IBMS BoneKEy. 2010 May;7(5):171-181
http://www.bonekey-ibms.org/cgi/content/full/ibmske;7/5/171
doi: 10.1138/20100443

PERSPECTIVES

PPARγ, an Essential Regulator of Bone Mass: Metabolic and Molecular Cues

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Abstract

Nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) is a transcription factor essential for adipocyte differentiation, insulin signaling, and processes of energy turnover in adipose, liver and muscle tissue. PPARγ also controls bone turnover and regulates bone cell differentiation of both mesenchymal and hematopoietic lineages. 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. Since PPARγ is positioned at the crossroads of the control of bone mass and energy metabolism, therapeutic manipulation of its activity may affect bone. Indeed, anti-diabetic thiazolidinedione (TZD) therapy, which targets PPARγ activity, is associated with both decreased bone mass and increased fracture risk. However, the anti-osteoblastic activity of PPARγ can be separated from its metabolic activity by using selective PPARγ agonists, which raises the possibility of the development of therapies beneficial for diabetes and safe for bone. This *Perspective* reviews current evidence for the role of PPARγ in bone metabolism and bone cell differentiation. It also discusses the role of bone fat in the modulation of the bone marrow microenvironment and a possible contribution of this fat compartment to the systemic regulation of energy metabolism. *IBMS BoneKEy*. 2010 May;7(5):171-181.
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Introduction

Nuclear receptor peroxisome proliferator-activated receptor gamma (PPARγ) is an essential regulator of lipid, glucose, and insulin metabolism (1). PPARγ is a key transcription factor for adipocyte differentiation and for the maintenance of the adipogenic phenotype. This nuclear receptor is also a target for a class of anti-diabetic drugs, thiazolidinediones (TZDs), which regulate adipose tissue capabilities to store fat and produce endocrine factors sensitizing peripheral tissues to insulin (1). Recent advances in understanding the role of bone in the systemic regulation of energy metabolism indicate that bone marrow cells, osteoblasts and adipocytes are involved in this process. Marrow adipocytes, the development and function of which are under PPARγ control, store significant quantities of fat and produce adipokines, leptin and adiponectin, involved in energy metabolism (2;3), whereas osteoblasts produce osteocalcin, a bone-specific hormone that has potential to regulate insulin production in the pancreas and adiponectin production in fat tissue (4). Perhaps not surprisingly, PPARγ plays an important role in the maintenance of bone mass. PPARγ regulates the lineage commitment of both marrow mesenchymal stem cells (MSCs) towards adipocytes and away from osteoblasts (5), and of hematopoietic stem cells (HSCs) towards osteoclasts (6). As a result, PPARγ controls both components of the bone remodeling process, bone formation and bone resorption.

The PPARγ Nuclear Receptor and Its Metabolic Activity

PPARγ in adipocytes regulates energy storage and insulin signaling. The dietary abundance of fatty acids increases the

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activity of PPARγ 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 PPARγ activity and allows for lipolysis, a process that mobilizes energy stored in fat tissue. Continuous over-nutrition leads to excessive upregulation of PPARγ activity, increased lipid storage, the development of obesity and insulin resistance (7).

The PPARγ protein belongs to the superfamily of nuclear receptors, which also includes the retinoic acid, estrogen, thyroid, vitamin D, and glucocorticoid receptors, and several other proteins involved in the metabolism of xenobiotics (1). PPARγ possesses a 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. Upon ligand binding and formation of the transcriptional complex, which includes a retinoic receptor heterodimerization partner and the assembly of co-activator proteins, PPARγ facilitates transcription by binding to *cis*-acting PPRE elements in a gene regulatory region. Transcriptional activity of PPARγ 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 ecosanoids. Synthetic ligands include anti-diabetic TZDs, of which rosiglitazone and pioglitazone have been used clinically since 1999. PPARγ transcriptional specificity is determined by a ligand-specific interaction, which introduces allosteric alterations in the AF-2 domain, and recruitment of co-activators in a ligand- and cell type-specific manner (1).

