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Skeletal integration of energy homeostasis: Translational implications[☆]

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Abstract

New evidence has recently emerged defining a close relationship between fat and bone metabolism. Adipose tissue is one of the largest organs in the body but its functions vary by location and origin. Adipocytes can act in an autocrine manner to regulate energy balance by sequestering triglycerides and then, depending on demand, releasing fatty acids through lipolysis for energy utilization, and in some cases through uncoupling protein 1 for generating heat. Adipose tissue can also act in an endocrine or paracrine manner by releasing adipokines that modulate the function of other organs. Bone is one of those target tissues, although recent evidence has emerged that the skeleton reciprocates by releasing its own factors that modulate adipose tissue and beta cells in the pancreas. Therefore, it is not surprising that these energy-modulating tissues are controlled by a central regulatory mechanism, primarily the sympathetic nervous system. Disruption in this complex regulatory circuit and its downstream tissues is manifested in a wide range of metabolic disorders, for which the most prevalent is type 2 diabetes mellitus. The aim of this review is to summarize our knowledge of common determinants in the bone and adipose function and the translational implications of recent work in this emerging field.

Keywords

PPARG; Brown fat; Osteocalcin; Adipocytes; Beige fat; Sympathetic tone

1. Introduction

The markedly increased fracture rate in patients with Type 1 (T1D) and Type 2 (T2D) diabetes mellitus, despite in most cases, normal bone mineral density, has led to substantial efforts to explain the pathophysiologic basis for the development of this unique but

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important diabetic complication [1]. On the other hand, this manifestation also has provided investigators with evidence of a close link between bone and energy metabolism. Diabetes is a complex disease defined by intracellular glucose starvation of muscle and fat cells. Besides impairment in cellular mechanisms for glucose utilization due to either insulin resistance (T2D) or insulin deficiency (T1D), this disease is accompanied by profound systemic changes that affect every organ system. These changes include, but are not restricted to hyperinsulinemia, hyperlipidemia, hyperglycemia, changes in hypothalamic determinants of energy metabolism, and enhanced reactive oxygen species and advanced glycation end products (AGEs) in many tissues.

Adipose tissue dysfunction is a major pathophysiologic component of T2D, but is also impacted by a cascade of diabetic pathologies including those that are bone-associated. In this review, we analyze bone and adipose relationships at the functional level with a premise that better understanding of these relationships will provide the basis for the development of therapies that improve the function of both organs and ultimately support maintenance of normal glucose levels and utilization.

2. Distinct adipocytes and their depots

The last two decades of research provided substantial evidence that adipose tissue consists of distinct morphological characteristics defined by local, systemic, and environmental signals. The major building blocks of adipose tissue are adipocytes, highly specialized cells that control fuel management through a continuous process of lipogenesis, lipid storage, and lipolysis. Adipose tissue also consists of other cell types, with macrophages playing an important role in managing the inflammatory environment that impacts adipocyte development, function, and insulin sensitivity. A unique feature of the adipose organ is that its distribution is directly associated with its function [2]. Thus, subcutaneous fat provides insulation for internal organs and is more insulin sensitive, while adipose tissue surrounding the heart has thermogenic characteristics, and visceral fat is more pro-inflammatory and insulin resistant. Adipose tissue is also present in the bone marrow and our understanding of its function is just emerging. In that context, we juxtaposed bone and fat because the regulation of mesenchymal stem cell (MSC) differentiation toward adipocytes vs. osteoblasts was considered as mutually exclusive, and because of the clinical finding of an inverse correlation between bone mass and marrow fat content. However, there is emerging evidence that bone marrow adipose tissue (MAT) is characterized by a high degree of plasticity. The regulation of MAT is still being explored but in some circumstances, marrow adiposity is influenced by the same modulators (e.g. sympathetic tone) as extramedullary adipose tissue, while in other circumstances, MAT is independent of peripheral adipose tissue regulators (e.g. anorexia nervosa) [3,4].

