THE UNIVERSITY of EDINBURGH

Edinburgh Research Explorer

Bone marrow adipose tissue as an endocrine organ: close to the bone?

Citation for published version:
Sulston, R & Cawthorn, W 2016, 'Bone marrow adipose tissue as an endocrine organ: close to the bone?', *Hormone Molecular Biology and Clinical Investigation*, pp. 1868-1883. https://doi.org/10.1515/hmbci-2016-0012

Digital Object Identifier (DOI):
10.1515/hmbci-2016-0012

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
Hormone Molecular Biology and Clinical Investigation

Publisher Rights Statement:
This is the author's final peer-reviewed manuscript as accepted for publication.

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

OPEN ACCESS

Download date: 24. Feb. 2022

Bone marrow adipose tissue as an endocrine organ: close to the bone?

Richard J. Sulston[1] and William P. Cawthorn[1,a]

[1]University/BHF Centre for Cardiovascular Science, The Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK.

[a]Corresponding author

William P. Cawthorn, Ph.D

University/BHF Centre for Cardiovascular Science

The Queen's Medical Research Institute, University of Edinburgh

47 Little France Crescent

Edinburgh, EH16 4TJ

Tel: +44 (0) 131 242 6691

Fax: +44 (0) 131 242 6779

Email: W.Cawthorn@ed.ac.uk

Running title: *Endocrinology of bone marrow adipose tissue*

I) Abstract

White adipose tissue (WAT) is a major endocrine organ, secreting a diverse range of hormones, lipid species, cytokines and other factors to exert diverse local and systemic effects. These secreted products, known as 'adipokines', contribute extensively to WAT's impact on physiology and disease. Adipocytes also exist in the bone marrow, but unlike WAT, study of this bone marrow adipose tissue (MAT) has been relatively limited. We recently discovered that MAT contributes to circulating adiponectin, an adipokine that mediates cardiometabolic benefits. Moreover, we found that MAT expansion exerts systemic effects. Together, these observations identify MAT as an endocrine organ. Additional studies are revealing further secretory functions of MAT, including production of other adipokines, cytokines and lipids that exert local effects within bone. These observations suggest that, like WAT, MAT has secretory functions with diverse potential effects, both locally and systemically. A major limitation is that these findings are often based on *in vitro* approaches that may not faithfully recapitulate the characteristics and functions of bone marrow adipocytes *in vivo.* This underscores the need to develop improved methods for *in vivo* analysis of MAT function, including more robust transgenic models for MAT targeting, and continued development of techniques for non-invasive analysis of MAT quantity and quality in humans. Although many aspects of MAT formation and function remain poorly understood, MAT is now attracting increasing research focus; hence, there is much promise for further advances in our understanding of MAT as an endocrine organ, and how MAT impacts human health and disease.

Keywords: Bone marrow adipose tissue, white adipose tissue, endocrinology, adiponectin, leptin, adipokine, paracrine, skeletal remodelling, haematopoiesis, bone metastases

Abbreviations: BADGE, bisphenol A diglycidyl ether; BAT, brown adipose tissue; BM, bone marrow; cMAT, constitutive MAT; CR, caloric restriction; CXCL1, chemokine (C-X-C motif) ligand 1; CXCL2,

chemokine (C-X-C motif) ligand 2; FABP4, fatty-acid-binding protein 4; FFA, free fatty acid; HMW, high-molecular-weight adiponectin; HSC, haematopoietic stem cell; IL-6, interleukin-6; LMW, low-molecular-weight adiponectin; MAT, marrow adipose tissue; M-CSF, macrophage colony-stimulating factor; MMW, middle-molecular-weight adiponectin; MSC, mesenchymal stromal cell; OPG, osteoprotegerin; PAI-1, plasminogen activator inhibitor-1; RANKL, receptor activator of nuclear factor κB; RANKL, RANK ligand; RBP4, retinol-binding protein 4; rMAT, regulated MAT; SPARC, secreted protein acidic and rich in cysteine; TNF-α, tumour necrosis factor-α; WAT, white adipose tissue.

II) Introduction

Adipose tissue is typically classified into two main subtypes, white adipose tissue (WAT) and brown adipose tissue (BAT), which exist in distinct depots throughout the body. WAT is found both subcutaneously and viscerally and has been the subject of extensive research, motivated largely by the global burden of obesity and associated metabolic diseases [1]. BAT is localised in more distinct sites and mediates adaptive thermogenesis through an abundance of uncoupled mitochondria [2]. Thus, while WAT plays a key role in energy storage, the fundamental function of BAT is associated with increased energy expenditure. In adult humans the amount of BAT is also far, far lower than that of WAT: in some subjects total BAT mass can exceed 100 grams, but it is typically much lower than this [3]. In addition to these well-researched fat depots, adipose tissue also exists within the bone marrow (BM) [4-6]. Whilst its existence has been known of for over a century, research into the function of this marrow adipose tissue (MAT) has remained relatively scarce. This is surprising given that MAT represents over 5% of total bone marrow mass [7] and around 10% of total adipose mass in lean, healthy humans [8]. Compare this to BAT, which is undetectable in many humans and yet is an area of intense research. Moreover, MAT increases further in ageing and many clinically relevant conditions, including osteoporosis; oestrogen deficiency; anorexia nervosa and caloric restriction (CR); type 1 diabetes; and glucocorticoid treatment [4]. These observations raise a key question: why has MAT been so ignored by modern biomedical research? Moreover, what are the mechanisms responsible for MAT formation, both in normal development and in pathological contexts? Finally, what is the function of this intriguing tissue?

The now-extensive understanding of WAT and BAT may provide insights into the function of MAT. Initially believed to exist as an inert store for lipids, WAT is now established as a major endocrine organ [1]. A myriad of reviews have discussed this endocrine function of WAT, highlighting a range of WAT-derived secreted factors, dubbed ‘adipokines’, that exert diverse local and systemic effects [9-

15]. More recent studies have explored the endocrine nature of BAT, showing that it too possesses the ability to secrete factors with a role in bioenergetics and metabolic homeostasis [16].

The recognition of WAT as an endocrine organ is largely underpinned by the study of two hormones, leptin and adiponectin, which were first discovered in the mid-1990s [17, 18]. Leptin is a key regulator of energy homeostasis that circulates in direct proportion to total WAT mass, thereby acting as an indicator of the body's long-term energy reserves. It does so by signalling in the hypothalamus to regulate satiety, energy balance, fertility and immune function, to name but a few of its effects [19]. Loss of WAT results in decreased circulating leptin, invoking a starvation response that causes increased hunger and decreased metabolic rate; this underlies every dieter's struggle to sustain fat loss, as well as the entrenchment of obesity at epidemic levels in most modern societies. Animal models and humans lacking leptin therefore have insatiable hunger, leading to profound obesity. Indeed, mice with mutations in the genes for leptin (*ob/ob* mice) or its receptor *(db/db* mice) have become key models of obesity and insulin-resistance, resulting from their insatiable hyperphagia [20]. Like leptin, adiponectin is a peptide hormone that is produced almost exclusively by adipocytes but, unlike leptin, circulating adiponectin concentrations typically correlate inversely with WAT mass [21]. Thus, obesity and insulin-resistance are associated with hypoadiponectinaemia, which may contribute to obesity-associated cardiometabolic dysfunction. Indeed, adiponectin can exert beneficial cardiometabolic effects including improved glucose tolerance, insulin sensitivity, β-cell survival and vascular function [22]. Decreased circulating adiponectin is now an established biomarker for increased risk of cardiometabolic diseases, and may have utility as a risk factor for cancer and other clinical conditions [23]. Adiponectin is also notable in that it circulates in distinct multimeric forms, including low-molecular-weight (LMW) trimers, middle-molecular-weight (MMW) hexamers, and high-molecular-weight (HMW) complexes that include dodecamers and even larger multimers. At the time of writing (February 2016), leptin and adiponectin have been mentioned in

over 39,000 Pubmed-indexed publications, emphasising the extensive study of WAT as an endocrine organ.

In contrast, study of MAT has been extremely limited, but this is beginning to change. Our recent research revealed that MAT is a source of increased circulating adiponectin during CR, identifying MAT as an endocrine organ that can exert systemic effects; however, many questions remain. For example, do the endocrine functions of MAT extend beyond adiponectin, and to contexts other than CR? What are the functions of MAT as a secretory organ, both within the BM environment and beyond? While recent research has begun to shed light on the endocrine and paracrine functions of MAT-secreted factors, many issues remain to be addressed. Herein, we summarise these developments and highlight areas that should provide fertile ground for future research.

III) MAT as an endocrine organ

i. Adiponectin

We first investigated the potential endocrine function of MAT in response to two surprising observations. The first of these is the so-called 'adiponectin paradox': despite being produced almost exclusively by adipose tissue, circulating adiponectin concentrations inversely correlate with peripheral adiposity. Thus, hypoadiponectinaemia occurs in obese and insulin-resistant subjects but hyperadiponectinaemia exists in states of leanness, including patients with anorexia nervosa and in animal models of CR [21, 24-26]. Hypoadiponectinaemia in obesity likely results from decreased production from WAT, but the mechanisms underlying hyperadiponectinaemia in CR have remained elusive [26]. Why might circulating adiponectin increase in states of leanness? A potential answer emerged in light of a second surprising observation: in stark contrast to WAT, MAT increases during CR in animals and humans [8, 27], i.e. the very states when circulating adiponectin is also increased.

Moreover, both MAT and circulating adiponectin are increased in many other clinical conditions, including estrogen deficiency, type I diabetes, ageing, and in response to pharmacological agents such as glucocorticoids, thiazolidinediones and fibroblast growth factor-21 [4, 25, 28-33]. Our findings have since extended this list, revealing that both MAT expansion and hyperadiponectinaemia occur in cancer patients undergoing chemotherapy or radiotherapy [8]. These observations led us to ask the question: does MAT contribute to circulating adiponectin?

Adiponectin production by MAT

Previous studies have demonstrated adiponectin expression in whole BM of the long bones of mice [34-38]; in adipocytes differentiated *in vitro* from mouse or human BM mesenchymal stromal cells (MSCs) [39, 40]; in BM adipocytes isolated from mouse tibiae and femurs [28, 41]; and in human femoral BM adipocytes, both *in situ* [42] and after isolation and culture *ex vivo* [43] (Table 1). We extended these findings by demonstrating that adiponectin is expressed in intact MAT depots, including caudal vertebrae of mice and in tibial MAT of rabbits and humans [8] (Table 1). We found that, relative to other typical adipocyte transcripts or proteins, adiponectin expression in these MAT depots is greater than that in WAT [8]. This finding is inconsistent with other results suggesting that adiponectin expression in adipocytes isolated from mouse or human BM is lower than that in WAT adipocytes [28, 44]. However, this discrepancy may reflect site-specific differences in MAT characteristics: while our initial analysis revealed greater adiponectin expression in tibial MAT than in WAT of humans [8], we have since found that, in human femoral MAT, adiponectin expression is not always higher than in WAT [26]. Indeed, we recently revealed that MAT exists in distinct subtypes, designated constitutive MAT (cMAT) and regulated MAT (rMAT), with different characteristics [45]. The former develops in the earlier postnatal stages and exists in more distal skeletal sites, such as the distal tibia and caudal vertebrae of rodents. This cMAT also contains few visible haematopoietic cells, thereby appearing histologically similar to WAT. In contrast, rMAT exists in more proximal regions, including the proximal femur, and is comprised of adipocytes interspersed

with haematopoietic populations. Lipid composition and response to external stimuli also differs between rMAT and cMAT [45]. Our findings in human tibial and femoral MAT thus suggest that these MAT subtypes may also differ in adiponectin expression [26].

