The obesity of bone

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Abstract: During the last decades, obesity and osteoporosis have become important global health problems, and the belief that obesity is protective against osteoporosis has recently come into question. In fact, some recent epidemiologic and clinical studies have shown that a high level of fat mass might be a risk factor for osteoporosis and fragility fractures. Several potential mechanisms have been proposed to explain the complex relationship between adipose tissue and bone. Indeed, adipose tissue secretes various molecules, named adipokines, which are thought to have effects on metabolic, skeletal and cardiovascular systems. Moreover, fat tissue is one of the major sources of aromatase, an enzyme that synthesizes estrogens from androgen precursors, hormones that play a pivotal role in the maintenance of skeletal homeostasis, protecting against osteoporosis. Moreover, bone cells express several specific hormone receptors and recent observations have shown that bone-derived factors, such as osteocalcin and osteopontin, affect body weight control and glucose homeostasis. Thus, the skeleton is considered an endocrine target organ and an endocrine organ itself, likely influencing other organs as well. Finally, adipocytes and osteoblasts originate from a common progenitor, a pluripotential mesenchymal stem cell, which has an equal propensity for differentiation into adipocytes or osteoblasts (or other lines) under the influence of several cell-derived transcription factors. This review will highlight recent insights into the relationship between fat and bone, evaluating both potential positive and negative influences between adipose and bone tissue. It will also focus on the hypothesis that osteoporosis might be considered the obesity of bone.

Keywords: adipocyte, adipokines, bone-derived factors, fat bone marrow, mesenchymal stem cell, obesity, osteoblast, osteoporosis

Introduction

During the last decades, obesity and osteoporosis have become important global health problems with an increasing prevalence and a high impact on both mortality and morbidity worldwide [Kado *et al.* 2004; Rossner, 2002; Hu, 2003; WHO, 2000], and the belief that obesity is protective against osteoporosis has recently come into question. In fact, the latest epidemiologic and clinical studies, have shown that a high level of fat mass might be a risk factor for osteoporosis and fragility fractures [Greco *et al.* 2010; Kim, 2010; Greco, 2013; Compston *et al.* 2014].

Age and female sex increase the risk of developing both obesity and osteoporosis, which affect millions of women [Hu, 2003; Albala *et al.* 1996; Reid, 2002; NIH, 2001]. In particular, age-related changes in body composition, metabolic factors, and hormonal levels after menopause, accompanied by a decline in physical activity,

may all provide mechanisms for the propensity to gain weight, which is often characterized by an increase in fat mass and a decrease in lean mass.

Several potential mechanisms have been proposed to explain the complex relationship between adipose tissue and bone [Cao, 2011].

Fat has long been viewed as a passive energy reservoir, but since the discovery of leptin and the identification of other adipose tissue-derived hormones and serum mediators [Kadowaki and Yamauchi, 2005; Steppan *et al.* 2000; Vendrell *et al.* 2004], it has come to be considered as an active endocrine organ involved in the modulation of the energy homeostasis. Adipose tissue, in fact, secretes various inflammatory cytokines, including interleukin (IL)-6 and tumor necrosis factor α (TNF α), which are thought to have adverse metabolic, skeletal, and cardiovascular consequences [Tilg and Moschen, 2008]. Moreover, as IL-6, other fat-derived mediators,

Ther Adv Endocrinol Metab

2015, Vol. 6(6) 273–286

DOI: 10.1177/

2042018815611004

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which include resistin, leptin, and adiponectin, affect human energy homeostasis and are involved in bone metabolism, contributing to the complex relationship between adipose and bone tissue [Magni *et al.* 2010]. Finally, fat tissue is one of the major sources of aromatase, an enzyme also expressed in the gonads, which synthesizes estrogens from androgen precursors. Estrogens are steroid hormones which play a pivotal role in the maintenance of skeletal homeostasis, protecting against osteoporosis by reducing bone resorption and stimulating bone formation. This extragonadal estrogen synthesis in fat tissue becomes the dominant estrogen source in postmenopausal women, due to the lack of ovarian function [Reid, 2002]. Additionally, in obese postmenopausal women, increased estrogen synthesis by adipose tissue has been suggested as one of the potential mechanisms for the protective effect of fat mass on bone.

Thus, the pathophysiological role of adipose tissue in skeletal homeostasis lies in the production of several adipokines and hormones which modulate bone remodeling *via* their effects on either bone formation or resorption.

