



Published in final edited form as:

J Intern Med. 2018 February ; 283(2): 121–139. doi:10.1111/joim.12718.

Clinical Implications of Bone Marrow Adiposity

Annegreet G Veldhuis-Vlug, MD PhD^{1,2} and Clifford J Rosen, MD²

¹Department of Endocrinology and Metabolism, Academic Medical Center, Amsterdam, the Netherlands ²Center for Clinical & Translational Research, Maine Medical Center Research Institute, Scarborough, Maine, USA

Abstract

Marrow adipocytes, collectively termed marrow adipose tissue (MAT), reside in the bone marrow in close contact to bone cells and hematopoietic cells. Marrow adipocytes arise from the mesenchymal stem cell and share their origin with the osteoblasts. Shifts in the lineage allocation of the mesenchymal stromal cell could potentially explain the association between increased MAT and increased fracture risk in diseases such as postmenopausal osteoporosis, anorexia nervosa and diabetes. Functionally, marrow adipocytes secrete adipokines, such as adiponectin, and cytokines, such as RANK-ligand and stem cell factor. These mediators can influence both bone remodeling and hematopoiesis by promoting bone resorption and hematopoietic recovery following chemotherapy. In addition, marrow adipocytes can secrete free fatty acids, acting as a energy supply for bone and hematopoietic cells. However, this induced lipolysis is also used by neoplastic cells to promote survival and proliferation. Therefore, MAT could represent a new therapeutic target for multiple diseases from osteoporosis to leukemia, although the exact characteristics and role of the marrow adipocyte in health and diseases remains to be determined.

Keywords

Bone marrow adipocyte; Hematopoiesis; Bone remodeling; Mesenchymal stem cell

INTRODUCTION

Marrow adipose tissue (MAT) as the bone marrow adipocytes are collectively termed, is virtually absent at birth, expands during growth and development of the skeleton and accelerates its expansion during aging and menopause. Historically, marrow adipose tissue was considered inactive filling of the bone marrow cavity when bone mass was low or hematopoiesis impaired. But, animal and human studies during the past two decades have

Corresponding Author: Prof. Clifford Rosen, Director of the Center for Clinical&Translational Research, 81 Research Drive, Scarborough, Maine, 04074, United States of America, rosenc@mmc.org.

DR. ANNEGREET VELDHUIS-VLUG (Orcid ID : 0000-0003-0277-2533)

Conflicts of Interest Statement

The authors declare no conflicts of interest.

Contributions

AGV and CJR contributed equally to the literature search, discussion, and writing of this manuscript.

shown that MAT is increased in diseases such as osteoporosis, obesity, diabetes, and paradoxically, anorexia nervosa. This increase in MAT is often associated with deterioration of bone mass resulting in increased fracture risk although this association may not be causal. Therapies such as bisphosphonates, estrogen and parathyroid hormone (PTH) that improve bone quality, coincide with a decrease in MAT and this has led to a growing interest in marrow adipose tissue as a therapeutic target for osteoporosis. More recently, the association between MAT and hematopoietic diseases such as multiple myeloma and myeloid leukemia has been recognized. Bone marrow adipocytes have been shown to play an active role in the support of neoplastic cells in the bone marrow niche and are now considered a potential therapeutic target. In addition, genetic mouse models targeting the bone marrow adipocyte, have shown surprising effects on other adipose tissue depots and whole body energy metabolism.

Emerging mechanisms for the interaction between bone marrow adipocytes and bone remodeling and hematopoiesis are 1] the regulation of differentiation of the mesenchymal stromal cell, which is the progenitor of both the bone marrow adipocyte and the bone-forming cell, the osteoblast 2] the supply of free fatty acids as a source of fuel to energy-demanding processes such as bone formation, hematopoiesis and neoplastic growth 3] the secretion of soluble adipocyte-specific mediators termed 'adipokines' such as adiponectin and factors such as RANK ligand (RANKL) that can directly or indirectly affect bone, tumour and systemic metabolism.

In sum, marrow adiposity is considered within the realm of bone metabolism, hematopoiesis, cancer and systemic energy metabolism. It remains a possible therapeutic target for bone disease, hematopoietic diseases, bone marrow neoplasms and obesity. Nevertheless, crucial questions regarding its origin, function and secretory capacity in health and disease remain to be answered.

BONE MARROW ADIPOSITY IN HEALTH

Growth

During fetal life, the skeleton is formed through endochondral and intramembranous ossification. Longitudinal growth continues after birth, during childhood and adolescence, while acquisition of peak bone mass occurs between ages 15-30. During skeletal growth, bones not only increase in length, but also in diameter, a process known as modelling. During modelling, the marrow cavity also increases. The marrow cavity becomes filled with hematopoietic marrow during fetal life and is fully populated with hematopoietic cells at the end of the first term of pregnancy¹. During fetal life, the main sites of hematopoiesis are firstly the yolk sac, followed by the liver and spleen during second and third trimester, when finally the hematopoietic bone marrow becomes the main site of hematopoiesis². Shortly before birth, the replacement of hematopoietic marrow by adipose marrow is initiated. The earliest conversion of hematopoietic to adipose marrow was studied in an infant autopsy study in 1964 which showed that, irrespective of term of birth, the conversion already started shortly before birth, accelerated following birth and was complete at 12 months of age in the proximal phalanx³. However, in the femur and tibia the appearance of adipose marrow starts later, around the age of 7 and is complete by the age of 18. In the ribs and vertebrae,

macroscopic fat is not detected at all until old age^{4,5}. These studies establish a pattern of peripheral to central replacement of hematopoietic marrow by adipose marrow in the skeleton; from the appendicular to the axial skeleton and from the diaphysis and distal metaphysis to the proximal metaphysis (figure 1). The same patterns of hematopoietic and adipose marrow development were observed using magnetic resonance imaging (MRI) scans in cross-sectional studies in infancy and adolescence^{6,7}. These studies showed that indeed marrow adiposity increases with age in 4- to 10-year old girls and is accompanied by an increase in total body fat and total bone mineral content in the femur⁸. Although total bone mineral content is positively associated with adipose marrow during childhood, other studies reported a negative association between adipose marrow and femoral cortical bone⁹ and pelvic bone mineral density¹⁰.

Aging

In healthy adults, marrow adiposity continues to increase with aging. Cross-sectional studies have shown that, depending on the MRI method used, marrow adiposity in the lumbar spine is 20–30% around the age of 20 and increases approximately 7% per 10 years to $\pm 50\%$ at age 50¹¹⁻¹⁵. During these years, men have higher marrow adiposity than women, but this difference reverses later in life¹⁶. From 50 years of age, women have accelerated marrow adiposity accumulation resulting in $>70\%$ of the marrow volume at age 80 years compared to 60% in men. In the femur, marrow adiposity also increases with age but the marrow adiposity is higher at younger age starting at 60% in the diaphysis and 80% in the femur head at age 20–30, increasing to 80% and 90% respectively at age 50–60¹⁷. After reaching peak bone mass between the age of 15–30 years, bone mass declines with aging. In men, this decline is gradual whereas in women the decline accelerates perimenopausally and continues postmenopause.

Menopause

The accelerated marrow adiposity accumulation in women over 50 years of age coincides with menopause. Menopause is characterized by ovarian follicle depletion leading to decreased estrogen and progesterone and increased luteinizing hormone (LH) and follicle-stimulating hormone (FSH) production¹⁸. Clinically, menopause is associated with bone loss and fat gain^{19,20}. The increase in marrow adiposity in women around age 50 is hypothesized to be due to the hormonal changes of menopause although marrow adiposity during menopause has not been investigated longitudinally. However, two weeks of estrogen supplementation in postmenopausal women decreases marrow adiposity by 5% demonstrating that estrogen is indeed a regulator of marrow adiposity²¹. Although menopause induces irreversible hormonal changes, during the follicular and luteal phase of the menstrual cycle concentrations of gonadal and gonadotropic hormones fluctuate on a smaller scale. Interestingly, marrow adiposity increases during the follicular phase and decreases during the luteal phase although the magnitude of the change is indeed much smaller compared to menopause²¹.

