciprocal relation between marrow adiposity and the amount of bone in the axial and appendicular skeleton of young adults. *J Clin Endocrinol Metab*. 2008;**93**(6):2281–2286.
108. Bredella MA, Daley SM, Kalra MK, Brown JK, Miller KK, Torriani M. Marrow adipose tissue quantification of the lumbar spine by using dual-energy CT and single-voxel ^{1}H MR spectroscopy: a feasibility study. *Radiology*. 2015;**277**(1):230–235.
109. Arentsen L, Yagi M, Takahashi Y, Bolan PJ, White M, Yee D, Hui S. Validation of marrow fat assessment using noninvasive imaging with histologic examination of human bone samples. *Bone*. 2015;**72**:118–122.
110. Custer RP. Studies on the structure and function of bone marrow. Part I. *J Lab Clin Med*. 1932;**17**:951–960.
111. Kricun ME. Red-yellow marrow conversion: its effect on the location of some solitary bone lesions. *Skeletal Radiol*. 1985;**14**(1):10–19.
112. Justesen J, Stenderup K, Ebbesen EN, Mosekilde L, Steiniche T, Kassem M. Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis. *Biogerontology*. 2001;**2**(3):165–171.
113. Schellinger D, Lin CS, Hatipoglu HG, Fertikh D. Potential value of vertebral proton MR spectroscopy in determining bone weakness. *AJNR Am J Neuroradiol*. 2001;**22**(8):1620–1627.
114. Griffith JF, Yeung DK, Ma HT, Leung JC, Kwok TC, Leung PC. Bone marrow fat content in the elderly: a reversal of sex difference seen in younger subjects. *J Magn Reson Imaging*. 2012;**36**(1):225–230.
115. Benvenuti S, Cellai I, Luciani P, Deledda C, Saccardi R, Mazzanti B, Dal Pozzo S, Serio M, Peri A. Androgens and estrogens prevent rosiglitazone-induced adipogenesis in human mesenchymal stem cells. *J Endocrinol Invest*. 2012;**35**(4):365–371.
116. Elbaz A, Rivas D, Duque G. Effect of estrogens on bone marrow adipogenesis and Sirt1 in aging C57BL/6J mice. *Biogerontology*. 2009;**10**(6):747–755.
117. Syed FA, Oursler MJ, Hefferanm TE, Peterson JM, Riggs BL, Khosla S. Effects of estrogen therapy on bone marrow adipocytes in postmenopausal osteoporotic women. *Osteoporos Int*. 2008;**19**(9):1323–1330.
118. Mistry SD, Woods GN, Sigurdsson S, Ewing SK, Hue TF, Eiriksdottir G, Xu K, Hilton JF, Kado DM, Gudnason V, Harris TB, Rosen CJ, Lang TF, Li X, Schwartz AV. Sex hormones are negatively associated with vertebral bone marrow fat. *Bone*. 2018;**108**:20–24.
119. Casazza K, Hanks LJ, Hidalgo B, Hu HH, Affuso O. Short-term physical activity intervention decreases femoral bone marrow adipose tissue in young children: a pilot study. *Bone*. 2012;**50**(1):23–27.
120. Pagnotti GM, Styner M. Exercise regulation of marrow adipose tissue. *Front Endocrinol (Lausanne)*. 2016;**7**:94.
121. Styner M, Thompson WR, Galior K, Uzer G, Wu X, Kadari S, Case N, Xie Z, Sen B, Romaine A, Pagnotti GM, Rubin CT, Styner MA, Horowitz MC, Rubin J. Bone marrow fat accumulation accelerated by high fat diet is suppressed by exercise. *Bone*. 2014;**64**:39–46.
122. Patel VS, Ete Chan M, Rubin J, Rubin CT. Marrow adiposity and hematopoiesis in aging and obesity: exercise as an intervention. *Curr Osteoporos Rep*. 2018;**16**(2):105–115.
123. Shen W, Velasquez G, Chen J, Jin Y, Heymsfield SB, Gallagher D, Pi-Sunyer FX. Comparison of the relationship between bone marrow adipose tissue and volumetric bone mineral density in children and adults. *J Clin Densitom*. 2014;**17**(1):163–169.
