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Nutrient regulation of bone marrow adipose tissue: skeletal implications of weight loss

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Abstract

Adipose tissue is a dynamic component of the bone marrow, regulating skeletal remodelling and secreting paracrine and endocrine factors that can affect haematopoiesis, as well as potentially nourishing the bone marrow during periods of stress. Bone marrow adipose tissue (BMAT) is regulated by multiple factors, but particularly nutrient status. In this Review we examine how bone marrow adipocytes originate, their function in normal and pathologic states, and how BMAT modulates whole body homeostasis through actions on bone cells, haematopoietic stem cells and extra-medullary adipocytes during nutritional challenges. We focus on both rodent models and human studies to help understand the unique marrow adipocyte, its response to the external nutrient environment and its effects on the skeleton. We finish by addressing some critical questions that to-date remain unanswered.

ToC blurb

This Review examines the origins of marrow adipocytes and their function under normal or pathological conditions. Regulation of bone marrow adipose tissue (BMAT) by nutrient status is considered, as well as the interactions between BMAT, haematopoietic cells of the bone marrow and bone.

Introduction

During the past 50 years our knowledge of adipose tissue has evolved, from purely a storage site for lipids to a complex endocrine organ with a potent secretory repertoire, responsive to systemic and local signals. Concomitantly, it also became apparent that the unchecked expansion of extra-medullary adipocytes was associated with a chronic, pro-inflammatory response that was systemic and has been termed ‘metainflammation’[1] . Similarly, bone marrow adipocytes (once thought to be inert) began receiving increased attention, in part owing to an appreciation for haematopoietic marrow function and its

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ability to reconstitute the bone marrow and peripheral blood following transplantation[2]. The most widely held tenet was that bone marrow adipocytes arose by default during mesenchymal progenitor cell (referred to hereafter as skeletal stem cells (SSCs)). SSCs refers specifically to stem cells and their progeny that are specific to the development and growth of bone and bone marrow differentiation to fill marrow space during loss of haematopoiesis[3]. Gradually, this hypothesis was replaced by the concept of bone marrow adipose tissue (BMAT) as a more dynamic organ, resembling extra-medullary adipose tissue in structure but with major functional differences[4]. Discoveries abounded regarding the site of origin of marrow adipocytes, their relationship to other cells in the bone marrow, and their unique responsiveness to environmental, genetic and nutritional determinants. Figure 1 shows a timeline of important discoveries in BMAT research.

Notwithstanding these advances, challenges remain in our understanding of bone marrow adipocytes, foremost of which are the absence of a tractable organoid system and the lack of an *in vivo* imaging technique to observe real time changes in lineage and responses to external stimuli. These technological advances are likely to be overcome in the next decade; in the meantime, some important new insights have provided fresh support for the dynamic nature of these cells. For example, the origin of the marrow adipocyte has been better defined by refinements in lineage tracing studies, particularly in animal models with genetic manipulations[5]. These techniques and single cell studies have opened new perspectives on where and how cells originate, as well as their function[6]. Importantly, these methodological breakthroughs have also led to new hypotheses about BMAT and the skeleton, some of which will be noted later.

In this Review, we examine how bone marrow adipocytes originate, what their function is in normal and pathologic states and how these cells influence skeletal remodelling, haematopoiesis and whole-body metabolism during nutritional challenges. We focus on both animal models and human studies to help understand this unique cell type, its response to the external nutrient environment and the resultant effects on the skeleton. We finish by addressing some critical unanswered questions particularly around weight loss from calorie restriction and its impact on the skeleton.

Origin of Bone marrow Adipocytes

Overview

The cellular composition of bone marrow Is quite heterogeneous, with mesenchymal and haematopoietic cells at different stages of differentiation. SSCs can give rise to chondrocytes, marrow stroma, osteoblasts and adipocytes (Figure 2), whereas haematopoietic stem cells (HSCs) give rise to all the blood and immune cell lineages. Haematopoietic cell development is supported by a tissue-network of marrow stroma, which provides the structure for different niches within the marrow space[7]. In addition, marrow adipocytes and osteoblast lineage cells regulate haematopoiesis. As an example, niches that support haematopoiesis form at the marrow–endosteal bone interface[8–12]. The marrow space is highly innervated and has an extensive blood supply. Outside of bone marrow, mesenchymal stem cells (MSCs) can be found in many organs, and extra-medullary white, brown and beige adipocytes derive from MSCs *in situ*. Marrow adipocytes possess

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characteristics that are distinct from these other types of adipocytes. In this discussion, SSCs and their progeny are specific to the development and growth of bone and bone marrow. This refinement helps to define this complex tissue as an organ.[7] Of note stem cells from the stromal vascular fraction of white adipose tissue (WAT) can differentiate into osteoblast-like cells with the appropriate in vitro stimulation although these cells are not SSCs and their chromatin landscape differs from SSCs[13–15]

Although the presence of marrow adipocytes has been recognized for more than a century, their origin and differentiation has only been described within the past decade. Early experiments showed that *in vitro* culture of plastic adherent, non-haematopoietic bone marrow cells lead to the development of discrete colony-forming unit-fibroblasts (CFU-F) , i.e. adherent individual colonies which proliferate in culture,and which could differentiate into the adipocyte, osteoblast and chondrocyte lineages[8,9,10]. These early observations have stood the test of time, being confirmed with our current understanding of lineage allocation and that SSCs do not express haematopoietic or endothelial cell markers[10–12].

Key findings from mouse studies

Leptin receptor is a marker of some SSCs.—SSCs in the bone marrow and the stromal–vascular fraction of other adipose depots have been delineated using fluorescent reporter constructs for lineage tracing, direct analysis of the cell surface phenotype by fluorescence-activated cell sorting (FACS) and single-cell RNA-sequencing (RNA-seq) (Table 1)[16–19] One key study used an antibody to the leptin receptor (LEPR) in conjunction with *Lepr-cre:tdTomato* conditional reporter mice to show LEPR expression around sinusoids and arterioles throughout the marrow space, consistent with the location of SSCs[19]. This population of cells were LEPR$^+$CD45$^-$TER119$^-$CD31$^-$ and uniformly expressed paired related homeobox transcription factor 1 (*Prrx1).* PDGFRα and CD51. This population of cells accounted for the majority (94%) of bone marrow CFU-Fs, were able to differentiate into adipocytes and chondrocytes, and were a major source of osteoblasts and marrow adipocytes in adult mice. Moreover, in bone marrow of adult mice, few CFU-Fs were Nestin$^+$ and able to become osteoblasts. These data indicated that SSCs are not in the haematopoietic (CD45$^-$) or endothelial (CD31$^-$) lineages, are multipotential, LEPR is a marker and they trace from very early mesenchymal progenitors as suggested by *Prx1* expression[16–19].

SSCs express very early mesenchymal markers.—Studies using *Prx1-Cre* mice showed that *Prx-1* is expressed in early mesenchymal progenitors of the head and limbs[20] and thus only labels bones and adipose depots that are associated with these developmental lineages. For example, *Prx1-Cre* labels 96% of white adipocytes in the inguinal depot (iWAT) and >95% of marrow adipocytes in tibia and femur[20,21]. In contrast, *Prx1-Cre* does not label visceral white adipocytes and brown adipocytes[21]. Thus, LEPR$^+$ marrow adipocytes, SSCs and iWAT progenitors share some cell surface markers (LIN$^-$CD34$^-$SCA1$^+$CD29$^+$CD24$^+$PDGFRα$^+$)[19,20]

Blocking marrow adipocyte development.—The protein encoded by the peroxisome proliferator activated receptor γ gene (*Pparg*) is considered a 'master' regulator of adipocyte

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differentiation. Both conditional *Pparg* deletion in adipocytes and global deletion of *Pparg* leaves mice devoid of adipose tissue[5,22,23]. To attempt to block BMAT development in long bones of mice, crossed *Prrx1-Cre* mice with *Pparg*$^{fl/fl}$ mice (to attempt to deplete marrow adipocyte progenitors) and irradiated the adult offspring to induce marrow adipogenesis[22,23] As expected, *Cre*$^{−}$ control mice had striking marrow adipogenesis in both the proximal and distal tibia and in the femur. In contrast, few if any marrow adipocytes could be seen even after irradiation in the long bones of the *Cre*$^{+}$ mice. Few adipocytes are seen in normal young and adult B6 mice bone marrow. Marrow adipogenesis can be induced by a variety of methods. Here we used sublethal irradiation.