The PPARγ nuclear receptor is expressed in mice and humans as two different isoforms, PPARγ1 and PPARγ2, due to alternative promoter usage and alternative splicing (1). In humans, PPARγ2 differs from PPARγ1 by the presence of 28 amino acids (30 amino acids in rodents) located in the AF-1 domain. PPARγ1 is expressed in a variety of cell types, including adipocytes, muscle, macrophages, osteoblasts and osteoclasts, whereas PPARγ2 expression is restricted to adipocytes, including marrow adipocytes, and is essential for the differentiation and maintenance of their phenotype and function (8;9).

PPARγ and Lipid Metabolism in Bone

The phenotype of marrow adipocytes 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. For years, marrow fat was considered merely a cellular component of bone that served a passive role by occupying space no longer needed for hematopoiesis. However, recent developments demonstrating that fat plays an essential role as an endocrine organ involved in energy metabolism place marrow fat under a new research spotlight (2;3).

A relatively well-characterized role of marrow adipocytes is in the support of hematopoiesis by producing the necessary cytokines and energy in the form of heat for hematopoietic cell development (10). 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 (*e.g.*, bone fracture healing) (2). Marrow adipocytes produce leptin and adiponectin, the expression of which are under PPARγ control and which are endocrine regulators of caloric intake and insulin sensitivity. Interestingly, cells of the osteoblastic lineage express receptors for both leptin and adiponectin and it was demonstrated that these adipokines may modulate osteoblast differentiation and function (11;12). Thus, it is reasonable to believe that bone fat has a local endocrine function, which modulates the marrow environment supporting bone remodeling.

By the third decade of human life, fat occupies almost the entire cavity of long bones. Similarly, TZD administration causes significant increase in bone fat, although its

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metabolic profile may differ from fat that accumulates with aging (13). Nevertheless, based on significant quantities of fat in bone, it is likely that adipokines produced in bone may enter the circulation and contribute to the regulation of systemic energy metabolism. Hence, it is reasonable to believe that the endocrine function of marrow fat has not only local, but also systemic significance.

PPARγ Controls Bone Stem Cell Differentiation

As shown in a number of *in vitro* models of MSC differentiation, as well as in primary bone marrow cells, the activation of the PPARγ2 isoform with either natural (fatty acids and ecosanoids) or artificial (TZD) ligands directs MSC differentiation toward the adipocyte lineage at the expense of osteoblast formation (5;14). Moreover, activation of PPARγ2 in cells of the osteoblast lineage converts them to terminally differentiated adipocytes and irreversibly suppresses their phenotype, including suppression of osteoblast-specific signaling pathways such as the Wnt, TGFβ/BMP and IGF-1 pathways and transcriptional regulators such as Dlx5, Runx2 and Osterix (9;15). Thus, PPARγ2 acts as a positive regulator of adipocyte differentiation and a dominant-negative regulator of osteoblast differentiation. Additionally, in mesenchymal cells PPARγ2 upregulates the expression of RANKL, a cytokine supporting osteoclastogenesis (13). Moreover, the PPARγ1 isoform has been shown to promote osteoclast differentiation from a pool of HSCs and bone resorption. In osteoclasts, PPARγ1 controls the expression of c-fos protein, an important determinant of osteoclast lineage commitment and development, and RANK signaling activity (6). Thus, in addition to the suppressive effect on osteoblast differentiation, PPARγ positively regulates osteoclastogenesis. The complexity of PPARγ effects on bone cell differentiation and bone remodeling is summarized in Fig. 1.