124. Beekman KM, Veldhuis-Vlug AG, den Heijer M, Maas M, Oleksik AM, Tanck MW, Ott SM, van 't Hof RJ, Lips P, Bisschop PH, Bravenboer N. The effect of raloxifene on bone marrow adipose tissue and bone turnover in postmenopausal women with osteoporosis. *Bone*. 2019;**118**:62–68.
125. Yang Y, Luo X, Xie X, Yan F, Chen G, Zhao W, Jiang Z, Fang C, Shen J. Influences of teriparatide administration on marrow fat content in postmenopausal osteopenic women using MR spectroscopy. *Climacteric*. 2016;**19**(3):285–291.
126. Somjen D, Katzburg S, Kohen F, Gayer B, Posner GH, Yoles I, Livne E. The effects of native and synthetic estrogenic compounds as well as vitamin D less-calcemic analogs on adipocytes content in rat bone marrow. *J Endocrinol Invest*. 2011;**34**(2):106–110.
127. Dane C, Dane B, Cetin A, Erginbas M. Comparison of the effects of raloxifene and low-dose hormone replacement therapy on bone mineral density and bone turnover in the treatment of postmenopausal osteoporosis. *Gynecol Endocrinol*. 2007;**23**(7):398–403.
128. Pop LM, Lingvay I, Yuan Q, Li X, Adams-Huet B, Maalouf NM. Impact of pioglitazone on bone mineral density and bone marrow fat content. *Osteoporos Int*. 2017;**28**(11):3261–3269.
129. Maciel JG, de Araujo IM, Carvalho AL, Simao MN, Bastos CM, Troncon LE, Salmon CE, de Paula FJ, Nogueira-Barbosa MH. Marrow fat quality differences by sex in healthy adults. *J Clin Densitom*. 2017;**20**(1):106–113.
130. Huovinen V, Viljakainen H, Hakkarainen A, Saukkonen T, Toiviainen-Salo S, Lundbom N, Lundbom J, Mäkitie O. Bone marrow fat unsaturation in young adults is not affected by present or childhood obesity, but increases with age: a pilot study. *Metabolism*. 2015;**64**(11):1574–1581.
131. Yeung DK, Griffith JF, Antonio GE, Lee FK, Woo J, Leung PC. Osteoporosis is associated with increased marrow fat content and decreased marrow fat unsaturation: a proton MR spectroscopy study. *J Magn Reson Imaging*. 2005;**22**(2):279–285.
132. Li X, Shet K, Xu K, Rodríguez JP, Pino AM, Kurhanewicz J, Schwartz A, Rosen CJ. Unsaturation level decreased in bone marrow fat of postmenopausal women with low bone density using high resolution magic angle spinning (HRMAS) ^{1}H NMR spectroscopy. *Bone*. 2017;**105**:87–92.
133. Patsch JM, Li X, Baum T, Yap SP, Karampinos DC, Schwartz AV, Link TM. Bone marrow fat composition as a novel imaging biomarker in postmenopausal women with prevalent fragility fractures. *J Bone Miner Res*. 2013;**28**(8):1721–1728.
134. Bredella MA, Fazeli PK, Daley SM, Miller KK, Rosen CJ, Klibanski A, Torriani M. Marrow fat composition in anorexia nervosa. *Bone*. 2014;**66**:199–204.
135. Machann J, Stefan N, Wagner R, Bongers M, Schleicher E, Fritsche A, Häring HU, Nikolaou K, Schick F. Intra- and interindividual variability of fatty acid unsaturation in six different human adipose tissue compartments assessed by ^{1}H-MRS in vivo at 3 T. *NMR Biomed*. 2017;**30**(9):e3744.
136. Baum T, Yap SP, Karampinos DC, Nardo L, Kuo D, Burghardt AJ, Masharani UB, Schwartz AV, Li X, Link TM. Does vertebral bone marrow fat content correlate with abdominal adipose tissue, lumbar spine bone mineral density, and blood biomarkers in women with type 2 diabetes mellitus? *J Magn Reson Imaging*. 2012;**35**(1):117–124.
137. Yu EW, Greenblatt L, Eajazi A, Torriani M, Bredella MA. Marrow adipose tissue composition in adults with morbid obesity. *Bone*. 2017;**97**:38–42.