There are two types of adipocytes categorized by their morphology and metabolic function, white and brown. Most recently, a third type has emerged, 'beige' adipocytes, cells that can be recruited from white progenitors in response to cold or β -adrenergic stimuli [5]. White adipose tissue (WAT) consists of adipocytes that expand in response to nutrient availability by incorporating fatty acids to store as triglycerides for later use. Classically, visceral adipocytes, particularly during expansion, have been associated with the elaboration of

inflammatory cytokines such as TNF α , IL-1, IL-6, and resistin, as well as several adipokines [6–10]. These peptides may have variable effects on the skeleton depending on time, site, and relative concentration. Although somewhat controversial, generation of new genetic models supports the precept that during high fat feeding, most of the increase in adipose volume is due to hypertrophy (i.e. expansion of existing adipocytes) rather than proliferation, particularly in the visceral compartment [11]. Once cellular expansion of the fat cell exceeds healthy boundaries (e.g. in obesity), adipocyte death, fibrosis and inflammation can occur, and storage of triglycerides begins in other tissues, particularly in the liver, muscle, and bone marrow; this ultimately leads to the development of insulin resistance.

Brown adipocytes are present in interscapular adipose tissue and regulate body temperature and glucose metabolism. These cells are innervated by the sympathetic nervous system (SNS) and are the major regulators of non-shivering thermogenesis in virtually all neonates [12]. Until recently, preformed brown adipose tissue (BAT) was thought to be present only in neonates; however with the advent of positron emission tomography using (18)F-fluorodeoxyglucose ((18)FDG-PET), metabolically active BAT has been detected in adults as discrete loci located in the neck and supraclavicular region [13,14]. Brown adipocytes are derived from a Myf-5-positive muscle-like cellular lineage and have a specific metabolic program [15]. They contain very high numbers of mitochondria as well as uncoupling protein 1 (UCP1) and Pgc1 α (ppar gamma co-activator 1 alpha) that are necessary for fatty acid oxidation and uncoupled heat generation [16,17]. That metabolic machinery also makes brown adipose tissue a target for insulin-mediated glucose uptake. Recent work suggests that the volume of BAT in adults may be positively related to BMD [18–20]. This may in part be due to endocrine secretory factors or confounding from the strong positive relationship of brown fat to muscle mass, likely from the shared transcriptional determinant, Myf5 [15]. Impairment in BAT function or ablation of beige fat in mice leads to development of insulin resistance and low bone mass [21–23]. In humans, the activity of BAT is attenuated in diabetes and during aging, both conditions associated with increased fractures [24].

The existence of beige adipocytes is an emerging concept; their contribution to regulation of energy metabolism in mammals is debatable. In rodents, beige adipocytes are present predominantly in the inguinal fat depots [25]. In humans, they are identified in the classical BAT depots but they may also develop as discrete loci in subcutaneous fat in response to distinct hormonal and environmental stimuli [5]. These include chronic cold exposure, adrenergic signaling, and pharmacologic and nutritional factors which increase mitochondrial number and enhance expression of brown-adipose specific proteins to uncouple respiration [26]. It remains controversial as to whether white adipocytes can trans-differentiate into beige cells or whether their progenitor is distinct [25]. Nevertheless, beige cells are controlled by specific transcription factors (Prdm16, Tbx15, FoxC2, others) and perhaps SNS tone, yet they can be distinguished from brown and white adipocytes by expression of unique sets of gene biomarkers [25,27,28]. The involvement of the SNS in beige fat development and bone remodeling is complex. In states in which SNS tone is high (e.g. cold exposure or FGF-21 excess), beige cells are found in the subcutaneous or inguinal depot [29]. On the other hand, bone mass is significantly reduced during high SNS tone,

principally due to activation of the $\beta 2$ receptor on osteoblasts. Activation of this receptor suppresses the key transcription factor, ATF4, and enhances RANKL production [30–35].