Importantly, similar numbers of marrow adipocytes could be seen in the caudal vertebra from *Cre*$^{−}$ and *Cre*$^{+}$ mice, suggesting caudal adipocytes do not express Prx1 and arise from different progenitors than adipocytes in long bones. These data show that *Prx1-Cre* traces the majority of marrow adipocyte progenitors in adult mouse tibia and femur[24,25].

Regulation of haematopoiesis by SSCs.—In addition to differentiating into their specific cell lineages, SSCs also function to regulate haematopoiesis in bone marrow niches[26]. Stromal cell-derived factor 1 (also known as CXCL12) is secreted by stromal cells and regulates the maintenance of HSCs and lymphoid progenitors[27]. Osterix (*Osx*) is a zinc-finger containing transcription factor specific to osteoblasts that functions early in osteoblast lineage differentiation and is essential for the development of mature, matrix-secreting osteoblasts[28]..*Prx1-Cre:Cxcl12*$^{fl/fl}$ mice were generated as a model of SSC depletion, whereas *Osx-Cre;Cxcl12*$^{fl/fl}$ mice were a model of early progenitor depletion[29] Bone marrow cellularity was reduced by ~50% in both models, in part due to the loss of B cells. A statistically significant decrease was observed in the number of HSCs in the *Prx1-Cre:Cxcl12*$^{fl/fl}$ mice, whereas only a modest decrease was seen in the *Osx-Cre;Cxcl12*$^{fl/fl}$mice[29]. Similarly, in transplantation studies, a defect occurred in multi-lineage long-term repopulation in recipient mice receiving bone marrow from *Prx1-Cre:Cxcl12*$^{fl/fl}$ mice but not *Osx-Cre;Cxcl12*$^{fl/fl}$ mice[31]. These data suggest that SSCs support HSCs, whereas osteoblast progenitors support the B cell niche and do not notably affect HSCs (Figure 2)

Variations in SSC phenotypes.—In a different set of experiments, cells were isolated from the femoral growth plate of Rainbow mice (a multicolor Cre recombinase mouse reporter strain that expresses multiple fluorescent proteins from a single genomic locus) and analyzed by FACS[30]. A population of stem cells was isolated (CD45$^{−}$TER119$^{−}$TIE2$^{−}$AlphaV^{+}THY$^{−}$6C3$^{−}$CD105$^{−}$CD200$^{−}$,which gave rise to eight discrete populations in a hierarchical manner. These postnatal stem cells differentiated into bone, marrow stroma and cartilage, but importantly not adipocytes[30]. Thus, AlphaV^{+} cells were multipotent and retained their self-renewing capacity *in vitro* and *in vivo*. As these SSCs differentiated, their progeny became more restricted in their cell type specificity, as expected. Some of the differentiated populations were LEPR^{+} and Nestin^{+}, consistent with some previous data[19]. As AlphaV^{+} cells do not give rise to marrow adipocytes, this cell population could be a different SSC population to LEPR^{+} SSCs.

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In a series of papers, cells were isolated from crushed tibiae and femurs of fluorescent reporter mice by collagenase digestion, and then subdivided based on expression of stem cell antigen 1 (SCA1, otherwise known as LY6A) by flow cytometry and expanded in culture[31]. These plastic adherent cells also expressed platelet-derived growth factor-α.[31]

Based on this approach, four distinct non-haematopoietic, non-endothelial populations were identified. First, $CD45^{-}CD31^{-}SCA1^{+}CD24^{+}$ stem cells, which gave rise to adipocytes, osteoblasts and chondrocytes. More cells of this first population OK resided in the metaphasis than the diaphysis, not on the endosteum but peri-vascularly. The second population were $CD45^{-}CD31^{-}SCA1^{+}CD24^{-}$ committed progenitor cells that gave rise unilaterateraly to adipocytes and were evenly distributed in the metaphysis and diaphysis and perivascularly. Third were $CD45^{-}CD31^{-}SCA1^{-}PDGFR\alpha^{+}$ committed progenitor cells that gave rise to osteoblasts and chondrocytes and had little adipogenic potential. Fourth, $CD45^{-}CD31^{-}SCA1^{-}ZFP423^{+}$ were a committed progenitor or precursor cell population that gave rise to adipocytes. Interestingly, less than 1% of $SCA1^{-}$ cells expressed ZFP423 (a zinc finger protein and transcriptional coregulator of PPARγ that regulates the potential of cells to undergo adipocyte differentiation, whereas ZFP423 was uniformly expressed by the $SCA1^{+}$ subset. These data suggested that $CD45^{-}CD31^{-}SCA1^{-}ZFP423^{+}$ cells were a more mature, adipocyte lineage committed population compared with the other three subsets. These cells suppressed competitive transplantation assays following lethal irradiation and inhibited fracture repair. When the adipogenic populations of cells were injected into the site of a stabilized tibial fracture, they inhibited fracture repair[31]. To test the effect of these population on hematopoiesis, recipient mice were lethally irradiated and the four populations of cells injected directly into the tibial medullary canal. The frequency of hematopoietic lineage ($Lin^{-}Sca1^{+}c\text{-}Kit^{+}$) cells was significantly reduced after adipogenic cell transplantation. In contract, transplantation of multipotent $CD45^{-}CD31^{-}Sca1^{+}CD24^{+}$ cells resulted in significantly increased repopulation of hematopoietic cells in the tibia. These data support the idea that bone marrow adipocyte inhibit bone repair and hematopoiesis.

In a follow up 2021 study tibiae and femurs were mechanically and enzymatically prepared and SSCs isolated by flow cytometry as described previously[31]. Two distinct population of SSCs were isolated by cell surface phenotype, CD45−Ter119−Tie2−CD51+Thy1−6C3−CD105−, which give rise to more restricted stromal and osteochondral lineages, and CD45−CD31−PdgfrαSca1+ CD24+, which can also give rise to bone, cartilage, and importantly to adipocytes[31,33] As these adipogenic progenitors differentiate, their cell surface phenotype changes to take on a more adipogenic phenotype. These progenitors are thought to be the source of all marrow adipocytes[31,33] Importantly, both these SSC subsets CD45−Ter119−Tie2−CD51+Thy1−6C3−CD105− and CD45−CD31−PdgfrαSca1+ CD24+ expressed LEPR determined by FACS, demonstrating that LEPR is a marker for the majority of SSCs[33,34]

Where you get your cells matters. .—Where in bone SSC populations are obtained from can dictate their phenotype and lineage development. This issue was illustrated in experiments that used the *Col2-Cre Rosa26* (Col:Td, tdTomato) fluorescent reporter mouse model, in which all CFU-F, marrow adipocytes and osteocytes were $tdTomato^{+}$ suggesting

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that all bone marrow SSCs were TdTomato+[35]. This tdTomato^{+} population of multi-potent progenitor cells was used for single cell RNA-seq analysis. In pilot experiments, two populations of bone marrow cells were isolated[35]. First, the epiphyses were removed from tibias and femurs and bone marrow flushed from the medullary canal (central bone marrow), which is a standard method of bone marrow collection. We know from our own experience that this method leaves cells adherent to the endosteal surface. For the second population, the exterior of the flushed bones was scraped and treated with collagenase and trypsin to remove periosteal cells. The bones were then cut in half, exposing the trabecular bone and endosteal surface, enzymatically digested and the cells collected (endosteal bone marrow). The enzymatic method enabled the capture of cells adherent to endosteal surfaces.