In conclusion, marrow adipose tissue appears in the bone marrow cavity shortly before birth and expands during growth of the skeleton in childhood and adolescence in a distal-to-proximal fashion, replacing the hematopoietic marrow almost completely in the long bones

by the age of 30 when reaching peak bone mass. During aging, vertebral marrow adipose tissue continues to increase and bone mass decreases. Men have higher marrow adiposity than women, but this difference reverses after menopause, implying that marrow adiposity is, at least partially, hormonally controlled. Further studies are needed to elucidate the exact role of marrow adiposity in growth and development and its regulation during the different stages of life.

EVALUATION OF MARROW ADIPOSITY

To measure bone marrow adiposity in humans, several methods have been developed (Figure 2). Historically, bone biopsies were performed to assess bone parameters and these biopsies included the marrow space. Bone biopsies are performed at the iliac crest bone. Marrow adipocytes are shown as holes in the biopsy, since the actual adipocytes are destroyed during the processing of the bone biopsies and therefore these are termed adipocyte ‘ghosts’. Marrow adipocytes can be quantified using imaging software manually or automatically. The parameters determined are the adipocyte total volume, individual volume (adipocyte size) and number.

Since bone biopsies are invasive and therefore not readily available, MRI methods have been developed. MRI using Quantitative Chemical Shift Imaging (QCSI) separates the water and the fat signals of the bone marrow and computes a fat fraction. MRI is mainly performed of the lumbar vertebrae in humans. Another MRI method, Magnetic Resonance Spectroscopy (MRS) determines the fat fraction and the fatty acid composition of the bone marrow by separating the signals from the different lipids.

Although these methods are able to determine the amount of bone marrow adipocytes and the lipid composition, it does not allow functional studies. Therefore, investigators are now exploring bone marrow aspiration to recover marrow adipocytes for culture and gene expression analysis *ex vivo*.

THE BONE MARROW ADIPOCYTE

Overview of adipocytes

Adipocytes are present in many tissues within the body and can be divided into several types based on their characteristics and their anatomical depots²². Three types of adipocytes are now widely recognized; white, brown and beige adipocytes. White adipocytes are the classic large, lipid droplet-rich cells, storing and releasing fatty acids. The white adipocytes make up the majority of the subcutaneous and visceral adipose tissue depots. Brown adipocytes are small, mitochondria-rich cells, capable of uncoupling energy by burning lipid and releasing heat. Although brown adipose tissue (BAT) was long considered to exist only in the neonatal period, it is now clear that BAT persists into adulthood in the interscapular region. Certain stimuli, such as cold exposure and adrenergic signaling, can induce some characteristics of brown adipocytes in white adipose depots and these are termed beige or bright adipocytes. These cells have thermogenic properties, although it is unclear whether beige adipocytes modulate body temperature *in vivo*. In addition to storing or burning fatty acids, adipocytes secrete mediators and hormones collectively called ‘adipokines’. The

classic adipokines are leptin and adiponectin which both regulate systemic energy metabolism, but many more adipokines have been identified.

Bone marrow adipocytes

The presence of adipocytes in the bone marrow has long been recognized, but these adipocytes were often considered a passive ‘filler’ in the absence of bone and hematopoietic cells. Interest in marrow adiposity was reignited in the 1970s when the dynamic interactions with hematopoiesis became evident after studies of bone marrow transplantation revealed a temporal relationship between marrow adiposity and hematopoietic reconstitution^{23,24,25}. Since then, many studies have investigated the association between marrow adipose tissue and clinical conditions such as osteoporosis, anorexia and obesity. But due to the location of the marrow adipocytes in the bone marrow, it has been more difficult to study its characteristics directly. Therefore, studies in animal models, especially in mice, have been pivotal in understanding the origin and function of the marrow adipocytes.

In 1996, Gimble and colleagues reported an increase in MAT in mice treated with thiazolidinediones (TZDs)²⁶. Subsequent studies by Jilka and Lecka-Czernik described the cellular and molecular signatures of marrow adipocytes^{27,28}. More recently, investigators have focused on inbred and mutant mice as models for describing the origin and development of bone marrow adipocytes, particularly using new lineage tracing methods^{29,30}. In addition, some investigators have proposed that different types of marrow adipocytes exist within the marrow cavity. Drugs (e.g. the TZDs), environmental factors (e.g. radiation), and nutritional determinants (e.g. high fat diet or calorie restriction) can induce premature marrow adiposity in the long bones of mice. This inducible marrow adiposity has been termed ‘regulated’ MAT (rMAT) in contrast to the distal sites that are referred to as ‘constitutive’ (cMAT) because of their presence at the time of birth³¹. In mouse models regulated MAT is dynamic and reversible in contrast to constitutive MAT. Whether constitutive and regulated MAT exists in humans is not clear, although what is certain is that marrow adiposity is also inducible in both men and women.

Origin and Regulation—Marrow adipocytes arise from the mesenchymal stromal cell (MSC) in the bone marrow. Mesenchymal stromal cells can also give rise to other cell types such as osteoblasts, the bone forming cells and chondrocytes, the cartilage forming cells. However, adipocyte and osteoblast differentiation is closely related and both types of cells share some common steps during their development (Figure 3). Several transcription factors are upregulated during early pre-osteoblast (Runt-related transcription factor 2 (Runx2), homeobox protein 1 (Prx1) and osterix (Sp7)) and pre-adipocyte differentiation (platelet-derived growth factor receptor- β (PDGFR β), CCAAT/enhancer binding protein alpha (C/EBP), Zinc finger proteins (Zfp) 423 and 467 and Prx1. For a particular window of time, plasticity might exist and the adipocyte and osteoblast progenitor cells may interconvert between phenotypes. The terminal differentiation is regulated by phenotype-specific transcription factors, in case of osteoblasts collagen type 1 (col1a1), osteocalcin (ocn), and RANKL (reviewed in³²). And in case of adipocytes peroxisome proliferator-activated receptors gamma (PPAR γ), fatty acid binding protein (FABP4), perilipin 2 (Pln2) and fatty acid synthase (FASN). Trans-differentiation in a later developmental stage has also been

suggested, but not established at this moment. Finally, osteoblasts terminally differentiate into osteocytes which secrete sclerostin (SOST) and fibroblast growth factor (FGF)-23³³.

The allocation of the mesenchymal stromal cell to either the adipocytic or the osteoblastic differentiation is affected by several factors. One of the earliest recognized factors is estrogen. Marrow adiposity increases after menopause or ovariectomy and estrogen prevents or reverses this increase. At the same time, bone loss accelerates following menopause and postmenopausal estrogen treatment increases bone mass. The effect of estrogen on marrow adipocytes is thought to be mediated through the estrogen receptor alpha (ERa) since ERa knockout mice show increased marrow adiposity with an increase in adipocyte number³⁴ and this effect was not observed in estrogen receptor beta knockout mice. In addition, mice with deletion of the ERa in mature adipocytes (through conditional adiponectin dependent cre-recombinase), also had normal marrow adiposity indicating the effect of estrogen must be before expression of adiponectin, a marker of late adipocytic differentiation. Indeed, in vitro studies show that estrogen promotes osteoblastic and suppresses adipogenic differentiation of the mesenchymal stromal cell^{35,36}.

Decreasing estrogen concentrations coincide with increasing FSH concentrations due to the endocrine feedback of the gonadal-pituitary axis. A recent study showed that blocking the binding of FSH to its receptor with an antibody prevented the increase in marrow adipose tissue after ovariectomy and treatment with this antibody in sham-operated mice, also decreased marrow adipose tissue³⁷. The authors showed that both adipocytes and MSCs express the FSH receptor and that FSH receptor deficient mice have increased MSC adipogenesis, suggesting that postmenopausal elevated FSH contributes to the increased adipogenic differentiation of the MSC.

Parathyroid hormone regulates calcium concentrations by influencing osteoclast activity, but intermittent PTH (iPTH) treatment can increase bone mass³⁸, among others through stimulation of osteoblastic differentiation of the MSC. In rats, intermittent PTH treatment prevented the increase in marrow adiposity induced by caloric restriction³⁹ and in mice intermittent PTH treatment reduced marrow adiposity. Genetic experiments in mice showed that loss of PTH receptor expression in mesenchymal stromal cells increased marrow adiposity⁴⁰ whereas overexpression of the PTH receptor in bone cells suppressed marrow adiposity⁴¹. These experiments show that PTH can influence the cell fate of the mesenchymal stromal cell to increase or decrease the development of adipocytes. The suppressing effect of PTH on adipogenesis of mesenchymal stromal cells was also shown in vitro previously⁴².