Besides their role in fatty acid oxidation and increased respiration, beige adipocytes secrete factors that may have anabolic effects on bone. Mice with adipocyte-specific expression of the FoxC2 transcription factor, which increases mitochondrial biogenesis and promotes “browning” of white adipocytes, have high bone mass [36]. A secretome of beige adipocytes isolated from an epididymal fat depot or from the bone marrow of these mice includes Wnt10b and IGFBP2 proteins, which increase osteoblast differentiation and function [36].

Although identified in humans, the volume of beige fat and its thermogenic function are still unclear. Moreover, it is not known whether the recruitment of human beige adipocytes by environmental or hormonal factors can enhance their thermogenic capacity. Recent efforts toward characterization of beige adipocytes led to the identification of specific biomarkers, which will undoubtedly constitute a very promising step toward determining their contribution to energy homeostasis and provide a basis for the development of new therapies to treat metabolic diseases and perhaps osteoporosis [37].

Marrow adipose tissue (MAT) represents a unique fat depot. It is an obvious candidate for a regulatory effect on the bone due to its proximity and juxtaposition to skeletal surfaces. Indeed, MAT likely modulates hematopoietic and skeletal turnover in several different ways [38,39]. In adult humans, MAT may constitute up to 1 kg of tissue, and its volume may increase in conditions of impaired energy metabolism including obesity, diabetes, aging, lipodystrophy and anorexia nervosa (reviewed in [36]). However, unlike in WAT, this increase is due to de novo differentiation rather than an increase in volume of existing adipocytes [40]. In some circumstances (e.g. aging and diabetes), particularly in humans, increased total adipocyte volume per marrow volume correlates with a decrease in bone mass/quality and increase in fractures (reviewed in [41]). But because the origin and function of marrow adipocytes are not known, our understanding of bone–fat interactions, particularly in the niche is lacking. It has been hypothesized that some marrow adipocytes develop from progenitors delivered during the process of vascularization of developing bone [42]. However, and unlike other marrow components, either mesenchymal or hematopoietic, these progenitors appear to be dormant during early bone growth. In humans, adipocytes start to populate marrow in the long bone after the first decade of life, whereas in mice marrow adipocytes start to appear at skeletal maturation between 1–3 months [43,44]. It is unclear which skeletal or systemic compartments signal to adipocytic progenitors to induce their differentiation. Also it should be noted that marrow adipocytes are not surrounded by fibrous or inflammatory components per se, unlike adipose depots in other sites. This may influence their secretory properties in vivo.

It is also unclear whether marrow adipocytes are directly related to osteoblasts. Several models suggest that both adipocytes and osteoblasts originate from a common Myf5-negative progenitor and determination of their fate is under control of retinoblastoma protein (pRB) [45,46]. However, in contrast to the periphery, a significant fraction of marrow adipocytes may express the osteoblast-specific lineage marker osterix [47]. This observation suggests that some adipocytes are in a closer developmental association with osteoblasts

than with peripheral adipocytes. Whether bone marrow adipocytes arise from bone-lining cells also remains a matter of debate since these cells also express osterix but appear to be more fibroblastic than pre-osteoblastic. Interestingly, it has been recently suggested that adipocytes do not share the same musculoskeletal ancestor as osteoblasts, chondrocytes, and muscle cells by demonstrating that Gremlin 1 positive mesenchymal progenitors can differentiate into the above lineages but not adipocytes [48].