However, since the demonstration that bone cells express several specific hormone receptors, the skeleton has come to be considered an endocrine target organ [Eriksen *et al.* 1988; Kim, 2010; Komm *et al.* 1988; Migliaccio *et al.* 1992], and since recent observations have shown that bone-derived factors, such as osteocalcin (OCN) and osteopontin (OPN), affect body weight control and glucose homeostasis [Gomez-Ambrosi *et al.* 2008; Takeda, 2008; Gimble *et al.* 2006], the bone has come to be considered an endocrine organ itself [Fukumoto and Martin, 2009]. These considerations suggest a possible role of bone as a player of a potential feedback mechanism between the skeleton and the other endocrine organs [Fukumoto and Martin, 2009]. Thus, the cross talk between fat and bone likely constitutes a homeostatic feedback system in which adipokines and bone-derived molecules represent the link of an active bone–adipose axis.

Finally, adipocytes and osteoblasts originate from a common progenitor, a pluripotential mesenchymal stem cell [Akune *et al.* 2004], which has an equal propensity for differentiation into adipocytes or osteoblasts (or other lines) under the influence of several cell-derived transcription factors. This process is complex, suggesting significant plasticity and multifaceted mechanisms of regulation within different cell lineages, among

which are adipocytes and osteoblasts [Gimble *et al.* 1996; Rodriguez *et al.* 2000].

Several human and animal studies have examined the function of adipocytes in bone marrow. Mesenchymal stem cells isolated from bone marrow in postmenopausal osteoporotic patients express more adipose differentiation markers than those from subjects with normal bone mass [Sekiya *et al.* 2004], and pronounced fatty infiltration in the bone marrow of rats following oophorectomy it has been observed, suggesting a pivotal role of estrogen in regulating adipocyte and osteoblast recruitment [Martin and Zissimos, 1991].

Then, since obesity and overweight have always been considered protective against osteoporosis and fragility fractures, this review will highlight recent insights into the relationship between fat and bone and we will focus on the fascinating hypothesis that osteoporosis might be considered the obesity of bone [Rosen and Bouxein, 2006].

Obesity and osteoporosis

The interplay between fat and bone

Obesity, which is due to an imbalance in which energy intake exceeds energy expenditure over a prolonged period, has always been known and recognized as a risk factor for metabolic and cardiovascular diseases [Rossner, 2002]. However, it has always been considered a protective factor for bone loss and osteoporosis, which is defined as a bone metabolic disease, characterized by a decrease in bone strength [for a decreased bone mineral density (BMD) and altered bone quality], leading to an increased risk of developing spontaneous and traumatic fractures. Interestingly, obese postmenopausal women are often affected by hypertension, dyslipidemia, diabetes mellitus, cardiovascular disease, and having an increased risk of developing some cancers, but always been considered protected against osteoporosis [Rossner, 2002; Albala *et al.* 1996; Reid, 2002]. Even though body fat and lean mass are correlated with BMD, with obesity apparently exerting protection against bone loss, especially after menopause, during the last decades some evidence has described an opposite event, suggesting that probably obesity, based on the amount of fat mass, can affect bone metabolism leading to osteoporosis (Table 1). In fact, recent studies have shown that increased abdominal fat tissue might be considered a risk factor for

Table 1. Clinical studies focused on the possible effects of adipose tissues on bone health.