Phosphate deficiency leads to impaired bone mineralization and the clinical condition is known as rickets. In mice, phosphate restriction during growth results not only in impaired bone mineralization, but also a decrease in osteoblasts and an increase in bone marrow adipocytes⁴³ although in vitro phosphate restriction did not change MSC differentiation, implying an indirect effect of phosphate availability on MSC differentiation.

Leptin is an adipokine produced by white adipocytes that regulates satiety, reproduction and energy homeostasis⁴⁴. In addition, leptin could play a role in hematopoiesis⁴⁵ and can also

influence bone mass both directly and indirectly through the central nervous system⁴⁶. These studies have also shown that both subcutaneous and hypothalamic leptin therapy decrease the obesity-induced marrow adiposity in two mouse models, the leptin receptor knockout mouse (ob/ob) and mice on a high fat diet^{47,48}.

Osteocytes produce sclerostin which stimulates bone resorption and inhibits bone formation. Recently sclerostin was shown to induce adipogenesis in vitro. A new osteoporosis drug in phase III trials, romosozumab, is an anti-sclerostin antibody, that enhances bone mass but can also suppress marrow adipose tissue volume in mice^{49,50}.

Bone marrow adipocyte function

1. Secretion of mediators: The first proposed role for the marrow adipocytes is the release of adipokines; peptides and fatty acids acting potentially in both a paracrine and endocrine manner. Adiponectin has been the most studied of the adipokines secreted from MAT, especially in animal models. Adiponectin is secreted primarily by adipocytes and it regulates among others insulin sensitivity and energy metabolism. Adiponectin concentration is lower in obese individuals, but it increases during energy deficit (reviewed in⁵¹). This could indicate that marrow adipocytes become a significant source of adiponectin during caloric restriction. Indeed, Cawthorn et al reported that in mice and rabbits adiponectin expression and secretion is higher in MAT compared to peripheral adipose tissue depots⁵². These same authors also demonstrated that mice with higher MAT due to calorie restriction had greater serum levels of adiponectin. In contrast, when a genetic manipulated mouse that had virtually no MAT (osteocalcin specific Wnt10b over-expressing mouse) underwent calorie restriction, adiponectin secretion was much lower. In the bone marrow, adiponectin has been linked to both bone metabolism and hematopoiesis. Although in vitro adiponectin promotes osteoblast differentiation, in clinical studies circulating adiponectin concentrations are associated with lower bone mass, possibly as a marker of increased marrow adiposity (reviewed in⁵³). Furthermore, increased adiponectin production by bone marrow adipocytes following myeloablative chemotherapy, promoted hematopoietic recovery in mice⁵⁴.

Recently another study in mice showed that the production of stem cell factor (SCF) by bone marrow adipocytes following myeloablative chemotherapy also promoted hematopoietic recovery and a mouse model with impaired adipogenesis (A-ZIP/F1 mouse) indeed had impaired hematopoietic regeneration⁵⁵.

In addition, Mattiucci et al showed that human bone marrow adipocytes secrete CXCL12 which is important for hematopoietic stem cell (HSC) maintenance in the bone marrow and that coculture of human bone marrow adipocytes can maintain HSCs in long term culture, suggesting that bone marrow adipocytes could play a role in steady state HSC maintenance⁵⁶.

Another cytokine produced by MAT is IL-6, an inflammatory protein that may suppress osteoblast activity. IL-6 was identified by Duque et al by comparing cytokines produced by marrow adipocytes with subcutaneous adipocytes. They found 53 proteins from the marrow adipocytes that were upregulated with aging and that could negatively impact neighboring osteoblasts leading to age-related bone loss⁵⁷. Similarly this same group reported that

palmitate, a saturated fatty acid, is released from adipocytes and can suppress osteoblast function⁵⁸. Moreover, treatment of mice with a fatty acid synthetase inhibitor prevented that osteoblast toxicity⁵⁹.

Another cytokine that might be released by adipocytes is RANKL. Increased MAT is often associated with decreased bone mass due to uncoupling of bone remodeling with a shift towards more bone resorption. In a study exploring the role of PTH in regulating marrow adiposity, Fan et al found that mice lacking the PTH receptor in MSCs had more marrow adipocytes and that these adipocytes were adjacent to sites of active bone resorption⁴⁰. Additional studies from that group revealed that marrow adipocytes express high levels of RANKL in the absence of the PTH receptor, and the protein is secreted into the marrow space potentially influencing bone resorption, as well as making its way into the systemic circulation. RANKL has also been shown to systemically impair hepatic glucose utilization and high levels of RANKL may be associated with glucose intolerance⁶⁰, although it is unclear whether the source of that RANKL is the marrow adipocyte. Intriguingly, peripheral adipocytes do not express significant amounts of RANKL.

Lecka-Czernik and colleagues showed that marrow adipocytes secrete growth factors including IGFBP-2 and Wnt 10b that enhance osteoblast differentiation and may also have endocrine properties in a mouse model of adipocyte beighing⁶¹.

Recently, Kousteni and colleagues⁶² reported that lipocalin 2, a protein which is made by adipocytes and osteoblasts, improves insulin sensitivity and reduces appetite through activation of the melanocortin 4 receptor in the hypothalamus. In that study, marrow adiposity was not reported, and expression of lipocalin 2 was significantly higher in bone cells than marrow adipocytes. However, it is conceivable that during states of increased MAT, lipocalin-2 is secreted by both cell types and targets whole body metabolism through changes in appetite.

2. Secretion of fatty acids as an energy source: A second hypothesis is that marrow adipocytes provide a fuel source for neighboring osteoblasts and hematopoietic stem cells by releasing lipids (lipolysis). It is striking that marrow fat is increased during periods when the osteoblast or hematopoietic stem cell are under extreme duress, including injury models, diet induced changes, calorie restriction and aging. Evidence to support that tenet has been difficult to prove in part because ex vivo model systems are challenging. An indirect measure of lipolysis can be the shrinkage of the adipocyte, since the size of an adipocyte is mainly determined by its lipid content. Preliminary studies have suggested that both estrogen and PTH treatment can reduce marrow adipocyte size in women with osteoporosis⁶³ and in mice on a calorie restricted diet (personal communication, C.J. Rosen). These data could implicate a role for estrogen and parathyroid hormone in mediating local lipolysis in addition to mesenchymal stromal cell fate. Whether this process is active during states of impaired bone cell function remains to be determined. Very recently though, Shafat et al showed in a series of in vitro experiments that adipocytes can actively transfer lipids (free fatty acids (FFAs)) to hematopoietic stem cells, and even more efficiently so to acute myeloid leukemia (AML) blasts which enhanced the survival and proliferation of the AML blasts. In addition, when these investigators blocked the lipolysis in a murine leukemia

model, this prolonged the survival of the mice⁶⁴. Tabe et al also report that bone marrow adipocytes promote the survival of AML cells in vitro by stimulating fatty acid oxidation using a genetic analysis⁶⁵. Previous studies have suggested that fatty acid oxidation through bone marrow adipocyte lipolysis could also play an important role in growth and survival of prostate cancer bone metastases^{66,67}.

In summary, these studies collectively show that the marrow adipocyte is heterogeneous both in terms of its function and its origin. Similarities between marrow adipocytes and peripheral adipocytes are the expression of the insulin receptor, the leptin receptor, and all the markers of mature adipocytes. Similarly, marrow adipocytes can store and release fatty acids and adipokines and cytokines. However, marrow adipocytes differ from peripheral adipocytes in several important ways as well both in function and origin. Functionally, marrow adipocytes can secrete RANKL which stimulates bone resorption⁴⁰ and adiponectin and stem cell factor which enhances hematopoiesis^{54,55}. As for the origin, some marrow adipocyte progenitors express Osterix (sp7), an early osteoblast transcription factor^{29,68} and others stain positively for Nestin, an early marker of neural progenitors⁶⁹ and not all the marrow adipocyte progenitors trace with platelet derived growth factor receptor alpha (PDGFRa) unlike visceral or subcutaneous adipocytes (Horowitz, personal communication); yet virtually all the marrow adipocytes trace with the leptin receptor⁷⁰. In addition, a direct comparison of the gene profiles of human adipose tissue derived and bone marrow derived adipocytes showed different transcriptomes, although the functional relevance of these differences has to be determined⁵⁶.

