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Bone Marrow Adipocyte Developmental Origin and Biology

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Abstract

a. Purpose of review: This review explores how the relationships between bone marrow adipose tissue (BMAT) adipogenesis with advancing age, obesity, and/or bone diseases (osteopenia or osteoporosis) contribute to mechanisms underlying musculoskeletal pathophysiology.

b. Recent findings: Recent studies have re-defined adipose tissue as a dynamic, vital organ with functions extending beyond its historic identity restricted solely to that of an energy reservoir or sink. “State of the art” methodologies provide novel insights into the developmental origin, physiology and function of different adipose tissue depots including. These include genetic tracking of adipose progenitors, viral vectors application and sophisticated non-invasive imaging modalities.

Summary—While constricted within the rigid bone cavity, BMAT vigorously contributes to local and systemic metabolic processes including hematopoiesis, osteogenesis, energy metabolism and undergoes dynamic changes as a function of age, diet, bone topography or sex. These insights will impact future research and therapies relating to osteoporosis.

Keywords

Beige cells; bone marrow; brown adipose tissue; Cytomegalovirus; white adipose tissue

Compliance with Ethical Guidelines

Conflict of Interest

Xiying Wu and Smith report a patent on use of adipose cells in therapy in the submission process from LaCell LLC.
Jeff Gimble is a co-founder and co-owner of Talaria Antibodies, a polyclonal antibody production company; Obatala Sciences, a fat on a chip technology; and is a co-founder, co-owner and employee at LaCell LLC. Xiying Wu is a co-founder, co-owner, and R&D Director at LaCell LLC; and a co-owner and co-founder of Obatala Sciences; and reports a patent on use of adipose cells in therapy in the submission process from LaCell LLC. Stanley Smith reports a patent on use of adipose cells in therapy in the submission process from LaCell LLC. Michelle McCarthy, Trivia Frazier, Joanna Bukowska, Theodore Brown, Robert Bender and Bruce Bunnell declare no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Introduction to the types of adipocytes and adipose tissues

Adipose tissue is found throughout the body and is distinguished morphologically by the presence of adipocytic cells containing prominent lipid containing vacuoles. Currently, adipocytes can be categorized based on their metabolic/physiologic function, developmental origins, and characteristic protein biomarkers (Table 1). The most abundant human adult or white adipocyte, localized to white adipose tissue (WAT), serve multiple functions including energy storage, adipokines secretion, immunomodulatory roles, and endocrine organ. Developmentally, white adipocytes are postulated to derive from resident adipose stromal/stem cells (ASC) or mesenchymal stromal/stem cells (MSC). These have been characterized based on flow cytometry as a hematopoietic lineage negative, $CD31^-$ (non-endothelial cell), $Sca1^+$, $CD29^+$ $CD34^+$ $CD24^+$ population [1, 2]. Multiple studies of extramedullary adipose depots have documented the expression of pericytic and perivascular markers such as CD146 and 3G5 on ASC and MSC [3–5]. This has led to the hypothesis that capillary mural wall associated pericytes are the original adipocyte progenitor cells, however, morphological and fate mapping studies now question this conclusion. Lineage tracing in transgenic mice using the pericytic-lineage specific Tbx18 transcription factor indicate that this cell population does not contribute directly to the formation of adipose and other tissues [6]. Furthermore, as there is *in vitro* and *in vivo* murine evidence associating adipogenic progenitors and adipose differentiation with the CD34 biomarker alone, exclusive of the CD146 pericytic antigen or a perivascular anatomical location [4, 7–9].

Brown adipocytes, localized to brown adipose tissues (BAT) located in the neck and around vital organs of newborn infants, are less abundant than white adipocytes and display thermogenic properties. With the advent of non-invasive metabolic imaging methods monitoring radiolabeled glucose, BAT can be routinely visualized and its persistence in adult humans is now accepted [10–12]. Due to their expression of uncoupling protein 1 (UCP1), brown adipocytes convert energy directly into heat by acting to short circuit the mitochondrial membrane [13, 14]. Developmentally, brown adipocytes are more closely related to skeletal muscle progenitors than to white adipocytes and ASC. Additional brown adipocyte biomarkers include the transcription co-factors PR domain 16 (Prdm16) and PPARγ Co-activator 1α (PGC1α) [15–17].

