Bone marrow fat: friend or foe in people with diabetes mellitus?

Running title: **Bone marrow adiposopathy**

Marianna Santopaolo, PhD.;[1] Yue Gu, BsC; [1] Gaia Spinetti, PhD; [2] Paolo Madeddu, MD[1]

1. Bristol Medical School (Translational Health Sciences), Bristol Heart Institute, University of Bristol, Upper Maudlin Street, Bristol BS2 8HW, UK.

2. Laboratory of Cardiovascular Research, IRCCS MultiMedica, Milan, Italy.

Total Words: 10896

Text Words: 5171

Corresponding author

Paolo Madeddu
Bristol Heart Institute
University of Bristol
Bristol UK
Email: mdprm@bristol.ac.uk
Tel 0044 0117 3423904

Abbreviations

adiponectin	ADIPOQ
bone marrow	BM
bone morphogenetic protein	BMP
BM adipose tissue	BMAT
C-C Motif Chemokine Ligand 2	CCL2
chemokine (C-X-C motif) ligand 12	CXCL12
glucagon-like peptide-1	GLP-1
leptin receptor	Lepr
magnetic resonance imaging	MRI
parathyroid hormone	PTH
peroxisome proliferator-activated receptor γ	PPARγ
subcutaneous adipose tissue	SAT
uncoupling protein-1	UCP-1
visceral adipose tissue	VAT

Abstract

Global trends in the prevalence of overweight and obesity put the adipocyte in the focus of huge medical interest. This review highlights a new topic in adipose tissue biology, namely the emerging pathogenic role of fat accumulation in bone marrow (BM). Specifically, we summarize current knowledge about the origin and function of BM adipose tissue (BMAT), provide evidence for the association of excess BMAT with diabetes and related cardiovascular complications, and discuss potential therapeutic approaches to correct BMAT dysfunction.There is still a significant uncertainty about the origins and function of BMAT, although several subpopulations of stromal cells have been suggested to have an adipogenic propensity. BM adipocytes are higly plastic and have a distinctive capacity to secrete adipokines that exert local and endocrine functions. BM adiposity is abundant in elderly people and has therefore been interpreted as a component of the whole-body ageing process. BM senescence and BMAT accumulation has been also reported in patients and animal models with type 2 diabetes, being more pronounced in those with ischaemic complications. Understanding the mechanisms responsible for excess and altered function of BMAT could lead to new treatments able to preserve whole-body homeostasis.

Introduction

Body fat exerts vital regulatory roles in systemic metabolic homeostasis, functioning as an energy storage organ (1) and a paracrine and endocrine entity regulating the function of neighbouring cells as well as influencing distant organs and tissues.(2-4)

During the development of obesity, adipose tissue expands through increases in cell number (adipocyte hyperplasia) and size (adipocyte hypertrophy). Fat accumulation is often associated with the acquisition of a pathogenic phenotype, for which the term “adiposopathy” has been coined.(5) Sick fat contributes to systemic inflammation, insulin resistance, and lipid accumulation in the liver and cardiovascular tissue (6-9) Research has mainly focused on the opposing influences of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) in these processes.(10, 11)

The observation that human bone marrow (BM) contains fat cells dates to over a century ago. Surprisingly, BM adipose tissue (BMAT) has attracted much less clinical attention than classical fat storing tissues. In the last 20 years, however, there has been an increasing number of publications on BMAT. Searching for “*bone marrow fat tissue*”, on PubMed, we retrieved 36 publications in year 2000; these increased sharply until 2014 (n=353) remaining stable to the present time. The first international meeting on BMAT was held in Lille, France, in 2015. Successful follow-up meetings culminated in the creation of a new scientific society, the International Bone Marrow Adiposity Society (BMAS, http://bma-society.org/), with the ambition to network and promote an interdisciplinary approach to the study of this emerging tissue. One important milestone of the BMAS has been the publication of guidelines for the standardisation, nomenclature, and harmonisation in BM adiposity research.(12)

Despite the growing interest on BMAT, there are still controversies regarding the relevance of this fat depot in the pathophysiology of diabetic cardiovascular complications. A critical review of BMAT accumulation in relation to global BM

remodelling in diabetes is also missing. Therefore, the overall scope of this review article is to put the BMAT into a more precise pathophysiology context, both at local and systemic level.

The review has three specific objectives: (1) Summarize current knowledge about the origin and function of BMAT and its association or direct contribution in a variety of pathological processes. (2) Provide evidence for the association of excess BMAT with diabetes mellitus and related cardiovascular complications. (3) Discuss potential therapeutic approaches to preserve BM function and whole-body health.

Review purpose 1: Current knowledge about the origin and function of BM adipose tissue

An extensive review of this topic has been provided by three recent publications. (13-15) Here, we summarize the most important concepts as a basis for subsequent interpretation of studies on the impact of diabetes on BMAT.