Furthermore, it is clear that marrow adipocytes secrete factors influencing processes within the bone marrow; bone remodeling and hematopoiesis, but they also secrete factors which are released into the circulation, potentially acting outside of the bone marrow. Bone remodeling and hematopoiesis are energy consuming processes which are tightly regulated to maintain bone mass and blood cell counts. Integration of information on energy availability and energy consumption is important to maintain whole body homeostasis. Previous studies in mice have shown that bone remodeling and whole body homeostasis are balanced through the combined actions of bone specific peptides and systemic hormones (e.g. osteocalcin, and insulin, reviewed in⁷¹), but marrow adipocytes could very well play a role in this balance as well and the secretion of adipokines could be the mechanism.

BONE MARROW ADIPOSITY IN DISEASE

Skeletal disease: osteoporosis

In healthy subjects an inverse relation between marrow adiposity and bone mineral density exists, independent of age, sex and total body fat^{14,16,72-75}. In addition, a wealth of studies showed that marrow adiposity is higher in osteopenic compared to healthy, and in osteoporotic compared to osteopenic women and men. Marrow adiposity ranged from 45 to 60% in healthy subjects around age 60, was 5% higher in osteopenia and again 5% higher in osteoporosis^{15,76-81}. Previously, biopsy and postmortem studies also showed 10% higher marrow adiposity in osteoporotic patients compared to healthy subjects^{23,25,82}. Moreover, several studies showed that marrow adiposity is higher in patients with vertebral fractures and in patients with signs of vertebral weakness^{76,83-85}. The lipid constituents of marrow

adiposity may also differ between healthy and osteoporotic patients in respect to the degree of saturated vs unsaturated fatty acids, although more studies are needed to assess the importance of this feature.

Bisphosphonates—Two randomized, double-blind, placebo-controlled trials investigated the effect of bisphosphonate therapy on marrow adiposity. A single injection of 5 mg zoledronic acid decreased marrow adiposity from 65% to 57% after a year compared to a 3% increase in the placebo group in 50 women per group as assessed by MRI in the lumbar vertebra⁸⁶. Daily administration of 5 mg of risedronate during 3 years decreased marrow adiposity by 20% compared to a 15% increase in the placebo group in 28 women as assessed by paired bone biopsies⁸⁷. These studies show that bisphosphonates indeed decrease marrow adiposity in humans. Studies in rats showed the same effect^{88,89} and in vitro bisphosphonates indeed suppress adipogenesis of mesenchymal stem cells^{90,91}, however a direct effect on the marrow adipocyte or an indirect effect via the osteoclast have not been investigated at the moment.

Estrogen—Estrogen supplementation decreases marrow adiposity in postmenopausal women. Daily administration of transdermal estradiol during one year prevented the increase in marrow adiposity as observed in the placebo-treated group of postmenopausal women with osteoporosis. Paired bone biopsies showed that during one year, both adipocyte size and number increased by 20% in untreated women and estradiol prevented the increase in number and even decreased adipocyte size⁶³. The same effect was observed in a short-term study of postmenopausal healthy women treated with oral estradiol for just two weeks. MRI scans of the lumbar vertebrae showed that the fat fraction decreased 5% during 2-weeks supplementation and increased again to baseline values in 2-weeks after withdrawal of treatment²¹. Also in animals, ovariectomy increases marrow adiposity⁹²⁻⁹⁴ and estrogen supplementation reverses this histological manifestation.

Parathyroid hormone—Parathyroid hormone is the most important calcium-regulating hormone in the body and is secreted by the parathyroid glands. PTH increases serum calcium concentrations by releasing calcium from the bone by stimulating bone resorption. Therefore continuous PTH oversecretion due to a parathyroid adenoma (primary hyperparathyroidism) causes bone loss leading to osteoporosis. However, intermittent treatment with PTH in contrast stimulates bone formation and iPTH is currently the only registered anabolic treatment for osteoporosis. Besides an increase in bone mass, iPTH treatment is also associated with a decrease in marrow adiposity in premenopausal osteoporotic women (as assessed by MRI)⁹⁵ and in osteoporotic men (as assessed by histomorphometry)⁴⁰.

Animal and in vitro studies show the same effect of PTH on marrow adiposity^{39,40,142}.

Energy imbalance: anorexia nervosa, obesity, and diabetes

Anorexia Nervosa—In 1949, Cheatum reported that bone marrow adiposity was a marker of malnutrition in deer⁹⁶. That seminal observation was confirmed in other studies of wildlife starvation but more importantly, it set the stage for the subsequent analysis of

women with anorexia nervosa. Several case reports and case series reported changes in biopsied bone marrow in anorexia patients, ranging from no changes to hypoplasia to gelatinous transformation⁹⁷⁻⁹⁹. More recently, MRI assessments showed that marrow adiposity is increased in anorectic compared to healthy age-matched controls and that the relative contribution of MAT as a percentage of total body fat almost triples^{52,100}. Marrow adiposity in recovered anorectic patients however, is comparable to healthy age-matched controls¹⁰¹, implying a reversible effect. No difference in marrow fatty acid composition was observed in anorectic compared to control subjects¹⁰². Interestingly, anorexia patients with amenorrhoea exhibited even higher marrow adiposity than anorexia patients with normal menstrual cycles¹⁰⁰, which might very well be due to the additive effect of estrogen withdrawal on marrow adiposity. Bone loss and increased incidence of fracture are well-established hallmarks of anorexia, and indeed the inverse association between bone mineral density and marrow adiposity was confirmed in these studies of anorexia patients.

Rodent models of caloric restriction report conflicting results; in some models marrow adiposity indeed increases^{52,103,104} whereas in others marrow adiposity remains constant^{105,106}. These differences might be explained by differences in sex, age and genetic background of the rodents, the degree of caloric restriction or the amount of weight loss achieved.

Obesity—Obesity is a major health issue leading to adipose tissue inflammation with adverse effects on systemic metabolism. Bone density is increased in obesity, which might be due to the higher load on the bones, but bone quality is impaired leading to increased fracture risk. Increased marrow adiposity could contribute to the impaired bone quality and increased fracture risk in obesity. Indeed, Cohen et al showed that marrow adiposity measured in bone biopsies was higher in obese compared to overweight and healthy subjects¹⁰⁷. These findings were confirmed in pre- and postmenopausal women and in young and older men using MRI¹⁰⁸⁻¹¹¹. In these studies, marrow adiposity was also inversely related to bone mass and measures of skeletal fragility and increased marrow adiposity was associated with a decrease in bone formation histologically. In contrast to these results, a recent study did not detect a difference in marrow adiposity in healthy-weight versus obese men and women¹¹². Also in rodents, high fat diet induced obesity increases marrow adiposity, independent of sex and genetic background¹¹³⁻¹¹⁶.

Treatment options for obesity are either lifestyle interventions (diet and exercise) or bariatric surgery and in all these the effect on marrow adiposity has been studied.

Diet: Three studies investigated the effect of a diet intervention on marrow adiposity. The first study included 55 premenopausal women and 12 men and subjected these to 12 weeks of low calorie diet resulting in 8 and 12 kilograms of weight loss respectively, accompanied by decreases in fat mass. Marrow adiposity decreased by 3.5%. Six months after the intervention, 24 subjects had >30% weight regain and marrow adiposity returned to the baseline levels¹¹⁷. The second study initiated a 12 week low calorie diet in 10 women resulting in 5 kilograms of weight loss and a decrease in subcutaneous and visceral fat depots. However, marrow adiposity did not change¹¹⁸. The third study initiated a 4 week low calorie diet in 20 women resulting in 7 kilograms of weight loss, a decrease in subcutaneous

and visceral fat, a dramatic decrease in liver fat of 40% and beneficial changes in serum lipid profiles. But again, marrow adiposity did not change¹¹⁹.