Beige or Brite (**Br**own/Wh**ite**) adipocytes are a metabolically intermediate population of adipocytes. Like brown adipocytes, beige/brite adipocytes express UCP1 and are capable of thermogenic function; however, they are localized to subcutaneous and other white-associated adipose tissues under inductive conditions such as cold exposure and are developmentally linked to ASC and MSC rather than skeletal muscle [18]. Furthermore, beige/brite adipocytes express distinct biomarkers including Tbx1, TMEM16, HoxC9, CIDEA, CITED1, and CD137 [19–21]. There is substantial interest in developing pharmacological methods to induce beige/brite adipocytes as a mechanism to combat obesity [22].

Bone marrow adipose tissue (BMAT) (also known as marrow adipose tissue (MAT) or yellow adipose tissue (YAT)) is an adipose depot with unique features distinguishing it from the better characterized extramedullary sites. While the mechanical constraints impacting extramedullary subcutaneous and visceral adipose depots are due to soft tissue compressive

forces, those faced by BMAT are more rigid due to the structural composition of trabecular and cortical bone. Furthermore, while extramedullary adipose depots contain cells capable of hematopoiesis, only BMAT supports this critical cell differentiation event on a daily high volume/turnover basis [23, 24]. There is a growing body of evidence indicating that the anatomical uniqueness of BMAT may be accompanied by a developmental origin distinct from both BAT and WAT [25, 26]. Bone marrow fat does not comprise a single, homogenous tissue. Indeed, BMAT may exist in two district populations: "regulated" (rMAT) that may influence hematopoiesis and "constitutive" BMAT (cMAT) important during early vertebrate development [27]. Although constricted within the rigid bone cavity, BMAT participates in both local and systemic metabolic processes [28]. Additionally, considering its contributions to hematopoiesis [27], osteogenesis [29], energy metabolism [30] and as a functional endocrine tissue [31], there is a growing appreciation that BMAT is a functional, dynamic organ. This review evaluates recent advances in studies of the anatomy, development, and function of BMAT with particular attention directed towards cell tracking/fate mapping with genetic models, brown/beige mechanisms and their contribution to marrow fat physiology. Additionally, the authors extrapolate from extramedullary adipose studies of viral infection to identify potential avenues for future investigations into the developmental origins and function of BMAT.

Age and disease dependent time course of marrow adipogenesis using non-invasive methods

While routine clinical bone imaging methods, dual-energy-X-ray-absorptiometry (DXA) or quantitative computed tomography (QCT), assess bone mineral density, they less effectively quantify BMAT and related non-mineralized bone components. To address this need, new imaging techniques have been developed to enable precise *in situ* characterization of BMAT. Positron emission tomography/computed tomography (PET/CT) scans determine tissue metabolic activity based on their uptake of radiolabeled pharmaceuticals (natural substrates including glucose and fatty acid, substrates analogs or drugs) that are injected intravenously or inhaled by the subject. By using PET and the glucose analog ^{18}F-fluoro-deoxy-glucose (^{18}F-FDG) as a tracer, it has been possible to extend studies concerning human BAT physiology in the context of body-mass index (BMI) and ambient temperature [32]. The PET techniques have been used to evaluate bone marrow composition and function in patients with multiple myeloma [33], follicular lymphoma [34] and related disorders [35]. Likewise, high-resolution magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) are alternative non-invasive techniques for quantification of BMAT [36, 37]. These techniques have provided new insights into bone pathophysiology in the context of metabolic diseases and cancers. MRI visualizes BM soft tissues based on their chemical composition and profile. The relative differences between fat, water and protein ratios observed between red bone marrow and BMAT serve as quantitative and identifiable signatures relevant to clinical diagnostic and therapeutic purposes. Furthermore, MRI has considerable advantage due to its lack of ionizing radiation, excellent tissue contrast and ability to quantify low amount of fat [38]. Both, MRI and MRS can be combed with bone mineral density (BMD) or DEXA to explore the bone-fat dynamic in laboratory animals. Scheller et al [39] quantified BMAT volume accurately by staining bone with osmium tetroxide followed by μCT scanning or