The cells isolated from endosteal bone marrow of tdTomato mice had more SSCs were more proliferative, gave rise to adipocytes, osteoblasts and chondrocytes, and decreased in number with age compared with cells isolated from central bone marrow[34]. The top 1% of the Td+ peak were isolated from 1-month-old Col2-Cre Rosa 26 mice by flow cytometry and analyzed by single cell RNA-seq[34] Gene expression profiling reveled nine clusters of mesenchymal cells, which included adipocytes, osteoblasts, osteocytes and chondrocytes. Cell trajectory analysis was used to identify cluster 1 cells as the progenitors to the other lineages, which expressed SCA1, CD34, THY1, LEPR and gave rise to adipocytes and osteoblasts. Compared to the other cluster, which are more lineage restricted, SCA1 alone preferentially marked cluster 1 cells. Using the femurs from 1-month-old Adipoq:Td:Col1a1-GFP, an adipocyte-specific (Adipoq-Cre) reporter mouse with or without Col1a1-GFP (that traces osteoblasts), a large cluster of Td+ cells could be seen histologically just below the growth plate of young, but not old mice[34] that expressed adipocyte markers, including PPARγ, CEBPα, adiponectin and lipoprotein lipase but notably did not express PLIN1 and FABP4, suggesting that these cells did not contain lipid droplets. Further analysis of this cell population indicated that these cells were a novel population of adipocyte lineage cells that did not contain lipid droplets and were intermediate between mesenchymal progenitor cells and classic lipid filled marrow adipocytes. They are known as marrow adipogenic lineage precursors (MALPs) (Figure 2)[35]. Moreover, MALPs did not express UCP1 (a brown or beige adipocyte marker), TNFRSF9 (a beige adipocyte marker) or leptin (a white adipocyte marker), consistent with the idea that marrow adipocytes are distinct from brown, beige and white adipocytes[19,35]. Deletion of MALPs in mice caused pathological changes to the marrow vasculature, which become dilated with decreased vessel density[35]. A distinct increase was observed in trabecular bone mass in the metaphysis extending into the diaphysis, with increased cortical bone thickness: consistent with an increase in osteoblasts. Haematopoietic cells in the bone marrow were unaffected. Whether the increase in bone is due to a shift in lineage allocation due to the loss of MALPs or is an indirect effect is not clear.

Current understanding of lineage

Our understanding of the lineage development of marrow adipocytes has made notable progress over the past 10 years. Evidence clearly shows that marrow adipocytes derive from a multipotential, self-renewing SSC population in the bone marrow. These cells differentiate to yield more lineage restricted intermediate progenitor or precursor cells, the result being

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mature functional marrow adipocytes, osteoblasts, chondrocytes and marrow stroma (Figure 2, Table 1). This progression from less restricted to more restricted cell types is similar in kind to the development of white adipocytes and haematopoietic cell lineages. Still, a number of challenges remain.

Many assume that a single true SSC population exists that gives rise to marrow adipocytes. Based on the available data, this hypothesis seems unlikely. As an example, in mouse long bones marrow adipocytes first appear in the distal tibia, with few if any adipocytes in the proximal tibia or femur[19,33–35]. Using *Prx-1-Cre:Ppar*$\gamma^{fl/fl}$ mice, we have shown that the long bone marrow adipocytes fail to develop if *Prx1*$^{+}$*Pparg*$^{+}$ progenitor cells are depleted[21,22]. By contrast, in adult mice, the progenitor that gives rise to marrow adipocytes in spine is *Prx1*$^{-}$, therefore from a different SSC than the one in tibiae and femurs[22]. The fact that x-irradiation, calorie restriction, methionine restriction high-fat diet (HFD, mice were fed either an isocaloric HFD (5.3 kcal/gm) consisting of 14% kcal protein, 26% kcal carbohydrate and 60% kcal fat) and Thiazolidinediones (TZD) diet feeding, and physical disruption of the marrow channel (via insertion of a needle) cause increased marrow adipogenesis suggests that marrow adipocytes are involved in the repair and recovery of bone marrow function[37]. TZDs are insulin sensitizing drugs, which increase insulin sensitivity and action by regulating gene expression by binding to PPARγ. These data suggest that the progenitor that gives rise to marrow adipocytes in tibias and femurs is *Prx1*$^{+}$ regardless if they appear early in postnatal life (for example, distal tibia), or are induced by a variety of treatments (for example, x-irradiation, calorie restriction or methionine restriction).[37]

Limitations and variations in phenotypes

The cellular phenotype of SSCs varies depending on the laboratory. As an example, *Osterix* and *Gremlin* expression by SSCs have been reported by some but not by others[38]. Even *Lepr* expression remains quite variable. The age of mice has a clear effect on the phenotype of the SSC population. Another explanation for these differences could be the starting population of cells used for analysis. *In vivo*, marrow adipogenesis in long bones is most prominent just distal to the growth plate trabecular bone extending into bone marrow in the primary spongiosa[34] This finding is consistent with the presence of increased marrow adipocyte progenitors. Upon stimulation, marrow adipocytes appear to move distally, filling the medullary canal[22,36]. Whether this finding simply reflects a need for more space as more cells differentiate or whether progenitors or precursors can be found in the metaphysis, or both mechanisms occur also remains unresolved. Thus, cells obtained from the endosteal bone marrow, especially in and around the growth plate, might be a superior starting population for lineage analysis compared with central bone marrow cells flushed from the medullary canal.

Open Research Questions

Much work still remains to be done on characterizing the interactions of marrow adipocytes and other cells in the marrow. In acute myeloid leukemia (AML), large numbers of dysfunctional leukemic blasts accumulate in the bone marrow resulting in the loss of myelocytes and erythrocytes, causing life threatening infections. *In vivo* and *in vitro*

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experiments show that AML causes a disruption in the adipocytic niche in bone marrow, resulting the failure of myeloid and erythroid lineage development[39]; thus, studying marrow adipocyte biology during AML is of clinical interest. HFD-feeding induces bone marrow adipogenesis and an increase in Ly6C^{High} monocytes in bone marrow, which was accompanied by a shift in monocyte metabolism, reducing oxidative potential and increasing glycolysis and mitochondrial function[40]. Finally, osteoclastogenesis requires colony stimulating factor 1 (CSF1); however, the cellular source of CSF1 in the bone marrow has been unclear. MALPs express much higher levels of CSF1 when compared with other mesenchymal cells. Deletion of MALPs in mice resulted in increased trabecular BMD, with decreases in TRAP$^+$ osteoclasts[41]

Nutrient regulation of BMAT

Adipose tissue is one of the largest organs in the body and 99% of the cells in this tissue are adipocytes[42]. These cells arise from several locations postnatally and their site of origin (for example, visceral, subcutaneous, brown, beige or breast adipose depots is thought to determine cellular function. Short-term and long-term changes in dietary regimens affect adipose depots in unique ways that are also location dependent. For example, in humans after a HFD, a modest 1–5% increase in thermogenesis occurs principally from brown adipose tissue (BAT) due to activation of the sympathetic nervous system in humans , while lipid storage ramps up in visceral and subcutaneous depots[43]. Like other depots, BMAT is unique in its origin and its response to dietary changes. For example, historic research from the 1970s showed that the bone marrow of individuals who have faced famine or starvation becomes laden with adipocytes and with persistent food deprivation, becomes gelatinous[44]. Similarly, most starving mammals are known to preserve adipose tissue in two sites: the bone marrow and the lymphatic tissues[45,46]. As haematopoiesis is such an energy consuming process, it follows that neighboring adipocytes could be used as a reservoir for energy during calorie restriction . This paradoxical response to extreme dietary restriction has been a focus of recent research efforts.