Bariatric surgery: Three studies reported the effect of bariatric surgery on marrow adiposity. Bredella included 18 women and 3 men, mean BMI 40, of whom 11 underwent roux-en-Y gastric bypass and 10 sleeve gastrectomy. Weight loss was 30 and 25 kilograms respectively and >30% decrease in subcutaneous and visceral fat 12 months following surgery. Marrow adiposity did not change following roux-en-Y gastric bypass and increased slightly following sleeve gastrectomy, bone mineral density decreases in both groups were as described before¹²⁰. Ivaska included 42 women and 4 men (19 diagnosed with diabetes), mean BMI 42, of whom 21 underwent roux-en-Y gastric bypass and 25 sleeve gastrectomy. Weight loss was 25 and 20 kilograms respectively 6 months following surgery and 13 of 19 diabetic patients reached remission of diabetes. Again, marrow adiposity did not change following surgery¹²¹. Finally, Schafer included 11 women, 6 with diabetes and mean BMI 40, all of which underwent Roux-en-Y gastric bypass. Weight loss was 25 kilograms 6 months following surgery and >30% decrease in fat mass and 5 of 6 diabetic patients reached remission of diabetes. Overall, marrow adiposity decreased 3.5%, however this effect was due to a 7.5% decrease in marrow adiposity in the diabetic patients, in the non-diabetic patients marrow adiposity increased 1%¹²². Recently Schafer et al published the extension of their data indeed showing that marrow adiposity decreases following weight loss after bariatric surgery in diabetic patients, but not in non-diabetic obese patients. The decrease in HbA1c and in marrow adiposity were significantly correlated. In addition, this study shows that bone loss following bariatric surgery is associated with increases in marrow adiposity¹²³.

Exercise: Exercise reduces body fat and body weight, and several studies have shown that exercise can also decrease marrow fat. In female athletes tibial marrow adiposity estimated by CAT (computerized axial tomography)-scanning was lower than in active control women¹²⁴. In young male wrestlers, spine marrow adiposity assessed by MRI was also lower compared to healthy controls¹²⁵. Two studies investigated the effect of an exercise intervention on marrow adiposity. In preschool children 10 weeks of school-based physical activity of 20 minutes 3 days a week reduced femoral marrow adiposity compared to an increase in age and sex matched control group¹²⁶. In older men (60 years) resistance training 3 days a week during 18 months reduced femoral marrow adiposity compared to controls in a randomized trial¹²⁷. In contrast, bed rest for prolonged periods of time increases marrow adiposity and this could be prevented by exercise^{128,129}. Studies in animals also show that exercise prevents the increase in MAT during high-fat diets¹¹⁵.

Diabetes—Diabetes, both type 1 (DMT1) and type 2 (DMT2), is associated with increased fracture risk and marrow adiposity is considered a potential contributing factor in both types of diabetes (recently extensively reviewed in¹³⁰).

Type 1: Diabetes type 1 is characterized by insulin deficiency due to auto-immune mediated beta cell dysfunction. Patients are in general diagnosed at younger age and are treated with lifelong insulin therapy. Two studies investigated marrow adiposity in type 1 diabetes. Slade

detected no difference between type 1 diabetes patients (HbA1c 7.5%) and age, sex and weight matched healthy controls in vertebral marrow adiposity (men T1DM 54% healthy 57%, women T1DM 55% healthy 52%) and marrow adiposity was not related to HbA1c. However marrow adiposity was positively correlated to serum lipids¹³¹. Abdalrahman recruited 30 young women with T1DM (HbA1c 9.8%) and age, sex and weight matched controls and did not find a difference in vertebral marrow adiposity (31% in T1DM and 26% in healthy controls)¹³².

Type 2: Diabetes type 2 is characterized by insulin resistance, adipose tissue inflammation and deranged metabolic profiles. Patients are generally overweight and are diagnosed later in life, although this is changing with the rising obesity rates in children. The cornerstone of treatment is metformin, but many patients eventually need insulin therapy. Several studies investigated marrow adiposity in type 2 diabetes with conflicting results. Sheu reported higher vertebral marrow adiposity in 38 older diabetic participants in the Osteoporotic Fractures in Men (MrOS) study compared to the 118 participants without diabetes (59 vs 55%)¹³³. Araujo did not find a difference in vertebral marrow adiposity in 28 type 2 diabetes patients (15 women, 13 men) compared to healthy controls (14 women, 10 men) or obese subjects (16 women, 10 men) (mean age 50 years, marrow adiposity 37% (T2DM) vs 36% (healthy) vs 32% (obese), but again marrow adiposity was positively related to HbA1c¹¹². Baum as well did not find a difference in 13 postmenopausal women with T2DM (HbA1c 7.6%) compared to 13 age, sex and weight matched controls in vertebral marrow adiposity (69% versus 67%), however lipid unsaturation was lower in diabetic subjects¹³⁴. Patsch compared vertebral marrow adiposity in four groups of 17 postmenopausal women per group; diabetic and nondiabetic with and without fragility fractures, and did not find an association with vertebral marrow adiposity (control 72%, T2DM 71%, fracture 65%, fracture and T2DM 69%), but again lipid unsaturation was negatively associated with diabetes and fracture¹³⁵.

Interventions: Three interventional studies reported marrow adiposity in type 2 diabetes. Schafer showed that diabetic patients had 10% higher marrow adiposity before bariatric surgery, which decreased 7.5% 6 months postsurgery, resulting in the same marrow adiposity as the non-diabetic subjects¹²², however in the extension of that study published more recently, diabetic subjects had the same marrow adiposity as non-diabetic subjects before surgery, although diabetic subjects lost marrow fat following surgery whereas non-diabetic subjects did not¹²³. In addition this study showed that the improvement in diabetes was associated with the decline in marrow fat. Ivaska reported 10% higher marrow adiposity in diabetes patients who reached remission following bariatric surgery, compared to the diabetes patients who remained diabetic¹²¹. Vogt showed that after 6 weeks of low calorie diet in 19 women and 10 men with type 2 diabetes, 13 kilograms of weight loss was accompanied by a 5% decrease in vertebral marrow adiposity (58% before and 53% afterwards), decrease in liver fat and serum lipids¹³⁶.

Studies in mouse models of diabetes show consistently increased marrow adiposity^{137,138} in hyperglycaemic mice. This discrepancy between mice and humans can be explained in several ways. Firstly, human studies are underpowered to show a difference; most of the

studies do show higher marrow adiposity in diabetes, but the group sizes might be too small to detect a significant difference. Secondly, hyperglycaemic mice remain hyperglycaemic, whereas humans are treated to reach normoglycaemia. Therefore the level of hyperglycaemia or the relative insulin deficiency can impact marrow adiposity. In addition, metformin is the first choice in therapy for type 2 diabetes and almost all diabetic patients are treated with metformin. Recently, the mechanism of its antidiabetic effect by reducing hepatic gluconeogenesis was elucidated¹³⁹, but interestingly, a recent study reports a suppressive effect of metformin on adipogenesis of bone marrow mesenchymal stem cells¹⁴⁰. Therefore metformin therapy can have a favorable effect on marrow adiposity in diabetic patients. Thirdly, mice have in general lower levels of marrow adiposity and this is usually assessed in the tibia and femur, whereas human marrow adiposity is mostly measured in the vertebrae. In addition, mice share the same genetic background mostly and indeed marrow adiposity is strain-specific, whereas humans have heterogeneous genetic backgrounds.

Neoplastic disease

Since it became clear that obesity increases cancer risk¹⁴¹, many studies have been conducted investigating the link between adiposity and tumour growth. These studies mainly focused on white adipose tissue depots and showed that adipocyte hypertrophy can promote neoplastic disease (reviewed in¹⁴²). Several solid tumour types have a preference to metastasize to adipocyte-rich environments and the adipocytes are thought to represent an important reason for this preference. Omental adipocytes for example have been shown to provide energy via direct lipid transfer to ovarian cancer metastases and to promote tumour growth via secretion of mediators¹⁴³; mammary adipocytes have been shown to release fatty acids to promote growth and stimulate invasion of breast cancer cells¹⁴⁴; and adipocytes surrounding the prostate have been shown to promote the migration of prostate cancer cells into the surrounding tissues¹⁴⁵. Since the bone marrow also frequently hosts cancer cells, both deriving from the bone marrow and metastasizing from different organs, some researchers have focused on bone marrow adipocytes and the interaction with neoplastic cells.