It is conceivable that MAT does not constitute a homogeneous population of adipocytes, but rather a mixture of lipid-accumulating cells, of different origins and of different functions and in distinct locations within the marrow. MAT has both WAT and BAT-like characteristics with respect to the expression of gene markers [49]. Whether that translates into functional activity remains to be determined since various depots can have a mix of metabolic expression signatures relative to brown or white adipocytes. In mice, BAT-like or beige fat features of MAT are compromised with diabetes and aging, suggesting a positive correlation between the beige metabolic profile of MAT and bone health [49]. An analysis of BAT gene markers in the bone marrow of either old (24 months) mice or yellow agouti A^{vy}/a mice, a murine model of human Type 2 diabetes, showed remarkable reduction in the basal expression of *Prdm16*, *FoxC2*, $\beta 3$ -ADR and *Dio2* transcripts despite the fact that the volume of MAT in old and in diabetic animals was almost 2-fold higher than in controls [49]. However, it remains to be established whether MAT's decreased expression of beige markers affects bone remodeling.

MAT may also impact the hematopoietic niche, which in turn is regulated by endosteal osteoblasts [50,51]. Some have proposed that MAT serves as a clearing site for circulating lipids (e.g. obesity, diabetes, anorexia nervosa), while other groups support its role as an endocrine organ [39], or a paracrine tissue providing energy for emergency situations requiring new bone formation [36]. Interestingly, MAT volume decreases substantially during lactation when skeletal calcium stores are mobilized and there is increased bone resorption [29]. Lineage tracing studies from several groups have now begun to address these various hypotheses, and it is anticipated that further work will uncover both the origin and function of marrow adipocytes. This is especially relevant for the question of whether increased marrow adipose tissue, present in patients with diabetes, affects osteoblast function and particularly skeletal fragility.

3. Molecular and metabolic determinants of adipogenesis and osteogenesis

3.1. PPAR γ — a master regulator of insulin sensitivity and adipogenesis

Differentiation toward WAT, BAT, MAT, or beige-type adipocytes is controlled by the transcription factor, PPAR γ . For its activation, PPAR γ requires heterodimeric formation with an RXR nuclear receptor and binding of a specific ligand, of which there are several both natural and artificial. Natural PPAR γ ligands consist of polyunsaturated fatty acids (PUFA) and their oxidized derivatives, certain phospholipids and oxidized forms of prostaglandin J2. Our understanding of PPAR γ 's biological function was expanded in the late 1990s with the discovery of artificial high-affinity agonists, thiazolidinediones (TZDs), which are potent insulin sensitizers. Two of them, rosiglitazone and pioglitazone, have been used successfully for the last decade in clinical settings to control hyperglycemia in diabetic

patients. TZDs are unique and their effect is most robust in combination with other anti-diabetic therapies, as they comprise PPAR γ -mediated activity to sensitize adipocytes to insulin. Despite the beneficial anti-diabetic effect of TZDs, this class of drugs possesses off target side effects, including weight gain, edema, cardiovascular events and fractures [52].

Upon ligand binding, PPAR γ undergoes post-translational modifications (PTMs) specifying its activities. These PTMs determine assembly of specific co-activators on the PPAR γ /RXR dimer. The functionally important PTMs include 1) de-phosphorylation of Ser273 by inhibiting Cdk5 activity and the de-sumoylation mediated by FGF21, which induce insulin sensitizing activity [53,54]; 2) de-phosphorylation of Ser112 by PP5 phosphatase, which induces adipogenic activity [55]; and 3) lysine de-acetylation by SirT1 which induces PPAR γ transcriptional switch to activate BAT-instead of WAT-specific gene expression [56].

The oft-cited ‘mutually exclusive’ hypothesis that MSCs can either go into one lineage or the other is questionable when considering the actions of the TZDs. Rosiglitazone forces MSCs toward the adipogenic lineage and blocks osteogenesis, in part by increasing β -catenin degradation, leading to enhanced marrow adiposity and bone loss [57–59]. This has led to the tenet that there must be exclusive commitment toward one cell type over another. However, other PPAR γ agonists that have pro-adipogenic effects, also have a neutral or even positive effect on bone forming cells, raising provocative questions about the role of PPAR γ in osteogenesis [60]. Notwithstanding, rosiglitazone also activates Pgc1 β , an important co-factor for energy utilization by the osteoclast and ultimately this pathway leads to bone resorption from cell autonomous actions on the differentiation of monocytic cells [61].