In mouse models, nutrient challenges (that is, a HFD or caloric restriction) both cause enhanced marrow adiposity, while the extra-medullary responses are quite distinct. Furthermore, oestrogen deficiency drives marrow adiposity in mice and can act as an interactive component associated with dietary changes[47,48]. In many studies, dietary interventions also cause bone loss, although exceptions do exist. A 2018 study of HFD-feeding in adult C57BL/6J mice demonstrated a very pronounced increase in BMAT and bone loss, with evidence suggesting that the bone marrow adipocytes in these mice were senescent and insulin resistant[49].The bone loss was due to reduced bone formation and enhanced adipogenesis from a shift in lineage allocation. Interestingly, those high calorie diet fed mice developed a pro-inflammatory response in peripheral adipose depots, yet such a response was not detected in the bone marrow or adjacent marrow adipocytes[49] Preliminary work in the Rosen laboratory confirmed that after mice were fed a HFD (45% calories from fat) for 8-weeks, expression of inflammatory cytokines was not observed in either marrow adipocytes or marrow macrophages. On the other hand, bone resorption was markedly increased in the HFD mice As such the mechanism of bone loss with HFD is

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likely to be uncoupled remodeling with higher rates of bone resorption and lower bone formation due to greater allocation of SSC into the adipocyte lineage.

In human studies, the effects of obesity on BMAT are not clear, with some reports showing an increase, whereas others demonstrating no change or less BMAT[45,46]. Similar findings have been noted for BMD in individuals with obesity[47]. In mice, as noted both a HFD and calorie restriction, lead to the higher BMAT and bone loss[48,49] (Table 2). However, the marrow responses that drive adipogenesis differ (see later). Taken together, most studies demonstrate that the marrow adipocyte response to external stimuli differs from extra-medullary adipocytes and that nutritional composition and intake directly affect the fate of marrow adipocyte precursors. Genetically engineered mouse models have provided major insights into these changes.

Clinical importance

The clinical importance of understanding the effects of nutritional challenges on the skeleton and the bone marrow cannot be emphasized enough[50–52]. Caloric restriction is the most common approach to treating obesity, but bone loss usually occurs with weight loss. For example, in long term human studies (for example, CALERIE) where 25% caloric restriction was attempted in healthy volunteers for 2 years, statistically significant bone loss was evident at the spine and femur[53]. The other effective therapeutic weight loss strategy in individuals with obesity, gastric bypass, causes bone loss but with variable effects on BMAT[54]. The most common procedure to treat obesity, vertical sleeve gastrectomy, consistently causes loss of bone mass as measured by DXA but this is not tied temporally to the weight loss or to a decrease in BMAT[55]. Other weight loss strategies such as time restricted eating, intermittent fasting and treatment with glucagon-like peptide 1 receptor agonists, have not been studied relative to the BMAT response, although bone loss has not been reported with GLP-1R agonist use. It is important to note that the effect on bone marrow adipocytes with weight loss seems to be sex dependent such that males have a greater increase in BMAT than females[54]. In human volunteers, after a 10 day fast and notable weight loss, BMAT increased rapidly, more so in men than women, and was reversed with an ad libitum diet[56] . By contrast, gastric bypass has been reported to either decrease, increase or not to change BMAT in humans[57].

Experimental Validation in Mice

For C57BL/6J and C3H/HeJ mice, 30% caloric restriction for as short as 4 weeks causes substantial bone marrow adipose infiltration, in both males and females across a wide age range. Cortical and trabecular bone loss and high bone marrow adiposity is more pronounced in young C57BL/6J mice that undergo caloric restriction[58]. However, in mature male and female mice, caloric restriction leads to cortical bone loss, with minimal or no change in trabecular bone volume fraction. Histomorphometric studies in young mice undergoing caloric restriction revealed a profound suppression in all aspects of bone remodelling, particularly bone formation, which is almost certainly a response to reduced substrate availability[59]. SSCs isolated from those young mice show an enhanced capacity to differentiate into adipocytes, although increased expression of tissue non-specific alkaline phosphatase (TNAP- (a key enzyme in skeletal mineralization) has also been noted in these

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cells (Li, unpublished observation) In mice undergoing vertical sleeve gastrectomy following a HFD, BMAT declines considerably and this change is associated with statistically significant bone loss[60]. Cold exposure also causes a similar skeletal phenotype in mice; that is, less BMAT and bone loss[36]. In dams undergoing lactation, bone loss is pronounced and marrow adiposity is reduced[61]. Thus, in rodent models, nutritional changes in diet can affect both skeletal remodelling and bone marrow adipose infiltration. However, the negative relationship between BMAT and bone loss is not always consistent or reproducible in these studies. In addition, notable sex differences have been observed in skeletal changes between male and female mice undergoing caloric restriction.

Observations from individuals with anorexia nervosa

One human disorder that recapitulates the effect of calorie deprivation in mice is anorexia nervosa. Severe bone loss and fractures are frequent and often difficult to treat in the face of very low body weight[62]. BMAT is consistently increased in both the peripheral and axial marrow and this increase has been related to both the duration and severity of anorexia[62]. BMAT is reversed by weight gain but bone mass is less easily restored and is dependent on the age of the individual. Oestrogen treatment can improve bone mass and reduce BMAT but does not fully restore body weightl[63,64]. Anabolic agents, such as parathyroid hormone and insulin-like growth factor 1 (IGF1), have been used to reverse the skeletal phenotype with mixed success[65]. No histomorphometric data on skeletal remodelling with anorexia have been published, although systemic biochemical markers suggest uncoupling, with suppressed bone formation and increased bone resorption. The paradoxical aspect of anorexia is that as adipose tissue mass declines with caloric restriction in peripheral adipose depots, BMAT increases. The mechanisms responsible for this process are probably multi-factorial and include paracrine, endocrine and neural factors. High levels of glucocorticoids, low circulating and marrow levels of IGF1 and changes in central mediators of appetite all probably contribute to the profound suppression in bone formation.[62,66 67] Whether the high numbers of marrow adipocytes contribute to the increase in bone resorption in humans is unclear, although this has been shown in other animal model systems.

Manipulating BMAT in mouse models

BMAT expansion is induced by injury, for example, radiation, drugs, mechanical ablation or chemotherapy[19,68,69]. Calorie deprivation could be considered injurious, that is, as an injury to cellular function due to substrate deficiency. In experimental mouse models caloric restriction is one of the most potent inducers of BMAT[59]. However, whether the marrow adipocyte response to nutrient challenges is primarily due to signalling directly to the adipocyte from neural or skeletal (paracrine) connections, or occurs as a secondary repair or compensatory response that somehow preserves skeletal function, has not been demonstrated. To address that question, several experimental approaches have been employed. These include deletion of marrow adipocytes using diphtheria toxin receptor or targeted *Pparγ* deletion, and/or bone marrow adipocyte targeted deletion (Table 1).

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Gene targeted deletions in mice

The first genetically modified animal model that examined the role of marrow adipocytes was the A-Zip or fat-less mouse, in which expression of the dominant negative A-ZIP/F protein was under control of the adipose-specific *Fabp4* promoter[69].This genetic construct suppressed *Pparγ* expression. The mice ha reduced adipose depots globally and in the marrow, increased HSC frequency in tail vertebrae and accelerated haematopoietic recovery after irradiation, as well as high bone mass, suggesting that BMAT was a negative regulator of haematopoiesis[69]. By contrast, a 2017 paper demonstrated using *Col1a1*2.3-cre; Scf$^{GFP/fl.}$* mice, that the presence of marrow adipocytes in long bones might be beneficial for haematopoiesis. This study was based on earlier observations in vitro that marrow adipocytes secreted stem cell factor **[** and this was essential for haematopoietic recovery[72] . Thus, marrow adipogenesis could theoretically be thought of as an emergency response to cytopenia and a rapid way to reconstitute the marrow after injury. A similar compensatory mechanism could be operative in bone, as noted in a model system of calorie restriction.