To investigate if MAT also secretes adiponectin, we cultured explants of MAT and WAT from rabbits and humans, *ex vivo*, and analysed adiponectin secretion into the explant-conditioned media. This revealed that, in both species, secretion of adiponectin was far greater from MAT than from WAT, with enhanced secretion from human explants also noted for LMW, MMW and HMW adiponectin multimers [8]. Importantly, these increases are not attributable to a general increase in MAT protein secretion or breakdown of the MAT explants [8]. These observations build on previous studies demonstrating that adiponectin is secreted from adipocytes differentiated *ex vivo* from human BM [46] and from primary adipocytes isolated from human femurs [43, 47] (Table 1). Indeed, measurement of adiponectin secretion has recently been suggested as a marker of adipogenesis in cultured BM-derived mesenchymal stem cells, further underscoring the capacity of BM adipocytes to secrete adiponectin [48]. Although the mechanisms underlying enhanced adiponectin secretion from MAT relative to WAT remain to be determined, there are many possibilities and technical approaches that are poised to shed further light on this issue [26].

While the above studies confirm that MAT expresses and secretes adiponectin, additional evidence suggests that MAT also influences circulating adiponectin levels. We originally investigated this role of MAT through studies in Ocn-Wnt10b mice, in which a Wnt10b transgene is overexpressed from the osteocalcin promoter. We found that Ocn-Wnt10b mice resist both CR-associated MAT expansion and hyperadiponectinaemia, demonstrating that MAT expansion is required for the full increases in circulating adiponectin [8]. Other recent studies further support this conclusion. For example, separation-based anorexia, a unique CR model, causes many effects typical of CR, including weight loss and hypoleptinemia, but without leading to hyperadiponectinemia [49]. Notably, MAT

expansion also fails to occur in this model (Christophe Chauveau, personal communication). Similarly, we recently revealed that CR in rabbits leads to decreased body mass, WAT mass, and circulating leptin, but without hyperadiponectinemia or MAT expansion [50]. Thus, findings in three distinct animal models collectively suggest that MAT expansion is required for hyperadiponectinemia during CR, supporting the conclusion that MAT contributes to increased circulating adiponectin in this context (Figure 1, Table 1).

Endocrine functions of MAT-derived adiponectin

What is the endocrine impact, if any, of MAT as a source of circulating adiponectin? Our studies in Ocn-Wnt10b mice revealed that CR-associated adaptations in skeletal muscle are impaired when MAT expansion and hyperadiponectinaemia are suppressed (Table 2). Specifically, we found that skeletal muscle expression of transcripts with roles in mitochondrial function, including *Ppargc1a, Tfam* and *Acadm,* is increased during CR in wild-type mice, but this response is completely absent in Ocn-Wnt10b mice [8]. This effect is consistent with adiponectin promoting mitochondrial biogenesis and function in skeletal muscle [51] (Figure 1). In contrast, skeletal muscle AMPK activity is elevated during CR in Ocn-Wnt10b mice but not in wild-types, which is at odds with the reported ability of adiponectin to activate AMPK [52]. Thus, it remains unclear if these systemic effects of impaired MAT expansion result from relative adiponectin deficiency, or if other MAT-derived endocrine factors are playing a role.

It is also possible that, as a source of adiponectin, MAT has systemic impacts beyond skeletal muscle. Indeed, adiponectin has diverse systemic actions, including increased systemic glucose tolerance, enhanced hepatic insulin sensitivity and beta-cell function, improved cardiovascular function, anti-inflammatory and anti-cancer effects [22] (Figure 1). We found that the ability of CR to improve glucose tolerance and modulate hepatic transcription did not differ between wild-type and Ocn-Wnt10b mice, despite resistance of the latter to MAT expansion and hyperadiponectinaemia [8].

However, although CR-associated MAT expansion and hyperadiponectinaemia are blunted in Ocn-Wnt10b mice, it is important to emphasize that these responses still occur [8]. It is therefore possible that the degree of hyperadiponectinaemia in Ocn-Wnt10b mice, while diminished, remains sufficient to impact glucose tolerance, hepatic transcription, and other systemic targets. This possibility underscores the need to develop improved animal models that more robustly resist MAT expansion, or in which MAT formation is prevented entirely.

ii. Leptin

Another possibility is that MAT exerts systemic effects independently of adiponectin, via production of other endocrine factors. While our research has focused primarily on adiponectin, BM adipocytes have also been found to produce leptin, which may extend the endocrine repertoire of MAT. Laharrague *et al* were the first to report leptin expression and secretion by adipocytes differentiated *in vitro* from human BM MSCs [53], an observation since repeated elsewhere [46, 54, 55]. It remains unclear if such BM-derived adipocytes faithfully recapitulate the characteristics of BM adipocytes *in vivo*; however, it is worth noting that leptin is also secreted by human primary BM adipocytes in culture [43, 56]. Moreover, microarray analyses by Liu *et al* have detected leptin expression in isolated BM adipocytes from mice [28], while we recently revealed leptin transcript expression in distal tibial MAT of rabbits [50] (Table 1). These latter findings demonstrate that leptin production by BM adipocytes is not limited to *ex vivo* culture, supporting the possibility that MAT contributes to circulating leptin *in vivo*. However, this possibility presents a paradox: if MAT does indeed contribute to circulating leptin, why does hypoleptinaemia occur during CR, when MAT volume is increased? Our recent findings provide a potential answer in that, as in WAT, leptin expression in MAT is decreased in CR [50]. This suggests that during CR, MAT expansion may be offset by decreased leptin production, thus ensuring that increased MAT does not undermine the hypoleptinaemia that is important for homeostatic adaptations to CR.

The above findings demonstrate that BM adipocyte express leptin and that, as in WAT, this is suppressed during CR. Laharrague *et al* found that the pro-inflammatory cytokines IL-1β, IL-6, TNF-α and interferon-γ reduce both expression and secretion of leptin from cultured BM adipocytes, further demonstrating that these cells produce leptin in a regulated manner [54]. These authors previously suggested that, given the relatively large amount of MAT in humans, the degree of leptin secretion detected from BM adipocytes is likely sufficient to contribute to circulating leptin concentrations [53]; however, in contrast to adiponectin, this remains to be determined (Figure 1). One possibility is that rather than exerting systemic endocrine functions, MAT-derived leptin acts predominantly within the BM as an autocrine and paracrine factor. Indeed, numerous studies report local effects of leptin within the skeleton, with potential consequences on skeletal remodelling, haematopoiesis and tumour progression (Figure 1). These possibilities are discussed further in section IV, below.

iii. Other adipokines

The identification of leptin and adiponectin presaged the discovery of numerous additional adipokines capable of exerting diverse local and systemic effects. Notable examples include retinol-binding protein 4 (RBP4), adipsin, resistin, chemerin, and cytokines such as tumour necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), monocyte chemoattractant protein-1 and plasminogen activator inhibitor-1 (PAI-1) (Table 1); the latter is implicated with elevated atherogenic risk (Figure 1). More recent findings suggest that fatty acid-binding protein 4 (FABP4), also known as adipocyte protein 2 (aP2), is an adipokine that can mediate systemic effects [57, 58]. Microarray analyses confirm that primary BM adipocytes express each of these adipokines, with *Il6* and *Tnf* transcripts expressed more highly in adipocytes from MAT than in those from WAT [28]. Another microarray study of whole mouse vertebrae further confirms that intact MAT expresses RBP4 and adipsin [59], while transcripts for chemerin and other secreted factors are expressed in BM MSC-derived adipocytes [60, 61] (Table 1). Moving beyond mRNA, several studies demonstrate expression and/or secretion

of these factors at the protein level (Table 1). For example, we found that FABP4 is expressed in lysates of intact rabbit MAT [8], while and TNF-α and PAI-1 are expressed and secreted by primary BM adipocytes in culture [47, 62]. Finally, BM MSC-derived adipocytes can also secrete chemerin [61], which, like TNF-α, can modulate inflammation within WAT (Figure 1). It remains to be determined if BM adipocytes secrete these adipokines *in vivo* and if this influences their concentrations in the circulation (Table 1); hence, the relevance of these adipokines to MAT's putative endocrine function requires further study. However, many of these factors have been suggested to mediate paracrine effects within the BM, highlighting another potentially important function of MAT as a secretory organ (Figure 1). This possibility is further discussed later.

iv. Lipid species

A hallmark of adipocytes is their ability to catabolise their stores of triacylglycerol in a process known as lipolysis. This leads to adipocyte release of glycerol, which is used by the liver for gluconeogenesis; and free fatty acids (FFAs), which are used in other tissues to produce ATP via beta-oxidation. However, the FFAs released by adipocytes can serve as more than just metabolic substrates: they are also able to play a signalling role to impact metabolic states, for example by causing changes in insulin signalling. It has been shown that toll-like receptor 4, which normally functions as a lipopolysaccharide receptor, can also respond to the presence of elevated levels of FFAs in the circulation, causing increased inflammatory signals in WAT [63] (Figure 1). Similarly, palmitoleate release from WAT has been suggested to inhibit hepatic steatosis and to promote insulin sensitivity in skeletal muscle [64], suggesting that adipose-derived circulating lipids can exert endocrine effects. Earlier research in dog models shows that MAT can release FFAs into the circulation *in vivo* in response to perfusion of epinephrine or isoproterenol, albeit to a lesser extent than omental WAT [65]. Follow-up research in dog tibiae also confirmed the release of FFA from MAT was regulated, in part, due to an interplay between α2- and β-adrenoreceptors [66]. Thus, MAT-derived FFA could contribute to circulating lipids and thereby act as a peripheral energy supply (Figure 1). However,

other studies suggest that BM adipocytes do not undergo lipolysis even during acute starvation [67], raising questions over whether MAT contributes to circulating lipids (Table 2).

The lipid composition of MAT is a subject that is attracting increasing attention. Early chromatographic analyses of human BM revealed that it consists almost exclusively of triglycerides, with free cholesterol and phospholipids the only other significant proportions [68]. A more recent study using ^{1}H NMR spectroscopy showed very similar degrees of fatty acid saturation between WAT and tibial MAT in humans [69], suggesting that the lipid content of MAT is similar to that of WAT. However, it has since been revealed that there are site-specific differences in MAT lipid composition, with the proportion of unsaturated fatty acids, such as palmitoleate, being greater in cMAT than in rMAT [45]. This is consistent with earlier observations [70] and suggests that the potential for MAT to exert lipid-mediated endocrine effects may vary among MAT subtypes. Increasing clinical evidence further underscores the potential importance of MAT lipid composition. For example, MRI studies show that increased fatty acid saturation in MAT can be used as a predictor for increased bone fracture risk [71], and that the degree of unsaturation is lower in patients with type 2 diabetes [72]. Thus, increased saturation in MAT of type 2 diabetics might contribute to increased fracture risk in type 2 diabetes. In addition, saturated fatty acids can suppress the expression and secretion of adiponectin [73]; hence, from an endocrine perspective, differential lipid saturation may influence adipokine production from rMAT and cMAT.

Collectively, these observations underscore the potential interplay between MAT location, lipid content, endocrine function and human health; however, as for the other adipokines discussed above, whether MAT contributes to systemic lipid metabolism, or if MAT-derived lipid species exert endocrine effects, remains to be firmly established (Figure 1). The accumulation of MAT during CR suggests that it is resistant to stimuli that drive lipolysis in WAT, consistent with the work of Bathija *et al* [67]. Nevertheless, MAT breakdown can occur if the degree and duration of CR is sufficient [50,

74-78], suggesting that BM adipocytes can undergo lipolysis in certain contexts. Therefore, the possibility that MAT contributes to circulating lipids remains an intriguing possibility (Figure 1).