In vitro studies, using a cellular model of PPARγ2-controlled MSC differentiation, showed that besides controlling MSC fate, PPARγ2 also regulates the expression of a number of genes associated with the stem cell phenotype and formation of a micro-environment supporting hematopoiesis (16). In general, PPARγ2 negatively regulates the expression of so-called "stemness" genes, which are involved in the recruitment and maintenance of the stem cell phenotype, and include *ABCG2*, *Egfr*, *CD44*, *Kitl*, *SDF-1*, *LIF* and *LIFR* (16). Interestingly, depending upon its activation status, PPARγ2 has a differential effect on the expression of genes involved in the support of hematopoiesis. Thus, the sole presence of PPARγ2, without activation with exogenous ligand, increased expression of hematopoiesis-supporting genes, as compared to cells that did not express PPARγ2. However, activation of PPARγ2 with rosiglitazone significantly decreased the expression of these genes and the support for hematopoiesis (16). On that note, prolonged TZD therapy in humans is associated with the development of anemia (17). Based on the above *in vitro* findings, one would suggest that activation of PPARγ in the bone marrow contributes to the decrease in blood cell number in diabetic patients on TZD therapy.

During the natural process of aging, bone mass declines and fat mass in bone increases (reviewed in (18)). This correlates with changes in the levels of phenotype-specific gene expression in MSCs; for example, levels of PPARγ2 increase, while levels of pro-osteoblastic Runx2 and Dlx5 decrease (19). The systemic and cellular levels of natural PPARγ activators, such as oxidized fatty acids, increase with aging as well (19-21). Thus, as a result of increased PPARγ expression with aging, marrow-borne MSCs lose their "stemness" and are more prone to adipocytic and less prone to osteoblastic differentiation.

An 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. In humans and animals, skeletal unloading results in bone loss, which is associated with increased expression of PPARγ and an increase in the marrow fat

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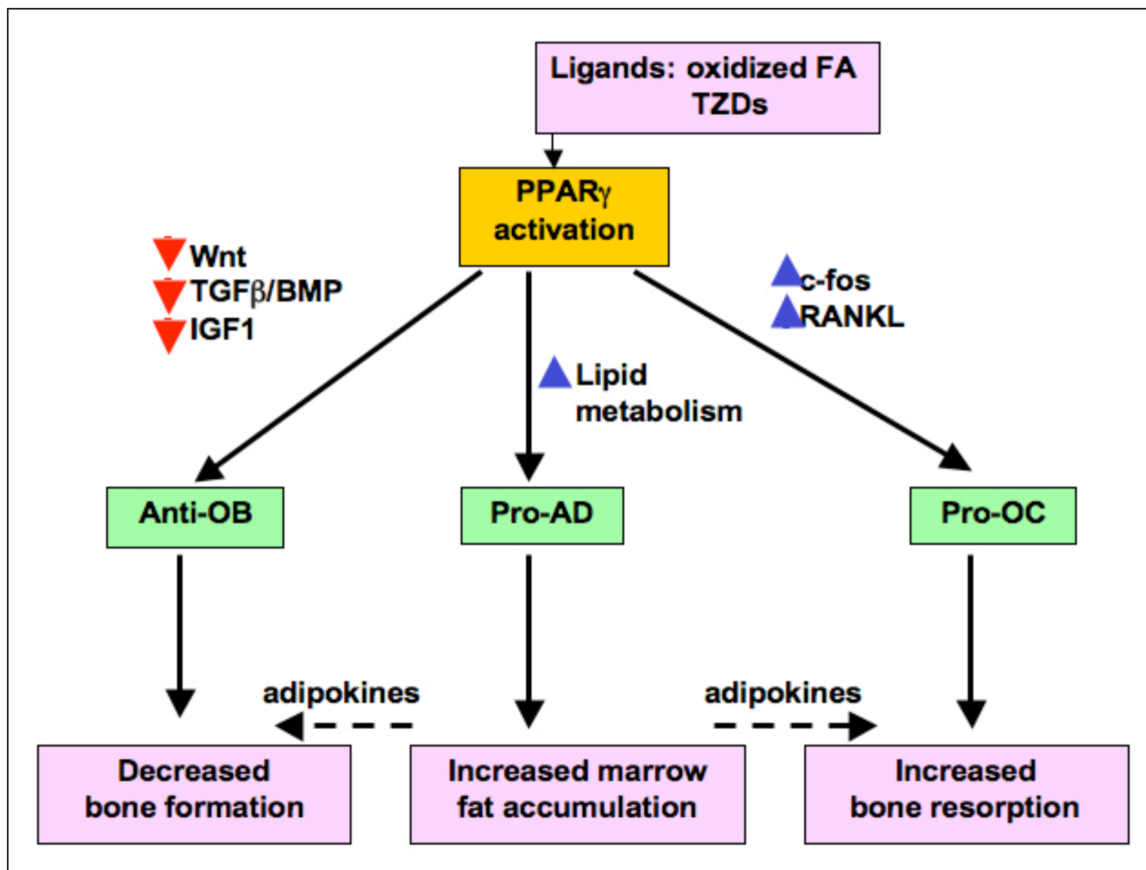

