PPARs in Bone: The Role in Bone Cell Differentiation and Regulation of Energy Metabolism

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Abstract Obesity, diabetes, and osteoporosis are major public health concerns. Current estimates indicate that the US population consists of 25% obese, 30% diabetic and prediabetic, and, among the elderly, 50% of all osteoporotic individuals. Mechanistically, these pathologies share several features including common regulators of bone homeostasis and energy metabolism. Peroxisome proliferator-activated receptors (PPARs) represent a family of proteins that control energy turnover in adipose, liver, and muscle tissue. These proteins also control bone turnover and regulate bone cell differentiation. Recent evidence suggests that bone is an organ integral to energy metabolism not only with respect to energy storage, but also as an organ regulating systemic energy homeostasis. In this article, we review current knowledge on the role of PPARs in bone metabolism and bone cell differentiation. We also discuss the role of bone fat in modulation of bone marrow microenvironment and its possible contribution to the systemic regulation of energy metabolism.

Keywords Bone metabolism · Bone cell differentiation · PPARs · Energy metabolism · Mesenchymal stem cells · Thiazolidinediones

Introduction

Recent advances in understanding of the role of bone in the systemic regulation of metabolism indicate that bone is an integral part of energy metabolism. It has become evident that bone cells of the mesenchymal lineage, osteoblasts and adipocytes, determine bone metabolic function. Osteoblasts produce osteocalcin, a bone-specific hormone that has a potential to regulate both insulin production in the pancreas and adiponectin production in fat tissue [1, 2]. Marrow adipocytes, which exhibit many features of extramedullary fat, store significant quantities of fat and produce adipokines (cytokines produced by adipose tissue), which play a role in the regulation of energy metabolism. Both osteoblasts and marrow adipocytes originate from marrow mesenchymal stem cells (MSCs) and their differentiation is under the control of systemic, as well as, intrinsic factors, including peroxisome proliferator-activated receptor (PPAR) transcription factors [3, 4].

PPARs are transcription factors that belong to the superfamiliy of nuclear receptors. Other members of this family include retinoic acid, estrogen, thyroid, vitamin D, and glucocorticoid receptors, and several other proteins involved in xenobiotic metabolism. PPARs act on DNA response elements as heterodimers with the retinoid X receptor. Their natural activating ligands are lipid-derived substrates. The family of PPARs is represented by three members: PPAR-α, PPAR-δ, and PPAR-γ. They play an essential role in energy metabolism; however, they differ in the spectrum of their activity—PPAR-γ regulates energy storage, whereas PPAR-α and PPAR-δ regulate energy expenditure [5].

The PPARs possess the canonical domain structure common to other nuclear receptor family members, including the amino-terminal AF-1 transactivation domain, followed by a DNA-binding domain, and a dimerization and ligand-binding domain with a ligand-dependent transactivation function AF-2 located at the carboxy-terminal region [6]. Upon ligand binding and formation of the transcriptional complex, which includes retinoic receptor
heterodimerization partner and assembly of coactivator proteins, PPARs facilitate transcription by binding to cis-acting peroxisome proliferator response element in the gene regulatory region. The PPARs’ transcriptional specificity is determined by a ligand-specific interaction, which introduces allosteric alterations in the AF-2 domain and recruitment of coactivators in the PPAR type- and cell type-specific manner [6, 7]. We review the metabolic activities of all three PPARs and the status of current knowledge regarding their role in bone cell differentiation and the maintenance of bone homeostasis.

**PPAR-γ**

PPAR-γ is an essential regulator of lipid, glucose, and insulin metabolism [7]. PPAR-γ represents a key transcription factor for adipocyte differentiation and for the maintenance of the adipogenic phenotype. This receptor is expressed in mice and humans as two different isoforms, PPAR-γ1 and PPAR-γ2, due to alternative promoter usage and alternative splicing [8]. In humans, PPAR-γ2 differs from PPAR-γ1 by the presence of 28 amino acids (30 amino acids in rodents) located on the AF-1 domain. PPAR-γ1 is expressed in a variety of cell types, including adipocytes, muscle, macrophages, osteoblasts, and osteoclasts, whereas PPAR-γ2 expression is restricted to adipocytes, including marrow adipocytes, and is essential for differentiation and maintenance of their phenotype and function [9, 10]. The transcriptional activity of PPAR-γ is controlled by binding of lipophilic ligands to the ligand binding pocket. The natural ligands are derived from nutrients or products of metabolic pathways and consist of polyunsaturated fatty acid derivatives and eicosanoids [7]. Synthetic ligands include a class of antidiabetic drugs, thiazolidinediones (TZDs), which bind to PPAR-γ with high affinity, activate its adipogenic activity, and act as insulin sensitizers [11].