IV) Close to the bone: paracrine functions of MAT

While the above observations underscore the potential of MAT as an endocrine organ, there has been a greater focus on the secretory properties of MAT from a more paracrine perspective, with particular interest on skeletal remodelling and bone health. This putative paracrine function of BM adipocytes has also been applied to other targets within the BM, including haematopoiesis and the progression of skeletal metastases. Several recent reviews have discussed these issues extensively [6, 79-81], and therefore herein we provide an overview of these potential paracrine effects, focusing on potential endocrine implications and whether existing findings truly reflect MAT function *in vivo*.

i. MAT and skeletal remodeling

MAT is often increased in conditions of decreased bone mass and increased fracture risk [4, 82], underscoring the possibility that MAT directly promotes bone loss and/or inhibits bone formation [82, 83]. Thus, many researchers have investigated the hypothesis that BM adipocytes directly regulate the formation and/or function of osteoblasts and osteoclasts. For example, several studies demonstrate that, during *in vitro* co-culture, primary or MSC-derived BM adipocytes stimulate osteoclastogenesis [84-88] and compromise osteoblast formation, proliferation or function [89, 90]. Treatment with BM adipocyte-conditioned media also enhances osteoclastogenesis [88]. These *in vitro* results suggest that BM adipocytes secrete factors that directly promote bone loss (Table 2). Potential candidates include conventional MAT-derived adipokines, cytokines, lipid species and

other secreted factors. For example, both leptin and adiponectin are implicated with the regulation of skeletal remodelling, and receptors for each of these adipokines are expressed by osteoblasts [34, 91], osteoclasts [35] or osteoclast precursors [92]. However, while some studies find that leptin or adiponectin stimulate the formation or function of osteoblasts and inhibit these processes for osteoclasts, other experiments show the opposite effects [79, 81, 92-95]; hence, precisely how these adipokines impact bone formation remains controversial (Figure 1). Other adipokines, including resistin, chemerin, apelin and visfatin, can also regulate the differentiation, function and/or survival of osteoclasts or osteoblasts [61, 96-98], but it remains unknown if MAT secretes these factors *in vivo* (Table 1). Similarly, FFA release from BM adipocytes may directly impair skeletal integrity by inhibiting the proliferation, differentiation or survival of osteoblasts while stimulating these processes for osteoclasts [81, 99-101] (Figure 1). This is consistent with the aforementioned finding that fatty acid saturation in MAT is positively associated with fracture risk in type 2 diabetic patients, supporting the possibility that BM adipocyte-derived fatty acids may directly impact bone integrity [71]. However, it remains unknown if this correlation reflects any causal role for MAT in compromising bone strength, a hypothesis that would benefit from further mechanistic studies *in vivo* (Table 2).

Beyond these adipokines, many cytokines also profoundly affect the formation and function of osteoblasts and osteoclasts. One essential regulator of osteoclastogenesisis is receptor activator of nuclear factor κ B ligand (RANKL), a member of the TNF family that signals via its receptor, RANK. Osteoblasts secrete RANKL to stimulate osteoclastogenesis from osteoclast precursors, and therefore RANKL production is strongly linked with bone resorption, osteoporosis and arthritis [102-104]. RANKL transcripts are upregulated during adipogenesis of BM MSCs, and pro-resorptive stimuli such as dexamethasone and TNF-α significantly increase RANKL mRNA in cultures of human primary BM adipocytes [84-86]. Similarly, one microarray has detected RANKL transcripts in mouse primary BM adipocytes [28]. While these findings are limited to BM adipocytes in culture, another report

reveals that BM of aged mice has increased RANKL expression [87], suggesting that BM adipocytes may also express RANKL *in vivo*. Functional studies *in vitro* further demonstrate that, in co-culture with osteoclast precursors, human primary BM adipocytes stimulate osteoclastogenesis in a RANKL-dependent manner [84, 85]; however, BM adipocyte production of RANKL was not assessed. MAT might further regulate RANKL activity by production of osteoprotegerin (OPG), an osteoblast-secreted protein that acts as a decoy receptor for RANKL, thereby inhibiting osteoclast-mediated bone resorption [102]. As for RANKL, primary BM adipocytes from mice and humans express OPG transcripts (Table 1). Some studies find that dexamethasone increases OPG expression in BM adipocytes [86], whereas others find no effect [84]; however, dexamethasone and TNF-α consistently increase the RANKL/OPG transcript ratio in human BM adipocytes [84-86], and one report suggests downregulation of OPG during BM MSC adipogenesis [87]. Thus, increased BM adiposity may augment the local RANKL/OPG balance and thereby contribute to osteoclast-mediated bone resorption (Figure 1).

In addition to RANKL, the other key regulator of osteoclastogenesis is macrophage colony-stimulating factor (M-CSF) [102]. Whereas RANKL stimulates osteoclast precursors to undergo osteoclastogenesis, M-CSF supports the proliferation and survival of these precursors, as well as upregulating their expression of RANK [102]; the latter is a defining feature of osteoclast precursor identity. As for RANKL and OPG, primary BM adipocytes from mice and humans express M-CSF mRNA (Table 1), suggesting that MAT may impact bone resorption via M-CSF secretion (Figure 1). The ability of BM adipocytes to secrete M-CSF awaits confirmation.

Other BM adipocyte-derived secreted factors may regulate skeletal remodelling by influencing RANKL or OPG production by neighbouring cell types. For example, in osteoblasts adiponectin can stimulate RANKL production and inhibit OPG expression [105]. TNF-α or IL-6 can also stimulate osteoclastogenesis and bone resorption by modulating the RANKL/OPG pathway [80] (Figure 1), and

therefore it is notable that primary BM adipocytes express these factors more highly than adipocytes from WAT [28] (Table 1). Other MAT-derived cytokines may also promote bone resorption. For example, BM MSC-derived adipocytes secrete chemokine (C-X-C motif) ligand 1 (CXCL1) and CXCL2 to promote osteoclastogenesis, at least during *in vitro* co-culture with osteoclast precursors [88]. In addition to these cytokines, BM adipocytes express cathepsin K [28], a protease implicated in bone resorption [102], while oxidised lipids can also stimulate RANKL production by T lymphocytes [106]. More recently, BM adipocytes were found to secrete extracellular vesicles allowing transfer of adipogenic RNAs to osteoblasts, which may impair osteoblast function [107]. Collectively, these findings support the possibility that BM adipocytes impact osteoblasts and osteoclasts and stimulate bone resorption by secretion of cytokines, proteases, lipid species and adipogenic RNAs (Figure 1); however, as above for RANKL, OPG and M-CSF, whether this occurs *in vivo* is unclear (Table 2).

In vivo relationships between MAT and skeletal remodelling*:*

While the above studies show that BM adipocytes can modulate skeletal remodelling under culture conditions *in vitro*, it is crucial to emphasize the limitations of these findings. For example, these observations are typically limited to transcript expression *in vitro*, and it remains unknown if BM adipocytes or intact MAT can secrete these factors *in vitro* or *in vivo* (Tables 1-2). Indeed, there is a telling lack of mechanistic studies *in vivo* that would provide more compelling evidence for the ability of BM adipocytes to impact skeletal remodelling via secretion of these factors (Table 2). The lipodystrophic A-ZIP/F1 mouse lacks MAT [108] and has increased bone mass as a result of enhanced osteoblast activity [109], supporting the concept that MAT suppresses osteoblast function *in vivo* (Table 2). However, A-ZIP/F1 mice also have complete loss of WAT, hypoleptinaemia, and marked insulin resistance, as well as other potentially confounding effects that might contribute to this bone phenotype [110]. Other clues that MAT regulates skeletal remodelling *in vivo* are based predominantly on conditions in which BM adiposity is negatively associated with bone mass; however, this relationship is not universal. For example, several animal models have increases in

both bone mass and MAT [45, 111], while MAT expansion during childhood and puberty coincides with peak bone acquisition [112, 113]. Indeed, one recent study of girls in early puberty found that BM adiposity positively associates with bone mineral content [114]. We have also observed that CR in rabbits causes bone loss but not MAT expansion [50], and others have shown that blocking MAT formation doesn't prevent bone loss in other animal models [115]. Collectively, these observations demonstrate that MAT expansion *per se* is not detrimental to bone formation, suggesting that BM adipocytes'ability to influence skeletal remodelling *in vivo* is likely context specific (Table 2). The development of improved *in vivo* models for MAT targeting would therefore be of great benefit in determining, mechanistically, the relationship between MAT and skeletal remodelling.

ii. MAT and haematopoiesis

It has been known for over 130 years that the fundamental function of BM is haematopoiesis [116]. This initial discovery motivated further studies of BM anatomy, which firmly established the existence of adipocytes within BM [112, 117]. It is therefore unsurprising that the earliest studies of MAT focused on its relationship with haematopoiesis [74, 112, 118-120]. Although MAT accumulation is a normal developmental process, clinical evidence clearly demonstrates that BM adiposity associates negatively with haematopoiesis. For example, MAT increases in aplastic anaemia [121] but regresses in response to increased erythropoietic demand, such as during hypertensive heart failure or haemolytic conditions [118, 122]. Radiation treatment for bone marrow transplantation is also associated with marked MAT expansion that subsides only after development of haematopoietic precursors [5], suggesting that the former may somehow stimulate the latter. Indeed, MAT expansion has often been reported as 'fatty degeneration' of the red marrow [123], reflecting the concept that increased MAT must necessarily compromise BM haematopoietic potential. While this could simply result from the gross balance between the amounts of haematopoietic 'red marrow' and non-haematopoietic 'yellow marrow' [108], it has also

been proposed that BM adipocytes directly modulate haematopoietic stem cell (HSC) differentiation through paracrine effects [82, 83].

Several lines of evidence support such a function. For example, many adipocyte-derived secreted factors can regulate haematopoiesis, including adiponectin, leptin, prostaglandins and sex steroids [53, 124, 125]. One earlier *in vitro* study suggested that leptin drives haematopoietic precursors toward myelopoiesis and away from other lineages [124], while subsequent work in leptin-deficient *ob/ob* mice suggests that leptin sustains both myelopoiesis and lymphopoiesis *in vivo* [126]. Adiponectin can also promote HSC proliferation *in vitro,* while HSCs cultured with adiponectin display enhanced reconstitution capacity after transplantation *in vivo* [36]. Fatty acid metabolism also plays a crucial role in HSC proliferation and function [127, 128], and therefore it has been proposed that BM adipocytes may influence haematopoiesis by acting as a local source of fatty acids. This is supported by the observations of Tavassoli and Scheller, showing that fatty acid content varies between adipocytes located within haematopoietic or non-haematopoietic BM (i.e. rMAT and cMAT, respectively) [45, 70, 122].

As for skeletal remodelling, co-culture approaches have been used to further investigate if BM adipocytes can directly impact HSC function. One study found that primary human BM adipocytes suppress granulopoiesis of $CD34^+$ HSCs, albeit only when co-culture allowed cell-to-cell contact; when cells were co-cultured using transwells, this effect did not occur [56]. While this is consistent with the concept that MAT suppresses haematopoiesis, it argues against a role for BM adipocyte-derived secreted factors. Similarly, an earlier co-culture study allowing direct cell-to-cell contact found that human BM MSC-derived adipocytes influenced haematopoiesis of $CD34^+$ progenitors, although in this case the adipocytes supported, rather than suppressed, both myelopoiesis and lymphopoeisis [55]. Thus, direct contact between BM adipocytes and HSCs may influence haematopoiesis, although this is stimulatory or suppressive remains unclear (Figure 1).