138. Mattiucci D, Maurizi G, Izzi V, Cenci L, Ciarlantini M, Mancini S, Mensà E, Pascarella R, Vivarelli M, Olivieri A, Leoni P, Poloni A. Bone marrow adipocytes support hematopoietic stem cell survival. *J Cell Physiol*. 2018;**233**(2):1500–1511.
139. Zhou BO, Yu H, Yue R, Zhao Z, Rios JJ, Naveiras O, Morrison SJ. Bone marrow adipocytes promote the regeneration of stem cells and haematopoiesis by secreting SCF. *Nat Cell Biol*. 2017;**19**(8):891–903.
140. Shafat MS, Oellerich T, Mohr S, Robinson SD, Edwards DR, Marlein CR, Piddock RE, Fenech M, Zaitseva L, Abdul-Aziz A, Turner J, Watkins JA, Lawes M, Bowles KM, Rushworth SA. Leukemic blasts program bone marrow adipocytes to generate a protumoral microenvironment. *Blood*. 2017;**129**(10):1320–1332.
141. Boyd AL, Reid JC, Salci KR, Aslostovar L, Benoit YD, Shapovalova Z, Nakanishi M, Porras DP, Almakadi M, Campbell CJV, Jackson MF, Ross CA, Foley R, Leber B, Allan DS, Sabloff M, Xenocostas A, Collins TJ, Bhatia M. Acute myeloid leukaemia disrupts endogenous myelo-erythropoiesis by compromising the adipocyte bone marrow niche. *Nat Cell Biol*. 2017;**19**(11):1336–1347.
142. Li G, Xu Z, Zhuang A, Chang S, Hou L, Chen Y, Polat M, Wu D. Magnetic resonance spectroscopy-detected change in marrow adiposity is strongly correlated to postmenopausal breast cancer risk. *Clin Breast Cancer*. 2017;**17**(3):239–244.
143. Templeton ZS, Lie WR, Wang W, Rosenberg-Hasson Y, Alluri RV, Tamaresis JS, Bachmann MH, Lee K, Maloney WJ, Contag CH, King BL. Breast cancer cell colonization of the human bone marrow adipose tissue niche. *Neoplasia*. 2015;**17**(12):849–861.
144. Herroon MK, Rajagurubandara E, Hardaway AL, Powell K, Turchick A, Feldmann D, Podgorski I. Bone marrow adipocytes promote tumor growth in bone

via FABP4-dependent mechanisms. *Oncotarget*. 2013;**4**(11):2108–2123.
145. Bredella MA, Torriani M, Ghomi RH, Thomas BJ, Brick DJ, Gerweck AV, Rosen CJ, Klibanski A, Miller KK. Vertebral bone marrow fat is positively associated with visceral fat and inversely associated with IGF-1 in obese women. *Obesity (Silver Spring)*. 2011; **19**(1):49–53.
146. Bredella MA, Lin E, Gerweck AV, Landa MG, Thomas BJ, Torriani M, Bouxsein ML, Miller KK. Determinants of bone microarchitecture and mechanical properties in obese men. *J Clin Endocrinol Metab*. 2012;**97**(11):4115–4122.
147. Scheller EL, Khoury B, Moller KL, Wee NK, Khandaker S, Kozloff KM, Abrishami SH, Zamarron BF, Singer K. Changes in skeletal integrity and marrow adiposity during high-fat diet and after weight loss. *Front Endocrinol (Lausanne)*. 2016;**7**: 102.
148. Tencerova M, Figeac F, Ditzel N, Taipaleenmäki H, Nielsen TK, Kassem M. High-fat diet–induced obesity promotes expansion of bone marrow adipose tissue and impairs skeletal stem cell functions in mice. *J Bone Miner Res*. 2018;**33**(6): 1154–1165.
149. Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes—a meta-analysis. *Osteoporos Int*. 2007;**18**(4):427–444.
150. McCabe LR. Understanding the pathology and mechanisms of type I diabetic bone loss. *J Cell Biochem*. 2007;**102**(6):1343–1357.
151. Botolin S, Faugere MC, Malluche H, Orth M, Meyer R, McCabe LR. Increased bone adiposity and peroxisomal proliferator-activated receptor-γ2 expression in type I diabetic mice. *Endocrinology*. 2005; **146**(8):3622–3631.