Fig. 1. The complexity of PPARγ effects on bone. PPARγ activities: Anti-OB – anti-osteoblastic; Pro-AD – pro-adipocytic; Pro-OC – pro-osteoclastic.

compartment (22;23). Conversely, mechanical signals of high frequency and low intensity have anabolic effects on bone and decrease marrow adiposity and PPARγ expression (24).

Selective Regulation of PPARγ Pro-adipocytic and Anti-osteoblastic Activity

The PPARγ ligand binding domain contains a large binding pocket capable of encompassing a variety of ligands. This provides a wide array of potential contact points that can result in various PPARγ conformations and differential recruitment of co-activators, which determine specificity of this nuclear receptor (1). Although anti-diabetic TZDs have a very beneficial profile in regard to insulin sensitization and lowering blood glucose levels, their adverse effects on weight gain, fluid retention, and bone fractures require development of selective PPARγ modulators (SPPARMs), which will have high potency to treat diabetic disease with minimal adverse effects.

In MSCs, the anti-osteoblastic and pro-adipocytic PPARγ2 activities can be separated by using ligands of different chemical structures, which suggests that PPARγ2 effects on the osteoblast and adipocyte phenotypes are mediated by distinct regulatory pathways (25). Indeed, gene expression profiling in response to rosiglitazone showed that PPARγ2 pro-adipocytic activity is transcriptional in nature and involves binding to PPRE elements in gene regulatory regions, whereas its anti-osteoblastic activity is PPRE-independent and involves changes in the activity of osteoblast-specific signaling pathways, such as the Wnt and TGFβ/BMP pathways, followed by suppression of osteoblast-specific transcriptional activators (15).

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The Role of PPARγ Coregulators in the Control of Bone Mass

It is well-appreciated that the activity and specificity of nuclear receptors are determined by coregulator proteins, which may either activate or repress their activity. It is also evident that proteins from the nuclear receptor family may share the same coregulators, which may lead to a form of competition for the same protein by two different nuclear receptors. An example of such competition and its effect on bone was recently reported between PPARγ and estrogen receptor (ER) for Src-2 coactivator (26). In animals with normal estrogen levels, Src-2 deficiency led to decreases in both ER and PPARγ activity, whereas in estrogen-deficient animals with normal levels of Src-2, PPARγ activity was enhanced due to increased availability of Src-2 coactivator for this transcription factor (26).

Recently, a member of the circadian clock system, the protein nocturnin, was identified as a new accessory protein for PPARγ. Nocturnin binds to PPARγ in the cytoplasm and facilitates its entry into the nucleus, which enhances PPARγ transcriptional activity. Mice deficient in nocturnin have higher bone mass and lower numbers of adipocytes in the marrow. Moreover, nocturnin overexpression in cells of the osteoblast lineage converts them to adipocytes and suppresses the osteoblast phenotype (27).

