The Effects of Adiponectin on Bone Metabolism

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Abstract

Osteoporosis and its related bone fractures are growing medical problems, especially in industrial countries, and thus the knowledge of regulation of bone metabolism is critical to develop therapeutic approaches. Bone adipocytes share common mesenchymal precursors with osteoblasts and chondrocytes and their numbers in bone marrow are altered in various pathophysiological conditions. Several findings suggest that accelerated adipogenesis in bone marrow, known as fatty marrow, is associated with the progression of osteoporosis and aging [1] [2]. It is now clear that adipose tissue is a complex, essential and highly active metabolic and endocrine organ [3] [4]. Adipocytes express and secrete various endocrine hormones such as leptin, adiponectin (ApN), TNF-α and...

Keywords

Adiponectin, Mesenchymal Stem Cells, Bone and Osteoporosis

1. Introduction

The relationship between bone and fat formation within the bone marrow microenvironment is complex and remains an area of active investigation. Clinical and experimental findings suggest that acceleration of adipogenesis in bone marrow, known as fatty marrow, is associated with the progression of osteoporosis and aging [1] [2]. It is now clear that adipose tissue is a complex, essential and highly active metabolic and endocrine organ [3] [4]. Adipocytes express and secrete various endocrine hormones such as leptin, adiponectin (ApN), TNF-α and...
Among these hormones, leptin is the first adipokine found to have function in both energy and bone metabolism [5] [6]. These observations suggest that energy metabolism and bone mass are regulated by the same hormones. Over the past decades, ApN and its receptors (AdipoR1 and AdipoR2) have been found to regulate energy homeostasis and have protective functions for metabolic and cardiovascular diseases [7]. In addition, accumulating evidences indicate that ApN measurement may serve as a useful screening tool for predicting osteoporosis [8]. ApN and its receptors are expressed in cells of osteoblastic and osteoclastic lineages, suggesting that they play roles in regulating bone metabolism [9] [10]. Here, we summarize the effects of ApN on bone metabolism and propose future research directions.

2. ApN and Its Receptors

ApN was originally identified by four independent groups and was named Acrp30 [11] and AdipoQ [12] in mice, and apM1 [13] or GBP28 [14] in humans. It belongs to the complement 1q family [15] and forms multimer complexes through the collagen-like domain in the circulatory system. Multi-mer complexes include trimers, hexamers and high-molecular-weight (HMW) forms [16]. ApN-deficient mice exhibit features of insulin resistance, dyslipidemia and hypertension [17]. ApN exerts its insulin-sensitizing effect by regulating glucose utilization and fatty acid metabolism [7]. Administration of ApN to mice decreases the plasma concentration of glucose, free fatty acids and triglycerides, increases muscular fatty acid oxidation, induces weight loss [18] and reverses obesity-associated insulin resistance [19].

Yamauchi et al. first cloned the cDNA encoding adiponectin receptors 1 (AdipoR1) and 2 (AdipoR2) from humans and mice [20]. Our group, then cloned the porcine counterparts [21]. AdipoR1 and AdipoR2 contain seven transmembrane domains with structure, topology and function distinct from those of the G-protein-coupled receptors [20]. AdipoR1 and AdipoR2 serve as the major AdipoRs in vivo, with the former activating the AMP kinase (AMPK) pathway and the latter activating the peroxisome proliferator-activated receptor alpha (PPARα) pathway in liver to enhance insulin sensitivity and decrease inflammation [7]. Disruption of both AdipoR1 and R2 exterminate ApN binding and downstream actions including abolishment of ApN-induced AMPK activation and decreased activity of the PPAR-α signaling pathway; The result of disruption is to increase tissue triglyceride content, inflammation, oxidative stress, insulin resistance and glucose intolerance [22]. Recent research indicates ApN has anti-proliferative effects on cancer cells and a cardio-protective role increasing longevity [23]. Figure 1 represents the effects of adiponectin on peripheral tissues and hypoadiponectinemia related diseases.