more precise nano-computed tomography (nanoCT). These studies revealed that single adipocytes that are spread with regions of active hematopoiesis ("regulated" MAT; rMAT) when exposed to cold temperature were reduced in size and number within the proximal tibia but remained unchanged in the distal tibia. This observation lends support to the hypothesis that region – specific differences exert regulatory control over adipogenesis within BMAT. Likewise, sophisticated proton magnetic resonance spectroscopy (^{1}H-MRS) evaluates not only total bone marrow fat content but also provides information about the fatty acid composition (assessment of saturated and unsaturated fatty acids) based on MR spectra [40, 41]. Using 3T full-body MRI scanner, Pansini et al [37] compared normal spectroscopic bone marrow fat content (FC) between different regions of the hip in the context of subject age and sex. They further calculated the spectroscopic conversion index (SCI) based on the FC neck/FC greater trochanter ratio. While SCI increased with age in both genders, it remained lower in women compared to men, consistent with a role for sex steroids in controlling BM conversion. Together, these non-invasive clinical imaging tools will provide a comprehensive picture of BMAT adipogenesis as a function of age and disease status in humans as well as pre-clinical animal models.

Developmental origin and fate map tracking of adipose progenitor cells

Bone marrow adipose tissue has long been recognized as the "yellow" bone marrow characterized by a metabolic profile and functions distinct from extramedullary fat depots. Current data reveal that BMAT plays vital role in bone remodeling; this is highlighted by impaired bone alterations observed in a spectrum of diseases associated with increased marrow adipogenesis [41–43]. However, in healthy subjects, development of BMAT, depends, among others, on age, bone topography and sex. Furthermore, recent data in C57BL/6J (B6) male mice indicates that high-fat diet induced obesity increased BMAT without altering bone mineral density (BMD) [44]. In humans, during intrauterine life and shortly after birth, bone cavities contain mainly hematopoietic red bone marrow. Deposition of BMAT starts during the childhood and lasts throughout adulthood. Adipose tissue expansion primarily occurs in the distal parts of the appendicular skeleton, then spreads towards more proximal portions whereas central bones (i.e., pelvis, ribs) retain red BM throughout life [37, 45]. By the age of 25, BMAT occupies approximately 70% of the BM volume and can reach up to 5% of total fat mass in adults [39]. Interestingly, a study by Griffith et al, shown that conversion on red BM into BMAT occurs with age and this process increases particularly sharply in women at menopause. Compared to age matched males, menopausal women displayed 10% higher levels of BMAT content [46].

Genetic tracking of adipose progenitors

Recent studies have employed inducible genetic lineage tracing systems to monitor and quantify adipose tissue formation *in vivo*. In order to track adipogenesis in mice fed a high fat diet (HFD; 60% of calories from fat) or in response to cold (6 °C) or β-3 agonist exposure Wang et al developed the "AdipoChaser" mouse model[47]. This triple transgenic system uses doxycycline to permanently label mature adipocytes in white adipose tissue (WAT). During the early stage of HFD exposure (1 month), hypertrophy accounted for the development of both gonadal and subcutaneous fat. In contrast, prolonged HFD feeding (2