Hematological cancers—Hematological malignancies induce changes in the bone marrow; it has long been known that marrow adiposity is increased in aplastic anemia and decreased in leukemia¹⁴⁶. This was thought to be due to the absence or accumulation of hematological cancer cells, but this has been revisited recently. In fact, the marrow adipocytes are now thought to play an active role in the pathophysiology of hematological neoplastic disease (extensively reviewed for multiple myeloma in¹⁴⁷). In vitro experiments have shown that human bone marrow adipocytes actively promote leukemic growth through transfer of lipids and secretion of mediators^{64,65}. In addition, pharmacological blocking of the lipid transfer between the bone marrow adipocyte and the leukemic blast through inhibition of FABP4, the fatty acid binding protein necessary for transport of the lipids, reduced the tumor survival in vitro and increased survival of leukemic mice in vivo⁶⁴ (Shafat). In addition, blocking carnitine palmitoyl transferase (CPT)1a which is necessary for the entry of the fatty acids into the mitochondria, decreased the survival of human leukemia cells in vitro¹⁴⁸, implying that indeed fatty acids are an important source of energy

for these cells. Although these animal and in vitro data are promising, no human clinical studies have been published yet.

Solid tumours—Breast cancer and prostate cancer are two solid tumours that preferentially metastasize to the bone marrow. Bone marrow adipocytes could be involved in the attraction and growth of these tumour types in the bone marrow. Previous in vitro studies provided some evidence that bone marrow adipocytes promote tumor growth of prostate cancer metastases through the secretion of fatty acids⁶⁶ and HIF (hypoxia inducible factor)-1 α activation⁶⁷. A study in mice showed that melanoma cells induce lipolysis in the bone marrow adipocytes and that bone marrow adipocytes secrete factors promoting melanoma cell proliferation¹⁴⁹. Supporting these findings in mice, a recent clinical study in humans showed that marrow adiposity was higher in breast cancer patients compared to age, sex and weight matched healthy controls (56 patients in both groups, 73% versus 60% marrow adiposity)¹⁵⁰, suggesting that breast cancer is associated with higher bone marrow adiposity, although these patients did not have metastasized disease.

Therapy—Neoplastic disease is treated with either chemotherapy, radiation therapy or both. Both therapies induce increased bone marrow adiposity and both therapies share bone marrow toxicity leading to decreased hematopoiesis as a frequent complication. Bolan showed that 6 months of treatment with chemo- or radiotherapy increased marrow adiposity from 45% to 63% in gynaecological cancer patients¹⁵¹. Indeed Schraml also found higher marrow adiposity, although not significant, in cancer patients (66%) previously treated with chemotherapy compared to untreated cancer patients (59%)¹⁵². And Mostoufi observed a vertebral marrow adiposity of 57% versus 27% in childhood cancer patients treated with an allogeneic hematopoietic stem cell transplantation (which includes chemotherapy and total body irradiation) compared to age-, sex and weight matched healthy controls¹⁵³. In mice, marrow adiposity is increased as well following chemo- or radiotherapy. However, the effect of the increased marrow adiposity on hematopoietic regeneration is complex according to new studies. The first study to report on bone marrow adiposity and hematopoietic reconstitution following radiation showed that the increase in marrow adiposity is related to the decrease in hematopoiesis following therapy. In addition, prevention of marrow adipogenesis pharmacologically or genetically, promoted hematopoietic repopulation of the bone marrow^{154,155}. However, more recently another study showed that indeed in the caudal vertebrae of the mouse, hematopoietic regeneration was better in genetically modified mice lacking bone marrow fat, but in the long bones (the femur and tibiae) hematopoietic regeneration was markedly impaired without adipocytes. This study also showed that the bone marrow adipocytes are an important source of stem cell factor, an important factor for hematopoietic stem cell survival, therefore these authors hypothesize that the increase in adipogenesis in the bone marrow following chemo- or radiotherapy, is promoting hematopoietic reconstitution and therefore could be beneficial⁵⁵. In humans, hematopoiesis is concentrated in the axial skeleton and not in the appendicular (long bones), therefore it remains to be determined what the role of the bone marrow adipocyte is in human hematopoietic reconstitution.

Another possible role of the bone marrow adipocytes could be to protect the neoplastic cells in the bone marrow from the effects of chemotherapy. One study showed that leukemic cells in the gonadal adipose tissue of mice were more resistant to conventional chemotherapy regimens than leukemic cells in other locations¹⁵⁶, possibly through the induction of fatty acid metabolism. Whether bone marrow adipocytes could also induce therapy-resistance in neoplastic remains to be determined.

CLINICAL IMPLICATIONS

In conclusion, bone marrow adipocytes appear shortly after birth and steadily increase during growth and aging. Bone marrow adipocytes reside in the bone marrow together with bone cells and hematopoietic cells and mounting evidence suggests that the adipocytes are actively involved in both bone remodeling and hematopoiesis. The adipocytes can provide fuel to the energy consuming processes of bone remodeling and hematopoiesis through the release of fatty acids which could be an important energy source in the hypoxic bone marrow environment. In addition bone marrow adipocytes secrete mediators which can have local paracrine, but perhaps also systemic, endocrine effects. The exact role of the bone marrow adipocyte in local and systemic energy metabolism will become clear from future studies.

Weight gain and weight loss are both associated with increased marrow adiposity. It remains to be determined whether these two conditions induce the same marrow adipocytes to increase, or that perhaps different adipocyte depots are present in the bone marrow as suggested in mice studies for example, ie constitutive versus regulated. A possible modifier of the relation between weight and marrow adiposity could be inflammatory status and insulin resistance, since some studies clearly show stronger associations with HbA1c than fat mass. Further studies are needed to resolve this issue and to understand the relation between peripheral and bone marrow adipose tissue depots. Some diseases are associated with increased marrow adiposity and it remains to be determined whether this is a physiological response, ie the increase in bone marrow adipocytes support hematopoietic reconstitution following chemotherapy by providing stimulating factors and fatty acids, or a pathological mechanism, ie the increase in bone marrow adipocytes provide fuel for neoplastic cells outgrowing their metabolic demands. This will be an important question to be answered, especially when considering the bone marrow adipocyte as a potential therapeutic target. Inhibiting bone marrow adipogenesis or blocking fatty acid metabolism could have detrimental effects if indeed the bone marrow adipocytes are rescuing metabolically stressed bone or hematopoietic cells. However, it could also be a very effective therapy possibly with few systemic side-effects if indeed the marrow adipocytes are supporting the pathological response.

In addition, marrow adiposity can now be measured non-invasively with MRI and this could add to the diagnostic or prognostic process for example in skeletal disease. Increased marrow adiposity has been shown to be an independent predictor of fracture and lipid saturation of the marrow adipose tissue also could be an important addition.

Over the last decades the bone marrow adipocyte has evolved from passive filler to key player in the bone marrow niche and many investigators are now exploring the characteristics, function and origin of the bone marrow adipocyte. These studies will provide more insight into its role in health and disease and the possibility for new treatments.

Acknowledgments

AGV is supported by the European Society of Endocrinology (Short-Term Fellowship 2017 and International Endocrine Scholars Programme 2017) and the Catharine van Tussenbroek Fund and CJR is supported by the NIH (R24: DK 092759, DK 112374, AR 071739).