3. Bone Remodeling

Bone remodeling is a continuous process throughout adult life consisting of the resorption of senescent bone
by osteoclasts (Oc) and the formation of new bone from osteoblasts (Ob). Differentiated Oc are derived from hematopoietic stem cells [24], whereas Ob are derived from mesenchymal stem cells [25]. In the process of bone remodeling, Oc adhere to bone to remove the exterior rigid structure by acidification and proteolytic digestion. Once the resorption site is created, Ob intrude and begin new bone formation along with the secretion of osteoid, a mineralizing factor. The process is completed by coverage of the bone surface by lining cells, a type of terminally differentiated Ob [26]. Bone disease is caused by an imbalance in bone remodeling. At the physiological level, mechanisms of bone remodeling are regulated locally by cytokines and systemically by hormones [27]. PTH and 1,5-dihydroxy vitamin D have anabolic actions, which are opposed by calcitonin [27]-[29]. Several systemic regulators, such as, insulin-like growth factor (also acting through local regulators) [30], glucocorticoids [31], thyroid hormone [32] and estrogen [33] contribute to bone formation and bone resorption. In addition, local factors derived from bone cells, like TGF-β, have inhibitory functions on osteoclastogenesis [34] regulate cell growth and differentiation and induce anabolic activity in Ob [35]. IL-1 and TNF-α serve to quiesce Oc and participate in bone resorption pathophysiology [36]. The receptor activator of NF-κB ligand (RANKL), RANK and osteoprotegrin (OPG) are essential for Oc differentiation. RANKL is expressed by Ob as a soluble factor that binds with RANK on Oc with recruitment of tumor necrosis factor receptor-associated factor 6 (TRAF6) and activation of the transcription factor, nuclear factor of activated T-cells cytoplasmic 1 (NFATc1) to induce of Ob differentiation related genes via the NF-κB pathway [37] [38]. OPG is a decoy receptor for RANKL which plays an inhibitory role in osteoclastogenesis [39]. OPG-deficient mice exhibit a decrease in total bone density and develop osteoporosis [40]. The regulation of bone remodeling not only involves osteoblastic and osteoclastic cell lineages but also other marrow cells. Adipocytes are derived from the same progenitor cells as Ob, and the equilibrium between the two cell types is important in bone remodeling [41] [42]. Several regulatory pathways of adipocyte differentiation from bone marrow mesenchymal stem cells interact with osteoblast differentiation pathway [43]. The major adipogenic transcriptional factors, C/EBPα and PPARγ, are regulated by extracellular signaling involved in osteoblastogenesis [43]. Mesenchymal stem cell differentiation into Ob requires Wnt signaling activation, which stimulates osteoblastogenesis and suppresses adipogenesis by blocking PPARγ and C/EBPα [44] [45]. Cell lineage allocation in the bone marrow microenvironment is shown in Figure 2.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Cell lineage allocation and regulation in the bone marrow microenvironment (modified from [30]). Osteoblasts and adipocytes are derived from the same progenitors, the mesenchymal stem cells. To achieve full differentiated status, multiple critical transcription factors must be activated. RANKL is expressed by osteoblasts as a soluble factor, which binds to RANK on osteoclasts. Further recruitment of TRAF6 and activation of NFATc1 induce osteoclast differentiation. Cytokines released from the adipocytes participate in the bone remodeling process. (CEBPα, CAAT/enhancer binding protein α; PPARγ, peroxisome proliferative activated receptor γ; RANK, receptor activator of nuclear transcription factor κB; RANKL, receptor activator of nuclear transcription factor κB ligand; OPG, osteoprotegrin; IL, interleukin; MCSF, macrophage colony stimulating factor; MSX, MSH homeobox homolog; RUNX, runt-related transcription factor; TRAF6, tumor necrosis factor receptor-associated factor 6; NFATc1, nuclear factor of activated T-cells cytoplasmic 1).
4. The Actions of Adiponectin on Bone Metabolism

4.1. Effects of ApN on Bone Metabolism and on Osteoblast and Osteoclast Differentiation

ApN stimulates osteoblastogenesis and chondrocytogenesis, but suppresses osteoclastogenesis and therefore promotes bone formation [9] [46]. There are three distinct ApN actions in bone formation: 1) a direct positive endocrine action through circulating ApN; 2) an autocrine/paracrine action; 3) indirect endocrine effects by interacting with other signaling pathways such as insulin and bone morphogenetic protein 2 (BMP2).