PPARγ Polymorphism and Skeletal Status

That PPARγ regulates skeletal homeostasis has been suggested by studies of single nucleotide polymorphisms (SNPs) in the PPARγ gene sequence. These studies showed that some relatively common SNPs, which are associated with the development of metabolic diseases and insulin resistance, are also associated with bone mass. In a population of Japanese women, a silent C161→T transition in exon 6, which is common for both PPARγ isoforms, results in lower bone mineral density (BMD) at the total hip and femoral neck, and a predisposition to develop osteoporosis with an odds ratio of 1.98 (28). The same SNP in a population of healthy middle-aged Korean women is associated with lower levels of circulating osteoprotegerin (OPG), a negative regulator of osteoclast development, but not changes in BMD (29). Similarly, the most frequently occurring polymorphism, a substitution of proline to alanine (Pro12Ala) in exon B of the PPARγ2 isoform, is associated with low levels of serum OPG in healthy Korean women (30).

Recently, an analysis of the Framingham Offspring Cohort revealed several novel polymorphic changes in the coding region of PPARγ, which indicate an interaction of the PPARγ gene by diet on areal (a)BMD in both men and women (31). The influence of the PPARγ interaction by dietary fat on bone mass was also confirmed in mice (31).

Genetic alterations in the coding sequence of lipoxygenases, enzymes responsible for the production of oxidized derivatives of fatty acids and ligands for PPARγ, are also associated with changes in bone mass in mice and humans. The disruption of either 5- or 15-lipoxygenase in mice leads to increased bone mass (32;33), whereas in humans polymorphic changes in the locus for 12- and 15-lipoxygenase correlate with changes in BMD in normal subjects and in postmenopausal women, respectively (34;35).

The Role of PPARγ in the Maintenance of Bone Mass

Since both cellular components of bone remodeling, osteoblasts and osteoclasts, are under the control of PPARγ, the status of its activity is important for maintaining the balance between bone resorption and bone formation. Indeed, several animal models, as well as human studies, have shown that changes in PPARγ activity, which are determined by both the level of protein expression and the level of PPARγ activation, lead to unbalanced bone remodeling (Fig. 1).

An essential role of PPARγ in the maintenance of adult bone homeostasis was demonstrated in animal models of either bone accrual or bone loss depending on the status of PPARγ activity. In models of bone accrual, a decrease in PPARγ activity in either heterozygous PPARγ-deficient mice or mice carrying a

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hypomorphic mutation in the PPARγ gene locus led to increased bone mass due to an increased number of osteoblasts (36;37). In addition, a decrease in PPARγ expression protected bone against age-dependent bone loss (36). Of interest, mice deficient in PPARγ expression in cells of the hematopoietic lineage developed high bone mass and were more resistant to TZD-induced bone loss than control mice (6). Thus, PPARγ deficiency has an anabolic effect on bone, which results from increased bone formation and decreased bone resorption.

In contrast, in rodent models of bone loss due to PPARγ activation, administration of the TZD rosiglitazone resulted in significant decreases in BMD and bone volume, and changes in bone microarchitecture (13;38;39). Due to the similarities with age-related bone loss, some speculate that TZDs may accelerate the aging of bone (13). The observed bone loss was associated with the expected changes in the structure and function of bone marrow, which 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 PPARγ2 expression in bone. In younger animals with lower levels of PPARγ, bone loss was less extensive than in older animals (13). 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 (13). Interestingly, in the absence of estrogen, rosiglitazone enhanced bone loss, mainly due to increased bone resorption, pointing to functional crosstalk between PPARγ and ER (40;41).

In addition to *in vitro* studies, the selective activation of PPARγ "bone" activities was also demonstrated *in vivo*. Administration of the TZD netoglitazone to normoglycemic mice resulted in extensive accumulation of marrow fat, but did not affect bone mass (42). Similarly, administration of troglitazone, to apolipoprotein E-deficient mice for 10 months, did not affect bone mass, although it increased the number of marrow adipocytes and appeared to affect the marrow hematopoietic compartment (43). Based on the finding that the pro-adipocytic and anti-osteoblastic activities of PPARγ2 can be separated, one can expect that specific SPPARMs can be identified with beneficial activities as insulin sensitizers and without adverse effects on bone.

Does Anti-diabetic TZD Therapy Lead to the Development of Secondary Osteoporosis?