Study	Number of patients	Results on bone mineral density (BMD) and fracture risk
Ribot [1987]	176 women	The results of this study suggest that even moderate obesity can play a protective role in postmenopausal bone loss
Reid <i>et al.</i> [1992]	140 normal postmenopausal women	The analysis indicated that total body BMD was positively related to fat mass ($p < 0.0001$), and similar relationships were found in the subregions of the total body scans and in the lumbar spine and proximal femur. The authors conclude that total body fat is the most significant predictor of BMD throughout the skeleton
Reid <i>et al.</i> [1992]	68 healthy premenopausal women and 51 men	In women, BMD was correlated with weight ($r = 0.69$), fat mass ($r = 0.60$), and lean mass ($r = 0.55$). In men, the respective correlations were 0.56, 0.26 (NS), and 0.51. It is concluded that bone density is closely related to fat mass in premenopausal women, but less so in men
Reid <i>et al.</i> [1995]	Premenopausal women with eumenorrhea, >36 years	Subjects with mean activity levels of more than 140 kJ/kg day (equivalent to undertaking vigorous physical activity for > 1.5 h/week) were classified as exercisers. In the nonexercising subjects ($n = 36$; age 36 ± 8 years), BMD was markedly weight dependent ($0.45 < r < 0.62$), and this was contributed to by both fat and lean tissue. It is concluded that bone density is only associated with fat mass in sedentary women
Migliaccio <i>et al.</i> [2013]	86 obese men	A direct correlation between T and LBMD and HBMD ($p < 0.001$, $r(2) = -0.20$; $p < 0.001$, $r(2) = -0.24$). Inverse correlation between E2 levels, LBMD, and HBMD ($p < 0.001$, $r(2) = -0.20$; $p < 0.001$, $r(2) = -0.19$)
Yang <i>et al.</i> [2013]	1126 (360 men and 766 women), >50 years	Lower abdominal fat was significantly associated with a higher fracture risk in women. There was no statistically significant association between aFM and fracture risk (HR 1.15; 95% CI 0.58–2.25) in men
Greco <i>et al.</i> [2013]	340 obese women	Trunk fat negatively correlates with BMD at lumbar spine and femoral neck independently from vitamin D levels
Premaor <i>et al.</i> [2013]	139,419 old men	Multiple rib fractures were more frequent in overweight (RR 3.42; 95% CI 1.03–11.37) and obese (RR 3.96; 95% CI 1.16–13.52) old men. Obesity was associated with a reduced risk of clinical spine, hip, pelvis, and wrist or forearm fracture and increased risk of multiple rib fractures when compared with normal weight or underweight men
Watts [2014]	60,393 obese women aged ≥ 55 years	Although obesity was thought to be protective against all fractures, it substantially increased the risk of fractures in the ankle or lower leg
Compston <i>et al.</i> [2014]	52,939 women	Inverse linear associations between BMI or weight and hip, spine, and wrist fracture
Ho-Pham <i>et al.</i> [2014]	Meta-analysis: 44 studies, 20,226 individuals (4966 men and 15,260 women), 18–92 years old	Lean mass exerts a greater effect on BMD than fat mass in men and women
Søgaard <i>et al.</i> [2015]	19,918 postmenopausal women; 23,061 men	Abdominal obesity (CV) was associated with an increased risk of hip fracture
Copès <i>et al.</i> [2015]	1057 women	Prevalence of fractures in obese and nonobese women was similar (17.3% versus 16.0%); 41.4% of all fractures occurred in obese women
Yang and Shen [2015]	5287 individuals, 8–69 years old	All obesity measures were positively associated with femoral neck BMD, but not with lumbar spine BMD. Greater BMI and hip circumference were the most important obesity measure in relation to BMD
BMI, body mass index; CI, confidence interval; CV, cardiovascular; HBMD, high bone mineral density; LBMD, low bone mineral density; NS, nonsignificant; RR, relative risk; aFM, abdominal fat mass.		

decreased BMD and osteoporosis both in women and in men [Holecki *et al.* 2012; Greco *et al.* 2010, 2013; Migliaccio *et al.* 2013; Bredella *et al.* 2011; Kim, 2010; Kim *et al.* 2010; Watts, 2014; Søgaard *et al.* 2015; Compston *et al.* 2014].

The mechanisms whereby increased central adiposity leads to metabolic alterations, cardiovascular morbidity, and probably to bone loss has been largely based on the demonstration that adipose tissue secretes a number of cytokines and

bioactive compounds, named adipokines. Interestingly enough, a recent study demonstrated that premenopausal women with increased central adiposity had poorer bone quality and stiffness and markedly lower bone formation [Cohen, 2013]. Furthermore, recent *in vitro* data supported the hypothesis of decreased osteoblast activity in subjects affected by abdominal obesity [Wannenes, 2014], due to a mechanism which involved intracellular modifications, such as alteration of the Wnt/ β -catenin pathway.

The adipokines, which include a variety of proinflammatory peptides, are involved in many physiological or pathological processes, including inflammation, endothelial damage, atherosclerosis, impaired insulin signaling, hypertension, and bone remodeling. Adipokine dysregulation is a strong determinant of the low-grade inflammatory state of obesity, which promotes a cascade of metabolic alterations leading to cardiovascular complications, insulin resistance, or diabetes mellitus and bone loss [Kadowaki and Yamauchi, 2005; Vendrell *et al.* 2004].