PPAR-γ is essential for the regulation of energy storage. The dietary abundance in fatty acids increases the activity of PPAR-γ and leads to the activation of lipogenesis—a program designed for energy storage in the form of accumulated lipids in fat tissue. A scarcity of nutrients results in a decrease in PPAR-γ activity and allows for lipolysis, a process that mobilizes energy stored in a fat tissue. Continuous overnutrition leads to excessive upregulation of PPAR-γ activity, increased lipid storage, and the development of obesity. In addition, PPAR-γ plays an essential role in the regulation of insulin signaling. Either deficiency or upregulation of PPAR-γ leads to the development of insulin resistance and subsequently diabetes [12].

Perhaps not surprisingly, PPAR-γ also plays an important role in the maintenance of bone mass. The PPAR-γ2 isoform regulates lineage commitment of MSCs toward osteoblasts and adipocytes [4•]. As shown in a number of in vitro models of MSC differentiation, as well as in primary bone marrow cells, the activation of the PPAR-γ2 isoform with natural (fatty acids and eicosanoids) or artificial (TZD) ligands directs MSC differentiation toward the adipocyte lineage at the expense of osteoblast formation [10]. Moreover, the activation of PPAR-γ2 in cells of the osteoblast lineage converts them to terminally differentiated adipocytes and irreversibly suppresses their phenotype, including suppression of phenotype-specific genes [10, 13]. Thus, PPAR-γ2 acts as a positive regulator of adipocyte differentiation and a dominant-negative regulator of osteoblast differentiation. In contrast, the PPAR-γ1 isoform has been shown to promote both osteoclast differentiation from a pool of hematopoietic stem cells and bone resorption, by controlling the expression of c-fos protein, an important determinant of osteoclast lineage commitment and development [14].

Because both cellular components of bone remodeling are under the control of PPAR-γ, the status of its activity is important for maintaining the balance between bone resorption and bone formation. Several animal models and human studies have shown that changes in PPAR-γ activity, which are determined by the level of protein expression and the level of its activation, lead to unbalanced bone remodeling [14, 15, 16•, 17].

During the natural process of aging, bone mass declines and fat mass in bone increases [18]. This correlates with changes in the phenotype of MSCs (eg, the increased expression of proadipocytic PPAR-γ2 and the decreased expression of osteoblast-specific transcription factors, Runx2 and Dlx5) [19]. Consequently, MSC differentiation potential shifts toward adipogenesis and away from osteoblastogenesis [19]. In addition, the availability of oxidized derivatives of fatty acids, which are potent activators of PPAR-γ, increases with aging [13, 20]. It was also demonstrated that bone marrow derived from old animals produces unknown PPAR-γ activator(s), which stimulate adipocyte differentiation and suppress osteoblast differentiation [19, 21].

The analysis of the gene expression profile in U-33/γ2 cells, a murine model of MSC differentiation under the control of PPAR-γ2, showed that the presence of PPAR-γ2, without activation by exogenous ligand, changes the expression of a large number of genes involved in the maintenance of MSC phenotype [22•]. Thus, with aging, marrow-borne MSCs lose their “stemness” and are more prone to adipocytic differentiation due to the increased expression of PPAR-γ2 and the increased availability of PPAR-γ activators. As a result, osteoblast number and bone formation decrease while fat accumulation in bone increases. In humans, the association between bone loss
and increased marrow adiposity is visible not only during aging, but also during conditions of skeletal disuse, such as microgravity or paraplegia [23, 24]. In animals, skeletal unloading results in bone loss, which is associated with increased expression of PPAR-γ and an increase in the marrow fat compartment [25, 26].