These inconsistent *in vitro* findings raise the question of whether MAT directly modulates haematopoiesis *in vivo*. One notable study in mice found that, post-irradiation, BM engraftment is enhanced when MAT formation is impaired [108]. This observation was based on two mouse models: A-ZIP/F1 mice, which, as mentioned above, lack the capacity to form adipose tissue; and mice treated with bisphenol A diglycidyl ether (BADGE), which inhibits adipogenesis in BM and other adipose depots. However, it is worth emphasizing that neither the A-ZIP/F1 transgene nor BADGE treatment is specific for MAT, such that each also affects WAT formation and other non-adipose cell types. This could have clear confounding effects, for example via markedly decreased circulating leptin concentrations. Thus, these models alone cannot conclusively establish whether MAT directly modulates haematopoiesis *in vivo*. Further uncertainty arises when other *in vivo* models are considered. For example, increased MAT in Ebf1-knockout mice is not associated with impaired haematopoiesis [129]. Conversely, Ocn-Wnt10b mice have decreased rMAT [26] but impaired haematopoiesis within the BM, as indicated by increased extramedullary haematopoiesis in the spleen (Erica Scheller, unpublished observations). While impaired haematopoiesis in Ocn-Wnt10b mice is likely influenced by their decreased BM volume, these observations demonstrate that increases or decreases in BM adiposity are not always associated with opposite effects on haematopoietic function *in vivo* (Table 2). Thus, the potential impact of MAT on haematopoiesis, including the contribution of BM adipocyte-derived secreted factors, remains to be firmly established.

iii. MAT and tumour progression

The above studies underscore the longstanding interest in MAT as a potential regulator of skeletal remodeling and haematopoiesis. More recently, researchers have begun to investigate the impact of BM adipocytes on tumour progression within bone, with most studies focusing on the hypothesis

that MAT promotes tumour development and invasiveness [80]. This concept has support from some clinical observations. For example, MAT is increased with ageing and, in some cases, during obesity [130], both of which are risk factors for aggressive prostate cancer (as discussed in [80]) (Table 2). Mechanistically, BM adipocytes may influence tumour progression by stimulating osteoclast activity, which is closely associated with the development of skeletal metastases [131]. In support of this hypothesis, increased BM adiposity is associated with elevated levels of pro-inflammatory factors such as CCL2, COX-2, and the chemokines CXCL1 and CXCL2, each of which is implicated in osteoclastogenesis and tumour growth within bone [80, 88, 132]. Based on these findings, Hardaway *et al* further investigated the role of BM adipocyte-derived CXCL1 and CXCL2 in prostate tumour-associated bone resorption. They found that, during transwell-based co-culture between BM MSC-derived adipocytes and prostate tumour cells, the adipocytes' expression and secretion of CXCL1 and CXCL2 was increased [88]. Thus, unlike many of the other putative regulators of bone remodelling, there is direct evidence that BM adipocytes can secrete CXCL1 and CXCL2, at least *in vitro* (Table 1; Figure 1). These authors further revealed that BM adipocyte-conditioned media stimulates osteoclastogenesis *in vitro*, an effect prevented by the inclusion of neutralising antibodies against CXCL1 or CXCL2, or by blocking CXCR2, the receptor for these chemokines [88]. This study therefore provides compelling *in vitro* evidence that BM adipocyte-derived chemokines may promote bone resorption and prostate tumour invasiveness (Figure 1).

In addition to these effects on osteoclast activity and bone resorption, BM adipocytes can also promote metastatic progression through direct effects on tumour cells. Fatty acid oxidation is a key pathway in prostate tumours [133], and therefore the local secretion of fatty acids from MAT may help to sustain their growth and proliferation within bone. Consistent with this, BM adipocytes can transfer lipids to prostate tumour cells during *in vitro* co-culture [134, 135] and this is associated with increased tumour cell invasiveness and FABP4 expression [135]. Notably, prostate tumour cells in mouse models and clinical samples also display increased FABP4 expression, particularly in those

cells within adipocyte-rich regions of the BM [135]. This is important, as it supports a role for this pathway in the progression of prostate tumour metastases within bone *in vivo* (Table 2; Figure 1).

Beyond chemokines and lipid species, classical adipokines such as leptin and adiponectin may also contribute to BM adipocytes' impact on skeletal tumours. *In vitro*, leptin can stimulate prostate cancer cell growth by modulating MAPK activity (as discussed in [80]), and BM adipocyte-derived leptin might also promote metastatic growth within bone by stimulating bone resorption. However, it is worth emphasizing that pro-proliferative effects of leptin have not been observed in all studies [136] and that, as noted above, leptin's impact on skeletal remodeling remains controversial [79]. A recent report by Templeton *et al* sheds further light on the relationship between leptin, MAT and tumour progression in humans. These authors found that conditioned media from human bone samples, which were highly enriched for MAT, stimulates migration of a breast cancer cell line and that the degree of migration is positively associated with leptin concentrations in the conditioned media [137]. This supports the possibility that BM adipocytes might promote breast cancer metastasis to bone via leptin production (Figure 1). However, the impact of MAT becomes less certain when adiponectin is also considered. Unlike leptin, most studies find that adiponectin suppresses proliferation and induces apoptosis in cancer cells *in vitro,* suggesting a role in limiting tumour progression [23] (Figure 1). Consistent with this, circulating adiponectin concentrations are inversely associated with the risk of developing endometrial cancer, postmenopausal breast cancer, colon cancer, renal cancer, leukaemia and other haematological malignancies [23]. As mentioned above, we recently revealed that both MAT and circulating adiponectin increase in cancer patients undergoing chemo- or radio-therapy [8], supporting not only the concept that MAT influences circulating adiponectin, but also raising another possibility: might MAT expansion actually exert anti-cancer effects?

iv. MAT as a paracrine and endocrine organ: why context matters

This question highlights an important point: the influence of MAT on tumour progression, as well as skeletal remodelling and haematopoiesis, is likely to be highly context dependent. For example, while ageing is associated with increases in both MAT and cancer risk, other conditions featuring MAT accumulation, such as CR, are associated with decreased cancer risk [138]. Microarray analyses suggest that BM adipocytes are inherently more pro-inflammatory than adipocytes in WAT, and that ageing or diet-induced obesity alters the global characteristics of these cells [28, 41]. Such changes have clear potential to impact MAT's ability to influence skeletal remodelling, haematopoiesis and tumourigenesis. For example, ageing is associated with decreased adiponectin expression in BM adipocytes [28], which might lessen their ability to suppress local tumour development and thereby contribute to age-associated increases in cancer risk. Conversely, we have shown that CR leads to decreased leptin expression in MAT [50], which may help to limit the progression of bone metastases during CR. As discussed above, rMAT and cMAT may have distinct secretory properties, with present observations suggesting increased adiponectin expression in cMAT [8, 26]. Thus, it is notable that bone metastases typically arise in the cMAT-deficient axial skeleton, whereas metastases in the ambulatory skeleton, which is cMAT-rich, seldom occur. Whether this relates to distinct secretory functions of rMAT vs cMAT, or to other context-dependent changes in BM adipocyte function, remains unknown. Future research must therefore address these important issues.

v. Endocrine consequences of MAT's paracrine actions

While many questions remain to be addressed, interest in the potential paracrine actions of MAT continues to increase. However, an unexplored possibility is that these local effects might have endocrine consequences and thereby mediate systemic effects. For example, it is now known that the skeleton serves not only a structural role, but also produces endocrine factors such as

osteocalcin, which has systemic effects on insulin sensitivity, glucose homeostasis and male fertility [139, 140]. In addition to osteocalcin, the bone-derived proteins SPARC and osteopontin also play important endocrine roles; SPARC (secreted protein acidic and rich in cysteine; also called osteonectin) influences bone mineralization but can also have broad systemic effects, including modifying cancer progression and wound healing [141-144]. Osteopontin is expressed by osteoblasts, osteocytes and other cell and tissue types and, like SPARC, can alter bone mineralization [145]; however, osteopontin can also act at an endocrine level, with effects on immune function, inflammation and tumour metastasis [146-148]. Thus, osteocalcin, SPARC and osteopontin have diverse systemic effects. Bone might also exert endocrine effects via secretion of factors such as bone morphogenetic proteins, or as an important site for systemic glucose disposal [140]. *In vitro* co-culture studies have shown that BM adipocytes can suppress osteocalcin expression in osteoblasts [90, 101, 107], which suggests that MAT might influence endocrine pathways as a result of its local effects within bone (Figure 1). This is an intriguing possibility that awaits further study.

V) Expert Opinion

The concept that MAT may exert a local influence over skeletal remodeling has existed since at least the 1980s [121, 149], presaging extensive research into the potential paracrine function of BM adipocytes. The number of such paracrine studies continues to increase to this day. In contrast, the concept that MAT is an endocrine organ, capable of exerting systemic effects, is still in its infancy. There is much evidence to suggest that MAT can produce adipokines, cytokines and lipid species that have known endocrine and/or paracrine effects (Table 1); however, this alone does not demonstrate that BM adipocytes contribute to these effects *in vivo.* This uncertainty exists at several levels. Firstly, evidence for BM adipocytes' ability to produce these secreted factors, and analysis of downstream effects on other cell types, is often based on *in vitro* studies using adipocytes differentiated from BM MSCs. While this approach is highly practicable, it remains unclear if these

BM-derived adipocytes truly reflect the properties of BM adipocytes *in vivo.* The same concern exists for cultures of primary adipocytes following isolation from the BM, given that such isolation techniques can alter cellular characteristics [150]. Indeed, the recent identification of rMAT and cMAT as distinct MAT subtypes raises fundamental questions about the relevance of primary or MSC-derived BM adipocytes to those that exist *in vivo.* Can this heterogeneity be modelled *in vitro?* Secondly, many studies find that BM adipocytes express transcripts for secreted factors, but this does prove that MAT secretes such factors in a biologically meaningful manner (Table 1). For these reasons, we have begun to characterize intact MAT, including analysis of adipokine secretion *ex vivo* [8, 50]. However, this approach is still subject to a third limitation: even if BM adipocytes or intact MAT can secrete a factor *ex vivo*, what does this tell us about MAT function *in vivo*? Earlier studies addressed these aspects of MAT function via studies in dogs [65, 66], while more recent *in vivo* approaches in mice have used the A-ZIP/F1 model or BADGE treatment to block MAT formation [108, 115]; however, a limitation of these mouse models is that they also disrupt WAT. Thus, we began to address MAT function *in vivo* through our studies in Ocn-Wnt10b mice, which resist MAT expansion during CR without the potentially confounding effects on WAT [8]. The finding that these mice also resist CR-associated hyperadiponectinaemia and systemic adaptations thus demonstrates that MAT can influence circulating adipokine concentrations and act beyond the skeleton to exert systemic effects. This is the strongest evidence yet that MAT is an endocrine organ. But even Ocn-Wnt10b mice have several limitations: they do not completely resist MAT expansion, which may explain why hyperadiponectinaemia is blunted, but not entirely prevented; they have increased bone mass owing to direct stimulation of osteoblastogenesis by Wnt10b [151], which prevents their use for investigating the interplay between MAT and skeletal remodeling; and transgene expression is constitutive, preventing the temporal dissection of MAT function that would be possible with an inducible model.