152. Botolin S, McCabe LR. Bone loss and increased bone adiposity in spontaneous and pharmacologically induced diabetic mice. *Endocrinology*. 2007;**148**(1): 198–205.
153. Botolin S, McCabe LR. Inhibition of PPARγ prevents type I diabetic bone marrow adiposity but not bone loss. *J Cell Physiol*. 2006;**209**(3):967–976.
154. Slade JM, Coe LM, Meyer RA, McCabe LR. Human bone marrow adiposity is linked with serum lipid levels not T1-diabetes. *J Diabetes Complications*. 2012;**26**(1):1–9.
155. Yu NY, Wolfson T, Middleton MS, Hamilton G, Gamst A, Angeles JE, Schwimmer JB, Sirlin CB. Bone marrow fat content is correlated with hepatic fat content in paediatric non-alcoholic fatty liver disease. *Clin Radiol*. 2017;**72**(5):425.e9–425.e14.
156. Di Iorgi N, Mittelman SD, Gilsanz V. Differential effect of marrow adiposity and visceral and subcutaneous fat on cardiovascular risk in young, healthy adults. *Int J Obes*. 2008;**32**(12):1854–1860.
157. Kim TY, Schwartz AV, Li X, Xu K, Black DM, Petrenko DM, Stewart L, Rogers SJ, Posselt AM, Carter JT, Shoback DM, Schafer AL. Bone marrow fat changes after gastric bypass surgery are associated with loss of bone mass. *J Bone Miner Res*. 2017; **32**(11):2239–2247.
158. Bredella MA, Greenblatt LB, Eajazi A, Torriani M, Yu EW. Effects of Roux-en-Y gastric bypass and sleeve gastrectomy on bone mineral density and marrow adipose tissue. *Bone*. 2017;**95**:85–90.
159. Ecklund K, Vajapeyam S, Feldman HA, Buzney CD, Mulkern RV, Kleinman PK, Rosen CJ, Gordon CM. Bone marrow changes in adolescent girls with anorexia nervosa. *J Bone Miner Res*. 2010;**25**(2): 298–304.
160. Singhal V, Tulsiani S, Campoverde KJ, Mitchell DM, Slattery M, Schorr M, Miller KK, Bredella MA, Misra M, Klibanski A. Impaired bone strength estimates at the distal tibia and its determinants in adolescents with anorexia nervosa. *Bone*. 2018;**106**:61–68.
161. Abella E, Feliu E, Granada I, Millá F, Oriol A, Ribera JM, Sánchez-Planell L, Berga LI, Reverter JC, Rozman C. Bone marrow changes in anorexia nervosa are correlated with the amount of weight loss and not with other clinical findings. *Am J Clin Pathol*. 2002; **118**(4):582–588.
162. Fazeli PK, Bredella MA, Freedman L, Thomas BJ, Breggia A, Meenaghan E, Rosen CJ, Klibanski A. Marrow fat and preadipocyte factor-1 levels decrease with recovery in women with anorexia nervosa. *J Bone Miner Res*. 2012;**27**(9):1864–1871.
163. Wiegelmann W, Solbach HG. Effects of LH-RH on plasma levels of LH and FSH in anorexia nervosa. *Horm Metab Res*. 1972;**4**(5):404.
164. Boyar RM, Katz J, Finkelstein JW, Kapen S, Weiner H, Weitzman ED, Hellman L. Anorexia nervosa. Immaturity of the 24-hour luteinizing hormone secretory pattern. *N Engl J Med*. 1974;**291**(17): 861–865.
165. Misra M, Miller KK, Kuo K, Griffin K, Stewart V, Hunter E, Herzog DB, Klibanski A. Secretory dynamics of leptin in adolescent girls with anorexia nervosa and healthy adolescents. *Am J Physiol Endocrinol Metab*. 2005;**289**(3):E373–E381.
166. Grinspoon S, Gulick T, Askari H, Landt M, Lee K, Anderson E, Ma Z, Vignati L, Bowsher R, Herzog D, Klibanski A. Serum leptin levels in women with anorexia nervosa. *J Clin Endocrinol Metab*. 1996; **81**(11):3861–3863.
167. Ambati S, Li Q, Rayalam S, Hartzell DL, Della-Fera MA, Hamrick MW, Baile CA. Central leptin versus ghrelin: effects on bone marrow adiposity and gene expression. *Endocrine*. 2010;**37**(1):115–123.