PPAR γ is one of two major transcription factors, (the other being β -catenin) that occupy a central role in determining the fate of bone marrow MSCs [62]. Both these proteins are modified not only by post-transcriptional events, but also by binding to other transcription factors such as FoxO1, as well as through the non-cell autonomous actions of reactive oxygen species (ROS) [63–65]. FoxO1 exerts multiple actions by regulating insulin sensitivity [66] and, by binding to Runx2 and ATF4, modulating oxidative stress. It also reduces osteocalcin activity through the upregulation of the Esp1 phosphatase, thereby altering glucose homeostasis and promoting glucose intolerance [65]. FoxO1 is also important for the early differentiation of adipocytes, although down-regulation occurs during later stages, particularly after the incorporation of free fatty acids [68,69]. Importantly, FoxO1 has been shown to regulate dopamine beta hydroxylase activity in neurons of the locus coeruleus and sympathetic ganglion, supporting its central role in modulating energy metabolism [70].

Rosiglitazone treatment favors activation of PPAR γ at the expense of β -catenin, and this is accompanied by up-regulation of FoxO1 [59]. However, PPAR γ is also expressed in early osteoblasts and may play some role in defining the ultimate fate of later stage progenitors [71]. In that same vein, conditional deletion of PPAR γ in osteoblasts results in high bone mass and greater bone formation via activation of mTOR, supporting the tenet proposed by

Krebsbach that there are likely to be both cell autonomous and cell non-autonomous actions of PPAR γ on bone cells [71].

3.2. Metabolic determinants: adipokines, serotonin, glucocorticoids and the skeleton

Two PPAR γ -regulated adipokines, leptin and adiponectin, have non-cell autonomous actions on adipose tissue and bone. Leptin is an adipokine secreted by adipocytes in response to storage of triglyceride and cell expansion. It circulates in relatively high concentrations, crosses the blood brain barrier, and has a multitude of effects on the brain, principally targeting the hypothalamus. As fat cells expand and release leptin, circulating concentrations increase, (although transport across the blood brain barrier may slow with higher circulating levels) to suppress orexigenic factors, alter hypothalamic reproductive factors, and enhance sympathetic tone [32]. Once sympathetic activity is increased, there is activation of the β 2 receptor on osteoblasts. This results in suppressed bone formation and enhanced bone resorption. On the other hand, there is some evidence, albeit preliminary, that peripheral leptin has an effect on osteoblasts, although it is not clear whether it acts directly through the leptin receptor or indirectly by regulating the myeloid lineage and the bone marrow milieu [72–74]. Interestingly however, in MAT, leptin expression is very low, highlighting the distinct phenotype of marrow adipocytes and the modest direct effect on osteoblasts and osteoclasts of locally generated leptin [75]. T2D is often associated with hyperlipidemia and development of leptin resistance. Although it is not known how this might affect bone it can be speculated that high leptin levels in type 2 diabetes will increase SNS tone and β -adrenergic signaling leading to bone loss.

Adiponectin, in contrast, has both direct and indirect effects on the bone, first by directly suppressing bone formation, and later by blocking SNS outflow from the CNS [76]. Hence, the global deletion of adiponectin results in a context specific skeletal phenotype; i.e. at a young age the animals have high bone mass due to the direct loss of adiponectin suppression on osteoblasts; at an older age (36 weeks) *adipo*^{−/−} mice have low vertebral trabecular bone volume due to the loss of antagonism of the SNS. This ‘yin and yang’ model proposed and validated by the Karsenty laboratory provides strong evidence that metabolic status, integrated in the brain but originating from the adipocyte, has a major impact on the state of the skeleton [76]. In contrast to leptin, adiponectin is relatively highly expressed in MAT and marrow adiponectin may contribute to the overall pool in circulation especially in states of WAT wasting and MAT expansion such as calorie restriction or anorexia nervosa [39]. On the other hand, in states of WAT and MAT expansion associated with development of insulin resistance such as diabetes and aging, circulating levels of adiponectin decrease suggesting that MAT is subjected to the same changes as WAT in respect to decrease in endocrine function [77].