Collectively, these limitations highlight the need to develop more sophisticated *in vivo* models in which BM adipocytes can be targeted specifically, independently of effects on adipocytes in WAT, BAT, or other cell types. Another method worth developing is analysis of arterio-venous differences in adipokine concentrations across BM and WAT depots, which has been used to study secretory properties of WAT [152]. This method could perhaps be applied to MAT by adapting previous techniques [65, 66], thereby enabling comparison of adipokine secretion from WAT and MAT *in vivo*. These approaches would allow us to comprehensively determine whether BM adipocytes contribute to circulating adipokine levels and to better establish the endocrine and paracrine functions of MAT.

We would also benefit hugely from more comprehensive MAT characterization, both in animal models and humans. Indeed, future studies should investigate if MAT properties in humans are associated with endocrine parameters, such as circulating adiponectin. One study finds a positive association between BM adiposity and circulating adiponectin in Caucasian girls [114], but this awaits confirmation in larger populations (Table 2). Recent findings also demonstrate that, clinically, MAT composition may be more informative than total MAT quantity [71], underscoring the benefits of characterising MAT at multiple levels. Whether MAT quantity and/or composition is linked to bone remodeling, haematopoiesis or skeletal tumour progression would also help to better establish if BM adipocytes really do influence these systems *in vivo.*

VI) Outlook

Despite recent advances in the field, the endocrine functions of MAT remain relatively unknown. However, MAT is attracting increasing research interest, encompassing both clinical perspectives and fundamental biological questions. This holds much promise for great advances over the next decade. In particular, we expect future research to establish the endocrine role of MAT beyond CR and adiponectin, revealing if MAT also contributes to circulating levels of other adipokines,

cytokines, lipid species, or other factors, and the physiological and pathological contexts in which this occurs. The application of advanced –omics approaches will reveal the global characteristics of MAT, including differences between cMAT and rMAT, and how these relate to other adipose depots. Such knowledge will facilitate development of new animal models for robust, specific inhibition of MAT formation, which will allow us to build upon previous studies in more limited animal models (e.g. A-ZIP/F1 mice, Ocn-Wnt10b mice). Other methodological advances may allow analysis of MAT's secretory output *in vivo*. Together, these improved approaches will greatly extend current evidence, largely derived from *in vitro* systems, to provide firm conclusions about the endocrine and paracrine properties of MAT *in vivo.* Finally, tools for non-invasive MAT analysis in humans will continue to improve, allowing clinical studies to assess MAT more accurately, comprehensively and, it is hoped, more routinely. This would greatly increase our knowledge of how MAT quantity and quality relates to numerous physiological and pathological parameters in humans, potentially shedding new light not only on the endocrine functions of MAT, but also more broadly on the relationship between MAT and human health and disease.

VII) Highlights

- MAT contributes to increased circulating adiponectin and skeletal muscle adaptations during conditions of caloric restriction, thus identifying MAT as an endocrine organ that can exert systemic effects.
- It is unclear if MAT also influences circulating adiponectin in conditions beyond caloric restriction, both in physiological and pathological contexts.
- MAT expresses and secretes leptin, but whether MAT influences circulating leptin *in vivo* requires further investigation.
- The secretory products of MAT remain to be characterised on a global level, and therefore it remains unclear how MAT compares to WAT as an endocrine organ.

- Secreted products of BM adipocytes can exert local effects on skeletal remodelling, haematopoiesis and tumour progression within bone, at least *in vitro*; however, the relevance of these relationships *in vivo* remains to be demonstrated conclusively.
- Recent studies suggest that MAT location may have a greater influence on health and disease than the amount of MAT *per se*, and therefore further research is required into whether endocrine properties of MAT differ in a site-specific manner.
- The local, paracrine actions of MAT might have endocrine consequences, for example by modulating production of bone-derived factors such as osteocalcin, osteopontin and SPARC.
- Development of new animal models, allowing more robust and specific targeting of MAT, will be important if we are to further dissect MAT function *in vivo.*
- Improved methods for non-invasive assessment of MAT quality and quantity would help to better establish if MAT influences physiological and pathological conditions in humans.
- The increasing research focus on MAT promises to yield many exciting discoveries over the next decade and beyond.

VIII) Acknowledgements

R.J.S is supported by a PhD studentship from the British Heart Foundation. W.P.C. is supported by a Career Development Award (MR/M021394/1) from the Medical Research Council (UK) and by a Chancellor's Fellowship from the University of Edinburgh. Both authors would like to acknowledge the generous support of the BHF Centre of Research Excellence Award. The authors have no conflicts of interest to disclose.

IX) References

1. Rosen, E.D. and B.M. Spiegelman, *What we talk about when we talk about fat.* Cell, 2014. **156**(1-2): p. 20-44.
2. Cannon, B. and J. Nedergaard, *Brown adipose tissue: function and physiological significance.* Physiological reviews, 2004. **84**(1): p. 277-359.
3. Cypess, A.M., et al., *Identification and importance of brown adipose tissue in adult humans.* N Engl J Med, 2009. **360**(15): p. 1509-17.
4. Fazeli, P.K., et al., *Marrow fat and bone--new perspectives.* J Clin Endocrinol Metab, 2013. **98**(3): p. 935-45.
5. Scheller, E.L. and C.J. Rosen, *What's the matter with MAT? Marrow adipose tissue, metabolism, and skeletal health.* Annals of the New York Academy of Sciences, 2014. **1311**(1): p. 14-30.
6. Hardouin, P., V. Pansini, and B. Cortet, *Bone marrow fat.* Joint Bone Spine, 2014. **81**(4): p. 313-9.
7. Hindorf, C., et al., *EANM Dosimetry Committee guidelines for bone marrow and whole-body dosimetry.* European Journal of Nuclear Medicine and Molecular Imaging, 2010. **37**(6): p. 1238-1250.
8. Cawthorn, W.P., et al., *Bone Marrow Adipose Tissue Is an Endocrine Organ that Contributes to Increased Circulating Adiponectin during Caloric Restriction.* Cell Metabolism, 2014. **20**(2): p. 368-75.
9. Ahima, R.S. and J.S. Flier, *Adipose Tissue as an Endocrine Organ.* Trends in Endocrinology & Metabolism, 2000. **11**(8): p. 327-332.
10. Galic, S., J.S. Oakhill, and G.R. Steinberg, *Adipose tissue as an endocrine organ.* Molecular and Cellular Endocrinology, 2010. **316**(2): p. 129-139.
11. Kershaw, E.E. and J.S. Flier, *Adipose Tissue as an Endocrine Organ.* The Journal of Clinical Endocrinology & Metabolism, 2004. **89**(6): p. 2548-2556.
12. Trayhurn, P. and J.H. Beattie, *Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ.* Proceedings of the Nutrition Society, 2001. **60**(03): p. 329-339.
13. Trayhurn, P. and I.S. Wood, *Adipokines: inflammation and the pleiotropic role of white adipose tissue.* British Journal of Nutrition, 2004. **92**(03): p. 347-355.
14. Vázquez-Vela, M.E.F., N. Torres, and A.R. Tovar, *White Adipose Tissue as Endocrine Organ and Its Role in Obesity.* Archives of Medical Research, 2008. **39**(8): p. 715-728.
15. Wozniak, S., et al., *Adipose Tissue: The New Endocrine Organ? A Review Article.* Digestive Diseases and Sciences, 2009. **54**(9): p. 1847-1856.
16. Villarroya, J., R. Cereijo, and F. Villarroya, *An endocrine role for brown adipose tissue?* Am J Physiol Endocrinol Metab, 2013. **305**(5): p. E567-72.
17. Zhang, Y., et al., *Positional cloning of the mouse obese gene and its human homologue.* Nature, 1994. **372**(6505): p. 425-32.
18. Scherer, P.E., et al., *A novel serum protein similar to C1q, produced exclusively in adipocytes.* J Biol Chem, 1995. **270**(45): p. 26746-9.
19. Rosenbaum, M. and R.L. Leibel, *20 years of leptin: role of leptin in energy homeostasis in humans.* J Endocrinol, 2014. **223**(1): p. T83-96.
20. Friedman, J.M. and J.L. Halaas, *Leptin and the regulation of body weight in mammals.* Nature, 1998. **395**(6704): p. 763-70.
21. Arita, Y., et al., *Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity.* Biochem Biophys Res Commun, 1999. **257**(1): p. 79-83.
22. Turer, A.T. and P.E. Scherer, *Adiponectin: mechanistic insights and clinical implications.* Diabetologia, 2012. **55**(9): p. 2319-26.
23. Dalamaga, M., K.N. Diakopoulos, and C.S. Mantzoros, *The role of adiponectin in cancer: a review of current evidence.* Endocr Rev, 2012. **33**(4): p. 547-94.
24. Delporte, M.L., et al., *Hyperadiponectinaemia in anorexia nervosa.* Clin Endocrinol (Oxf), 2003. **58**(1): p. 22-9.
25. Combs, T.P., et al., *Sexual differentiation, pregnancy, calorie restriction, and aging affect the adipocyte-specific secretory protein adiponectin.* Diabetes, 2003. **52**(2): p. 268-76.

26. Scheller, E.L., et al., *Inside out: Bone marrow adipose tissue as a source of circulating adiponectin.* Adipocyte, 2016. **[Paper in Press]**.
27. Devlin, M.J., *Why does starvation make bones fat?* Am J Hum Biol, 2011. **23**(5): p. 577-85.
28. Liu, L.F., et al., *Characterization of age-related gene expression profiling in bone marrow and epididymal adipocytes.* BMC Genomics, 2011. **12**: p. 212.
29. Isobe, T., et al., *Influence of gender, age and renal function on plasma adiponectin level: the Tanno and Sobetsu study.* Eur J Endocrinol, 2005. **153**(1): p. 91-8.
30. Imagawa, A., et al., *Elevated serum concentration of adipose-derived factor, adiponectin, in patients with type 1 diabetes.* Diabetes care, 2002. **25**(9): p. 1665-6.
31. Berg, A.H., et al., *The adipocyte-secreted protein Acrp30 enhances hepatic insulin action.* Nat Med, 2001. **7**(8): p. 947-53.
32. Kharitonenkov, A., et al., *The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21.* Endocrinology, 2007. **148**(2): p. 774-81.
33. Wei, W., et al., *Fibroblast growth factor 21 promotes bone loss by potentiating the effects of peroxisome proliferator-activated receptor gamma.* Proc Natl Acad Sci U S A, 2012. **109**(8): p. 3143-8.
34. Berner, H.S., et al., *Adiponectin and its receptors are expressed in bone-forming cells.* Bone, 2004. **35**(4): p. 842-9.
35. Shinoda, Y., et al., *Regulation of bone formation by adiponectin through autocrine/paracrine and endocrine pathways.* J Cell Biochem, 2006. **99**(1): p. 196-208.
36. DiMascio, L., et al., *Identification of adiponectin as a novel hemopoietic stem cell growth factor.* J Immunol, 2007. **178**(6): p. 3511-20.
37. Thorrez, L., et al., *Using ribosomal protein genes as reference: a tale of caution.* PloS one, 2008. **3**(3): p. e1854.
38. Lattin, J.E., et al., *Expression analysis of G Protein-Coupled Receptors in mouse macrophages.* Immunome Res, 2008. **4**: p. 5.
39. Ugarte, F., et al., *Notch signaling enhances osteogenic differentiation while inhibiting adipogenesis in primary human bone marrow stromal cells.* Experimental hematology, 2009. **37**(7): p. 867-875 e1.
40. Xiao, L., et al., *Disruption of the Fgf2 gene activates the adipogenic and suppresses the osteogenic program in mesenchymal marrow stromal stem cells.* Bone, 2010. **47**(2): p. 360-70.
41. Liu, L.F., et al., *Age-related modulation of the effects of obesity on gene expression profiles of mouse bone marrow and epididymal adipocytes.* PloS one, 2013. **8**(8): p. e72367.
42. Yokota, T., et al., *Paracrine regulation of fat cell formation in bone marrow cultures via adiponectin and prostaglandins.* J Clin Invest, 2002. **109**(10): p. 1303-10.
43. Uchihashi, K., et al., *Organotypic culture of human bone marrow adipose tissue.* Pathol Int, 2010. **60**(4): p. 259-67.
44. Poloni, A., et al., *Molecular and functional characterization of human bone marrow adipocytes.* Experimental hematology, 2013. **41**(6): p. 558-566 e2.
45. Scheller, E.L., et al., *Region-specific variation in the properties of skeletal adipocytes reveals regulated and constitutive marrow adipose tissues.* Nat Commun, 2015. **6**: p. 7808.
46. Ryden, M., et al., *Functional characterization of human mesenchymal stem cell-derived adipocytes.* Biochem Biophys Res Commun, 2003. **311**(2): p. 391-7.
47. Hozumi, A., et al., *Dexamethasone-induced plasminogen activator inhibitor-1 expression in human primary bone marrow adipocytes.* Biomed Res, 2010. **31**(5): p. 281-6.
48. Martella, E., et al., *Secreted adiponectin as a marker to evaluate in vitro the adipogenic differentiation of human mesenchymal stromal cells.* Cytotherapy, 2014. **16**(11): p. 1476-85.
49. Zgheib, S., et al., *Long-term physiological alterations and recovery in a mouse model of separation associated with time-restricted feeding: a tool to study anorexia nervosa related consequences.* PLoS One, 2014. **9**(8): p. e103775.
50. Cawthorn, W.P., et al., *Expansion of bone marrow adipose tissue during caloric restriction is associated with increased circulating glucocorticoids and not with hypoleptinemia.* Endocrinology, 2016. **157**(2): p. 508-21.