- ***Assessment, characterisation, and imaging of BMAT.*** Various analytic methods are available to study BMAT. Histology is a standard approach to determine adipocyte size and the association with other marrow cells, but is not suitable to assess the absolute volume of fat and can be affected by bias due to anatomical heterogeneity.(16)

New flow cytometry methods have resolved the problems associated with the large volume and frailty of adipocytes as well as lipoprotein clogging in the apparatus. It is now possible to use flow cytometry to distinguish adipocyte subpopulations with high precision.(17). Sorting methods have been also improved with resolution at a single cell level (18, 19) Recently, Schwalie and colleagues have performed single-cell RNA sequencing to determine the diversity of gene expression in each cell.(20) Using bioinformatic approaches, they identified two major adipocyte populations associated with stemness or the early steps of adipocyte formation. A rarer population was attributed with adipogenesis regulatory function (20) However, there is a paucity of

single-cell RNA sequencing studies to define the heterogeneity and adipogenic commitment of BM cells. Tikhonova et al have mapped the transcriptional landscape of mouse BM vascular, perivascular, and osteoblast cell populations at single-cell resolution, under normal conditions and following stress-induced haematopoiesis. This analysis demonstrated a dynamic and heterogeneous molecular landscape of the BM niche, including an adipocytic skewing of perivascular cells in conditions of stress (21)

Imaging methodologies to visualize and quantify the BMAT *in vivo* include proton magnetic resonance spectroscopy, magnetic resonance imaging (MRI) (22-24) and osmium-based μCT.(25, 26)

- ***Origin of BM adipocytes.*** Lineage tracing studies indicate BM adipocytes derive from resident mesenchymal progenitor cells,(27, 28) renamed recently as skeletal stem cells,(29) which also have osteo/chondrogenic potential.(30, 31) Although osteoporosis often associates with BMAT accumulation, recent work indicated the latter can develop in the setting of preserved or increased bone density.(32-34) This observation suggests the existence of distinctive populations of stromal cells specifically committed to differentiate into osteoblasts and chondrocytes, or adipocytes. Candidate adipogenic populations comprise perisinusoidal mesenchymal cells expressing the leptin receptor (Lepr) and platelet-derived growth factor receptor α (35-37), the chemokine (C-X-C motif) ligand 12 (CXCL12), (38) or neural/glial antigen 2 and nestin.(39) On the other hand, expression of the bone morphogenetic protein antagonist gremlin 1 defines a population of BM osteo-chondro-reticular stem cells, endowed with capacity to self-renew and generate osteoblasts and chondrocytes, but not adipocytes.(40)
- ***Comparison of BMAT with other fat depots.*** Adipocytes from classical depots have been divided into two subtypes derived from distinct precursors (**Figure 1A**). The first subtype consists of unilocular white adipocytes that derive from $Pax7^{-}/Myf5^{-}$ stromal cells and represent the bulk of fatty tissue in most animals. White adipocytes store

excessive energy supply in a triglyceride droplet and release fatty acids in periods of energy depletion. In addition, white adipocytes exert endocrine activity through the secretion of adipokines that regulate metabolism and inflammation. The second subtype comprise thermogenic brown adipocytes and beige adipocytes. They derive from $Pax7^{+}/Myf5^{+}$ stromal cells, are multilocular, rich in mitochondria, and highly specialized in dissipating stored energy in the form of heat through a mitochondrial mechanism involving the uncoupling protein-1 (UCP-1). For the sake of conciseness, we refer the reader to a recent review about the similarities and distinct features of white, brown and beige adipocytes.(41)

BMAT shares some phenotypic similarities with but constitutes a distinct category from both white and brown adipose tissue. In term of morphological features, BM adipocytes tend to be smaller than those in other depots are, are filled with a large unilocular lipid vacuole, a typical feature of white adipocytes, but appear isolated and scattered among haematopoietic cells, which is a striking difference compared with well-packed adipocytes forming subcutaneous or visceral fat lobules (**Figure 1B**).(4, 42, 43) The molecular phenotype of BM adipocytes reportedly varies according to the localisation. In the long bones, they show gene expression features typical of the white lineage, whereas in the vertebrae brown-like thermogenic characteristics seemingly prevail. (4) Differences are also known regarding functional modulation by aging, caloric and amino acid intake, and cold or beta-adrenergic stimulation (**Table 1**).(44)

- ***BMAT plasticity.*** A distinction has been proposed for the existence of constitutive and regulated BMAT (**Figure 1C**). The former appears early during postnatal development in distal skeletal regions including the hands, feet, distal tibia and tail (in rodents). The latter accumulates in areas of red, haematopoietic marrow throughout life and changes in quantity and quality in response to environmental or pathological factors.(14, 26, 45) This topographical distinction is more evident in rodents than in

larger animal species, where the two subtypes tend to overlap. Moreover, constitutive BMAT contains more unsaturated lipids than the regulated BMAT in proximal/central skeletal regions.(26)

In an adult subject weighing 65 kg, the average weight of BM is about 2.6 kg, of which half is fat tissue. In the same individual, total body fat is about 10 kg; hence, BMAT is estimated to be 10-15% of the total fat mass.(4, 46) BMAT increases with ageing more sharply in female subjects between 55 and 65 years of age, while male subjects continue to increase marrow fat at a more gradual steady rate.(30, 47, 48)