Serotonin is a neurotransmitter and gut secretory peptide that can affect the bone and fat in two distinct but opposing ways. Brain-derived serotonin, catalyzed by *Tph2*, binds to the serotonin receptor (5-HTR) in the ventral medial hypothalamus (VMH), inactivates tone from the SNS, and enhances skeletal acquisition. Leptin suppresses *Tph2* expression in the brain stem, resulting in decreased serotonin in the brain, and bone loss due to suppressed serotonin signaling in the VMH. Gut-derived serotonin, catalyzed by *Tph1*, is taken up by

platelets through the serotonin transporter (5-HTT) and has been shown to suppress skeletal accrual. Osteoblasts and osteoclasts express 5-HT receptors and 5-HTT, and thus the serotonin network is likely to be operative in the skeletal environment [78]. Moreover it has been demonstrated that Lrp5 in the gut can inhibit serotonin synthesis which in turn has been shown in several studies to block osteoblast proliferation [79].

Serum serotonin levels are elevated in diabetes probably due to hyperreactivity of platelets, which are storage cells for peripheral serotonin [80]. In contrast, prolonged use of selective serotonin reuptake inhibitors (SSRIs) is often associated with increased body weight and obesity [81]. It has been shown recently that peripheral serotonin plays an important role in the regulation of energy homeostasis by acting directly on adipose tissue [82]. In mice, pharmacologic inhibition of serotonin synthesis or its production in adipocytes inhibits lipogenesis in WAT, induces browning in inguinal WAT, and increases thermogenic activity in BAT. In humans, pharmacologic inhibition of serotonin receptor by the drug sarpgrelate increases insulin sensitivity and adiponectin production [83]. Serotonin is implicated in the degradation of insulin substrate 1 (IRS-1) in adipocytes, a factor essential for insulin signaling [84]. Thus, serotonin may stimulate lipogenesis in WAT implicating its contribution to WAT expansion. Hence, theoretically, serotonin levels in diabetics may increase MAT expansion and suppress insulin signaling in both adipocytes and osteoblasts, the latter resulting in decreased bone turnover [82,85].

Glucocorticoids (GCs) are the factors that contribute to the development of diabetes, while having profound effects on fat and bone metabolism [86]. Excessive pharmacologic exposure to GCs or elevated levels of endogenous cortisol (due to either hyperactivity of hypothalamic–pituitary–adrenal axes or activity of 11 β -hydroxysteroid dehydrogenase-1 at the tissue level) is recognized as a risk for development of T2D and osteoporosis [87,88]. GCs predispose to development of central obesity and metabolic syndrome. GCs regulate adrenergic response by regulating the expression of all three forms of the β -adrenergic receptors. GCs inhibit the transcriptional response of the UCP1 gene to adrenergic stimulation in brown adipocytes by inhibiting the expression of β 1 and β 3 adrenergic receptors and suppress brown or beige adipocyte activity in the periphery, and perhaps in the bone marrow. In epididymal WAT, increased levels of endogenous cortisol correlate with down-regulation of β 1 and β 3 adrenergic receptor expression and upregulation of β 2 adrenergic receptor expression [89]. Taken together, glucocorticoids have a dual effect on adrenergic signaling. They decrease adrenergic receptor-mediated metabolic activity of brown fat and increase catabolic activity in the bone. Thus, the activation of glucocorticoid signaling in marrow adipocytes may lead to a decrease in β 3 adrenergic receptor expression and loss of brown-like potential of marrow adipocytes, which is seen with aging and diabetes. Alternatively, GCs' effects on energy metabolism may be mediated through the skeleton. As demonstrated in rodents, GCs suppress directly osteoblast function and osteocalcin synthesis and, through the skeleton, contribute to development of insulin resistance in fat and enhance marrow adipogenesis [88,35].