51. Civitarese, A.E., et al., *Role of adiponectin in human skeletal muscle bioenergetics.* Cell Metab, 2006. **4**(1): p. 75-87.
52. Zhou, L., et al., *Adiponectin activates AMP-activated protein kinase in muscle cells via APPL1/LKB1-dependent and phospholipase C/Ca2+/Ca2+/calmodulin-dependent protein kinase kinase-dependent pathways.* Journal of Biological Chemistry, 2009. **284**(33): p. 22426-35.
53. Laharrague, P., et al., *High expression of leptin by human bone marrow adipocytes in primary culture.* FASEB J, 1998. **12**(9): p. 747-52.
54. Laharrague, P., et al., *Regulation by cytokines of leptin expression in human bone marrow adipocytes.* Horm Metab Res, 2000. **32**(10): p. 381-5.
55. Corre, J., et al., *Human bone marrow adipocytes support complete myeloid and lymphoid differentiation from human CD34 cells.* Br J Haematol, 2004. **127**(3): p. 344-7.
56. Belaid-Choucair, Z., et al., *Human bone marrow adipocytes block granulopoiesis through neuropilin-1-induced granulocyte colony-stimulating factor inhibition.* Stem Cells, 2008. **26**(6): p. 1556-64.
57. Cao, H., et al., *Adipocyte lipid chaperone AP2 is a secreted adipokine regulating hepatic glucose production.* Cell Metab, 2013. **17**(5): p. 768-78.
58. Wu, L.E., et al., *Identification of fatty acid binding protein 4 as an adipokine that regulates insulin secretion during obesity.* Mol Metab, 2014. **3**(4): p. 465-73.
59. Kindblom, J.M., et al., *Increased adipogenesis in bone marrow but decreased bone mineral density in mice devoid of thyroid hormone receptors.* Bone, 2005. **36**(4): p. 607-16.
60. Sekiya, I., et al., *Adipogenic differentiation of human adult stem cells from bone marrow stroma (MSCs).* J Bone Miner Res, 2004. **19**(2): p. 256-64.
61. Muruganandan, S., et al., *Chemerin neutralization blocks hematopoietic stem cell osteoclastogenesis.* Stem Cells, 2013. **31**(10): p. 2172-82.
62. Sakamoto, K., et al., *Simvastatin suppresses dexamethasone-induced secretion of plasminogen activator inhibitor-1 in human bone marrow adipocytes.* BMC Musculoskelet Disord, 2011. **12**(1): p. 82.
63. Shi, H., et al., *TLR4 links innate immunity and fatty acid-induced insulin resistance.* J Clin Invest, 2006. **116**(11): p. 3015-25.
64. Cao, H., et al., *Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism.* Cell, 2008. **134**(6): p. 933-44.
65. Tran, M.A., T.L. Dang, and M. Berlan, *Effects of catecholamines on free fatty acid release from bone marrow adipose tissue.* J Lipid Res, 1981. **22**(8): p. 1271-6.
66. Tran, M.A., et al., *Interplay of alpha-2 and beta adrenoceptors in the control of free fatty acid release from bone marrow adipose tissue.* Journal of Pharmacology and Experimental Therapeutics, 1984. **230**(1): p. 228-231.
67. Bathija, A., S. Davis, and S. Trubowitz, *Bone marrow adipose tissue: response to acute starvation.* Am J Hematol, 1979. **6**(3): p. 191-8.
68. Lund, P.K., D.M. Abadi, and J.C. Mathies, *Lipid composition of normal human bone marrow as determined by column chromatography.* Journal of Lipid Research, 1962. **3**(1): p. 95-98.
69. Ren, J., et al., *Composition of adipose tissue and marrow fat in humans by 1H NMR at 7 Tesla.* J Lipid Res, 2008. **49**(9): p. 2055-62.
70. Tavassoli, M., D.N. Houchin, and P. Jacobs, *Fatty acid composition of adipose cells in red and yellow marrow: A possible determinant of haematopoietic potential.* Scand J Haematol, 1977. **18**(1): p. 47-53.
71. Patsch, J.M., et al., *Bone marrow fat composition as a novel imaging biomarker in postmenopausal women with prevalent fragility fractures.* J Bone Miner Res, 2013. **28**(8): p. 1721-8.
72. Baum, T., et al., *Does vertebral bone marrow fat content correlate with abdominal adipose tissue, lumbar spine bone mineral density, and blood biomarkers in women with type 2 diabetes mellitus?* Journal of magnetic resonance imaging : JMRI, 2012. **35**(1): p. 117-24.
73. Karki, S., et al., *The multi-level action of fatty acids on adiponectin production by fat cells.* PLoS One, 2011. **6**(11): p. e28146.
74. Dietz, A.A. and B. Steinberg, *Chemistry of bone marrow. VIII. Composition of rabbit bone marrow in inanition.* Arch Biochem Biophys, 1953. **45**(1): p. 10-20.

75. Cohen, P. and F.H. Gardner, *Effect of Massive Triamcinolone Administration in Blunting the Erythropoietic Response to Phenylhydrazine Hemolysis.* J Lab Clin Med, 1965. **65**: p. 88-101.
76. Vande Berg, B.C., et al., *Distribution of serouslike bone marrow changes in the lower limbs of patients with anorexia nervosa: predominant involvement of the distal extremities.* AJR Am J Roentgenol, 1996. **166**(3): p. 621-5.
77. Thouzeau, C., C. Duchamp, and Y. Handrich, *Energy metabolism and body temperature of barn owls fasting in the cold.* Physiol Biochem Zool, 1999. **72**(2): p. 170-8.
78. Abella, E., et al., *Bone marrow changes in anorexia nervosa are correlated with the amount of weight loss and not with other clinical findings.* Am J Clin Pathol, 2002. **118**(4): p. 582-8.
79. Motyl, K.J. and C.J. Rosen, *Understanding leptin-dependent regulation of skeletal homeostasis.* Biochimie, 2012. **94**(10): p. 2089-96.
80. Hardaway, A.L., et al., *Bone marrow fat: linking adipocyte-induced inflammation with skeletal metastases.* Cancer Metastasis Rev, 2014. **33**(2-3): p. 527-43.
81. Muruganandan, S. and C.J. Sinal, *The impact of bone marrow adipocytes on osteoblast and osteoclast differentiation.* IUBMB Life, 2014.
82. Devlin, M.J. and C.J. Rosen, *The bone-fat interface: basic and clinical implications of marrow adiposity.* Lancet Diabetes Endocrinol, 2015. **3**(2): p. 141-147.
83. Gimble, J.M., et al., *The function of adipocytes in the bone marrow stroma: an update.* Bone, 1996. **19**(5): p. 421-8.
84. Goto, H., et al., *Human bone marrow adipocytes support dexamethasone-induced osteoclast differentiation and function through RANKL expression.* Biomedical Research, 2011. **32**(1): p. 37-44.
85. Goto, H., et al., *Primary human bone marrow adipocytes support TNF-α-induced osteoclast differentiation and function through RANKL expression.* Cytokine, 2011. **56**(3): p. 662-668.
86. Hozumi, A., et al., *Bone marrow adipocytes support dexamethasone-induced osteoclast differentiation.* Biochem Biophys Res Commun, 2009. **382**(4): p. 780-4.
87. Takeshita, S., et al., *Age-related marrow adipogenesis is linked to increased expression of RANKL.* J Biol Chem, 2014. **289**(24): p. 16699-710.
88. Hardaway, A.L., et al., *Marrow adipocyte-derived CXCL1 and CXCL2 contribute to osteolysis in metastatic prostate cancer.* Clin Exp Metastasis, 2015. **32**(4): p. 353-68.
89. Maurin, A.C., et al., *Influence of mature adipocytes on osteoblast proliferation in human primary cocultures.* Bone, 2000. **26**(5): p. 485-9.
90. Clabaut, A., et al., *Human osteoblasts derived from mesenchymal stem cells express adipogenic markers upon coculture with bone marrow adipocytes.* Differentiation, 2010. **80**(1): p. 40-5.
91. Lee, Y.J., et al., *Leptin receptor isoform expression in rat osteoblasts and their functional analysis.* FEBS Lett, 2002. **528**(1-3): p. 43-7.
92. Holloway, W.R., et al., *Leptin inhibits osteoclast generation.* J Bone Miner Res, 2002. **17**(2): p. 200-9.
93. Luo, X.H., et al., *Adiponectin stimulates human osteoblasts proliferation and differentiation via the MAPK signaling pathway.* Exp Cell Res, 2005. **309**(1): p. 99-109.
94. Liu, L.F., et al., *Adipocytes decrease Runx2 expression in osteoblastic cells: roles of PPARgamma and adiponectin.* J Cell Physiol, 2010. **225**(3): p. 837-45.
95. Kanazawa, I., *Adiponectin in metabolic bone disease.* Curr Med Chem, 2012. **19**(32): p. 5481-92.
96. Xie, H., et al., *Insulin-like effects of visfatin on human osteoblasts.* Calcif Tissue Int, 2007. **80**(3): p. 201-10.
97. Thommesen, L., et al., *Expression and regulation of resistin in osteoblasts and osteoclasts indicate a role in bone metabolism.* J Cell Biochem, 2006. **99**(3): p. 824-34.
98. Wattanachanya, L., et al., *Increased bone mass in mice lacking the adipokine apelin.* Endocrinology, 2013. **154**(6): p. 2069-80.
99. Maurin, A.C., et al., *Role of polyunsaturated fatty acids in the inhibitory effect of human adipocytes on osteoblastic proliferation.* Bone, 2002. **31**(1): p. 260-6.

