



## Commentary

## New insights into bone marrow adipocytes: Report from the First European Meeting on Bone Marrow Adiposity (BMA 2015)☆



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### 1. Introduction

A two-day meeting on bone marrow adiposity (BMA) was held in Lille (France) on 28–29 August 2015 (<http://bma2015.sciencesconf.org>). The meeting was co-organized by P. Hardouin and P.J. Marie and was hosted by the University of Lille. This is the first time that an international meeting covering all aspects of this emerging field was organized. The underlying idea was to give the opportunity for physicians and scientists interested in BMA but from different fields (cell biology, fat, bone, cancer, imaging, obesity and diabetes) to share their results and to exchange their views on this subject. There were over 120 delegates from fifteen countries who attended the two-day meeting. This paper represents a compendium of the presentations as summarized by individual authors. A list of contributors can be found in [Appendix A](#).

### 2. Overview

The basic premise of the meeting centered on the precept that bone marrow adipocytes are not simply fillers to occupy space in the bone marrow; rather that marrow adipose tissue represents a dynamic depot, intimately involved in bone remodeling, hematopoietic stem

cell differentiation, whole body energy homeostasis and neoplastic homing and metastases [1]. Recent advances in understanding the origin and function of BMA have been dramatic and some of these were highlighted at this meeting, as was the need for more research.

### 3. The bone marrow adipocyte and its molecular characteristics

Bone marrow adipose tissue is composed of cells that have molecular characteristics consistent neither with white nor brown fat, and likely not beige fat; however marrow adipocytes may not be homogeneous; furthermore these cells may be unique in their surface markers and represent a combination of several cell types such as white and beige cells. All the adipocytes in the marrow express PPARG2 and studies have suggested that other downstream markers of mature white adipocytes are present. Whether these cells have a beige-like function is not clear, although some of these cells have a brown-like molecular signature [2]. In addition, preliminary evidence presented at this meeting suggested that some of the cells containing lipid droplets have osteoblast-like characteristics. Furthermore, in elegant transplantation studies Arner and colleagues showed that a proportion of marrow adipocytes (possibly as many as 10%) after transplantation find their way to peripheral depots as lipid storing cells [3].

Location and metabolic profiling imply there are at least two distinct types of bone marrow adipocytes in mice, one that is 'constitutive' and one that is 'regulated' [4]. Although identical in morphology they differ in other characteristics. In C57BL6J mice, BMA appears during the first two weeks of life in caudal vertebrae and distal tibiae. Those adipocytes are static, have high levels of unsaturated fatty acids, and are resistant to insulin and  $\beta$  adrenergic stimuli. 'Regulated' bone marrow adipocytes are located in the proximal long bones, composed primarily of saturated fatty acids and can expand or contract in response to  $\beta$  adrenergic cues, dietary changes and injury [4]. Regulated bone marrow adipocytes may modulate bone remodeling although the effects of changes in this depot may also be independent of the skeleton [5]. A decrease in bone mass/quality during aging and in diabetes correlates with bone marrow adipose tissue expansion and loss of a beige-like markers. On the other hand, genetic or pharmacologic induction of a 'beiging' phenotype in marrow adipocytes correlates with increased bone mass [2] [6].

☆ The symposium summary is dedicated to the memory of Dr. Paolo Bianco who was a great scientist and contributed to the success of this meeting.

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The genetic programming of marrow adipocytes remains a controversial area of investigation in part because their origin is not clear. As noted, bone marrow adipocytes express *Pparg2* as well as other typical white adipogenic markers, regulatory genes important in thermogenesis, stem cell surface antigens and genes involved in pluripotency, cell reprogramming, and survival of hematopoietic cells. Although bone marrow adipocytes are derived from mesenchymal stem cells (MSCs), they have long-term hematopoietic supporting capabilities and express similar levels of negative and positive regulators of hematopoiesis seen with white adipocytes [7,8]. Emerging data support the novel concept of a higher susceptibility of the osteogenic rather than the adipogenic progenitors to oxidative stress and apoptosis, preferentially triggered in pre-osteoblasts rather than pre-adipocytes by activated PPAR $\gamma$  [9,10]. Interestingly, MSCs derived from induced pluripotent stem cells (hiPSCs) and from bone marrow display a similar immunophenotype. hiPSC-MSCs have the potential to differentiate towards brown and white adipocytic and osteoblastic lineages. However, hiPSC-MSCs have a low adipogenic capacity as compared to bone marrow-MSCs. This low differentiation capacity is limited to adipogenesis, suggesting that MSCs derived from adult tissues and from embryonic-like cells are likely to be very different, making the task of defining the origin of the marrow adipocyte, that much more difficult [11,12].