Leptin, the first identified adipose tissue-derived factor, is an anorexigenic hormone secreted by adipocytes in proportion to body fat content [Thomas *et al.* 1999]. Leptin levels are typically elevated in obesity, which is considered a leptin-resistant state [Considine *et al.* 1996]. In obese subjects, hyperleptinemia has been widely recognized as an independent cardiovascular risk factor associated with hyperinsulinemia and insulin resistance [Martin *et al.* 2008], while its effect on bone is complex, and both negative and positive actions have been reported on BMD [Kontogianni *et al.* 2004; Goulding and Taylor, 1998]. Leptin-deficient ob/ob mice and leptin-receptor-deficient db/db mice are extremely obese, with increased vertebral trabecular bone volume due to increased bone formation [Ducy *et al.* 2000]. Interestingly, intracerebroventricular infusion of leptin in both ob/ob and wild-type mice was shown to decrease vertebral trabecular bone mass [Ducy *et al.* 2000]. *In vivo* studies indicate that the effect of leptin might depend on its site and mode of action [Thomas, 2004], and it has been proposed that peripheral administration of leptin could increase bone mass by inhibiting bone resorption and increasing bone formation, while inhibiting bone formation through a central nervous system effect [Goulding and Taylor, 1998]. *In vitro* studies also found that leptin can act directly on bone marrow derived mesenchymal stem cells (BMSCs) to

enhance their differentiation into osteoblasts and to inhibit their differentiation into adipocytes [Thomas, 2004]. Finally, leptin also inhibits the expression of neuropeptide Y (NPY), a hypothalamus-derived peptide, essential for the regulation of food consumption, energy homeostasis, and bone remodeling [Baldock *et al.* 2002]. Specific NPY-knockout mice show a significant decrease in body weight, a significant increase in food intake, and a twofold increase in trabecular bone volume compared with wild-type animals [Sainsbury *et al.* 2002].

Adiponectin exerts a protective role on the cardiovascular system and glucose metabolism, and in contrast with leptin, serum adiponectin levels are reduced in subjects with obesity and diabetes, and increase after weight loss [Pajvani *et al.* 2003]. Low levels of adiponectin are a common feature of obesity and correlate with insulin resistance [Yamauchi *et al.* 2001]. Adiponectin levels are inversely related to the circulating levels of C reactive protein (CRP), TNF α and IL-6, which are, especially the latter two, powerful inhibitors of adiponectin expression and secretion in cultured human adipose cells [Fasshauer *et al.* 2002]. Human osteoblasts express adiponectin and its receptors, and *in vivo* and *in vitro* studies show that adiponectin increases bone mass by suppressing osteoclastogenesis and activating osteoblastogenesis [Jurimae *et al.* 2005], likely indicating that a rise in adiponectin levels, caused by fat reduction, could have a beneficial effect on BMD.

Resistin is produced by macrophages and visceral adipocytes, it is elevated in obesity, regulates insulin sensitivity in skeletal muscle and liver and is positively associated with insulin resistance and glucose tolerance in both human and animal models [Ukkola, 2002]. Resistin might also play a role in bone remodeling since it is expressed in mesenchymal stem cells, osteoblasts, and osteoclasts in bone marrow, and appears to increase osteoblast proliferation, cytokine release, and osteoclast differentiation [Thommesen *et al.* 2006].

TNF α is a proinflammatory cytokine, which plays important regulatory effects on lipid metabolism, adipocyte function, insulin signaling, and bone remodeling [Fasshauer *et al.* 2003]. Its expression has been shown to correlate with percent body fat and insulin resistance in humans [Hotamisligil *et al.* 1995], and it was further recognized that inflammatory processes predispose people to bone

loss, giving rise to speculation that inflammatory cytokines, such as IL-6 and TNF α , may play critical roles in osteoclast activity [Pacifi *et al.* 1991]. Osteoclasts are the unique cells of the body tasked with resorbing bone and in the late 1990s the identification of three different molecules built the bases of the modern bone biology: an osteoclastogenic cytokine, the receptor activator of nuclear factor κ B ligand (RANKL), its receptor (RANK), and its inhibitor osteoprotegerin (OPG) [Simonet *et al.* 1997]. It is now clear that RANKL is the key osteoclastogenic cytokine effector, inducing osteoclast formation and promoting osteoclast resorptive activity, while OPG functions as a decoy receptor, preventing association of RANKL with RANK receptor, thus moderating osteoclastogenesis and bone resorption [Anderson *et al.* 1997].

It has also become clear that TNF α promotes RANKL production by BMSCs and mature osteoblasts, reduces OPG production, and upregulates the receptor RANK on osteoclast precursors, increasing their sensitivity to prevailing RANKL concentrations [Wei *et al.* 2005]. Additionally, TNF α turns out to have another property that is relatively unique among the inflammatory cytokines, it has potent effects on osteoclastogenesis as it not only promotes RANKL production but also synergizes with RANKL to amplify osteoclastogenesis, and to intensify osteoclastic resorption by directly modulating RANKL-induced signal transduction pathways [Cenci *et al.* 2000]. These effects are likely a consequence of the fact that RANKL is a TNF superfamily member and functions through many of the same pathways induced by TNF α itself.