Adipocyte specific deletion models

Several investigators reported that specific deletion of marrow adipocytes in mice could drive high bone mass, supporting the notion that these cells negatively affect the skeleton[71]. Ablation of adiponectin-expressing cells in the bone marrow of mice yielded a rapid and profound increase in systemic bone mass[5]. This enhancement in bone formation was due to induction of bone morphogenetic protein signaling as a result of the elimination of its inhibitors produced by marrow adipocytes, combined with epidermal growth factor receptor stimulation. The two inhibitors found in marrow adipocytes were chordin 1 and gremlin 1, both expressed fairly early in lineage allocation, consistent with the adiponectin Cre temporal expression[5]. The genetic modification was accomplished using a conditional expression of the diphtheria toxin receptor (DTR) crossed to an adiponectin Cre mouse. Although extra-medullary adipocytes were also deleted in addition to marrow adipocytes, the authors established that white adipose transplants in those mice did not affect the osteogenic response[5]. Similarly, transplants of DTR-null adipose tissue into wild-type mice did not affect the skeleton. Notwithstanding, concerns were raised that the whole body lipoatrophic phenotype and insulin resistance of the conditional-DTR mice might contribute to the skeletal changes. A new model system was developed and findings published in 2022, using the FLPo-dependent-Adipo-Cre (FAC) mouse which deletes genes in marrow adipocytes only, further supported earlier work[71,23,73]. That FAC mouse model, discussed below has the potential for providing future insights into the functional importance of BMAT

FAC mice.—The creation of the FAC mouse is worth noting. Studies published around 9–10 years ago from several laboratories showed that *Osterix* traces to osteoblasts and bone marrow adipocytes, but not to white adipocytes[28,29]. Thus, CRISPR–Cas9 was used to create *Osterix-FLPo* mice, with an in-frame fusion of *Osterix* and optimized *FLPo (the flippase enzyme)* separated by a *P2A* self-cleaving sequence, which enabled independent functioning of the two proteins[73]. Using eGFP, these mice had positive staining in osteocytes, osteoblasts, bone marrow adipocytes and a subset of marrow stromal cells within

the bone. FAC mice were then created, which contained an internal ribosome entry sequence followed by FLPo-dependent *Cre* in reverse orientation within the 3'-untranslated region of endogenous *Adipoq*[23]. FLPo expressed from the *Osterix* locus recombines *Cre* to the correct orientation in progenitors of osteoblasts and bone marrow adipocytes. However, as *Adipoq* is selectively expressed in adipocytes, *Cre* was expressed in bone marrow adipocytes, but not in osteoblasts or other adipose depots[23]. *Cre* efficiency was ~80% in both male and female mice over 16 weeks of age with one *Cre* allele and over 90% at 12 weeks of age in mice with two *Cre* alleles.

In subsequent experiments FAC mice were then crossed with floxed *Pparg* mice to delete *Pparg* in marrow adipocytes only[23]. The FAC *Pparg*$^{-/-}$ mice exhibited markedly reduced BMAT but high cortical bone mass, similar in magnitude to what was observed with the targeted DTR. However, no change was noted in the trabecular compartment[23]. In a second set of experiments, FAC mice were crossed with ROSA DTA mice harboring a LoxP-flanked STOP cassette proximal to the DTA sequence. In this model, Cre recombinase excises the STOP cassette to enable expression of cytotoxic DTA in bone marrow adipocytes, thus inducing cell death[23,72]. In the DTA conditional mice, cortical bone mass and trabecular bone volume fraction were increased and haematopoiesis was markedly impaired. In addition to a reduction, but not loss of, bone marrow adipocytes at the distal and proximal tibia, a statistically significant increase was observed in bone formation. Intriguingly, the DTA mice were resistant to cortical bone loss with 30% caloric restriction. Taken together, marrow adipocytes clearly have a supportive role for the haematopoietic niche in trabecular bone of the proximal femur, whereas the reduction in marrow adipocytes distally was associated with a concomitant increase in bone formation and mass[73].

FAC mice were also used to test the hypothesis that marrow adipocytes might have a supportive role for the skeleton as well as the haematopoietic niche[73]. Thus, FAC mice were crossed with *Pnpla2*$^{fl/fl}$ mice to delete adipose triglyceride lipase (the rate limiting enzyme for lipolysis, but also a transacylase that drives synthesis of fatty acyl esters of hydroxy fatty acids) only in marrow adipocytes[73]. FAC *Pnpla*2$^{-/-}$ mice fed a regular or HFD exhibited a bone marrow adipocyte phenotype comparable with wild-type mice; BMD was also not different in the mutants compared with *Pnpla*2$^{+/+}$ mice[73]. However, during 30% caloric restriction, trabecular bone volume fraction was statistically significantly reduced in male mice, although not in females. The latter finding contrasts with the absence of trabecular bone loss In *Pnpla2*$^{+/+}$ mice after 30% caloric restriction. Also, haematopoiesis was impaired in mice that had absence of ATGL in bone marrow adipocytes , as was trabecular and cortical bone regeneration using a wound defect in the tibia. Bulk RNA-seq of the bone marrow in male FAC *Pnpla2*$^{+/+}$ mice revealed that adipogenic genes were the most up-regulated pathway by 30% caloric restriction, but osteogenesis was the second most differentially expressed network[73] . On the other hand, in FAC *Pnpla2*$^{-/-}$ mice, both pathways were not activated. Thus, 30% caloric restriction probably drives adipogenic and osteogenic responses in bone marrow, the latter of which might be essential for trabecular bone maintenance. Similarly, genes associated with de novo lipogenesis (for example, *Fasn, Acaca, Acacb, Echdc2* and *Echdc3*) were also up-regulated with caloric restriction in the FAC *Pnpla2*$^{+/+}$ mice, but not in the FAC *Pnpla2*$^{-/-}$ mice[73].

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Further support for the tenet that marrow adipocytes are a source of substrate comes from Lion enrichment analysis of lipids in marrow sera after 10 days of fasting in healthy volunteers[56]Lipid storage and synthesis were the top two networks in marrow sera compared with pre-fasting values. Furthermore, RNAseq of marrow adipocytes isolated from those fasting individuals revealed that lipoprotein lipase (*LPL)* was one of the most highly expressed genes with fasting Costa, unpublished observation . Hence, marrow adipocytes could be extracting fatty acids from the circulation for recycling and for later use by other marrow components, such as osteoblasts or haematopoietic cells. Thus, much like the ‘support’ hypothesized for haematopoiesis, increased BMAT might be a compensatory response for maintaining skeletal integrity during nutritional stress. By contrast, under normal conditions, marrow adipocytes secrete factors that tend to suppress bone formation and the osteogenic response[73] (Figure 3).

Amino acid deficiency and BMAT

Evidence also suggests that amino acid deficiency might phenocopy 30% caloric restriction. First reported in 1992, sulfur amino acid restriction (especially methionine restriction) extends lifespan across many species, from yeast and flies to worms and mice . Mice live up to 45% longer than controls, with less age-related disease including; adiposity, reduced insulin resistance, cancer and improved over-all metabolic status[74] .Mice placed on a continuous methionine restriction (0.12%) versus control (0.86%) diet for 8 weeks lose body weight due to a loss of subcutaneous and visceral adipose tissue, although this effect is more pronounced in male mice[75]. Concomitantly, the mice have a striking increase in long-bone marrow adipogenesis, with increased beige adipogenesis in the iWAT depot (MC Horowitz and GP Ables: unpublished observation.

Trabecular and cortical bone mass are significantly decreased and the bones are weaker biomechanically[75,76]. This phenotype is strikingly similar to that seen with caloric restriction in mice and anorexia nervosa in young women. Importantly, the beneficial effects of methionine restriction, including improved glucose metabolism and protection against HFD-induced obesity, can be achieved by feeding a methionine restriction diet intermittently, which reduces the increased BMAT and bone loss phenotype seen with continuous methionine restriction

To assess whether humans could benefit from a methionine restriction diet, healthy men and women were placed on an methionine restriction diet or a diet restricted in both methionine and cysteine for three 4-week feeding periods, separated by 3–4 week wash-out periods[77]. Volunteers in the methionine and cysteine restricted group had reductions in body weight and plasma levels of cholesterol, LDL, blood urea nitrogen, IGF1 and insulin, and increased FGF21 after 4-weeks[77]. These data support the idea that the beneficial effects of sulfur amino acid restriction, seen in numerous animal models, are translatable to humans.