months) resulted in high capacity adipogenesis exclusively within the gonadal (epididymal) fat depots. Furthermore, exposure to cold or β-3 agonists induced gonadal WAT formation and the *de novo* appearance of beige/brite adipocytes within subcutaneous fat depots [47]. Similar genetic lineage tracing approaches examining cold-induced brown adipocytes (BA) have determined a distinct origin for newly appearing BA in the interscapular BAT (iBAT) and subcutaneous inguinal WAT. While iBAT derive from undifferentiated resident PDGFRα+ progenitors, multilocular BA in the inguinal WAT derive exclusively from preexisting "white" adipocytes [48]. Focusing on committed osteoblast progenitors, Mizoguchi et al used both Lepr/Tomato^{+} and P5-iOsx/Tomato+ transgenic mice to evaluate marrow adipogenesis following irradiation or physical injury. Adipogenesis increased significantly following a pulsed 6 Gy radiation injury while P5-iOsx/Tomato+ were detected in the newly formed chondrogenic tissue of the fracture area that also expressed the chondrocyte marker Sox9. Together, this suggests that progenitor cells expressing Lepr or P5-iOsx can be a source of osteo-, adipo-, and chondro-precursors and they contribute to granulation tissue formation and healing of the injured area [49]. In an independent study, Zhou et al determined a role for leptin receptor LepR+ (*Lepr-cre; tdTomato*) mesenchymal stem cells (MSC) in adipogenesis and osteogenesis of adult mice; however, their role in prenatal bone formation was restricted [50] showing rare distribution in the bone marrow at embryonic day (E) 19.5. However, as early as postnatal day (P) 0.5 a sharp increase appeared in the number of Tomato+ cells that further increased with age.

While fate mapping showed that Lep-cre MSC cells were a rare population in adult murine bone marrow, representing ~0.3% of cells, their number increased considerably within the callus formed immediately following a bone fracture. Moreover, intrafemoral injection of LepR+ cells led to the appearance of Tomato signal positive osteoblasts, adipocytes and chondrocytes. These findings support the hypothesis that LepR+ derived adipocytes and osteoblast contribute to bone marrow renewal and regeneration [50, 51]. However, studies conducted with mice expressing a conditional deletion of LepR in bone marrow MSC (*Prx1-Cre;Lepr$^{fl/fl}$*) indicate that while LepR is required for increased bone marrow adipogenesis in response to an HFD, the receptor is not required for bone marrow adipose tissue formation following radiation injury [51]. Indeed, LepR deficiency in *Prx1-Cre;Lepr$^{fl/fl}$* mice correlated with increased femoral trabecular bone volume relative to the controls. While irradiation increased adipocyte numbers in the marrow, the impact was comparable between LepR deficient mice and their controls. While HFD feeding increased marrow adipogenesis at the expense of osteogenesis in control mice, bone marrow LepR deficient mice displayed improved bone formation and reduced adipogenesis. This suggests that HFD exhibits leptin-dependent effects not only through the central hypothalamic system but also at the level of the MSC. Evidence supports a mechanistic role for leptin-dependent downstream activation of the *Jak2/Stat3* signaling pathway and *Cebpα* transcription resuling in *Wnt4* downregulation as a regulator of bone marrow adipogenesis [51]. Ambrosi et al. have examined BMAT adipogenesis in the context of aging and high fat diet using lineage tracing based on reporters driven by the promoters of identifying hematopoietic (*Vav1*), endothelial (*Cdh5/Tie2*), MSC (*PDGFRα*), and committed adipocytes (*Adipoq*) [52]. They noted that high fat diet with or without aging increased marrow adipogenesis and did so at the expense of hematopoiesis and osteogenesis [52]. The authors further validated their findings based on

flow cytometric analysis and in vitro tri-lineage differentiation analyses [52]. Together this body of work confirms the value of genetic lineage cell tracking in dissecting the developmental dynamics of bone marrow adipogenesis under physiological and pathological conditions. Further studies with these *in vivo* genetic-based tools in the context of tumor formation and metastasis are warranted.