3.3. Skeletal determinants of metabolic homeostasis

Osteocalcin was the first bone specific protein to be identified as a modulator of peripheral adipose sensitivity and insulin secretion. Osteocalcin is produced by osteoblasts and circulates both in its native form, and after release from the skeletal matrix during bone resorption. Beyond its recognition as a marker of differentiated osteoblasts, its functional significance for the bone remodeling unit has been difficult to clarify. Under-carboxylated osteocalcin (unOC) that is generated by resorption of the matrix and osteoblastic synthesis has been shown to enhance glucose uptake in peripheral fat depots and increase insulin secretion in mice. In addition, insulin promotes the release of unOC by stimulating resorption; thus there is a fast-forward system whereby release of osteocalcin from bone promotes glucose sensitivity. Loss of *esp1*, a phosphatase, leads to enhanced insulin sensitivity and low glucose, in part by preventing the de-phosphorylation of the insulin receptor [90]. In contrast, deletion of the insulin receptor in osteoblasts leads to an insulin resistance state due to lower levels of unOC and increased free fatty acids resulting in insulin receptor ubiquitination [85]. More recent work confirms the feedback circuit between bone and the beta cell of the pancreas. Abdallah et al. reported that unOC stimulates DLK1 production in the beta cells of the pancreas, which in turn circulates and inhibits further osteoblast production of unOC [91].

Despite the vast support for this critical metabolic regulatory loop in mice, studies in humans are conflicting with some showing a positive correlation between unOC and glucose control, and others showing no effect. Other matrix bound proteins may also influence whole body homeostasis. IGF-I is another peptide synthesized and stored in the bone, although the vast majority of circulating IGF-I is derived from the liver. IGF in the bone is bound to a series of IGF binding proteins and can be released during bone resorption. IGF-I can regulate glucose and fatty acid synthesis through the type I IGFR as well as hybrid insulin/IGF receptors. Recent evidence in humans shows a strong correlation between low levels of IGF-I and high circulating fatty acids as well as reduced GLUT1 expression in skeletal muscles [92]. It is also likely that there are other bone proteins that influence glucose homeostasis.

3.4. Bioenergetic determinants of adipogenesis and osteogenesis

Recent attention has focused on mechanisms related to mesenchymal cell fate, and in particular, the metabolic program of MSC progenitors. There is now preliminary evidence that adipocytes and osteoblasts have distinct energy utilization pathways as they move down their respective lineages during differentiation. During these stages, ATP demand drives the type of substrate utilization that is operative, and thus is very context specific. Early progenitors and pluripotent stem cells utilize glucose as their primary fuel, even in aerobic states, in a manner analogous to cancer cells (i.e. the Warburg effect) [93]. But pre-adipocytes and newly differentiated adipocytes primarily use mitochondrial respiration to supply energy for their metabolic needs [94,95]. The process of glucose entry and fatty acid oxidation through the Krebs Cycle generates more molecules of ATP per mole of glucose (36:1) than glycolysis (2:1) but it comes at a cost, as mitochondrial respiration leads to the generation of reactive oxygen species (ROS) from the electron transport chain (ETC.). ROS compounds (e.g. H_2O_2 , superoxides) can further suppress mitochondrial respiration and

promote an adipogenic program that is associated with more insulin resistance and less lipolysis [94,95]. Excess ROS in adipocytes may also cause mitochondrial DNA damage or further changes to complex I in the ETC, leading to metabolic dysfunction.