100. Moerman, E.J., et al., *Aging activates adipogenic and suppresses osteogenic programs in mesenchymal marrow stroma/stem cells: the role of PPAR-gamma2 transcription factor and TGF-beta/BMP signaling pathways.* Aging Cell, 2004. **3**(6): p. 379-89.
101. Elbaz, A., et al., *Inhibition of fatty acid biosynthesis prevents adipocyte lipotoxicity on human osteoblasts in vitro.* J Cell Mol Med, 2010. **14**(4): p. 982-91.
102. Asagiri, M. and H. Takayanagi, *The molecular understanding of osteoclast differentiation.* Bone, 2007. **40**(2): p. 251-64.
103. Boyce, B. and L. Xing, *Biology of RANK, RANKL, and osteoprotegerin.* Arthritis Research & Therapy, 2007. **9**(Suppl 1): p. 1-7.
104. Wada, T., et al., *RANKL–RANK signaling in osteoclastogenesis and bone disease.* Trends in Molecular Medicine, 2006. **12**(1): p. 17-25.
105. Luo, X.H., et al., *Adiponectin stimulates RANKL and inhibits OPG expression in human osteoblasts through the MAPK signaling pathway.* J Bone Miner Res, 2006. **21**(10): p. 1648-56.
106. Graham, L.S., et al., *Oxidized lipids enhance RANKL production by T lymphocytes: implications for lipid-induced bone loss.* Clin Immunol, 2009. **133**(2): p. 265-75.
107. Martin, P.J., et al., *Adipogenic RNAs are transferred in osteoblasts via bone marrow adipocytes-derived extracellular vesicles (EVs).* BMC Cell Biol, 2015. **16**: p. 10.
108. Naveiras, O., et al., *Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment.* Nature, 2009. **460**(7252): p. 259-63.
109. Ducy, P., et al., *Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass.* Cell, 2000. **100**(2): p. 197-207.
110. Kim, J.K., et al., *Mechanism of insulin resistance in A-ZIP/F-1 fatless mice.* J Biol Chem, 2000. **275**(12): p. 8456-60.
111. Ackert-Bicknell, C.L., et al., *Strain-specific effects of rosiglitazone on bone mass, body composition, and serum insulin-like growth factor-I.* Endocrinology, 2009. **150**(3): p. 1330-40.
112. Piney, A., *The anatomy of the bone marrow: With special reference to the distribution of the red marrow.* British Medical Journal, 1922. **1922**: p. 792-795.
113. Kricun, M.E., *Red-yellow marrow conversion: its effect on the location of some solitary bone lesions.* Skeletal radiology, 1985. **14**(1): p. 10-9.
114. Newton, A.L., et al., *The relationships among total body fat, bone mineral content and bone marrow adipose tissue in early-pubertal girls.* BoneKEy Rep, 2013. **2**(4).
115. Botolin, S. and L.R. McCabe, *Inhibition of PPARgamma prevents type I diabetic bone marrow adiposity but not bone loss.* Journal of cellular physiology, 2006. **209**(3): p. 967-76.
116. Neumann, E., *Das Gesetz der Verbreitung des gelben und roten Knochenmarkes.* Centralblatt Fur Die Medicinischen Wissenschaften, 1882. **20**: p. 321-322.
117. Muir, R. and W.B. Drummond, *On the Structure of the Bone-Marrow in Relation to Blood-Formation.* Journal of anatomy and physiology, 1893. **28**(Pt 1): p. 125-41.
118. Shillingford, J.P., *The red bone marrow in heart failure.* J Clin Pathol, 1950. **3**(1): p. 24-39.
119. Huggins, C., J. McFadyen, and E. Wiege, *A quantitative study of neutral fat, solids and water in the bone marrow of normal and anemic limbs and the effect of centrifugation on marrow.* The Anatomical Record, 1940. **76**(3): p. 309-317.
120. Huggins, C. and B.H. Blocksom, *Changes in Outlying Bone Marrow Accompanying a Local Increase of Temperature within Physiological Limits.* J Exp Med, 1936. **64**(2): p. 253-74.
121. Burkhardt, R., et al., *Changes in trabecular bone, hematopoiesis and bone marrow vessels in aplastic anemia, primary osteoporosis, and old age: a comparative histomorphometric study.* Bone, 1987. **8**(3): p. 157-64.
122. Tavassoli, M., *Marrow adipose cells. Histochemical identification of labile and stable components.* Arch Pathol Lab Med, 1976. **100**(1): p. 16-8.
123. Schnitzler, C.M. and J. Mesquita, *Bone marrow composition and bone microarchitecture and turnover in blacks and whites.* J Bone Miner Res, 1998. **13**(8): p. 1300-7.

124. Umemoto, Y., et al., *Leptin stimulates the proliferation of murine myelocytic and primitive hematopoietic progenitor cells.* Blood, 1997. **90**(9): p. 3438-43.
125. Yokota, T., et al., *Adiponectin, a fat cell product, influences the earliest lymphocyte precursors in bone marrow cultures by activation of the cyclooxygenase-prostaglandin pathway in stromal cells.* J Immunol, 2003. **171**(10): p. 5091-9.
126. Claycombe, K., L.E. King, and P.J. Fraker, *A role for leptin in sustaining lymphopoiesis and myelopoiesis.* Proc Natl Acad Sci U S A, 2008. **105**(6): p. 2017-21.
127. Ito, K., et al., *A PML-PPAR-delta pathway for fatty acid oxidation regulates hematopoietic stem cell maintenance.* Nat Med, 2012. **18**(9): p. 1350-8.
128. Kinder, M., et al., *Hematopoietic stem cell function requires 12/15-lipoxygenase-dependent fatty acid metabolism.* Blood, 2010. **115**(24): p. 5012-22.
129. Fretz, J.A., et al., *Altered metabolism and lipodystrophy in the early B-cell factor 1-deficient mouse.* Endocrinology, 2010. **151**(4): p. 1611-21.
130. Bredella, M.A., et al., *Ectopic and Serum Lipid Levels Are Positively Associated with Bone Marrow Fat in Obesity.* Radiology, 2013.
131. Olechnowicz, S.W. and C.M. Edwards, *Contributions of the host microenvironment to cancer-induced bone disease.* Cancer Res, 2014. **74**(6): p. 1625-31.
132. Craig, M.J. and R.D. Loberg, *CCL2 (Monocyte Chemoattractant Protein-1) in cancer bone metastases.* Cancer Metastasis Rev, 2006. **25**(4): p. 611-9.
133. Liu, Y., *Fatty acid oxidation is a dominant bioenergetic pathway in prostate cancer.* Prostate Cancer Prostatic Dis, 2006. **9**(3): p. 230-4.
134. Gazi, E., et al., *Direct evidence of lipid translocation between adipocytes and prostate cancer cells with imaging FTIR microspectroscopy.* J Lipid Res, 2007. **48**(8): p. 1846-56.
135. Herroon, M.K., et al., *Bone marrow adipocytes promote tumor growth in bone via FABP4-dependent mechanisms.* Oncotarget, 2013. **4**(11): p. 2108-23.
136. Garofalo, C. and E. Surmacz, *Leptin and cancer.* J Cell Physiol, 2006. **207**(1): p. 12-22.
137. Templeton, Z.S., et al., *Breast Cancer Cell Colonization of the Human Bone Marrow Adipose Tissue Niche.* Neoplasia, 2015. **17**(12): p. 849-61.
138. Meynet, O. and J.E. Ricci, *Caloric restriction and cancer: molecular mechanisms and clinical implications.* Trends Mol Med, 2014. **20**(8): p. 419-27.
139. Lee, N.K., et al., *Endocrine regulation of energy metabolism by the skeleton.* Cell, 2007. **130**(3): p. 456-69.
140. Oldknow, K.J., V.E. MacRae, and C. Farquharson, *Endocrine role of bone: recent and emerging perspectives beyond osteocalcin.* J Endocrinol, 2015. **225**(1): p. R1-19.
141. Lane, T.F. and E.H. Sage, *The biology of SPARC, a protein that modulates cell-matrix interactions.* FASEB J, 1994. **8**(2): p. 163-73.
142. Guweidhi, A., et al., *Osteonectin Influences Growth and Invasion of Pancreatic Cancer Cells.* Annals of Surgery, 2005. **242**(2): p. 224-234.
143. Yiu, G.K., et al., *SPARC (secreted protein acidic and rich in cysteine) induces apoptosis in ovarian cancer cells.* Am J Pathol, 2001. **159**(2): p. 609-22.
144. Wang, H., et al., *Secreted protein acidic and rich in cysteine (SPARC/osteonectin/BM-40) binds to fibrinogen fragments D and E, but not to native fibrinogen.* Matrix Biology, 2006. **25**(1): p. 20-26.
145. Addison, W.N., et al., *Phosphorylation-dependent inhibition of mineralization by osteopontin ASARM peptides is regulated by PHEX cleavage.* Journal of Bone and Mineral Research, 2010. **25**(4): p. 695-705.
146. Wang, K.X. and D.T. Denhardt, *Osteopontin: Role in immune regulation and stress responses.* Cytokine & Growth Factor Reviews, 2008. **19**(5–6): p. 333-345.
147. Giachelli, C.M. and S. Steitz, *Osteopontin: a versatile regulator of inflammation and biomineralization.* Matrix Biol, 2000. **19**(7): p. 615-22.
148. Rodrigues, L.R., et al., *The role of osteopontin in tumor progression and metastasis in breast cancer.* Cancer Epidemiol Biomarkers Prev, 2007. **16**(6): p. 1087-97.

149. Wronski, T.J., J.M. Smith, and W.S. Jee, *Variations in mineral apposition rate of trabecular bone within the beagle skeleton.* Calcif Tissue Int, 1981. **33**(6): p. 583-6.
150. Hayman, D.M., et al., *The effects of isolation on chondrocyte gene expression.* Tissue engineering, 2006. **12**(9): p. 2573-81.
151. Bennett, C.N., et al., *Wnt10b increases postnatal bone formation by enhancing osteoblast differentiation.* J Bone Miner Res, 2007. **22**(12): p. 1924-32.
152. Frayn, K.N. and S.W. Coppack, *Assessment of white adipose tissue metabolism by measurement of arteriovenous differences.* Methods Mol Biol, 2001. **155**: p. 269-79.
153. Zhang, H., et al., *Adipocytes derived from human bone marrow mesenchymal stem cells exert inhibitory effects on osteoblastogenesis.* Curr Mol Med, 2011. **11**(6): p. 489-502.
154. Schwartz, A.V., et al., *Vertebral Bone Marrow Fat Associated With Lower Trabecular BMD and Prevalent Vertebral Fracture in Older Adults.* J Clin Endocrinol Metab, 2013. **98**(6): p. 2294-300.