IL-6 is a cytokine that has a wide range of actions: it is secreted by several cell types, including fibroblasts, endothelial cells, and adipocytes, and its plasma levels are significantly upregulated in human obesity and in individuals affected by insulin resistance [Vozarova *et al.* 2001]. Like TNF α , IL-6 is also a well recognized stimulator of osteoclastogenesis and bone resorption. Data show that IL-6 mRNA is expressed in preosteoblasts and osteoblasts [Dodds *et al.* 1994] and that it stimulates osteoblast proliferation and differentiation by controlling the production of local factors [Taguchi *et al.* 1998]. In addition, IL-6 may play a role in bone formation in conditions of high bone turnover [Sims *et al.* 2004].

Plasminogen activating inhibitor (PAI-1), produced by liver and adipose tissue, inhibits the

activity of tissue plasminogen activator favoring thrombus formation over ruptured atherosclerotic plaques. PAI-1 expression is elevated in visceral obesity, insulin resistance, and hypertriglyceridemia, and its levels appears to predict risk for future development of both type 2 diabetes and cardiovascular disease [Festa *et al.* 2002]. Some data suggest that the plasminogen activator/plasmin system is not required for osteoclast formation, or for the resorption of the mineral phase, but it is involved in the removal of noncollagenous proteins of the nonmineralized bone matrix [Mao *et al.* 2014]. In addition, in an animal PAI-1 knockout model, this molecule acts as important regulator of bone size during developmental growth and plays a modulatory role in the determination of fracture callus and resorption during fracture repair [Rundle *et al.* 2008].

Ghrelin is a 28-amino-acid acylated peptide mainly secreted by stomach and represents the principal endogenous ligand for growth hormone secretagogue receptor type 1a, whose expression is observed in the hypothalamo-pituitary region [Van der Lely, 2013]. Although ghrelin does not meet the definition of adipokine, it is involved in the regulation of glucose metabolism and lipogenesis both directly and through interactions with adipokines. Ghrelin is known to stimulate the differentiation of preadipocytes into adipocytes and antagonize lipolysis, and its levels are inversely correlated with body mass index and insulin resistance [Van der Lely, 2004]. Ghrelin exerts anti-inflammatory and cardioprotective effects through its inhibitory actions on TNF α , IL-1, and IL-6, and exerts a protective role on bone metabolism acting both directly and indirectly on bone cells function, inhibiting osteoclastogenic precursors and osteoclastogenic cytokines such as TNF α , IL-1 and IL-6, and modulating osteoblast differentiation and function, through regulation of the growth hormone–insulin-like growth factor axis [Soeki *et al.* 2007]. In addition, ghrelin interacts with leptin in modulating bone structure in an age-dependent manner, as recently shown [Van der Velde *et al.* 2013].

The interplay between bone tissue, fat, and energy balance

Emerging evidence points to a critical role for the skeleton in several homeostatic processes, including energy balance and adipose metabolism. In addition, the connection between fuel utilization and skeletal remodeling seems to begin in the

bone marrow with lineage allocation of mesenchymal stromal cells into adipocytes or osteoblasts.

Mature bone cells secrete factors that modulate insulin sensitivity and glucose metabolism, such as OCN, by which the skeleton could function as an endocrine organ itself [Lee *et al.* 2007]. OCN is an osteoblast-specific protein and a major non-collagenous protein in the extracellular matrix. Karsenty and colleagues recently demonstrated that uncarboxylated OCN, acting as a prohormone, can increase β -cell proliferation, insulin secretion, insulin sensitivity, and adiponectin expression [Ferron *et al.* 2008]. Thus, osteoblasts may be able to regulate glucose metabolism by modulating the bioactivity of OCN. In addition, more recent studies showed that OCN bioactivity is modulated by enhanced sympathetic tone driven by leptin, which has been shown to suppress insulin secretion by β cells [Hinoi *et al.* 2008], and three recent studies have demonstrated an inverse correlation between serum OCN and plasma glucose levels, supporting a role for this pathway in humans [Covey *et al.* 2006]. Thus, a novel picture has emerged linking glucose metabolism, adipose stores, and skeletal activity.