Epigenetic programming of mesenchymal progenitors may lead to an imbalance between adipocytes and osteoblasts and BMA. For example, conditional deletion of *Hdac3* in pre-osteoblasts causes osteopenia and marrow adiposity. *Hdac3* mRNA is also reduced in bone marrow from elderly women and aged mice. Lipid droplets (LD) are prevalent in osteogenic cultures of *Hdac3*-CKO BMSC with increased expression of genes regulating lipid storage (*Cidec* and *Plin1*) and glucocorticoid (GC) metabolism (*Hsd11b1*). *Runx2*<sup>+</sup>, LD<sup>+</sup> cells were increased 3-fold in the *Hdac3* CKO BMSCs. Thus, *Hdac3* promotes bone formation and prevents LDs by regulating intrinsic activation of GCs and lipid storage in pre-osteoblasts. The precise number of bone marrow cells with lipid droplets that have this signature is not clear but may be close to 10% of the total stromal cell population [13,14].

#### 4. Bone marrow adiposity and its anatomical niche

The current paradigm is that the more vessels, the less marrow fat, the more bone. This may be true for mechanical challenges. However, experiments (hypoxia, aging, and PTH treatment) show that the marrow fat/vessel/bone relationships are more complex [15]. The various phenotypes reported for both marrow adipocytes and bone marrow vessels are likely to be functionally linked, which needs to be investigated further [16]. With aging, while the number and size of adipocytes increase in the medullary cavities, hematopoiesis decreases. Studies have shown that adipocytes play an active role in hematopoiesis regulation by blocking granulopoiesis and erythropoiesis through cell–cell contact implicating neuropilin-1 (NRP-1), by inhibiting G-CSF synthesis in stromal cells and by an additional but unknown mechanism [17].

The paradigm of ‘transdifferentiation’ also relates to BMA. For example, a 48 h coculture of human mesenchymal stem cell-derived adipocytes and osteoblasts is enough to induce osteoblast transdifferentiation, suggesting a negative impact of secretory products of mature adipocytes on relatively mature bone cells. By contrast, a co-differentiation model of mouse bone marrow stromal cells looking at early interactions showed the presence of adipocytes in the mineralized matrix. Thus, the nature of interactions between the two lineages depends on the context [18] [19]. Bone marrow adipocyte differentiation from multipotent marrow stromal progenitors is tightly controlled to avoid exhaustion of progenitors. Provocative new data show that proteases may play an important role in regulating lineage. *Mt1-mmp* expressed by mature osteocalcin-positive cells regulates this adipogenic differentiation via *DLK1* (Delta like-1, or *Pref-1*) shedding from the cell surface thereby suppressing *Dll4* Notch-mediated adipogenesis.

#### 5. Phenotyping bone marrow adiposity

The study of MAT has been hindered by the presence of bone, the previous histological approach to counting adipocytes and the heterogeneity of bone marrow cells. New techniques have been developed to study MAT and quantify marrow tissue volume. MAT can now be measured *ex vivo* using osmium tetroxide staining and micro-CT [20]. To determine the origin of BM adipocytes, lineage tracing studies using the fluorescent mT/mG reporter mouse in concert with lineage specific promoters are now being used. Nano-CT and 2-photon microscopy are also being utilized. *In vivo* studies in humans with MR spectroscopy and 1.5 and 3 Tesla magnets have been shown to reliably measure marrow adipose volume in the vertebrae and in the proximal femur and tibia [20]. More modern magnetic resonance techniques such as chemical shift imaging, enhanced spectroscopy and perfusion sequences are suited for bone marrow assessment: tailor-made sequences can be adapted to the objectives of clinical studies. Yet few studies describe the normal features of bone marrow, and especially its adiposity. Research efforts are necessary to improve this knowledge and to better understand the pathophysiological mechanisms involved in bone diseases [21,22].

#### 6. Endocrine and paracrine aspects of bone marrow adiposity

Bone marrow adipose tissue accounts for over 10% of total adipose mass in healthy adults, but research into its function and integration with whole body metabolism has only recently accelerated. Marrow adipose tissue may be a key source of adiponectin, a hormone associated with improved cardio-metabolic health. Therefore, like white adipose depots, marrow adipose tissue can be considered an endocrine organ that may exert systemic effects [23]. It has been established that the mouse skeleton also plays an important role in systemic glucose and fatty acid (FA) clearance. FA profiles of tibial bone marrow resembles that of BAT and WAT. Mice lacking lipoprotein lipase in adipocytes display alterations of the FA composition in bone marrow and cortical bone, indicating that systemic fatty acid metabolism is closely linked to local FA metabolism of bone marrow and bone matrix.

In respect to Type I and Type II diabetes mellitus, BMA is enhanced in the former and often in the latter. Its role in the dysregulation of glucose homeostasis is not well defined. However, *DLK1* is an inhibitor of osteoblast and adipocyte differentiation of skeletal stem cells. There is now an emerging endocrine role for *Dlk1* in regulating whole body energy metabolism. *Dlk1* is produced in response to under-carboxylated osteocalcin (Glu-OCN) and antagonizes the stimulatory effects of insulin on osteoblastic production of Glu-OCN. This is a potential mechanism preventing Glu-OCN-induced hypoglycemia and may have relevance to BMA since some studies have identified *Dlk1* as a marker on marrow adipocytes and the circulating level of *Dlk1* is directly related to marrow adipose volume in anorexic individuals [24,25].