BMAT and nutrient stress in humans

Since the original description of the relationship between BMAT and bone by Meunier[78], subsequent analyses of bone marrow biopsies in postmenopausal women OK have supported

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the negative relationship between BMD density and BMAT volume[78,79] Within the last two decades, MR spectroscopy has been used to quantitate BMAT in the spine and proximal femur in females and to provide relative estimates of percent saturated fat versus unsaturated fat[56,62,64,79,80]. Increased fat volume and saturated fat in the marrow by MR spectroscopy have both been negatively associated with BMD, trabecular bone volume and cortical bone thickness measured by either areal measurements or volumetric CT[79,80]. Furthermore, this technique, MR spectroscopy, provided *in vivo* evidence that oestrogen supplementation in post menopausal women rapidly reduced BMAT and ultimately improved bone density[81]. Subsequent studies also showed that intermittent administration of parathyroid hormone for osteoporosis in men led to a reduction in the number and size of marrow adipocytes, as measured by histomorphometry[82]. Thus, under certain circumstances in humans, a clear negative relationship exists between BMAT and bone mass. However, those studies do not provide a causal mechanism and as illustrated in mouse studies, the marrow adipogenic response could conceivably also be supportive of bone formation and serve as a source of fatty acids rather than as a cause of bone loss (Table 2).

Work from our laboratory has lent further credence to the dynamic nature of marrow adiposity in human volunteers, using MR spectroscopy, lipidomics, and RNAseq[56]. We have also provided a window into possible mechanisms that drive marrow adipogenesis. Healthy young men and women volunteers were admitted to the clinical research center and given a high calorie diet (HCD) for 10 days. They subsequently went home for two weeks and returned for a 10 day fast in the research center. Bone marrow aspirates were performed pre-HCD and post-HCD and pre-fasting and post-fasting. Not surprisingly, the volunteers gained weight with the HCD and lost weight with fasting[56]. But after just 10 days, BMAT measured by MR spectroscopy increased statistically significantly [with both fasting and HCD, and the increase was greater in men than women. These increases were reversed after returning to an ad libitum diet after the fast or HCD intervention. Surprisingly, trabecular bone volume measure by microCT was higher after the 10 day fast than at baseline, while no significant changes were noted in BMD measurements after the HCD. Bulk RNA-seq of marrow adipocytes isolated from the volunteers revealed that the three top gene ontology networks up-regulated after fasting were the alternative complement activation pathway, response to stress and regulation of IGF transport (Costa, unpublished observation) . The most up-regulated genes were complement factor D (adipsin) and lipoprotein lipase and by lipidomic analysis, lipid storage and lipid droplet pathways were enhanced. Similarly, complement factor D has been shown to be markedly up-regulated in bone marrow adipocytes from mice undergoing caloric restriction and global deletion of *Cfd* results in rescue of bone mass and the diet-induced increase of BMAT[83].

Unbiased approaches using tools such as single cell and single nuclear RNAseq, as well as lipidomics and proteomics can provide novel insights into the importance of BMAT relative to the marrow during states of nutritional stress[84]. However, single cell RNA-seq studies of bone marrow adipocytes are challenging, owing to cell fragility. Single nuclear RNA-seq has been accomplished in adipocytes and provides a window into lineage trajectories, although this analysis has not been accomplished yet in marrow adipocytes. Nevertheless, despite the nearly identical cellular phenotype of high BMAT with fasting or HCD, functional

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differences clearly exist in the bone marrow adipocyte Importantly, caloric restriction drives an injury response in mice that can have major implications for both haematopoiesis and skeletal remodelling[73]. The recruitment of skeletal progenitors to both adipocytes and osteoblasts also occurs through IGF-I and its carrier proteins the IGF binding protein regulatory system[85,86]. High expression of the leptin receptor possibly reflects a recruitment of progenitors in the adult and is consistent with mouse studies of caloric restriction[19,73]

Conclusions

Although progress has been made in understanding the origin of marrow adipocytes, as well as the major regulatory determinants of BMAT, many unanswered questions remain about the functionality of marrow adipocytes under distinct conditions. For example, is long term caloric restriction detrimental to the skeleton because of increased marrow adiposity and if so, what is the mechanism? Importantly, if in fact there is a detrimental effect from long-term intermittent fasting, time-restricted eating or sustained caloric restriction on the skeleton, how is this manifested clinically? Studies of gastric bypass patients show that both bone loss and fractures can occur late after surgery[87,88] .

Hence, longer term calorie restriction trials are needed with bone density and fractures as primary or secondary end points. Mechanistically, we still do not know the signal that drives the adipogenic and the osteogenic response to caloric restriction. For example, is there a centrally mediated signal or does it arise locally from the hematopoietic marrow or possibly from compromised bone cells? In other words, what comes first: the adipogenic response to caloric restriction driven by a hormonal or neural signal; or a paracrine factor originating from a nutrient compromised osteoblast or hematopoietic cell?

Another key question is what is the colour and function of the marrow adipocyte: beige, brown, white or other? Marrow adipocytes have a phenotype that distinguishes them from WAT and BAT. However, whether beige adipocyte precursors reside in the bone marrow and are able to differentiate into functional cells is unclear, as is the unlikely possibility that the precursor of marrow adipocytes can also give rise to beige adipocytes. Beige precursor cells can be found embedded in WAT depots, most notably in the inguinal depot, whereas they are almost never seen in the perigonadal depot in mice[89].

Only a small hand full of reports suggest that human bone marrow can beige[90,91]. For example, a bone marrow biopsy was taken from a 74 year-old man with untreated lymphoplasmacytic lymphoma. The marrow contained large numbers of lymphoma cells and “a large area of loosely aggregated adipose cells containing multiple cytoplasmic vacuoles, suggestive of brown adipocytes”. Whether these cells were brown or beige adipocytes is unknown. Moreover, whether these cells arose in the bone marrow or migrated from another site is equally unknown. The authors conclude that, “this is an incidental intraosseous hibernoma[90]. Other investigators have used in vitro culture systems and in some experiments gene expression has been used to show the presence of genes associated with beige adipocytes in bone marrow[92,93]. However, none of these experiments have used histology of freshly prepared bone to determine if multilocular cells (indicative of brown or beige adipocytes with multiple small lipid droplets) are present in bone marrow.[94]

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Regulation of the beige adipocyte phenotype is complex. Inactivation of ZFP423 in mature adipocytes triggers a conversion of differentiated adiponectin-expressing iWAT and gonadal (gWAT) white adipocytes into beige adipocytes[95]. We have taken advantage of this effect to determine whether marrow adipocytes have the capacity to express a beige-like phenotype similar to that seen in iWAT and gWAT in this model. A terminally differentiated adipocyte-specific inducible *Zfp423*-knockout (KO) mouse model was generated[95]. Marrow adipogenesis after a sub-lethal dose of whole-body X-irradiation was measured in the tibia and femur of *Zfp423*-KO and control mice using osmium staining with micro-CT[95]. As expected, irradiated *Zfp423*-KO mice and control mice had a marked increase in marrow adipocytes. However, no multilocular marrow adipocytes could be observed in either the tibia or femurs of irradiated *Zfp423*-KO mice. Irradiated control mice also had no multilocular cells (M.C, Horowitz and R.K.Gupta, unpublished observation). By contrast, the loss of *Zfp423* in iWAT and gWAT results in the appearance of numerous beige adipocytes in those depots[95].

Preliminary findings have been obtained in adipocyte-specific *Zfp423*-KO mice that were irradiated and then treated with CL-316243 (a potent β3adrenergic agonist) to induce beiging This protocol induces obvious beiging in iWAT and even in gWAT. However, no multilocular cells were observed in marrow adipocytes (M.C.Horowitz and R.K.Gupta; unpublished observation) Thus, using the same inducible adipocyte-specific *Zfp423*-KO mouse model, even under the most forceful conditions of beige cell induction, marrow adipocytes fail to develop multilocular beige adipocytes.