An essential role of PPAR-γ in the maintenance of bone homeostasis was demonstrated in several animal models of bone accrual or bone loss depending on the status of PPAR-γ activity [15, 16•, 17, 27–29]. In models of bone accrual, a decrease in PPAR-γ activity in heterozygous PPAR-γ–deficient mice or mice carrying a hypomorphic mutation in the PPAR-γ gene locus led to increased bone mass due to increased numbers of osteoblasts [17, 27]. Interestingly, mice deficient in PPAR-γ expression in cells of the hematopoietic lineage develop high bone mass and are more resistant to the TZD-induced bone loss than control mice [14]. In rodent models of bone loss due to PPAR-γ activation, an administration of the TZD rosiglitazone resulted in significant decreases in bone mineral density (BMD), bone volume, and changes in bone microarchitecture [15, 16•, 28]. The observed bone loss was associated with the expected changes in the structure and function of bone marrow, which included decreased number of osteoblasts, increased number of adipocytes, and increased production of receptor activator for nuclear factor-κB ligand (RANKL), the cytokine physiologically responsible for osteoclastogenesis.

The TZD-induced structure/function changes in the bone marrow led to alterations in the ratio between the number of osteoblasts and osteoclasts, suggesting an imbalance between bone resorption and bone formation. The degree of bone loss in response to rosiglitazone treatment correlated with age and the level of PPAR-γ expression in bone. In younger animals with lower levels of PPAR-γ, bone loss was less extensive than in older animals [16•]. Moreover, age determines the mechanism by which bone loss occurs. In younger animals, rosiglitazone administration decreased bone formation, whereas in older animals it increased bone resorption [16•]. Interestingly, in the absence of estrogen, rosiglitazone enhanced bone loss, mainly due to increased bone resorption pointing to the functional cross-talk between PPAR-γ and estrogen receptor [29]. Such cross-talk has been recently described in osteoblasts as a form of competition between PPAR-γ and the estrogen receptor for a common coactivator, SRC-2 [30]. It would be interesting to examine whether a similar mechanism is responsible for the TZD-induced increase in bone resorption in a condition of estrogen deficiency and during aging.

An analysis of gene expression in MSCs following rosiglitazone treatment showed reduced expression of genes essential for activity of signaling pathways controlling bone homeostasis and MSC commitment to the osteoblast lineage, among them Wnt, transforming growth factor-β/bone morphogenetic protein, and insulin-like growth factor-1 [31•]. The effect of TZDs on the expression of genes essential for osteoblast development was strikingly similar to changes observed during aging. Due to the similarities with age-related bone loss, some speculate that TZDs may accelerate the aging of bone [16•, 19].

TZDs increase insulin sensitivity via activation of PPAR-γ. Two TZDs, rosiglitazone and pioglitazone, have been used clinically since 1999. Despite TZDs’ beneficial antihyperglycemic profile, their use is associated with adverse effects on human bone [32]. Recently published clinical evidence suggests that TZD therapy is associated with a decrease in BMD and an increase in fracture risk. The first evidence of increased fracture risk in TZD users came from ADOPT (A Diabetes Outcome Progression Trial), which compared the antihyperglycemic effects of three different antidiabetic therapies with rosiglitazone, metformin, or glyburide, for a median of 4.0 years in randomly assigned individuals (1,840 women and 2,511 men) [33•]. Surprisingly, the effects were gender-specific, and the fracture rates in men did not differ between treatment groups and did not demonstrate a significant difference in overall risk. The cumulative incidence of fractures in women was 15.1% (11.2–19.1) with rosiglitazone, 7.3% (4.4–10.1) with metformin, and 7.7% (3.7–11.7) with glyburide, representing hazard ratios of 1.81 and 2.13 for rosiglitazone compared with metformin and glyburide, respectively. Fractures were seen predominantly in the lower and upper limbs; however, vertebral fractures were not assessed. There was no correlation between rosiglitazone use and estrogen status because both premenopausal and postmenopausal women demonstrated increase in fractures [33•]. Similarly, meta-analyses of data from 10 randomized controlled trials showed that long-term TZD use doubles the risk of fractures exclusively in women but not in men with type 2 diabetes mellitus [34•]. Finally, the observational studies based on the United Kingdom General Practice Research Database, which included a large population of older individuals, concluded that TZD therapy and its duration are associated with significant increase in nonvertebral fractures independently of patient age and sex. The adjusted odds ratio of fracture occurrence for hip/femur was 4.54, for humerus was 2.12, and for wrist/forearm was 2.90 [35].