ABBREVIATIONS

MAT	marrow adipose tissue
PTH	parathyroid hormone
RANKL	RANK ligand
MRI	magnetic resonance imaging
LH	luteinizing hormone
FSH	follicle stimulating hormone
QCSI	Quantitative Chemical Shift Imaging
MRS	Magnetic Resonance Spectroscopy
BAT	brown adipose tissue
TZD	thiazolidinediones
MSC	mesenchymal stromal cell
RUNX2	Runt-related transcription factor 2
PRX1	homeobox protein 1
Sp7	osterix
PDGFR	platelet-derived growth factor receptor- β (b),
C/EBP	CCAAT/enhancer binding protein alpha
Zfp	Zinc finger proteins
Col1a1	osteoblasts collagen type 1
Ocn	osteocalcin
PPARγ	peroxisome proliferator-activated receptors gamma
FABP	fatty acid binding protein
Pln	perilipin

FASN	fatty acid synthase
SOST	sclerostin
FGF	fibroblast growth factor
ER	estrogen receptor
iPTH	intermittent PTH treatment
SCF	stem cell factor
HSC	hematopoietic stem cell
FFA	free fatty acids
AML	acute myeloid leukemia
PDGFRα	platelet derived growth factor receptor alfa
CAT	computerized axial tomography
T1DM	type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus
CPT	carnitine palmitoyl transferase
HIF	hypoxia inducible factor

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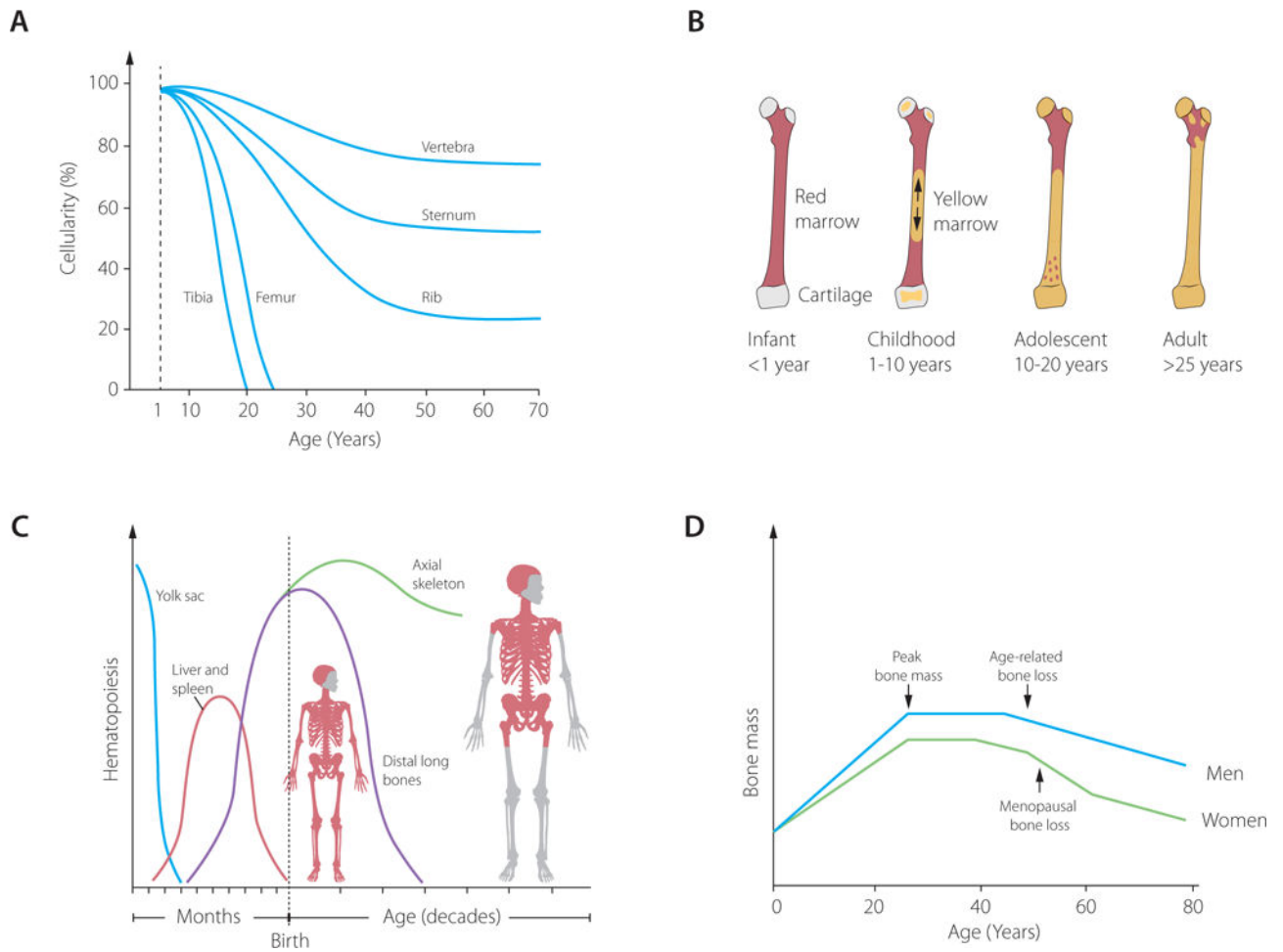


Figure 1. Changes in bone marrow hematopoietic cellularity per bone (A), conversion of hematopoietic to adipose marrow in the femur (B) development of hematopoiesis (C) and bone mass (D) during human life.

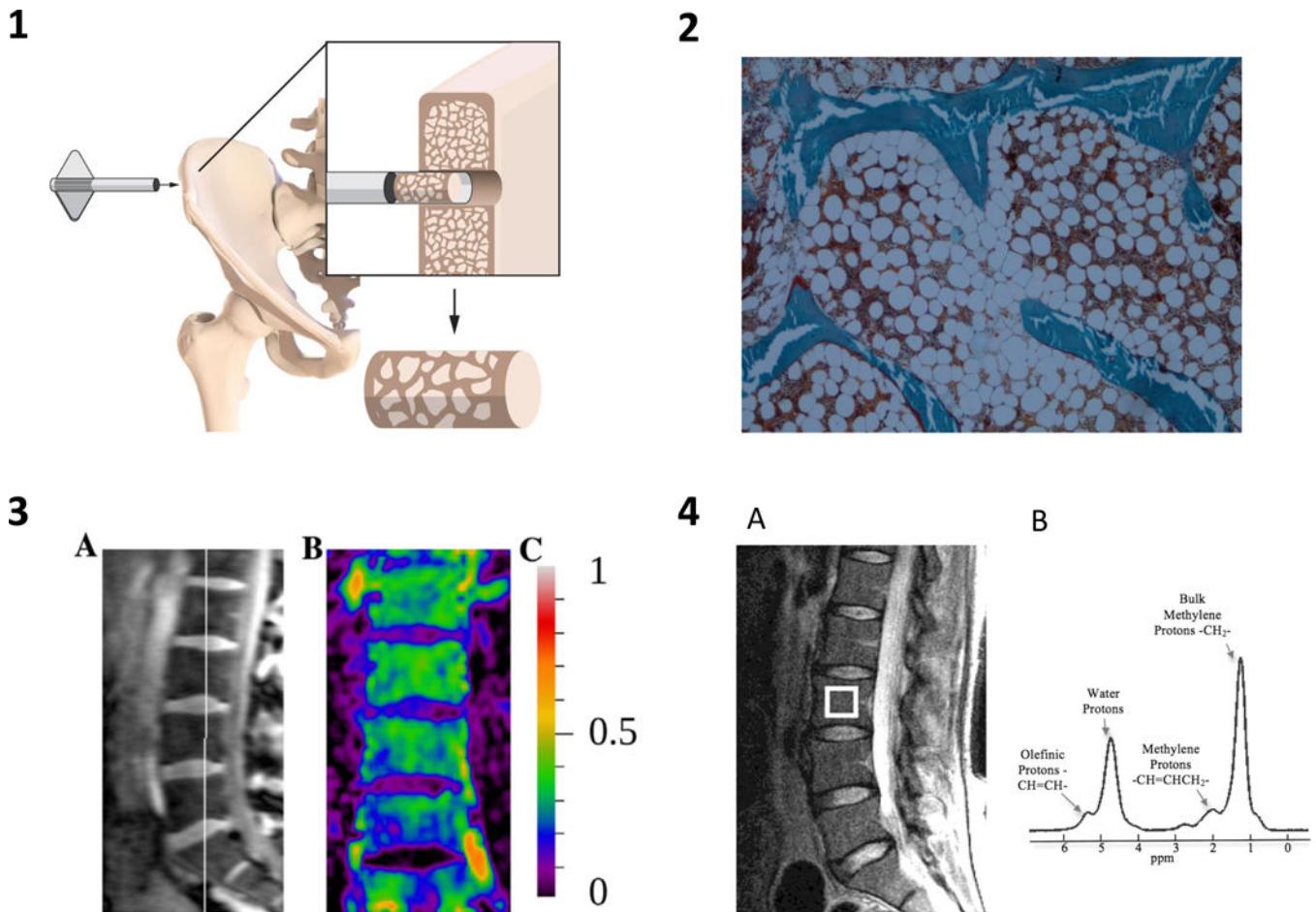


Figure 2.