One of the molecular hallmarks of beige and brown adipocytes is the expression of UCP1[96] A fluorescent double reporter mT/mG mouse was used to trace *Ucp1* expressing cells, demonstrating that demonstrating that *Ucp1* is not expressed in marrow adipocytes[97].Taken together, these data strongly suggest that mature marrow adipocytes in mouse tibia and femur are unable to undergo beiging. These data also suggest that beige precursors are not present in mouse bone marrow and support the idea that marrow adipocytes are distinct from the other adipocyte lineages.

Another provocative question is whether BMAT has any importance or relevance in human evolution. It is generally accepted that the consumption of meat from large animals was instrumental in human evolution, enabling the development of large brains in early hominins, such as the australopithecines 3.4 million years ago[98]. These early ancestors of ours probably gathered meat from the kills of predatory animals because hunting large animals, with few tools, was dangerous. Scavenging carcasses would enable the hominins to break open the bones to access the nutrient-rich fatty bone marrow (which was not available to most other carnivores) and help support their high metabolism, body heat and brain development[98,99]. Although we have made great progress in understanding the origin, development, physiology and function of marrow adipocytes, we still retain some of the same traits of our hominin ancestors. Nowadays, we don’t have to root through carcasses and bone marrow is now better prepared, but we are still eating BMAT after millions of years of human evolution. As we look back across centuries, much progress has been made in understanding the bone marrow milieu and so, the marrow adipocyte can rightly take its place as an important functional component of bone marrow.

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Key points

- Adipocytes are critical cellular components of the bone marrow that are regulated by local and systemic factors.
- Bone marrow adipocytes have unique origins and distinct functions that are distinguishable from extra-medullary adipocytes.
- In mice and humans, both axial and appendicular bone marrow adipose tissue (BMAT) increase with age and in response to environmental, nutritional and endocrine factors.
- Both a high fat diet and caloric restriction enhance the recruitment and differentiation of marrow adipocytes, although their function might differ by nutrient stores.
- A unique marrow adipocyte like precursor (MALP) probably serves as source of mature bone marrow adipocytes
- Increased BMAT can drive bone loss during high dietary intake or can protect the skeleton during caloric restriction.

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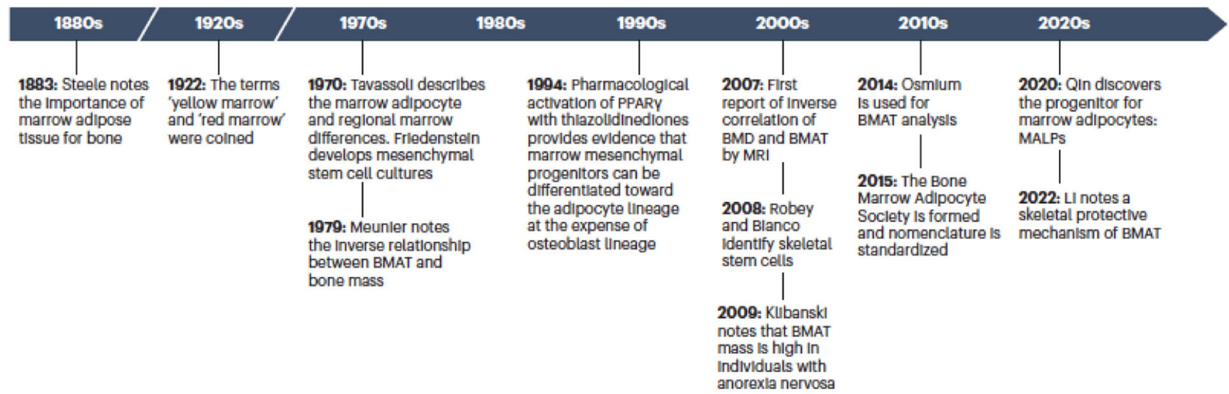

Figure 1: A timeline in the evolution of our knowledge on bone marrow adipose tissue.
Pathologists noted in the 1880s that abundant adipocytes were found in the marrow of healthy older individuals. Bone marrow adipose tissue (BMAT) was speculated as a nutrient source for haematopoietic elements and a cushion for the skeleton[100]. In the 1920s, certain blood disorders and heavy metal intoxication were associated with florid marrow adiposity and impaired haematopoiesis. The term 'yellow marrow' was designated to distinguish that tissue from red haematopoietic marrow[101]. .An important report by Tavassoli and colleagues delineated anatomic and functional differences between yellow and red marrow, and described for the first time, the marrow adipocyte[102]. In the latter part of the twentieth century, investigators noted that marrow ablation following chemotherapy or radiation was associated with a fibrotic response and the appearance of large numbers of adipocytes[103] Similarly, others reported that post-menopausal women with osteoporosis had abundant marrow adipocytes and decreased trabecular bone tissue[78]. A seminal study on anorexia nervosa noted an association between an increase in marrow adiposity and skeletal fragility[104]. With the advent of MR spectroscopy and dual energy CT imaging, a strong negative association between BMAT and bone quantity was reported[105,106]. BMAT was then postulated to regulate bone turnover by increasing bone resorption and suppressing bone formation[107,108]. *In vitro* studies confirmed that isolated skeletal stem cells (SSCs) could commit to either the adipocyte or bone lineage. Furthermore, activation of PPARγ shifted SSCs into an adipocyte-like phenotype. Some in vitro evidence showed that cold temperatures could induce expression of markers of thermogenesis in marrow adipocytes[109]. Subsequent studies using *in vivo* models confirmed that lineage allocation enables early progenitors to shift between osteoblasts and adipocytes, providing another mechanism for altered bone remodeling after hormonal, nutritional or environmental injury,[110]. Ablation of BMAT in mice using global and conditional genetic deletions drives increased cortical and trabecular bone, in part due to the absence of growth factors secreted by marrow adipocytes[5,23,]

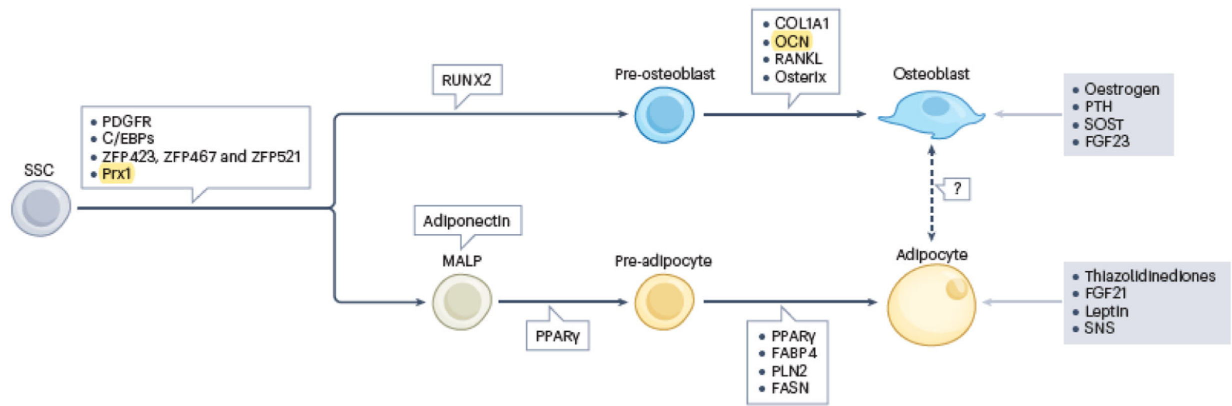

Figure 2: Skeletal stem cells can differentiate into osteoblasts, chondrocytes, stromal cells or adipocytes.

Skeletal stem cells (SSCs) differentiate to early mesenchymal progenitor cells, which can give rise to the adipocyte or osteoblast lineages. One of the earliest progenitors identified that can become an adipocyte is the marrow adipocyte-like progenitor (MALP)[27]. However, we cannot rule out the possibility that other early progenitor cells can differentiate into marrow adipocytes. Transcriptional, paracrine and endocrine factors ultimately define the fate of progenitors. Of note, with activation of PPARγ early in the progenitor lineage, adipocyte differentiation occurs at the expense of osteoblastogenesis. The zinc finger proteins (ZF423, ZFP467 and ZFP521) are expressed early in mesenchymal lineage differentiation both in SSCs and in peripheral adipose depots, but have direct lineage regulation[82,95,] In the pre-osteoblast lineage, RUNX2 (not shown) is the critical transcriptional regulator that sets the stage for osterix activation, followed by differentiated markers of the terminal osteoblast.