X) Figure Legends

Figure 1 – Endocrine and paracrine functions of bone marrow adipose tissue. BM adipocytes and/or intact MAT can express and/or secrete a diverse range of secreted factors. In some cases expression has been confirmed but secretion has not yet been demonstrated; these factors are shown in thin-bordered boxes at the end of arrows with dotted borders. Those factors for which secretion has been demonstrated *in vitro* or *ex vivo,* are shown in thick-bordered boxes at the end of arrows with solid borders. At present, adiponectin is the only endocrine factor for which MAT has been shown to influence circulating concentrations; hence, adiponectin is highlighted in a black box. MAT-derived adiponectin is implicated in regulating CR-associated adaptations in skeletal muscle, suggestive of effects on mitochondrial function. Other putative systemic effects of adiponectin or other MAT-derived endocrine factors remain to be determined, although it is possible that MAT may have indirect effects by regulating osteocalcin production. It is unclear how local production of leptin or adiponectin might influence osteoblasts and osteoclasts *in vivo*, although positive and negative effects for other MAT-secreted paracrine factors have been demonstrated more conclusively. These positive and negative effects are indicated by red or green circles containing '+' or '-', respectively. Inconclusive effects are indicated by question marks in grey circles. Few of the studies supporting these findings have yet distinguished between rMAT and cMAT, and therefore future research must investigate if these paracrine and endocrine functions of MAT vary in a site-specific manner. Abbreviations are defined in the text.

Table 1 – Endocrine and paracrine factors expressed and/or secreted by BM adipocytes. Protein expression is based on immunoblots or immunohistochemistry. Transcript expression is based on the indicated methods. 'Other factors' lists microarray-detected transcripts, some of which encode proteins that are better known by the following names: *Cfd,* adipsin; *Apln,* apelin; *Ctsk,* cathepsin K; *Nampt,* visfatin; *Ccl2,* MCP-1; *Ptgs2,* COX-2; *Cxcl8,* interleukin 8; *Fgf7,* keratinocyte growth factor; *Serpinf1,* pigment epithelium-derived growth factor.

Table 2 – Clinical observations, *in vitro* and *in vivo* evidence for or against the putative endocrine and paracrine functions of MAT. Green and red boxes denote evidence for or against each putative function, respectively. Decreased haematopoiesis in Ocn-Wnt10b mice is based on unpublished observations by Erica Scheller.

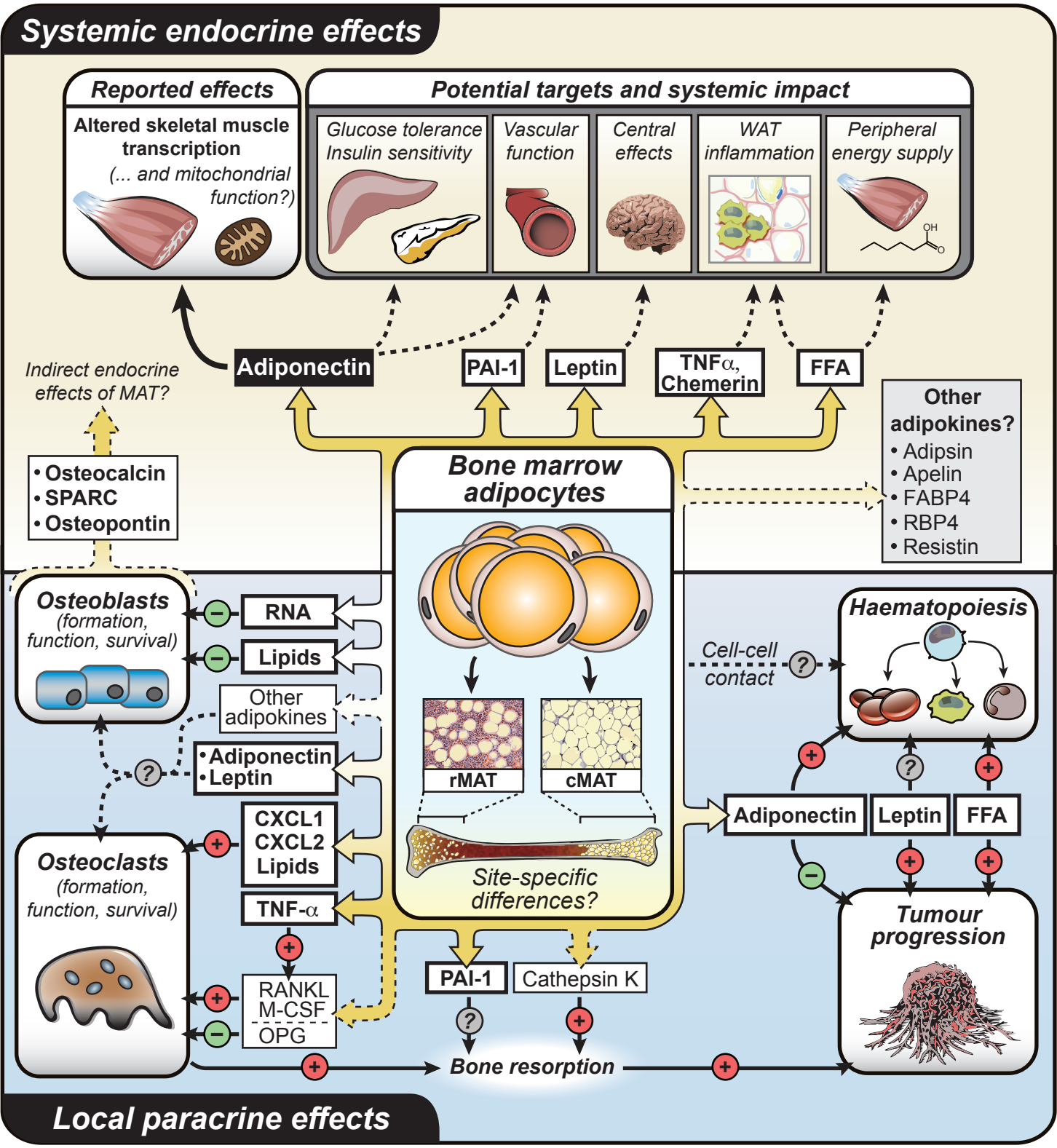
	BM MSC-derived adipocytes		Primary BM adipocytes		Intact MAT or whole bones	
Secreted factor	**Expression**	**Secretion**	**Expression**	**Secretion**	**Expression**	**Secretion**
Adiponectin	**qPCR** [39, 40, 87], **Microarray** [60]	[46, 48]	**qPCR** [43, 62], **Microarray** [28]	[43, 47, 62]	**Protein** [8, 42], **qPCR** [8], **Northern blot** [42], **Microarray** [59]	[8]
Leptin	**Northern blot** [53, 54]	[46, 53-55]	**qPCR** [43] **Microarray** [28]	[43, 56]	**qPCR** [8, 50]	
FABP4	**Protein** [40], **qPCR** [40], **RT-PCR** [60], **Microarray** [60]		**RT-PCR** [56] **Microarray** [28]		**Protein** [8], **qPCR** [8]	
TNF-α			**Microarray** [28]	[47]		
Chemerin		[61]	**Microarray** [28]			
PAI-1			**qPCR** [47, 62] **Microarray** [28]	[47, 62]		
CXCL1	**qPCR** [88], **Microarray** [60]	[88]	**Microarray** [28]			
CXCL2	**qPCR** [88]	[88]	**Microarray** [28]			
RBP4	**Microarray** [60]		**Microarray** [28]		**Microarray** [59]	
IL-6			**Microarray** [28]			
Resistin			**Microarray** [28]			
RANKL	**qPCR** [87]		**qPCR** [84-86], **Microarray** [28]			
OPG	**qPCR** [87]		**qPCR** [84-86] **Microarray** [28]			
M-CSF			**qPCR** [84-86], **Microarray** [28]			
Other factors	*Igf1, Igf2, Igfbp2, Igfbp5, Cxcl8, Fgf7, Angpt1, Serpinf1* [60]		*Cfd, Apln, Ctsk, Igf1, Igf2, Igfbp2, Igfbp5, Nampt, Ccl2, Ptgs2, Il10, Cxcl8, Fgf7, Angpt1, Serpinf1* [28]		*Cfd* [59]	

Table 1 – Endocrine and paracrine factors expressed and/or secreted by BM adipocytes. Protein expression is based on immunoblots or immunohistochemistry. Transcript expression is based on the indicated methods. 'Other factors' lists microarray-detected transcripts, some of which encode proteins that are better known by the following names: *Cfd,* adipsin; *Apln,* apelin; *Ctsk,* cathepsin K; *Nampt,* visfatin; *Ccl2,* MCP-1; *Ptgs2,* COX-2; *Cxcl8,* interleukin 8; *Fgf7,* keratinocyte growth factor; *Serpinf1,* pigment epithelium-derived growth factor.

Putative function	*In vitro* evidence	*In vivo* evidence	Clinical evidence
Systemic endocrine effects		- Altered responses to CR in Ocn-Wnt10b mice [8] - Contribution to circulating lipids [65].	- Positive association between MAT and circulating adiponectin in Caucasian girls [114]
		- Sustained lipogenesis and no lipolytic breakdown of MAT in acute starvation [67]	
Bone loss (decreaased formation and/or increased resorption)	- Inhibition of osteoblast differentiation, function or surivival [89, 90, 99, 101, 107, 153] - Enhanced osteoclast formation/function [84-88]	- High bone mass and increased osteoblast activity in A-ZIP/F1 mice [109]	- Positive association between fracture risk and MAT volume or lipid composition [71, 154]
		- Bone loss occurs independently of MAT expansion in some models of CR and type 1 diabetes [50, 115]	- Positive association between MAT and bone formation or bone mineral content during puberty [112-114]
Suppression of haematopoiesis	BM adipocytes suppress granulopoiesis of CD34+ cells [56]	Increased post-BMT haematopoietic recovery in A-ZIP/F1 mice [108]	- MAT loss during hypertensive heart failure [118] - Increased MAT in aplastic anaemia [121]
	- BM adipocytes support haematopoiesis of CD34+ cells [55]	Increased MAT but intact haematopoiesis in Ebf1 KO mice [129] - MAT loss but impaired haematopoiesis in Ocn-Wnt10b mice [26]	
Enhanced tumour growth, survival or invasiveness	- Increased invasiveness and migration [135, 137] - Lipid transfer to tumours [134, 135] - Enhance tumour-induced bone resorption [88]	- Increased FABP4 expression in MAT-proximal metastases *in vivo* [135]	- Increased MAT and increased cancer risk with ageing and obesity
	- Anti-tumourigenic effects of adiponectin [23]		- Increased MAT during cancer treatment [8] - Increased MAT and decreased cancer risk with CR [138]

Table 2 – Clinical observations, *in vitro* and *in vivo* evidence for or against the putative endocrine and paracrine functions of MAT. Green and red boxes denote evidence for or against each putative function, respectively. Decreased haematopoiesis in Ocn-Wnt10b mice is based on unpublished observations by Erica Scheller.

Figure 1


Systemic endocrine effects
Reported effects
Altered skeletal muscle transcription
(... and mitochondrial function?)
Potential targets and systemic impact
Glucose tolerance Insulin sensitivity
Vascular function
Central effects
WAT inflammation
Peripheral energy supply
OH
O
Indirect endocrine effects of MAT?
Adiponectin
PAI-1
Leptin
TNFα, Chemerin
FFA
Other adipokines?
• Adipsin
• Apelin
• FABP4
• RBP4
• Resistin
• Osteocalcin
• SPARC
• Osteopontin
Bone marrow adipocytes
rMAT
cMAT
Site-specific differences?
Osteoblasts
(formation, function, survival)
RNA
Lipids
Other adipokines
• Adiponectin
• Leptin
CXCL1
CXCL2
Lipids
TNF-α
RANKL
M-CSF
OPG
Osteoclasts
(formation, function, survival)
PAI-1
Cathepsin K
Bone resorption
Cell-cell contact
Haematopoiesis
Adiponectin
Leptin
FFA
Tumour progression
Local paracrine effects