**PPAR-α**

PPAR-α is expressed predominantly in the liver, and, to a lesser extent, in muscle, in the heart, and in bone. In the liver, it plays a crucial role in fatty acid oxidation, which provides energy for peripheral tissues [36]. Mice deficient
in PPAR-α are unable to meet energy demands during fasting and suffer from hypoglycemia, hyperlipidemia, and hypoketonemia. Conversely, PPAR-α activation with fibrates, specific agonists, reduces adiposity, improves hepatic and muscle steatosis, and improves insulin sensitivity [36]. PPAR-α regulates plasma lipid levels through the control of fatty acid oxidation process. Fibrates are wildly used in clinics to lower plasma triglyceride levels in patients with hypertriglyceridemia [37].

PPAR-α is also expressed in bone, in cells of the hematopoietic and mesenchymal lineages [38]. PPAR-α–null animals do not exhibit an apparent bone phenotype [39]. Bone mass is not altered and bone cortical area is not different from control animals. In addition, the differentiation potential of the bone marrow toward osteoblasts and adipocytes measured in ex vivo primary bone marrow culture is not altered in PPAR-α–deficient mice [39]. However, PPAR-α deficiency has an effect on bone morphology in male animals, which results in a significantly larger medullary space [39]. Although PPAR-α does not regulate marrow mesenchymal cell differentiation, it does regulate bone marrow myeloid cell commitment toward the B cell lineage [40], implicating a role in the bone marrow environment. In vitro studies demonstrated that bezafibrates, which are PPAR-α and PPAR-δ agonists, stimulate rodent osteoblast differentiation [41] and inhibit human osteoclast development [42]. Moreover, in rats, bezafibrates stimulated periosteal bone formation [41] and increased femoral BMD while decreasing medullary area [43]. Although these studies suggest anabolic effect of bezafibrates on bone, therapeutic use of fibrates does not appear to have an effect on the quality of human bone because there is no change in the rate of fractures in patients on fibrate therapy [44].

Thus, the available studies suggest that although PPAR-α does not play a critical role in differentiation of bone cells, it may be important for bone metabolism by providing energy through fatty acid oxidation and for the regulation of the bone marrow milieu by regulating cell commitment within hematopoietic lineages. Based on the evidence that PPAR-α deficiency does not lead to changes in the energy metabolism under normal conditions, it is possible that the role of PPAR-α in bone is manifested in challenging conditions such as high-fat diet or caloric restriction. Both of these conditions lead to bone loss; however, how these environmental challenges affect the status of PPAR activity in bone is not known.

**PPAR-δ**

PPAR-δ, also called PPAR-β, is ubiquitously expressed in many tissues including bone [38]. For years, the role of this nuclear receptor was obscure because of a lack of identified selective agonists. Recently, animal models of genetically altered PPAR-δ activity and availability of selective PPAR-δ agonists revealed a powerful role of this nuclear receptor in fat-burning processes [45]. Transgenic expression of PPAR-δ in adipose tissue produces mice that are resistant to high-fat diet-induced obesity, hyperlipidemia, and tissue steatosis. In contrast, mice deficient in PPAR-δ show reduced energy uncoupling and are prone to obesity [46]. In skeletal muscle, PPAR-δ upregulates fatty acid oxidation and energy expenditure to a far greater extent than PPAR-α [45]. Taken together, PPAR-δ appears to perform a unique function in the regulation of energy metabolism; it exceeds the fatty acid oxidative activity of PPAR-α and, as a fat burner, it opposes the fat-storing function of PPAR-γ.

The role of PPAR-δ in the regulation of bone homeostasis remains largely unknown. Although the bone status in mice overexpressing or knocked down PPAR-δ was not studied, one in vitro study suggests that PPAR-δ may regulate bone resorption. The PPAR-δ selective agonist L165041 dose dependently inhibited osteoclast differentiation in human peripheral blood mononuclear cell cultures, but stimulated resorption, the activity of mature osteoclast, as measured in an in vitro assay on dentine slices [42]. In light of recent developments regarding the significance of PPAR-δ for the regulation of energy metabolism and the potential for the development of antiobesity therapies based on selective activation of this nuclear receptor, more studies are needed to assess the role of this regulator in fatty acid metabolism in bone.