Role of viral vectors in promoting or inhibiting adipogenic development

There is a growing appreciation of the potential role of viral vectors as etiological agents responsible for adipogenesis and the development of obesity. The term 'infectobesity', first introduced by Dhurandhar [53], reflects the concept of close association between certain infections and obesity in human [54], non –human primate [55] and rodents [56]. Multiple viral agents have been reported to influence adipose tissue biology [57, 58]. To date, the most extensive studies have focused on human adenovirus type 36 (Ad-36) which increases adiposity and paradoxically reduces the concentrations of cholesterol and triglycerides in experimental infected animals [56]. Infection with Ad-36 had multiple effects on differentiation of 3T3-L1 preadipocytes cell line including increase in the cells numbers that differentiate into adipocytes, enhanced lipid accumulation, and upregulation of the adipocyte marker enzyme GPDH [58]. The *in vitro* induction of the adipogenic program of both rodent (3T3-L1 cell line) and human (hASC) adipocyte progenitors involved the Ad-36 viral E4 open reading frame (orf)-1 gene. Introduction of this gene was sufficient to upregulate cAMP levels, phosphatidylinositol 3-kinase (PKB) activity and C/EBP-β expression [59]. In contrast, infection with human cytomegalovirus (HCMV) significantly reduced the adipogenic differentiation capacity of hASC [60]. Accordingly, HCMV-infected cells developed fewer lipid droplets when compared with mock-infected cells cultured under adipogenic differentiation conditions. Furthermore, the presence of HCMV altered lipid droplet formation and/or stability. While vacuoles in infected cells aggregated in the perinuclear region, these organelles were distributed regularly throughout the cytoplasm of un-infected control hASC. Parallel analyses by RT-PCR confirmed the downregulation of adipogenic-associated genes e.g. peroxisome proliferator-activated receptor gamma (*PPARγ)*, adiponectin, fatty acid binding protein 4 (*FABP4*), lipoprotein lipase *(LPL*) and perilipin 1. Moreover, HCMV infection inhibited ASCs expression of transcripts of anti-inflammatory mediators including *TSG-6* and *IL-6.* These studies emphasize the importance of determining the HCMV seropositivity of both ASC-donors and recipients since viral status can impact hASC immunomodulatory function and subsequent clinical outcome. Additionally, they raise questions about the role of adenoviral and cytomegaloviral infections as contributors to the regulation of bone marrow adipogenesis.

Conclusions and future directions

Studies during the past few years have substantially advanced the understanding of BMAT and its developmental origins; however, additional questions remain unanswered and merit further consideration. These include the following:

Are beige/brite mechanisms responsible for bone marrow adipogenesis seen with advancing age, osteopenia, and osteoporosis? There is a growing body of data indicating that bone

marrow contains beige/brite adipocytes. Studies of heterotopic ossification in human and rodent models have detected UCP1, consistent with the presence of beige/brite or brown adipocytes [61, 62].

1. These findings indicate that beige/brite cells are associated and co-localized with peripheral nerves [63, 64]. These are intriguing observations and further work is necessary to determine if the same progenitor population contributes to age associated adipogenesis. Are beige/brite adipocytes an intermediate step in the pathway towards the acquisition of a yellow marrow? Can the endocrine/bone community re-purpose drugs developed for the treatment of obesity and metabolic syndrome targeting the beige/brown pathway to redirect or commandeer the BMAT differentiation pathway, thereby promoting bone growth rather than age-associated osteopenia and osteoporosis?

2. Are viral agents, including but not limited to adenovirus and cytomegalovirus, contributors to bone marrow adipogenesis? The ability of viral vectors to alter adipogenesis presents an alternative view of extramedullary obesity as a possible infectious disease. This may have an equivalent impact on BMAT formation. Since Ad-36 and CMV induce adipogenic markers associated with a white or beige/brown phenotype, respectively, this raises questions concerning the role of viral infection or reactivation in the acquisition of marrow adiposity and bone loss. Additionally, it suggests that studies examining the contribution of other common viral infections, such as hepatitis and HIV, to BMAT pathophysiology at the progenitor cell level.