- ***Molecular regulators of adipocyte turnover.*** The increase of fat mass in obesity and diabetes is attributed to two mechanisms: adipocyte hypertrophy, the process where pre-existing fat cells increase in size due to lipid accumulation, and adipocyte hyperplasia, occurring through differentiation from preadipocytes. Preadipocytes are replicative cells, but they are also committed to differentiation under the regulatory influence of a cascade of transcription factors, which cause growth arrest and promote adipogenesis. (49) Adipocytes are instead considered terminally differentiated cells, though recent research suggests that a low level of turnover occurs at this cellular level.(50-52) Moreover, aged adipocytes can be cleared through apoptosis.(53) Activation of adipocyte apoptosis/necrosis could result in increased blood lipid concentrations, ectopic lipid storage and detrimental metabolic effects.(54)

In peripheral fat, preadipocyte differentiation is regulated by transcription factors with most belonging to the C2H2-type zinc finger and HOX families,(55) and including the peroxisome proliferator-activated receptor γ (PPARγ).(56) These work together, directly or indirectly, to regulate the expression of hundreds of downstream protein-coding genes and noncoding RNAs responsible for adipogenesis. (57)

The molecular machinery controlling adipocyte turnover in BM is not different from other fat depots.(58) However, BM adipogenesis seems to be particularly dependent on

the leptin-dependent activation of a downstream signaling encompassing Jak2/Stat3 and Cebpα.(59) This mechanism was verified by investigating mice with conditionally deleted leptin receptor and using Jak2/Stat3 inhibitors, which blocked the effect of leptin on preadipocyte differentiation. (59) In contrast, the mechanisms triggering BMAT apoptosis and the contribution of BMAT in increasing blood lipid concentrations have not been deeply explored as for other tissue depots.

- ***Putative BMAT functions and implication in disease states.*** BMAT plays functional roles in energy storage, bone metabolism, and haematopoiesis and exerts systemic endocrine effects. Here, we will discuss the aspects in relation to health and disease, while the implication for obesity and diabetes is discussed in a subsequent section.

Energy storage. Lipids released by BM adipocytes represent a fuel source for neighbouring cells, including for bone and haematopoietic cells.(60) When cellular energy is requested, either by the BM adipocytes themselves or neighbouring cells, lipid droplets are broken down to free fatty acids through lipolysis. This process is regulated in a way like other adipose depots, but responsiveness to stimuli can be different, for instance following beta-adrenergic stimulation.(61) *Implication in disease states:* BMAT induced lipolysis is exploited by proliferating neoplastic cells thereby facilitating cancer spreading. For instance, BM adipocytes reportedly transfer lipids to leukemic cells; contrasting this mechanism can be useful to inhibit cancer growth.(62-64)

Haematopoiesis. A keystone contribution from Naveiras et al. reported BM adipocytes act as negative regulators of the haematopoietic microenvironment.(16). However, contrasting findings have been reported in humans and animal models and even in different anatomical locations in the same individual, suggesting a complex interplay between BM adipocytes and haematopoietic cells.(65-69) *Implication in disease states:* Changes in BM haematopoietic cellularity with aging seemly reflects a

pattern of peripheral to central replacement of the haematopoietic component with adipose tissue.(60) Excessive expansion and dysfunction of BMAT, as in aging, obesity, or exogenous adipocyte transplantation, reportedly results in inhibition of haematopoiesis, and bone frailty. (30, 70) BMAT accumulation in aging mice and humans is associated with an inflammatory environment referred to as inflammaging.(71) Increased levels of inflammatory cytokines, including IL-1, IL-6, and TNFα, found in aged BM, can negatively regulate B lymphopoiesis, and enhance myelopoiesis. Early arrest of B lymphopoiesis in rabbit BM corresponds with increases in fat and myeloid cells, through a mechanism involving inflammatory molecules IL-1β and S100A9.(72) The inhibition of B lymphopoiesis by adipocytes could be partially prevented by an inhibitor of the NLRP3 inflammasome.(72)

Endocrine functions. A recent review by Li et al. summarized the endocrine functions of BMAT.(46) BM adipocytes release different molecules either in a soluble form or packaged in microvesicles and exosomes.(73, 74) Although an overlap exists in the adipokine repertoire with other fat depots, BMAT surpasses the more widespread WAT as a source of adiponectin.(75) Moreover, BMAT secretes an extensive number of cytokines, such as leptin, resistin, and C-C Motif Chemokine Ligand 2 (CCL2).(76, 77)

Implication in disease states: Mounting evidence indicates that paracrine/endocrine activity of BMAT is altered in myeloproliferative disease and can favour metastatic tumour growth in the bone through induction of a local inflammatory microenvironment (77-80)

Review purpose 2: Association of excess BMAT with diabetes and related cardiovascular complications.

Excess VAT is widely acknowledged to be a pathogenic factor in the metabolic syndrome and to increase the risk of cardiovascular complications. In contrast, an

association between BMAT, diabetes, and cardiovascular disease is still matter of debate.