**Bone Fat and Its Role in Local and Systemic Energy Metabolism**

All three PPARs are involved in adipocyte biology, either by regulating adipocyte development and/or regulating energy metabolism. The marrow adipocyte phenotype is similar to that of adipocytes present in white and brown fat, but the unique location of these cells in bone presumably directs their more specialized functions [47]. For years, marrow fat was merely considered a cellular component of bone that served a passive role by occupying space no longer needed for hematopoiesis. However, recent developments suggesting that marrow fat plays an essential role as an endocrine organ involved in energy metabolism, places marrow fat under a new research spotlight [47].

A relatively well-characterized role of marrow adipocytes is in the support of hematopoiesis by producing the necessary cytokines and heat for hematopoietic cell development [48]. In addition, marrow fat may participate in lipid metabolism by clearing and storing circulating...
triglycerides thereby providing a localized energy reservoir for emergency situations affecting, for example, osteogenesis (eg, bone fracture healing) [47]. Marrow adipocytes produce a number of adipokines, but two of them (leptin and adiponectin), whose expression is under PPAR-γ control and which regulate caloric intake and insulin sensitivity, are the focus of increased attention as possible mediators of marrow fat endocrine function. Based on the fact that cells of osteoblastic lineage express receptors for leptin and adiponectin and the evidence that these adipokines may modulate osteoblast differentiation and function [49, 50], it is reasonable to believe that bone fat has a local endocrine function and that it modulates the marrow environment supporting bone remodeling. Moreover, based on the quantity of bone fat, which by the third decade of human life occupies almost the entire cavity of long bones, one can speculate that adipokines produced in bone may enter the circulation and contribute to the systemic energy metabolism. Thus, it is reasonable to propose that marrow fat serves a number of endocrine functions not only of local, but also systemic, significance. With advancing age, fat cells infiltrate bone marrow cavities. It has even been suggested that osteoporosis is an obesity-like disease of bone [18]. Interestingly, with aging and other metabolic diseases, a profile of cytokine expression changes in both, in extramedullary fat depots and in bone [51, 52]. Thus, it is possible that with aging, marrow fat undergoes changes similar to visceral fat leading to the development of insulin resistance and changes in insulin-dependent glucose and fatty acid metabolism.

An analysis of the marrow fat response to TZDs presents a paradigm that may unravel additional function(s) of marrow fat. Microarray analysis of the PPAR-γ transcriptome in U-33/γ2 cells showed that rosiglitazone significantly increased the expression of genes involved in carbohydrate and fat metabolism, and inhibited the proinflammatory phenotype [31*]. Furthermore, the response of marrow fat to TZDs is strikingly similar and distinctive from that of extramedullary fat. There is a significant upregulation of genes essential for fatty acid metabolism, including fatty acid synthase, fatty acid-binding proteins, hormone-sensitive lipase, uncoupling protein 2, and cholesterol transporter CD36. Interestingly, although a large number of genes involved in carbohydrate metabolism are upregulated, there is no change in the expression of any of the important insulin-dependent glucose transporters, including GLUT4. Most importantly, in marrow adipocytes, rosiglitazone induces the expression of genes involved in insulin signaling, among them the insulin receptor, insulin receptor substrate-1 and FoxO1, while suppressing the expression of negative regulators of this signaling network such as Socs3 [31*]. This profile suggests that upon rosiglitazone activation, marrow fat is sensitized to insulin and has increased fatty acid metabolism.

Thus, marrow fat may acquire different physiologic functions: 1) it may serve as an energy and heat provider; 2) it may provide energy storage by accumulating an excess of fat; 3) it may play a protective role and serve as a “sink” for circulating triglycerides in pathologic conditions; and 4) it may serve as an endocrine tissue sensitive to insulin. Therefore, it is important to unravel the different functions of marrow fat and to develop the means to manipulate this tissue compartment for improving the outcome of therapies for energy metabolism disorders.

Conclusions

Osteoporosis, obesity, and diabetes are the most common pathologies occurring in highly industrialized countries (US enters for Diseases Control and Prevention; http://www.cdc.gov). Because PPARs are positioned at the crossroads of the control of bone mass and energy expenditure, the therapeutic manipulation of their activities may affect the skeleton, in both a positive and a negative fashion. Conversely, pharmacologic harnessing of the metabolic properties of marrow fat is an attractive possibility for increasing our armamentarium to fight metabolic disease.

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