In contrast, pre-osteoblasts utilize a distinct metabolic program that features oxidative phosphorylation and glycolysis, although the latter predominates especially during differentiation. Pre-osteoblasts differentiate under the influence of various ligands, particularly the Wnts, TGF-beta, and IGFs. Long and colleagues first demonstrated that glycolysis is a major feature of Wnt3a-induced osteoblast differentiation [96]. But remarkably, much older *ex vivo* studies from Neumann and colleagues demonstrated that PTH produced lactic acid in calvarial osteoblasts supporting the tenet that osteoblasts utilize glycolysis to generate lactate during collagen synthesis and mineralization [97]. Guntur et al. also showed that glycolysis is essential for terminal differentiation of osteoblasts and that oxidative phosphorylation is more important early in the differentiation scheme [98]. The Long group reported that HIF1 α is a critical transcriptional regulator of glycolysis, triggered in part, by relative hypoxia in the bone marrow niche [99]. More recently, Karsenty and colleagues demonstrated the importance of the Glut1 transporter in Runx2-mediated early osteoblast differentiation [100]. In that same vein, Karner et al. demonstrated that glutaminolysis also is essential for osteoblast differentiation through the Wnt signaling system [101].

In sum, there are at least three substrates for ATP generation and differentiation of MSCs: Glutamine — which then enters the Krebs Cycle through alpha ketoglutarate, glucose — which via glycolysis can generate lactate as well as ribose nucleotides via the pentose phosphate shunt, and fatty acids — which are metabolized via acetyl CoA in the mitochondria. Osteoblasts have the machinery to use all three substrates but their relative utilization likely depends on their availability. Notwithstanding, the bioenergetics of the niche must be defined in order to fully understand lineage allocation and the role transcription factors play in defining the ultimate fate of MSCs in the marrow [102]. ATP demand is at the forefront of what biochemical program is activated, and that in turn is determined by the differentiative function of that cell. It is remarkable, however, that a picture is emerging of a metabolic program in osteoblasts that resembles tumor cell bioenergetics. The relative hypoxic environment, the additive effects of glutaminolysis with glycolysis on ATP generation and the evolutionary importance of substrate availability that is intimately tied to glycolysis in single cell organisms, provide us with potential clues about progenitor cells and their ultimate fate either to store energy (i.e. adipocytes) or to use sufficient energy to build and mineralize matrix (i.e. osteoblasts). What is still not known is how osteoblasts handle a high glucose load *in vivo*, and what bioenergetic pathways are utilized particularly in states of chronic hyperglycemia.

4. Translational implications

The bone and fat arise from progenitors but have distinct metabolic programs. Their physiologic interactions are critical for overall metabolic homeostasis. Hence any alterations starting at the molecular level and proceeding all the way to the brain (e.g. hypothalamus and SNS), can result in metabolic syndromes, and in particular T2D. Our knowledge of

adipocyte biology has grown immensely and somewhat in parallel with our understanding of the osteoblast as an energy-demanding cell. Mesenchymal cells have a common origin and share common physiologic integrators (i.e. SNS, cytokines, adipokines), but are distinct in many ways. Importantly we still know little about the intricate cell-cell communication that must take place, whether in the marrow niche, in the circulation or in the bone, between adipocytes and osteoblasts. Furthermore, to better understand the skeletal defects in T2D, we must define fuel preferences by differentiating osteoblasts, the role of the insulin receptor in mediating substrate utilization in humans and the importance of endocrine (e.g. adiponectin, leptin) and paracrine mediators during chronic states of hyperglycemia. Newer therapies for T2D aimed at enhancing glucose utilization, such as agents that can brown white adipocytes or enhance the function of preformed brown adipose tissue, may have off target effects on the skeleton requiring careful phenotyping in both humans and animal models. Notwithstanding, in this era of personalized medicine, the development of new drugs to treat chronic conditions will demand an even greater understanding of the molecular and cellular underpinnings of the disease itself.

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Highlights

- This paper reviews the fundamental control over the bone-adipose connection, with a particular focus on the sympathetic nervous system, the regulation of brown and beige fat, and the release of bone specific proteins that regulate adipose tissue insulin sensitivity.