- ***Data regarding BMAT expansion.*** Several studies investigated BMAT in type 2 diabetes with conflicting results. Sheu reported an increased adiposity in the vertebral marrow of 38 older diabetic participants in the *Osteoporotic Fractures in Men Study* in comparison with the 118 participants without diabetes.(81) Other authors did not find an association between BMAT volume and diabetes; yet, marrow adiposity was positively related to HbA1c levels and lower lipid unsaturation in patients with diabetes.(82, 83) Studies in mouse models showed consistently increased marrow adiposity;(84-86). The discrepancy between patients and experimental models could be attributed to the limited group size of clinical studies and the confounding influence of background factors, such as comorbidities and drug treatment.(60)

Our group has performed an extensive investigation of the impact of diabetes on the general BM structure and function, including animal models and human subjects.(43, 86-90) Results indicate that type 2 diabetes causes a profound remodelling of the marrow, including rarefaction of microvessels and sensory nerves, apoptotic pauperization of CD34 haematopoietic progenitor cells and fat accumulation (**Figure 2A**). Morphometric analysis of the human BM showed a remarkable increase in the area covered by adipocytes compared with controls without diabetes, due to an increase in both adipocyte number and size (**Figure 2B**).(43, 87) The average adipocyte volume was 1.7-fold larger in patients with type 2 diabetes compared with non-diabetic controls. In a multiple regression model, the abundance of BMAT was associated with diabetes, independently of age, gender and body mass index (**Table 2**). (43, 87) This suggests that diabetes has an impact on BMAT accumulation that is autonomous from other major determinants of marrow adiposity. The mechanisms underpinning excess fat in the BM of patients with diabetes remains in large part unknown. Recent evidence suggests

hyperinsulinemia, microangiopathy, neuropathy and adipokine-mediated processes could be implicated.

Activation of aging mechanisms. The expansion of BMAT occurring in diabetic people is regarded as a facet of the whole-body accelerated senescence process.(91) On the other hand, BMAT aging could amplify senescence at systemic levels through direct endocrine mechanisms, but also indirectly impinging upon regenerative mechanisms presided by hematopoietic and mesenchymal stromal cells.(92) The intricate nature of this reciprocal interaction requires further investigation. **Table 3** summarizes the typical features of senescence in peripheral fat and BMAT. The chronological aging of adipocytes from peripheral depots has been defined as a propensity to undergo apoptosis, accumulate reactive oxygen species, (93) and manifest an inflammatory profile. (94-97) Moreover, aged adipocytes show increased levels of p53, p21 and p16, and a decrease in SIRT-1 protein compared to younger cells. (97-99) Studies in established obesity and type 2 diabetes indicated that the number of adipogenic progenitor cells is not altered, whereas signalling responsible for adipogenic differentiation is suppressed (98-102)

In apparent contrast with peripheral fat, we found that in BM of diabetic patients preadipocytes expressed higher transcript levels of the differentiation markers C/EBPα, PPARγ, adiponectin, and fatty acid–binding protein 4 (FABP4) and were further stimulated to differentiate under the influence of mature adipocytes (see below). (Ferland-McCollough et al., 2018). It is therefore probable that newly formed adipocytes fill the space left by necrotic and apoptotic adipocytes.(43, 87) In addition, BMAT secretes a unique repertoire of chemokines(103) A recent study comparing BM and peripheral adipocytes from the same healthy subject showed that the former express increased levels of pro-inflammatory molecules concomitant with an elevated generation of reactive oxygen species.(104) This characteristic is exacerbated in

diabetes. BM-adipocytes from diabetic patients secrete higher quantities of MCP-1, leptin, resistin, MMP2 and interferon-γ, but lower quantities of adiponectin.(43)

Hyperinsulinemia and glycation. Insulin signaling role in regulation of mitochondrial function has been investigated in liver, muscle, or adipose tissue, but only marginally in BM adipocytes and pre-adipocytes. A recent report indicates that enhanced insulin signaling increases the activity of the mitochondrial oxidative phosphorylation system and production of reactive oxygen species in BM pre-adipocytes of obese subjects. (Tencerova, Frost et al., 2019) The authors posited that the insulin-mediated hypermetabolic status induces pre-adipocyte conversion to adipocytes but also senescence and pro-inflammatory phenotype.

RAGE, the receptor for advanced glycation end products, is a candidate inducer of BMAT. Deletion of RAGE prevents the upregulation of adipogenic factors induced by type 1 diabetes in BM stromal cells and their differentiation into adipocytes. (105)

- ***Microangiopathy and hypoxia.*** The BM is considered a constitutively low-oxygen tension tissue, a characteristic instrumental in maintaining haematopoietic stem cells quiescence. Using two-photon phosphorescence lifetime microscopy, Spencer et al have determined the absolute pO2 of the BM.(106) The lowest pO2 (~9.9 mmHg, or 1.3%) was found in deeper peri-sinusoidal regions; whereas the endosteal region, which is perfused by small arteries, was less hypoxic. In mice, constitutive BMAT is reportedly more abundant in the zone with higher vascular density while regulated BMAT is prevalent in the hypoxic zone.(107)

Jiang et al. have shown that extreme hypoxia enhanced adipogenic differentiation of BM stromal cells, through a mechanism involving the HIF-1A dependent activation of adipocyte-specific genes.(108) However, these findings are in contradiction with an earlier report showing no effect of hypoxia on differentiation.(109) In peripheral fat depots, chronic hypoxia and nutrient starvation may also result in the induction of

several inflammation-related adipokines as well as adipocyte degeneration and death with liberation of the lipid cargo.(110) However, to the best of our knowledge, this mechanism has not been explored in the BMAT. In BM of people and models with diabetes, we observed a remarkable association between microvascular rarefaction and BMAT accumulation, which indirectly supports the hypothesis of hypoxia being an inducer of adipogenesis.(43, 87) Nonetheless, the demonstration of a cause effect relationship between these phenomena warrants further investigation.