T2D is often treated with metformin, which has diverse actions in several tissues. It has been shown that metformin has a direct osteogenic effect by promoting *Runx2* activity, as well as an anti-adipogenic effect in suppressing the protein expression and activity of PPAR $\gamma$ , through a novel AMPK-independent suppression of p70s6-kinase in murine mesenchymal stem cells. This reciprocal action on mesenchymal stem cell differentiation may be the mechanism whereby metformin reduces fracture risk among T2D patients [26,27]. Consistent with the findings in mice that there are regions within marrow that have distinct adipose tissue functions, data has emerged that marrow fat in humans also exhibits insulin sensitivity and in T2D appears to be insulin resistant, much like other adipose depots. The Dresden team showed that T2D leads to impaired bone quality in ZDF rats and this is characterized by a specific osteoblastic defect with an accumulation of bone marrow fat. Whether the increase in BMA is related to the osteoblastic defect has not been established. It was also noted by the Turku team that free fatty acid (FFA) uptake is higher in vertebrae than in femur

bone marrow adipose tissue both in healthy as well as obese type 2 diabetics. Interestingly, there is evidence that bariatric surgery may reverse BMA insulin resistance. FFA uptake in the entire vertebral bone marrow is not altered after bariatric surgery but the uptake in bone marrow adipose tissue is decreased significantly after surgery. While bone-anabolic treatment with the anti-sclerostin antibody reversed the adverse effects of diabetes on bone, insulin treatment was not as effective. Thus, in addition to controlling blood glucose levels, specific osteoporosis therapies may be required to treat diabetic bone disease [28,29].

Postmenopausal osteoporosis is a major public health problem. In a case-control study reported by Bjornerem and colleagues, 77 women with non-vertebral fractures and 155 controls aged 40–73 years had images of distal tibia and radius obtained using high-resolution peripheral quantitative computed tomography, and analyzed using StrAx1.0 software. Fracture cases had higher fat proportion within the marrow cavity and higher cortical porosity than controls. Bone marrow adiposity and cortical porosity were independently associated with higher odds for fracture. Surprisingly, in one study, Raloxifene, a widely used SERM for the treatment of osteoporosis, was associated with a longitudinal increase in BMA. On the other hand, bone loss in early postmenopausal women is commonly associated with enhanced marrow adipogenesis due largely to the reduction in circulating estradiol. Administration of 17 $\beta$ -estradiol to postmenopausal women reduced the marrow fat fraction by 10% within 2 weeks, indicating that 17 $\beta$ -estradiol regulated bone marrow fat independent of bone mass [30].

Age-related bone loss is ubiquitous and is characterized by reduced osteoblastogenesis, increased resorption and excessive bone marrow adipogenesis in mice and humans. It is well known that bone marrow stromal cells are *in vitro* capable of differentiating into osteoblasts and adipocytes, but the mechanisms regulating the cell fate decisions are poorly understood. A direct role of adipocytes to induce dysfunctional osteoblasts has been surmised but never proven. A hint that this might be the case comes from studies in newly generated, first-in-class mouse models of a genetic disease of bone, fibrous dysplasia (OMIM174800) where changes in osteoblast function might originate from altered adipocyte programming in the marrow [31,32].

Within the tumor-bone microenvironment, the bone marrow adipocyte is one of the most poorly understood cell types. Emerging evidence suggests that bone marrow adipocytes are located close to tumor cells and can promote tumor cell migration, and regulate tumor growth *in vitro* and *in vivo*. Therefore, bone marrow adipocytes are ideally placed to interact with tumor cells and likely contribute to the pathogenesis of both tumor growth and cancer-induced bone disease. In that same vein, enhanced osteoclastogenesis promotes tumor growth and RANKL may be expressed in marrow adipocytes. Transgenic RANKL overexpression in mice develop severe early-onset osteoporosis in both sexes and features trabecular bone loss, cortical porosity, decreased bone strength, progressively increased bone marrow adiposity and bone marrow leucopenia. Ongoing studies aim to investigate the mechanisms that lead to marrow adiposity in the TgRANKL osteoporotic mouse model [33].

## 7. Conclusions

In summary, BMA is not homogeneous but is characterized by differences in distribution (*i.e.* proximal versus distal), evolution (*i.e.* constitutive versus inducible), function (*i.e.* local versus general), and composition (*i.e.* saturated versus unsaturated lipids). BMA is a dynamic tissue relative to bone, metabolism and the immune system. More studies are required to understand these relationships and to explain unexpected and sometimes paradoxical evolution (such as the increase of BMA during anorexia nervosa and high fat feeding, but a decrease with starvation). The current advances in imaging, cell based analyses, and model systems open new avenues to understand and to treat

frequent and serious diseases such as osteoporosis, cancer-induced bone diseases, and metabolic related diseases.

**The Second International Meeting on Bone Marrow Adiposity (BMA 2016) will be held in Rotterdam, August 25–26th, 2016.**

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