Since its first description more than 20 years ago, OPN has emerged as an active player in many physiological and pathological processes, including biomineralization, tissue remodeling, and inflammation. As an extracellular matrix protein and proinflammatory cytokine, OPN is thought to facilitate the recruitment of monocytes and macrophages, and to mediate cytokine secretion in leukocytes. Modulation of immune cell response by OPN has been associated with various inflammatory diseases and may play a pivotal role in the development of adipose tissue inflammation and insulin resistance [Scatena *et al.* 2007]. Several studies have described OPN as a critical regulator of adipose tissue inflammation, insulin resistance, and diabetes mellitus. OPN expression is drastically upregulated 40- and 80-fold in adipose tissue from diet-induced and genetically obese mice, respectively [Kiefer *et al.* 2008]. It has been demonstrated that OPN expression in adipose tissue as well as circulating OPN levels were substantially elevated in patients with obesity, diabetes, and insulin resistance compared with lean subjects, and conversely that dietary weight loss significantly decreased OPN concentrations [Sarac *et al.* 2011; You *et al.* 2013]. Moreover, it has been observed that OPN expression in human macrophages is upregulated by a variety of

proinflammatory mediators known to be elevated in type 2 diabetes and cardiovascular disease, including TNF- α , IL-6, and oxidized low-density lipoprotein [Zeyda *et al.* 2011]. Finally, more recently, it has been reported that there is simultaneous upregulation of IL-18 and OPN in peripheral blood mononuclear cells (PBMCs) from obese individuals compared with a lean group. Intriguingly, treatment with a neutralizing IL-18 antibody diminished OPN secretion from PBMCs, indicating that IL-18 regulates OPN expression [Ahmad *et al.* 2013]. These findings point towards a specific pathophysiological role of OPN in human inflammatory processes linked to obesity-induced adipose inflammation (Figure 1), insulin resistance, type 2 diabetes, and its complications.

Fat bone marrow and skeletal homeostasis

Adipocytes and osteoblasts originate from a common progenitor, a pluripotential mesenchymal stem cell [Akune *et al.* 2004], which has an equal propensity for differentiation into adipocytes or osteoblasts or other lines, such as chondrocytes, fibroblast, and endothelial cells, under the influence of several cell-derived transcription factors. This process is complex, suggesting significant plasticity and multifaceted mechanisms of regulation within different cell lineages, among which are adipocytes and osteoblasts [Gimble *et al.* 1996; Rodriguez *et al.* 2000].

Transdifferentiation is the irreversible switching of differentiated cells that sometimes occurs during disease [Burke and Tosh, 2005], and it interests partially differentiated cells (e.g. preosteoblasts) that switch to another lineage (e.g. adipocytes) [Schilling *et al.* 2008].

Fat bone marrow is indicative of aging and it is frequently observed in the presence of osteoporosis, especially in postmenopausal women [Menagh *et al.* 2010]. One possible cause of bone marrow fat deposition is the aberrant commitment of bone marrow derived stem cells (BMMSCs) into adipocytes because of their inability to differentiate into other cell lineages, such as osteoblasts. There exists an inverse relationship between bone marrow fat production and bone formation during osteoporosis; in fact, inhibited adipogenesis in patients with a high bone mass has been observed [Gao *et al.* 2014].

Recently, a correlation between the osteoadipogenic transdifferentiation of bone marrow cells

Figure 1. Interaction between adipose and skeletal tissue. Adipocytes are a source of bioactive molecules (adipokines) that act in either a paracrine or endocrine manner to modulate insulin sensitivity locally as well as in the liver and skeletal muscle. Adipose tissue is also a source of inflammatory mediators. Thus, adipose tissue promotes atherosclerosis through a number of pathologic mechanisms. IGF, insulin-like growth factor; IL, interleukin; OPN, osteopontin; TNF, tumor necrosis factor.

and numerous bone metabolism diseases has been established. Human BMMSC-derived osteoblasts, adipocytes, and chondrocytes had the potential to transdifferentiate to each lineage, and these findings provided new insights into the pathogenesis of skeletal diseases such as osteoporosis [Song and Tuan, 2004].