Recent clinical evidence suggests that anti-diabetic TZD therapy is associated with a decrease in BMD and an increase in fracture risk in humans (44;45). Observational studies using data from the Health, Aging, and Body Composition cohort reported that older postmenopausal TZD users experience additional bone loss at the rate of -0.61% annually as compared to non-TZD users (46). A randomized controlled trial performed in New Zealand followed postmenopausal nondiabetic women over 14 weeks of rosiglitazone administration. The results showed a decrease of serum biochemical bone formation markers, unchanged bone resorption markers, and a decrease in hip BMD by -1.9% as compared to BMD at the beginning of treatment (47). This study concluded that short-term therapy with rosiglitazone exerts detrimental skeletal effects by inhibiting bone formation. Similarly, a randomized placebo-controlled study of the effect of pioglitazone on bone in polycystic ovary syndrome patients in Denmark demonstrated that 16 weeks of treatment with pioglitazone resulted in a significant decline in BMD of the lumbar spine (-1.1%) and femoral neck (-1.4%) (48).

The first evidence of increased fracture risk in TZD users came from A Diabetes Outcome Progression Trial (ADOPT), which compared the anti-hyperglycemic effects of three different anti-diabetic therapies with rosiglitazone, metformin, or glyburide, for a median of 4.0 years in randomly assigned women and men (49). Surprisingly, the effects were gender-specific and the fracture rates in men did not differ between treatment groups and did not show a significant difference in overall risk. The calculated hazard ratio for women receiving rosiglitazone was 1.81 and 2.13 compared with metformin and glyburide,

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respectively. Fractures were seen predominantly in the lower and upper limbs; however, vertebral fractures were not assessed in this study. There was no correlation between rosiglitazone use and estrogen status since both pre- and postmenopausal women exhibited an increase in fractures (49). 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 (T2DM) (50). In contrast, 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 a significant increase in non-vertebral fractures independently of patient age and sex. The adjusted odds ratio of fracture occurrence was 4.54 for the hip/femur, 2.12 for the humerus, and 2.90 for the wrist/forearm (51). Similarly, retrospective studies of 84,339 patients from British Columbia, Canada, showed that both men and women receiving TZDs are at risk of increased fractures by 28%, and that the TZD pioglitazone may be more strongly associated with fractures than rosiglitazone (52).

The issue of increased fracture risk in patients on TZD therapy is further underscored by the fact that the incidence of bone fractures in individuals with non-insulin-dependent T2DM is higher at a given BMD and increases with the duration of diabetic disease (53). A number of findings suggest that diabetic bone is altered in quality. Highly reactive glucose metabolites (AGEs), of which circulating levels are increased in T2DM, are implicated in forming cross-links between collagen fibers and affect bone biomechanical properties by increasing bone's stiffness and fragility (54). Recent observational studies showed that increased levels of the AGE pentosidine positively correlate with increased fracture risk in T2DM (55;56). The abnormal rate of bone remodeling may in addition contribute to the decrease in bone quality. In a murine L-SACC1 model of hyperinsulinemia, high bone mass correlates with a decreased bone remodeling rate (57), while histomorphometric studies of human bone suggest that bone turnover is compromised in older T2DM patients (58).

Conclusion

Osteoporosis, obesity, and diabetes are the most common pathologies occurring in highly industrialized countries (Centers for Disease Control and Prevention; http://www.cdc.gov). Since PPARγ is positioned at the crossroads of the control of bone mass and energy expenditure, the therapeutic manipulation of its activities may affect the skeleton, in both a positive and a negative fashion. On the other hand, there is increasing interest in the function of marrow fat including its capability to contribute to insulin-dependent glucose and fatty acid metabolism. If marrow fat plays such a role, then pharmacologic harnessing of the metabolic properties of bone fat is an attractive possibility for enlarging our armamentarium to fight metabolic diseases.

Acknowledgments

This work was supported by funds from NIH/NIA AG028935 and the American Diabetes Association's Amaranth Diabetes Fund 1-09-RA-95.

Conflict of Interest: None reported.

Peer Review: This article has been peer-reviewed.

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