Methods of MAT evaluation

1. Iliac crest bone biopsy or marrow aspiration procedure.
2. Bone biopsy of the iliac crest (Goldner trichrome staining) showing trabecular bone (green), hematopoietic marrow (red) and the adipocyte ghosts (white). Image courtesy of Dr. N. Bravenboer.
3. Magnetic Resonance Imaging (MRI) Quantitative Chemical Shift Imaging (QCSI) of the lumbar vertebrae (L1-4). A shows the MRI image of the lumbar vertebrae with the white line indicating the QCSI localizer, B shows the fat signal fraction image, with (C) color coding: from black (no fat), to white (all fat). Image courtesy of Dr. E.M. Akkerman³³.
4. Magnetic Resonance Spectroscopy (MRS). A shows the MRI image of the lumbar vertebrae with the white box indicating the site of measurement B is an example spectrum showing four peaks: olefinic, double bond -CH=CH- protons at 5.31 ppm, water protons at 4.65 ppm, the CH₂methylene protons α- to a double bond (-CH=CHCH₂-), at 2.03 ppm, and the bulk CH₂methylene protons at 1.3 ppm. Image from Li JMIR 2011¹³².

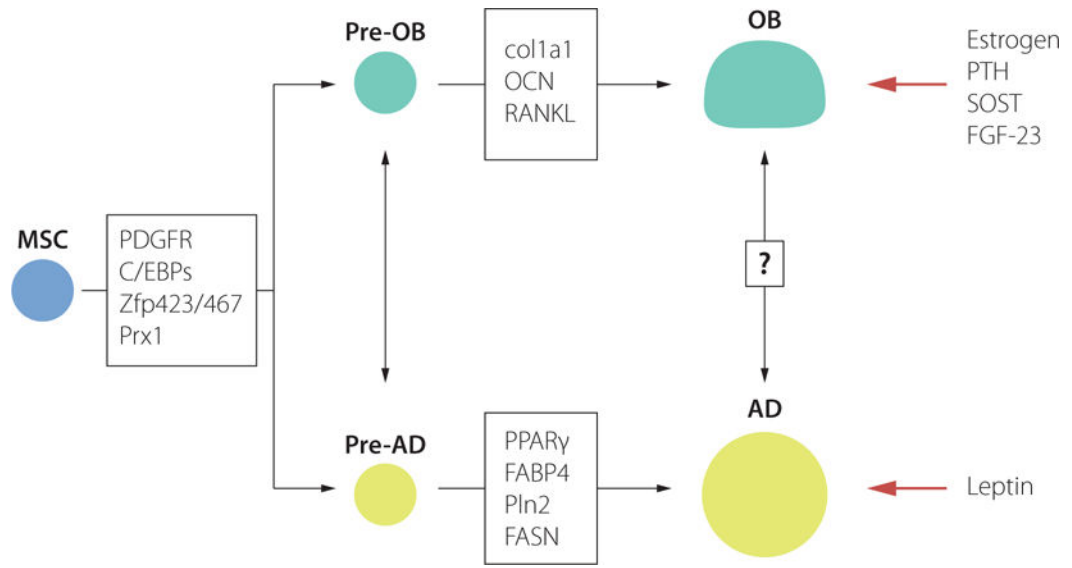


Figure 3.
Bone marrow mesenchymal stem cell differentiation

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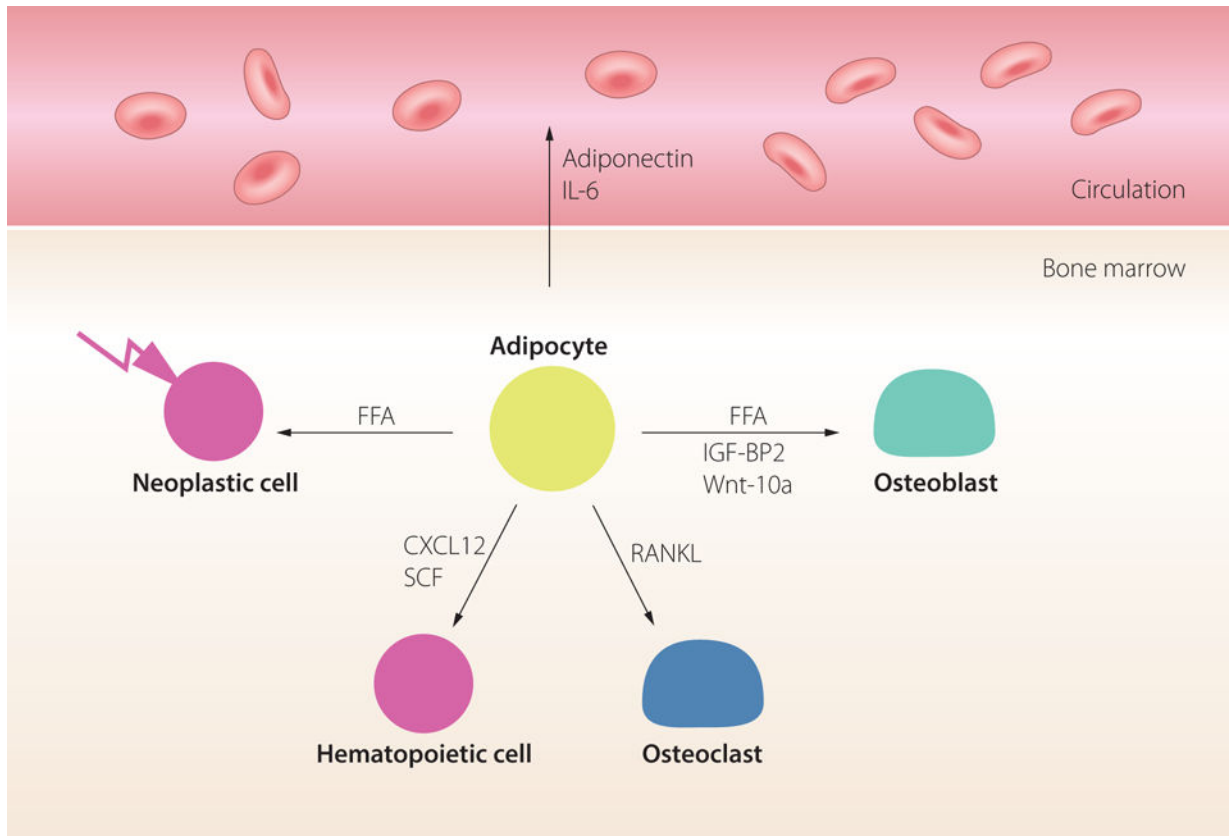


Figure 4.
Function of the marrow adipocyte

TABLE 1

Effects of interventions on marrow adipose tissue and bone mass, fracture risk and fat mass

	MAT	Bone Mass	Fracture Risk	Fat Mass
Bisphosphonate (86, 87)	↓	↑	↓	—
Estrogen (21,63)	↓	↑	↓	↓
Parathyroid hormone (95, 40)	↓	↑	↓	
Diet (117, 118, 119)	↓/—			↓
Bariatric surgery (120,121, 122)	↓/—*	↓		↓
Exercise (124, 125, 126, 127)	↓	↑	↓	↓
Metformin (140)	↓			
Chemo-/radiotherapy (151,152, 153)	↑			

*
depending on HbA1c

references between brackets

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TABLE 2

Possible Clinical Implications of MAT

1	Skeletal Health Improve diagnosis of skeletal fragility and fracture prediction by measuring amount and lipid saturation of vertebral MAT by MRI Develop new therapies for osteoporosis by reducing amount of MAT or changing secretory profile of MAT
2	Hematopoiesis Improve hematopoietic recovery following chemo- or radiotherapy by stimulating MAT production of SCF
3	Cancer Develop new therapies to prevent growth of cancer cells in the bone marrow by inhibiting induced lipolysis from MAT
4	Systemic energy metabolism Identify new treatment targets such as adiponectin

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