Estrogens can regulate several molecular signals within bone metabolism (Figure 1) and play an important role in the development of bone marrow fat [Abdallah *et al.* 2011; Kamiya *et al.* 2013; Song *et al.* 2013; Pierroz *et al.* 2009]. In fact, after menopause, an increase in adipogenic switch in bone marrow and a decrease in bone mass have been observed [Justesen *et al.* 2001; Gambacciani *et al.* 1997]. Several human and animal studies have examined the function of adipocytes in bone marrow. Mesenchymal stem cells isolated from bone marrow in postmenopausal women with osteoporosis express more adipose differentiation markers than those from subjects with normal bone mass [Sekiya *et al.* 2004]. In addition, pronounced fatty infiltration in the bone marrow of rats following oophorectomy has been observed, suggesting a pivotal role of estrogen in regulating adipocyte and osteoblast recruitment [Martin and Zissimos,

1991]. More recent studies have shown that estrogens are negative regulators of adipogenesis and essential for osteogenic commitment. In particular, it seems that estrogens simultaneously induce osteogenesis and inhibit adipogenesis both *in vivo* and *in vitro* [Guo *et al.* 2006; Mani *et al.* 2007; Grant *et al.* 2006], and it has been demonstrated that estrogens modulate osteoadipogenic transdifferentiation in a dose-dependent manner [Gao *et al.* 2014]. Specifically, Gao and colleagues have demonstrated that estrogens inhibit osteoadipogenic transdifferentiation of MC3T3-E1 cells, treated with 10^{-7} M of 17β estradiol and ICI, an estrogen receptor inhibitor. Alkaline Phosphatase (ALP) activity assay, alizarin red, and oil red staining showed 17β estradiol suppressed osteoadipogenic transdifferentiation. The authors observed that the mineralized matrix area of MC3T3-E1 cells cultured in the presence of 17β estradiol was larger than that of cells cultured in the presence of ICI, and that 17β estradiol also affected the morphology of fully differentiated adipocytes and decreased the number and size of lipid droplets. However, they observed that this effect could be neutralized when ICI was used to inhibit the effect of 17β estradiol, indicating that estrogen inhibits osteoadipogenic transdifferentiation [Gao *et al.* 2014]. In addition, the

authors observed that 17β estradiol increased and decreased the levels of osteogenic (ALP, Collagen-1, Runx-2, and OCN) and adipogenic (Dlk1, Gata2, and Wnt10b) markers, respectively, whereas there were no changes in cells simultaneously treated with 17β estradiol and ICI. Moreover Gao and colleagues demonstrated that estrogens regulate the osteoadipogenic transdifferentiation of primary BMMSCs, obtained using the ovariectomized C57BL/6 mouse model, in a dose-dependent manner. The authors have investigated the ability of BMMSC-derived osteoblasts to transdifferentiate under osteoporotic conditions, treating the cultured cells with increasing doses of hormone. They found that the transdifferentiation potential of BMMSC-derived osteoblasts from the ovariectomized model group was higher than that of control cells. In addition, they observed that the expression levels of osteogenic markers in BMMSC-derived osteoblasts from the ovariectomized model group were lower than those in cells from the control group, while the expression levels of adipogenic markers were increased [Gao *et al.* 2014]. Finally, Gao and colleagues demonstrated that estrogens suppress osteoadipogenic transdifferentiation *via* canonical Wnt signaling, an important system which regulates bone development, adipogenic differentiation, and gene expression in the whole process of bone metabolism [Gao *et al.* 2014].

Specifically, canonical Wnt/ β -catenin signaling is highly expressed in mesenchymal precursor cells and pluripotent cells, especially toward the osteoblast lineage, while it inhibits adipogenic differentiation [Krishnan *et al.* 2006]. In fact, canonical Wnt signaling stabilizes and promotes cellular and nuclear β -catenin levels, which inhibit adipogenesis [Krishnan *et al.* 2006], and the suppression of Wnt signaling is indispensable for peroxisome proliferator activated receptor γ (PPAR γ) induction and preadipocyte differentiation [Qiu *et al.* 2011].

The importance of canonical Wnt signaling in bone development is well known. Furthermore, noncanonical Wnt members may also be involved in regulating osteogenesis, and a reversal process, from noncanonical Wnt signaling to canonical Wnt signaling or vice versa, drives the progression into the differentiation stage. Indeed, during early adipogenesis, a prompt activation–inactivation of the Wnt pathway is crucial for the induction of PPAR γ , which regulates differentiation and insulin sensitivity of mature adipocytes [Colaianne *et al.* 2014].