- ***Neuropathy.*** The central nervous system is a key mediator of adipose tissue function through sympathetic adrenergic signalling. BM adipocytes are surrounded by neuronal fibers, with a density that increases from proximal to distal along the length of the tibia.(111, 112) Moreover, BMAT autonomic innervation shares common central pathways with peripheral adipose tissue. These include putative “command” neurons that may facilitate an interplay between the BM, BMAT and peripheral adipose tissue.(111) Several groups, including ours, have demonstrated the occurrence of sympathetic and nociceptive neuropathy in the BM of mice and humans with type 2 diabetes, characterized by the dysfunctional release of neurokinins, such as substance P.(88, 113-115) This altered nociceptor signalling may contribute to BMAT accumulation. In line with this, substance P is capable of inducing lipid accumulation in 3T3-L1 cells during their differentiation into adipocytes in response to a high concentration of glucose.(116) We confirmed the occurrence of sensory neuropathy in BM of type 1 diabetic mice and the possibility of preventing BM adverse remodelling with nerve growth factor gene therapy.(117)

- ***Differentiation bias of BM stromal cells.*** Diabetes could also influence the fate of mesenchymal progenitors, the initial precursor and major source of adipocytes and osteoblasts, leading to an imbalance between adipogenesis and osteogenesis.(118) A recent study in cells isolated from the BM of type 1 diabetic rats suggests the activation

of Notch2 may have a role in this imbalance.(119) We recently reported that BM stromal cells from type 2 diabetic patients have an increased propensity to differentiate into mature fat cells in an *in-vitro* adipogenesis assay as compared with cells from nondiabetic controls.(43) This was associated with the upregulation of PPARγ and Adiponectin (ADIPOQ), which are master regulators of adipocyte differentiation and metabolism. The increase of adipogenic inducers observed in diabetic BMAT is an apparent contrast with findings from a study in elderly people, in which BM fat accumulation was attributed, at least in part, to repression of transcription factors in adipocyte precursors.(44) Understanding the difference between conditions that induce BMAT formation is crucial to develop personalised therapeutic strategies.

Interestingly, the secretome of diabetic adipocytes contains factors that can stimulate BM stromal cells specification to adipogenesis, thereby creating an incremental feedback loop by which existing fat stimulates other fat to accumulate. (43) To identify pathogenic components of the secretome, we measured the expression of multiple adipokines and cytokines in freshly harvested human BM adipocytes (**Figure 3A**). The combined assessment of intracellular mRNA levels and secreted factors indicates diabetic BM adipocytes express and release leptin, CCL2, metalloproteinase 2, and interferon-γ more abundantly than nondiabetic BM adipocytes.(43) Moreover, resistin, which is known to contribute to insulin resistance, was increased, whereas adiponectin, which promotes insulin sensitivity, was decreased in the conditioned medium of diabetic BM adipocytes. A STRING analysis of differentially regulated factors denoted they were associated in a functional network PPI enrichment p-value < 1.0e-16 (**Figure 3B**)**.** Among KEGG pathways hsa04933 AGE-RAGE signalling pathway was enriched with a false discovery rate of 3.42e-9.

The pathogenic relevance of CCL2, a key notch in the network, was further explored by using the CCR2 antagonist RS504393. *In-vitro*, the antagonist abrogated the ability of the conditioned medium from BM adipocytes of type 2 diabetic patients to promote adipogenesis in BM stromal cells.(43) This was associated with inhibition of the transcription factor, CCL2-induced protein, which mediates inflammatory actions of CCL2 *via* sequential indunction of oxidative stress, endoplasmic reticulum stress and autophagy.(120) Likewise, *in-vivo* treatment of type 2 diabetic mice with RS504393 reduced BM fat abundance and adipocyte quantities and size. Interestingly, RS504393 treatment also improved the glycaemic control and reduced the quantity of epididymal fat, which is considered equivalent to white adipocytes of human VAT, but not inguinal fat, which is comparable in terms of location to the large gluteofemoral subcutaneous depot in humans.(43) This data suggests that blocking CCL2 signaling could be a viable option to blunt BMAT and VAT adiposopathy in type 2 diabetes.

- ***Induction of adipogenic microRNA.*** MicroRNAs (miRNAs) are evolutionarily conserved small non-coding RNAs of ~22 nucleotides in length involved in a range of pathophysiological processes. Several miRNAs have implicated in the regulation of adipocyte function, diabetes, obesity, and cardiovascular pathologies.(121) We found that five adipo-miRNAs were significantly upregulated and three downregulated in BM adipocytes from patients with type 2 diabetes (**Figure 4A**). Interestingly, the analysis of candidate targets showed all the downregulated miRNAs converge towards the CCL2 pathway (**Figure 4B**). MiRNA-124a (-19.4-fold vs. ND, $P<0.01$) directly inhibits CCL2 thereby regulating the fate of M2 tissue-resident macrophages,(122-124) and the suppression of FoxP3 Tregs.(125) Likewise, miRNA-193b (-18.4-fold, $P<0.01$ vs. ND) and miRNA-126 (-6.1-fold vs. ND, $P<0.05$ vs. ND) inhibit CCL2 expression in adipocytes and M1 macrophages, either directly or through the transcription factors ETS1 and MAX.(126)

Moreover, as illustrated in **Figure 4B**, three miRNAs that we found upregulated in BM adipocytes from type 2 diabetic patients, miRNA-15a, miRNA-16, and miRNA-223 converge in inhibiting IKKα, a suppressor of the NF-κB pathway (127-130) Altogether, these data provide evidence for the activation proadipogenic and inflammatory signalling in the BM of diabetic patients and rodents.