3. Is extramedullary obesity linked directly to bone marrow adiposity? (Corollary: How does nutrition alter bone marrow adipogenesis?) This concept is by no means new as evidenced by the intriguing title of highly cited review article: “Mechanisms of Disease: Is Osteoporosis Obesity of the Bone Marrow?” [65].

4. There remains considerable interest in the links between high fat diets and marrow adipogenesis [52, 66].

   Studies have found that male C57BL6 mice fed a high fat diet displayed increased bone mass relative to controls secondary to the mechanical strain of an increased body mass index; however, it was also associated with a reduced rate of bone formation and turnover [66].

5. High fat diet studies have been found to increase the number of lineage committed adipogenic progenitors in murine marrow and this is enhanced with advancing age [52] The production of dipeptidyl peptidase 4 (Dpp4) by the marrow adipocytes exerted a negative impact on bone repair and osteogenesis during fracture healing in this model [52]. These findings further confirm the importance of bone marrow adipocytes as a therapeutic target in the treatment of osteopenia, osteoporosis, and their co-morbidities of fracture and sarcopenia.

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Table 1

Adipose tissue categories and their characteristic features

Tissue type	Tissue Function	Developmental Origin	Specific markers*	References
White Adipose Tissue (WAT)	Energy storage as triglycerides; Coordination of systemic metabolism (i.e. glucose metabolism); Insulation; Mechanical support for internal organs Endocrine organ (secretes adipokines: leptin, adiponectin, resistin, apelin, visfatin); Immunomodulation; Role in toxicokinetics of a persistent organic pollutant (POP);	Derived from a *Myf5-* precursors	Leptin, Resistin, Adiponectin	1–3, 66–73-
Brown Adipose Tissue (BAT)	Energy dissipation and heat production (transferring energy from nutrients to heat in the process involving UCP1 as a main agent that collapses the electron gradient to generate heat rather than ATP); Body weight regulation; Counteracting metabolic diseases (obesity, type 2 diabetes); Critical to newborn infants survival;	Derived from *Myf5+* and *Pax7+* progenitor cells that also give rise to skeletal muscle	Uncoupling protein 1 (UCP1), PPARγ coactivator-1α (PGC-1α), PR domain zinc finger protein 16 (PRDM16), Type 2 iodothyronine deiodinase (DIO2), V-like antigen 1 (EVA1), also known as myelin protein zero-like 2 (MPZL2)	1–3, 66–73-
Beige/Brite Adipose Tissue	Thermogenic function; Counteracting metabolic diseases (obesity, type 2 diabetes);	Derived from a *Myf5-* precursors	Homeobox C9 (HOXC9), Transmembrane protein 26 (TMEM26), T-box 1 *(Tbx1),* Tumor necrosis factor receptor superfamily member 9 (TNFRSF9) also known as 4-1BB or CD137; Cytochrome c oxidase subunit 8B (COX8B), Cell death activator (CIDEA), Fatty Acid Elongase 3 (ELOVL), Fibroblast Growth Factor 21 (FGF21), -	15, 19–21, 67–71, 73–75
Bone Marrow Adipose Tissue (BMAT; MAT)	Regulates hematopoietic stem cell biology; Contributes to hematopoiesis; participates in bone remodeling; bone lipid metabolism; Secretory tissue (releases leptin, adiponectin).	Some BMAT derived from a *Myf5-* precursors than can differentiate into osteoblasts and WAT, however both BAT and beige are present in the bone marrow	Specific markers no identified, however it has been reported high expression of BAT markers including PRDM16, PGC1a, DIO2 and Low levels of WAT markers (adiponectin and leptin) BAT-like characteristics decrease with aging and diabetes.	25, 27, 30, 31

*Adipose tissues share transcriptional cascade including peroxisome proliferator-activated receptor (PPAR)-γ and CCAAT/enhancer-binding proteins (C/EBPs) [68].