PPAR γ plays a central role in initiating adipogenesis and mutations of the PPAR γ gene are associated with an altered balance between bone and fat formation in the bone marrow [Gimble *et al.* 1996]. The nuclear hormone receptor family of transcriptional regulatory proteins is activated by a range of ligands, including steroid hormones, naturally occurring metabolites, synthetic chemicals, and as yet unidentified endogenous compounds (orphan receptors). However, *in vitro* analyses demonstrate that various PPAR γ ligands not only induce murine bone marrow stromal cell adipogenesis but also inhibit osteogenesis [Lecka-Czernik *et al.* 2002]. In particular, PPAR γ -2 is the dominant regulator of adipogenesis, and ligand activation of PPAR γ -2 favors differentiation of mesenchymal stem cells into adipocytes rather than into osteoblasts [Aubin, 1998]. Akune and colleagues showed that PPAR γ insufficiency led to increased osteoblastogenesis *in vitro* and higher trabecular bone volume *in vivo*, confirming the key role of mesenchymal stem cell lineage allocation in the skeleton [Akune *et al.* 2004]. Interestingly, aged mice exhibit fat infiltration into bone marrow and enhanced expression of PPAR γ -2, along with reduced mRNA expression of bone differentiation factors [Moerman *et al.* 2004]. In addition, mice with premature aging (the SAM-P/6 model) show nearly identical patterns of adipocyte infiltration, with impaired osteoblastogenesis [Kajkenova *et al.* 1997], indicating that aging, or events that accelerate aging, results in significant bone marrow adiposity and a defect in osteoblastogenesis in mice [Duque *et al.* 2004].

It has also been observed that after exposure to transforming growth factor β , human bone marrow mesenchymal stem cells increase their expression of various Wnt signaling receptors and ligands [Duque *et al.* 2004], and members of the epidermal growth factor family, such as protein Pref-1, influence both adipogenesis and osteogenesis. In fact, *in vitro* analysis of human bone marrow mesenchymal stem cells has shown that Pref-1 overexpression blocks both adipogenesis and osteogenesis, suggesting the hypothesis that Pref-1 maintains mesenchymal stem cells in a multipotent state [Abdallah *et al.* 2004].

Recent studies have found that 1,25(OH) $_2$ vitamin D treatment inhibits adipogenesis and enhances osteogenesis in SAM-P/6 mice, with a 50% reduction in PPAR γ mRNA and protein levels [Duque *et al.* 2004]. In addition, gene microarray analyses demonstrated coordinated induction

of osteoblastogenic genes and a reduction of adipogenic genes after $1,25(\text{OH})_2$ vitamin D treatment, which stimulates not only bone formation but also bone resorption, according to circulating biomarkers of bone turnover [Duque *et al.* 2005].

Finally, other factors, such as total caloric intake, type of nutrients, alcohol consumption, oxygen tension, and cellular oxidation–reduction pathways influence bone marrow adipogenesis despite osteoblastogenesis [Gimble *et al.* 2006], showing that the bone marrow mesenchymal stem cell may consider multiple differentiation pathways during its lifetime and, indeed, may dedifferentiate and transdifferentiate in response to changes in the microenvironment.

Conclusion

Obesity and osteoporosis are two major global health problems worldwide, with obesity probably interfering with bone health. In fact, the belief that obesity is protective against osteoporosis has recently come into question on the basis of the latest epidemiologic and clinical studies, which have shown that a high level of fat mass might be a risk factor for osteoporosis and fragility fractures.

Body fat and bone interplay through several adipokines and bone-derived molecules, which modulate bone remodeling and adipogenesis, body weight control, and glucose homeostasis. Thus, the existence of a cross talk between fat and the skeleton suggests a homeostatic feedback system in which adipokines and bone-derived molecules form part of an active bone–adipose axis.

The bone–adipose axis, due to its peculiarity in the common origin of osteoblasts and adipocytes, which are derived from pluripotent mesenchymal stem cells, has an equal propensity for differentiation into adipocytes or osteoblasts, or other lines under the influence of several cell-derived transcription factors. In particular, when specific conditions occur, such as aging, menopause or diseases such as osteoporosis, obesity, or metabolic alterations, causing a status of low-grade inflammation, an osteoadipogenic transdifferentiation has been observed, an aberrant commitment of BMMSCs into adipocytes because of their inability to differentiate into other cell lineages, such as osteoblasts.

Many factors, such as estrogens, cytokines, growth and transcription factors, vitamin D, total

caloric intake, type of nutrients, alcohol consumption, oxygen tension, and cellular oxidation–reduction pathways could influence bone marrow adipogenesis despite osteoblastogenesis and modulate fat–bone interplay. However, the mechanisms by which all these events occur remain unclear, and this molecular control could be crucial to understanding the pathogenesis of both obesity and osteoporosis. Finally, since there are a large number of clinical studies that show a positive and protective effect of fat tissue on bone health, to better understand the relationship between the adipose and bone tissues, we need further clinical and molecular studies in the future to fully characterize the complex and fascinating interplay between fat and bone tissue.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work received grant from MIUR (PRIN 2011 052013 to SM).

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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