Review purpose 3: Therapeutic approaches to inhibit BM adiposity

Restoring proper fat content in BM of diabetic patients may benefit hematopoiesis, bone strength, and metabolic homeostasis. In the next paragraphs, we will provide a brief prospect of available and new candidate treatments. Mechanisms underpinning the effect of the described therapies are also reported in the subheadings, if this was supported by robust experimental evidence. In fact, for several proposed treatments, only observational data are available, with insufficient understanding of the underpinning mode of action. In addition, given the difficulties in a direct appreciation of the amount of BMAT, the graded approach used in obesity is generally not applicable.

- *Metabolic control.* There is a consensus on the fact that BMAT formation is affected by glycaemic control; nonetheless, further studies are needed to determine whether these effects are independent of insulin action. BM adiposity is directly related with serum glucose and HbA1c.(103) Improvement in the glycaemic control induced by gastric bypass surgery and pharmacological treatment is reportedly associated with reduced BM fat content.(131)

Glucagon-like peptide-1 (GLP-1) is an intestinal hormone implicated in the regulation of glucose homeostasis. Exendin-4, a stable GLP-1 analogue currently used for the treatment of type 2 diabetes, promotes adipocytic and osteoblastic differentiation by influencing the number of committed progenitors.(132) Exendin-4 also stimulates lipolysis without affecting osteoblast metabolic activity.(133) The effect of GLP-1 on obesity- or diabetes-associated BMAT expansion remains to be determined.

Thiazolidinediones such as troglitazone, rosiglitazone and pioglitazone are PPARγ agonists that improve glucose control in patients with type 2 diabetes by enhancing insulin sensitivity. Side effects include induction of adipogenesis, osteoporosis and lowering of blood counts. These effects could be partially reversed by the selective PPARγ antagonist bisphenol A diglycidyl ether.(134)

- *Parathyroid Hormone.* Treatment with Parathyroid Hormone (PTH) promotes bone formation, which may be mediated by a shift in the differentiation fate of BM stromal cells from adipocytes toward osteoblasts.(135, 136) This mechanism is seemingly implicated in suppression of BMAT by PTH in ovariectomized mice. Nonetheless, PHT treatment in mice with type 1 diabetes increases trabecular bone mass and suppresses osteoblast apoptosis without reducing BMAT.(137)
- *Exercise regulation of BMAT.* Exercise can burn BMAT according to research conducted by Steiner et al. (138) Using obese mice, the authors challenged the established concept of BMAT incapability to fuel energy during exercise. Two groups of mice were randomly allocated to be fed a normal diet (lean mice) and or a high-fat diet (obese mice) starting a month after birth. When they were four months old, half the mice in each group were offered to exercise using a running wheel for the next six weeks. As expected, the obese mice showed more and larger fat cells in their marrow. After exercising, both obese and lean mice had a significant reduction in the overall size of fat cells and the overall quantity of BMAT. There was however a remarkable difference in the number of BM adipocytes, which remained unchanged in lean mice but fell by more than half in exercised obese mice compared with sedentary obese mice. Additionally, exercise improved the thickness of bone, with this effect being more pronounced in obese mice. The authors speculated that BMAT was burned to generate the energy necessary to build more bone.

Interestingly, exercise is effective in preventing the side effects of PPARγ agonists.(139) In mice, the PPARγ agonist rosiglitazone induced BMAT extension to the femoral diaphysis, while superimposing exercise suppressed BMAT accumulation, induced adipocytes browning, and supported bone formation.(25)

Observational studies comparing athletes and sedentary people, as well as interventional studies in children and older men confirm a benefit of exercise on BM adiposity.(140, 141) Moreover, prolonged bed rest increases BMAT and this could be inhibited by exercise.(142)

- *Caloric restriction.* In a follow-up of the above study, the same group demonstrated that adding caloric restriction to exercise can be detrimental to the bone health. This time, the authors split mice into two groups: one given a regular diet and the other with 30% less calories than the regular diet but with supplements of minerals and vitamins to match the content in the normal diet. Then, mice were assigned into sedentary and exercise subgroups, and monitored for 6 weeks. Interestingly, adding exercise to calorie restriction consistently reduced BMAT volume but also made bones more fragile.(143) Therefore, a balanced diet seems to be necessary to take full advantage from exercise.