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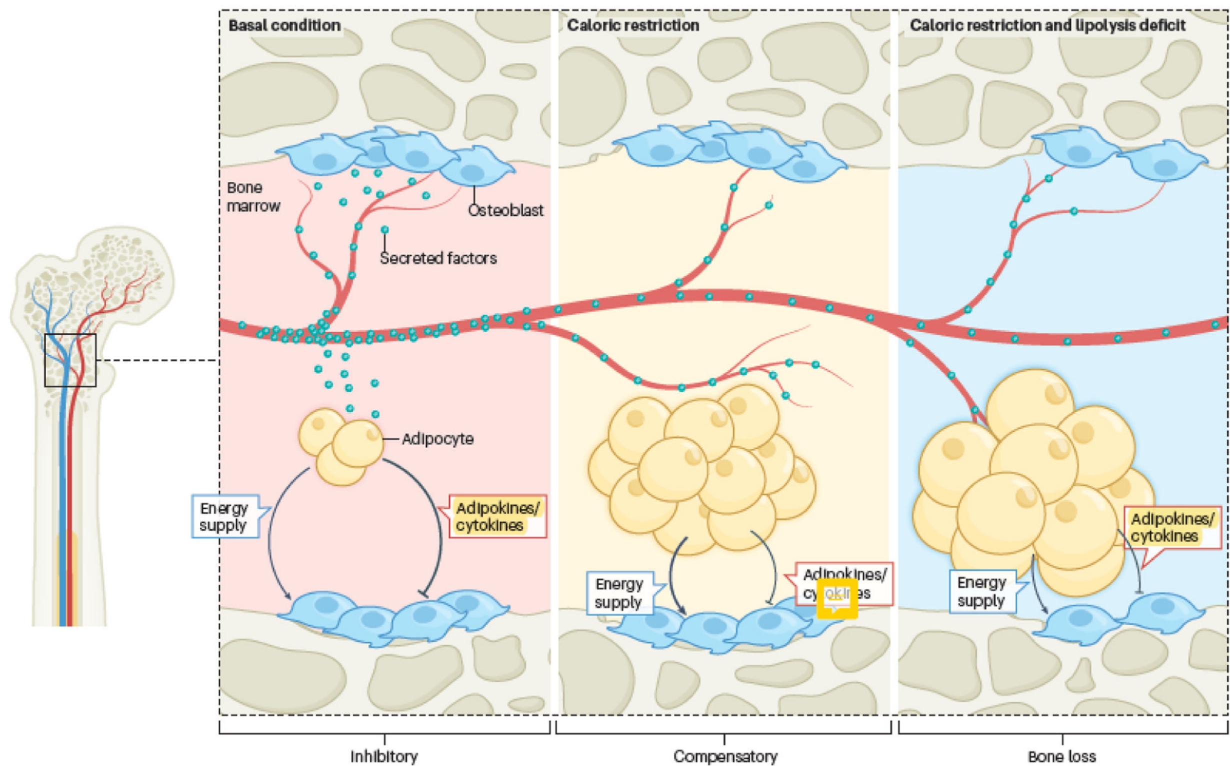

Figure 3: Multi-functional marrow adipocytes.

Bone marrow adipose tissue (BMAT) is dynamic and responsive to nutrient cues. BMAT can be a source of inhibitory cytokines or a fuel depot for use by haematopoietic and skeletal cells in the niche. In the basal condition with adequate energy, marrow adipocytes restrict osteoblast differentiation and function. During caloric restriction. the adipocytes can release free fatty acids and glycerol which could provide energy for osteoblast differentiation. However, if adipose triglyceride lipase (the rate limiting lipolysis enzyme) is inhibited, osteoblasts cannot differentiate and bone formation is reduced.

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Table 1:

Genetically engineered and inbred mouse models to elucidate the effects of diet on marrow adipocytes

Model	Key findings	Limitations	Ref
AdipoCre DTR: elimination of adipocytes	Loss of BMAT and high bone mass with normal diet	Global deletion of all adipocytes	[5]
Prx1-Cre;*Cxcl12*: targeting cells expressing *Prx1* and *Cxcl12*	Used to trace mesenchymal progenitor cells	Prx1 and Cxcl12 Expressed by other cell types in bone marrow and inguinal adipocytes	42,43
Osx-Cre;*Cxcl12*: targeting cells expressing *Osx* and *Cxcl12*	Used to trace osteoblast lineage cells that express osterix	Osterix expression is variable	[28,29]
LEPR conditional	Expressed by the majority of bone marrow CFU-Fs LEPR is a marker of multi-potential SSCs	LEPR is expressed on other SSC progenitors	[19]
FAC-DTA: enables the deletion of genes in marrow adipocytes only	Depletion of marrow adipocytes led to loss of constitutive BMAT and high bone mass in distal tibia	Distinct responses between constitutive and regulated BMAT	[73]
Global adipsin$^{-/-}$	Protection from bone loss and induction of BMAT expansion with 30% caloric restriction	Global deletion could limit utility	[84]
Col2-Cre;Rosa 26: osteoblasts, osteocytes and marrow adipocytes are labelled with tdTomato	Traces osteoblasts, osteocytes and marrow adipocytes.	These papers provide an important method for isolating SSCs	[34]
Rainbow mice	Enables the expression of multiple fluorescent markers from one genetic locus [	Identified postnatal stem cells, but not marrow adipocyte precursors	[30]
AdipoCre *Zfp423*dox inducible: *Zfp423*-KO in terminally differentiated adipocytes	Marrow adipocytes do not undergo beiging	There are likely other determinants that regulate beiging	[32,95]
C57BL/6J wild-type mice	30% caloric restriction led to high marrow adipose tissue	Strain specific	[58,59]

BMAT, bone marrow adipose tissue; DTA, **diphtheria toxin** ; DTR, diphtheria toxin receptor; FAC, FLPo-dependent-Adipo-Cre; LEPR, leptin receptor; SSC, skeletal stem cell.

Table 2:

Clinical disorders associated with changes in BMAT volume in humans and their relation to mouse studies

Disorder or condition	Key findings	Quality of evidence	Ref
Ageing	High BMAT mass and bone loss in humans; high BMAT mass in mice	Cross-sectional studies only	[3,31,78,79,83,]
Gastric bypass, including vertical sleeve gastrectomy	High BMAT mass in some human studies, but not in others; bone loss observed in most human studies; in mice, decreased BMAT	Mostly cross sectional, might be time dependent and might not be a cause of bone loss	[87,88]
Oestrogen deficiency	High BMAT mass and bone loss in humans with short term and long term deficiency; high BMAT mass in mice	Strong evidence, both cross sectional and longitudinal of mice and humans	[78,79,80,81]
Anorexia nervosa	High BMAT mass with, bone loss in humans;no mouse model	Strong evidence, but bone loss ismultifactorial	[62–65]
Chemotherapy or radiation	High BMAT mass with or without bone loss in humans; high BMAT mass in mice, also with bone loss	Cross-sectional studies	[35,68]
Diabetes mellitus	Increased BMAT mass in individuals with T1DM, unclear in T2DM; bone loss in individuals with T1DM and greater fracture risk in mouse models of T1DM, high BMAT; mouse models of T2DM with obesity,high BMAT mass and bone loss	Few longitudinal studies in humans of BMAT; BMD in T2D may be increased, the same or decreased	[50,54,]
Fasting, caloric restriction or starvation	Increased marrow adiposity in femur and spine in humans, but bone loss is not certain; in mice increased BMAT mass and bone loss	Cross-sectional and short-term studies only	[44–46,56,58,59,73]
High-fat diet	Increased marrow adiposity in the short term, with or without bone loss in humans; In mice, increased BMAT mass and bone loss	No extensive longitudinal studies	[56,58,59,]

BMAT, bone marrow adipose tissue; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus.

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