168. Devlin MJ, Brooks DJ, Conlon C, Vliet M, Louis L, Rosen CJ, Bouxsein ML. Daily leptin blunts marrow fat but does not impact bone mass in calorie-restricted mice. *J Endocrinol*. 2016;**229**(3): 295–306.
169. Li S, Jiang H, Wang B, Gu M, Zhang N, Liang W, Wang Y. Effect of leptin on marrow adiposity in ovariectomized rabbits assessed by proton magnetic resonance spectroscopy. *J Comput Assist Tomogr*. 2018;**42**(4):588–593.
170. Fazeli PK, Bredella MA, Misra M, Meenaghan E, Rosen CJ, Clemmons DR, Breggia A, Miller KK, Klibanski A. Preadipocyte factor-1 is associated with marrow adiposity and bone mineral density in women with anorexia nervosa. *J Clin Endocrinol Metab*. 2010;**95**(1):407–413.
171. Boyar RM, Hellman LD, Roffwarg H, Katz J, Zumoff B, O'Connor J, Bradlow HL, Fukushima DK. Cortisol secretion and metabolism in anorexia nervosa. *N Engl J Med*. 1977;**296**(4):190–193.
172. Lawson EA, Donoho D, Miller KK, Misra M, Meenaghan E, Lydecker J, Wexler T, Herzog DB, Klibanski A. Hypercortisolemia is associated with severity of bone loss and depression in hypothalamic amenorrhea and anorexia nervosa. *J Clin Endocrinol Metab*. 2009;**94**(12):4710–4716.
173. Greenberger JS. Corticosteroid-dependent differentiation of human marrow preadipocytes in vitro. *In Vitro*. 1979;**15**(10):823–828.
174. Mayo-Smith W, Rosenthal DI, Goodsitt MM, Klibanski A. Intravertebral fat measurement with quantitative CT in patients with Cushing disease and anorexia nervosa. *Radiology*. 1989;**170**(3 Pt 1): 835–838.
175. Maurice F, Dutour A, Vincentelli C, Abdesselam I, Bernard M, Dufour H, Lefur Y, Graillon T, Kober F, Cristofari P, Jouve E, Pini L, Fernandez R, Chagnaud C, Brue T, Castinetti F, Gaborit B. Active Cushing syndrome patients have increased ectopic fat deposition and bone marrow fat content compared to cured patients and healthy subjects: a pilot ^{1}H-MRS study. *Eur J Endocrinol*. 2018;**179**(5):307–317.
176. Fazeli PK, Klibanski A. Determinants of GH resistance in malnutrition. *J Endocrinol*. 2014;**220**(3): R57–R65.
177. Abdallah BM, Jensen CH, Gutierrez G, Leslie RG, Jensen TG, Kassem M. Regulation of human skeletal stem cells differentiation by Dlk1/Pref-1. *J Bone Miner Res*. 2004;**19**(5):841–852.
178. Faje AT, Fazeli PK, Katzman D, Miller KK, Breggia A, Rosen CJ, Mendes N, Misra M, Klibanski A. Inhibition of Pref-1 (preadipocyte factor 1) by oestradiol in adolescent girls with anorexia nervosa is associated with improvement in lumbar bone mineral density. *Clin Endocrinol (Oxf)*. 2013;**79**(3):326–332.
179. Agarwal AK, Arioglu E, De Almeida S, Akkoc N, Taylor SI, Bowcock AM, Barnes RI, Garg A. AGPAT2 is mutated in congenital generalized lipodystrophy linked to chromosome 9q34. *Nat Genet*. 2002;**31**(1): 21–23.
180. Chen W, Yechoor VK, Chang BH, Li MV, March KL, Chan L. The human lipodystrophy gene product Berardinelli-Seip congenital lipodystrophy 2/seipin plays a key role in adipocyte differentiation. *Endocrinology*. 2009;**150**(10):4552–4561.
181. Simha V, Garg A. Phenotypic heterogeneity in body fat distribution in patients with congenital generalized lipodystrophy caused by mutations in the *AGPAT2* or *Seipin* genes. *J Clin Endocrinol Metab*. 2003;**88**(11):5433–5437.