Studies in humans are contradictory regarding the effect of caloric restriction. Bosy-Westphal et al investigated 55 premenopausal women and 12 men undergoing 12 weeks of low-calorie diet, which was followed by return to the usual diet.(144) Results showed a reduction of fat mass and marrow adiposity (~4%) during the low-calorie diet and regain of body weight and marrow adiposity with return to the usual diet. In line with this, Vogt at al showed obese patients with type 2 diabetes had a significant weight loss that was accompanied by a 5% decrease in vertebral marrow adiposity.(145) However, other two studies on obese subjects did not show any effect of diet on BMAT, despite evident reduction of SAT and VAT depots.(146, 147)

- *Adipocyte dedifferentiation.* Recent research has shown that adipocytes can dedifferentiate into fibroblast-like cells *in-vitro* and thus re-acquire proliferation and multipotent capacities. A physiological example is represented by the capacity of adipocytes in mammary glands to undergo lipid loss and acquire a preadipocyte-like cell phenotype during late pregnancy and lactation. The dedifferentiated cells can re-differentiate to generate adipocytes during the involution of the gland after lactation.(148) However, an earlier study in rodents discounted the possibility that lactation is responsible for decreased adipocyte content in BM during post-partum.(149) Therefore, mechanisms activated during mammary gland dedifferentiation could be organ specific.
- *Targeting the inflammasome*. Danger signals that accumulate during aging, obesity, and diabetes can trigger active IL-1 production by myeloid lineage cells through paracrine signaling by BM adipocytes.(150) Blocking the NLRP3 inflammasome with glybenclamide indeed, inhibited accumulation of myeloid-derived suppressor cells, and stimulated B lymphopoiesis *in vitro*.(72) Similarly, in mice, deletion of NLRP3 prevented the decline of T lymphopoiesis.(151) These findings and data from our recent study, showing a CCL2 antagonist halted BMAT accumulation and re-established the abundance of long-term haematopoietic stem cells in type 2 diabetic mice, suggest that targeting the inflammasome pathway could be an effective means to restore BM health in diabetes. (43) Strategies to reduce the inflammasome activation diabetes could prove useful as a treatment to contrast the myelopoiesis/lymphopoiesis imbalance in patients with type 2 diabetes.(152, 153)
- *Senolytic agents.* A breakthrough study from Kirkland's group showed that intermittent administration of the senolytic drugs dasatinib and quercetin promoted the

elimination of senescent preadipocytes and increased lifespan in recipient mice.(154) The effect of such a radical treatment on BMAT needs to be demonstrated.

- *Miscellanea.* Several bioactive molecules may stimulate osteogenic differentiation while inhibiting BM adipogenesis. For instance, resveratrol, a phytoalexin acting as a caloric mimetic, can contrast the adipogenic commitment of BM stromal cells,(155) and promote anti-aging functions on lipolysis, mitochondrial biogenesis and thermogenesis, in part through activation of SIRT1.(156) Vitamin D combined with genistein, quercetin, and resveratrol showed inhibitory effects on BMAT.(157) Puerarin, a phytoestrogen used in Chinese traditional medicine, in association with zinc supported BM stromal cells proliferation, and induced the expression of alkaline phosphatase, while decreasing BMAT mass.(158) Similar effects have been reported for genistein, epigallocatechin and various oxysterols; all of them being capable of inhibiting PPARγ expression and repressing adipogenic differentiation of BM stromal cells.(159-161) Strontium ranelate is used for the treatment osteoporosis; it also reportedly exerted a dose-dependent inhibitory effect on BM adipocyte differentiation and lipotoxicity within the BM environment.(162) In senescent mice, strontium ranelate treatment increased bone mass and reduced BMAT through stimulation of Wnt and NFATc/Maf pathways, and consequent inhibition of adipogenesis due to inhibition of PPARγ expression.(163)
- *Authors' view regarding therapeutic intervention.* Evidence from literature is not enough to determine when, how, and to which extent treating excess BMAT. Difference and even contraindication exist for the use of drugs that benefit the general metabolic control. Moreover, imaging techniques may not be available or justified on a cost-benefit basis to the general population. Surrogate molecular and cellular markers in peripheral blood reflecting the state of the BM niche could be useful in this respect.(164, 165)

Concluding remarks

Studies from our group and others clearly indicate that diabetes impinges upon the BM integrity, one major characteristic being the accumulation of fat and depletion of the vascular and hematopoietic niche. Excess BMAT goes in parallel with cardiovascular complications, suggesting possible causative relationship between these pathological processes. Several therapeutic options are available, and others may be developed soon as underpinning mechanisms of BM adiposopathy are unravelled. If BM adiposopathy can be halted or reversed, then next question is whether the empty space left by fat loss will be replaced by functional hematopoietic and stromal tissue or become a scar. Future research should address optimal windows for treatments in relation with the patient's specific BMAT profile.

Figures

Figure 1: Characteristics of different fat depots. (A) Histology of white (WAT), brown (BAT), and bone marrow adipose tissue (BMAT). (B) Distribution of WAT, BAT and beige adipose tissue in mice and humans. (C) distribution of constitutive and inducible BMAT.

Figure 2: Association of BMAT with vascular complications. (A) Histology of human BM from non-diabetic controls and type 2 diabetic patients with or without critical limb ischaemia. Relative proportion of fat increases in diabetes and even more in the case of associated limb ischaemia. (B) Shift of BM adipocyte (ADPC) toward larger sizes in type 2 diabetic patients.