182. Kim CA, Delépine M, Boutet E, El Mourabit H, Le Lay S, Meier M, Nemani M, Bridel E, Leite CC, Bertola DR, Semple RK, O'Rahilly S, Dugail I, Capeau J, Lathrop M, Magré J. Association of a homozygous nonsense caveolin-1 mutation with Berardinelli-Seip congenital lipodystrophy. *J Clin Endocrinol Metab*. 2008;**93**(4):1129–1134.
183. Hayashi YK, Matsuda C, Ogawa M, Goto K, Tominaga K, Mitsuhashi S, Park YE, Nonaka I, Hino-Fukuyo N, Haginoya K, Sugano H, Nishino I. Human *PTRF* mutations cause secondary deficiency of caveolins resulting in muscular dystrophy with generalized lipodystrophy. *J Clin Invest*. 2009;**119**(9):2623–2633.
184. Lo JC, Mulligan K, Tai VW, Algren H, Schambelan M. "Buffalo hump" in men with HIV-1 infection. *Lancet*. 1998;**351**(9106):867–870.
185. Carr A, Miller J, Law M, Cooper DA. A syndrome of lipoatrophy, lactic acidaemia and liver dysfunction associated with HIV nucleoside analogue therapy: contribution to protease inhibitor-related lipodystrophy syndrome. *AIDS*. 2000;**14**(3):F25–F32.
186. Huang JS, Mulkern RV, Grinspoon S. Reduced intravertebral bone marrow fat in HIV-infected men. *AIDS*. 2002;**16**(9):1265–1269.
187. Rauch A, Haakonsson AK, Madsen JGS, Larsen M, Forss I, Madsen MR, Van Hauwaert EL, Wiwie C, Jespersen NZ, Tencerova M, Nielsen R, Larsen BD, Röttger R, Baumbach J, Scheele C, Kassem M, Mandrup S. Osteogenesis depends on commissioning of a network of stem cell transcription factors that act as repressors of adipogenesis (published correction appears in *Nat Genet*. 2019; **51**(4):766). *Nat Genet*. 2019;**51**(4):716–727.

Acknowledgments

Financial Support: This work was supported by National Institutes of Health/National Institute of Diabetes and Digestive and Kidney Diseases Grants R24DK092759 (to M.C.H. and D.M.L.), R01DK090489 (to M.S.R.), and R03DK106410 (to P.K.F.); National Institutes of Health/National Institute of Arthritis and

Musculoskeletal and Skin Diseases Grants R01AR068345 (to M.C.H.) and K01AR072123 (to E.R.-R.); the Department of Orthopaedics and Rehabilitation, Yale University School of Medicine (to M.C.H. and D.M.L.); National Science Foundation Fellowship DGE1122492 (to Z.L.S.); and the Orentreich Foundation for the Advancement of Science (to G.P.A.).

Correspondence and Reprint Requests: Mark C. Horowitz, PhD, Department of Orthopaedics and Rehabilitation, Yale University School of Medicine, Box 208071, New Haven, Connecticut 06520. E-mail: mark.horowitz@yale.edu.

Disclosure Summary: M.C.H. is a consultant for the Orentreich Foundation for the Advancement of Science. The remaining authors have nothing to disclose.

Abbreviations

ACS, acyl–coenzyme A synthetase; AV/TV, adipose volume/tissue volume; B6, C57BL/6; BAT, brown adipose tissue; BM, bone marrow; BMD, bone mineral density; CGL, congenital generalized lipodystrophy; C3H, C3H/HeJ; DAG, diacylglycerol; DM, diabetes mellitus; HbA1c, hemoglobin A1c; HSC, hematopoietic stem cell; IWAT, inguinal WAT; LepR, leptin receptor; MAT, marrow adipose tissue; mG, membrane-targeted eGFP; MRS, magnetic resonance spectroscopy; mT, membrane-targeted dTomato; PGC-1α, PPARγ coactivator 1α; PLIN, perilipin; PPAR, peroxisome proliferator–activated receptor; Pref-1, preadipocyte factor-1; PTHrP, PTH-related protein; RYGB, Roux-en-Y gastric bypass; SWAT, subcutaneous WAT; TAG, triacylglycerol; VWAT, visceral WAT; WAT, white adipose tissue.