Figure 3: Alteration of the BMAT secretory phenotype in diabetes. (A) T2D modulates the expression and secretion of adipokines and cytokines in BM adipocytes. Bars graphs showing secreted factors in conditioned media from BM adipocytes of subjects without diabetes (ND) and with T2D, as assessed by ELISA. (B) Network analysis of secreted factors.

Figure 4: microRNA profile of diabetic BM adipocytes. (A) differentially modulated microRNAs. (B) cartoon highlighting alterations in lipid flux, storage, and efflux and microRNA convergence on inflammatory targets.

Table 1: Difference in modulation of adipogenesis in BMAT versus other depots. an up arrow indicates increased numbers of cells; and adown arrow indicates decreased numbers of cells

Type	White	Brown	Beige	BMAT
Aging	↑	↓	↓	↑
High fat diet	↑	↑	↑	↑
Methionine restriction	↓	↓	↑	↑
Cold/beta adrenergic	↓	↑	↑	↓

Table 2: BMAT abundance in type 2 diabetes

	Controls	T2D	T2D+CLI
Number	41	19	10
Sex (Male)	24	12	6
Age (years)	71±1	70±1	73±2
	(39-83)	(47-80)	(56-88)
BM Fat (%)	50.2 ±2.3	65.5 ±2.6*	75.5 ±4.3**
	(23.4-80.5)	(48.0-95.9)	(61.7-92.7)
BMI	28.4±0.8	34.1±2.2*	26.6±1.5
	(20-46)	(22-53)	(21-35)

T2D, Type 2 diabetes; CLI, Critical limb ischaemia. Values are mean±SEM with min-max in parenthesis; *p<0.01 and **p<0.001 vs. Controls. Results of Covariance analysis: Diabetes p<0.001, Sex p=0.38, Age p=0.62, BMI p=0.34

Table 3: Comparison of aging features in BM of diabetic patients and peripheral fat depots

Peripheral fat senescence	***BMAT senescence in diabetes***
Senescent adipocytes present progressive changes with aging typically showing roundish shape, greater diameters, smaller peripheral nuclei and a large central lipid droplet.(166)	*Increased in BM adipocyte number and size. In our study, the average size was 4,487 ± 675 and 7,812 ± 666 μm^2 in subjects without or with diabetes, respectively, corresponding to a 1.74-fold increase. (43)*
Senescent preadipocytes/adipocytes produce more inflammatory cytokines, such as interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1/CCL2), and less anti-inflammatory cytokines, such as adiponectin. This associates with an increased infiltration of macrophages. Another feature of the so-called senescence-associated secretory phenotype (SASP) is the secretion of extracellular matrix proteins, such as COL1α1 and MMP-11. Proinflammatory phenotype can be propagated to neighbouring cells. (94-96)	*BMAT secretes a unique repertoire of chemokines(103) A recent study comparing BM and peripheral adipocytes from the same healthy subject showed that the former express increased levels of pro-inflammatory molecules concomitant with an elevated generation of reactive oxygen species.(104) We showed BM-adipocytes from diabetic patients have upregulated levels of adipokines leptin, resistin TNFα, and MCP-1, while IGF1, PEDF, IL18, adiponectin and angiopoietin are downregulated. Diabetic BM adipocytes secrete high quantities of MCP-1, leptin, resistin, MMP2 and interferon-γ, but less adiponectin. They propagate the inflammatory phenotype to BM stromal cells. (43)*
In obese individuals with type 2 diabetes, SAT cells showed to have reduced proadipogenic signals, with downregulation of CCAAT/enhancer binding protein α (C/EBPα) and peroxisome proliferator-activated receptor gamma (PPARɣ) leading to larger fat cells, ectopic fat deposition and insulin resistance. (100-102)	*In contrast to peripheral adipocytes, diabetic BM-preadipocytes expressed higher transcript levels of the differentiation markers C/EBPα PPARγ, adiponectin, and fatty acid–binding protein 4 (FABP4). (Ferland-McCollough et al., 2018)*
Obesity is characterised by chronic low-grade inflammation with permanently increased oxidative stress. The number of apoptotic events is significantly higher in adipocytes and related with intracellular oxidative stress levels.(167) Oxidative stress reportedly Inhibits adipose expansion through suppression of lipogenic pathway.(93)	*Oxidative stress, assessed using the CM-H_2DCFDA fluorophore, is increased in total BM cells together with p-H2AX (Ser139), a marker of double DNA strand breaks.(86, 87)*
Studies of hypertrophic obesity and diabetes indicate the number of adipogenic progenitor cells is not altered. Instead, a key mechanism for the impaired adipogenesis is an increased progenitor cell senescence, characterised by induction of BCL-2, BAX and LITAF, dysregulated p53 and P16ink4 resulting in inhibition of cell adipogenic differentiation. (98, 99)	*Stromal cells were reportedly not reduced in the BM of diabetic patients. Cell cycle was not assessed in preadipocytes. However, we could not find defects in the proliferation of BM preadipocytes from diabetic patients. In contrast, adipocyte precursors seem to be committed to adipogenic differentiation. It is therefore probable that new adipocytes fill the space left by necrotic and apoptotic adipocytes in BM.(43, 87)*

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