Several studies confirm the positive endocrine role of ApN in bone formation. Challa et al. suggest that ApN increases chondrocyte proliferation, proteoglycan synthesis and matrix mineralization by upregulating the expression of type II collagen and Runx2 and increasing the activities of alkaline phosphatase [46]. Similarly, Oshima et al. (2005) demonstrated that ApN-treated C57BL/6J mice have increased trabecular bone mass and enhanced mineralization of Ob, accompanied by a decreased number of Oc and bone resorption activity [47]. Potential mechanisms for circulating ApN to modulate Oc differentiation are that ApN augments gene expression of several osteogenic markers and increases Ob differentiation in mesenchymal progenitor cells [48]. Moreover, ApN activates p38 mitogen-activated protein kinase via AdipoR1, which results in c-Jun activation and up-regulation of the target gene, cyclooxygenase-2 (COX-2). ApN also stimulates BMP2 expression in a COX2-dependent manner and therefore increases Ob differentiation [48]. An alternative action of ApN, discovered by Huang et al. is that ApN stimulates osteoblast differentiation by increasing BMP-2 expression with involvement of AMPK, p38 and NF-κB [49].

ApN regulates bone formation in an autocrine/paracrine manner. ApN and its receptors are expressed in osteoblastic and osteoclastic cells, indicating participation in bone metabolism not only through an endocrine pathway, but also locally in the bone [9]. The authors analyzed cultures of bone marrow cells from ApN-knockout (Ad−/−) and WT mice and find a significant decrease of osteogenesis in Ad−/− marrow cell culture, compared to WT. Collectively, these results suggest a positive autocrine/paracrine action of ApN on bone formation.

ApN also has indirect effects on bone, possibly through modulating growth factor action or insulin sensitivity. In the presence of insulin and BMP2 (but not IGF-1), ApN stimulates Ob differentiation in bone marrow cells [9]. Co-culture of Ob with the secretory products of adipocytes produces an inhibitory effect on Ob differentiation, which can be reversed by knockdown of adipoR1 [50].

4.2. Involvement of Insulin Signaling, Long Term Adaption and Compensation in ApN-Modulated Bone Metabolism

Most studies indicate that the effects of ApN and its receptors have a positive role on bone metabolism, but some opposite aspects are also described [51]. One confounding factor is insulin signaling. Shinoda et al. (2006) treated a bone marrow cell culture with insulin or recombinant mouse ApN and found that insulin impairs the effect of ApN treatment on osteogenesis and restores the number of colonies. In addition, long term adaption and compensation in transgenic or knockout mice may modify ApN-modulated bone metabolism. Analyses of bone characteristics by radiological and histological examination of the femur, tibia and vertebrae in male WT and Ad−/− littermates indicates no difference between WT and Ad−/− marrow cell culture, compared to WT. Collectively, these results suggest a positive autocrine/paracrine action of ApN on bone formation.

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5. Involvement of AMPK in ApN-Modulated Bone Metabolism

Bone remodeling is an energy intensive process and bones need to balance energy constantly in response to nutrient availability during growth and bone turnover. During the last decade adenosine 5’-monophosphate-activated protein kinase (AMPK) emerged as a key in the regulation of energy homeostasis and as the mediator of
Table 1. Effects of ApN on bone metabolism.

| Main idea | Animal model | Cell model | Approach | Ref. |
|-----------|--------------|------------|----------|------|
| **In vivo:** | ↑ trabecular bone mass | C57BL/6j mice treated with adenovirus expressing lacZ or adiponectin | Treat MC3T3-E1, CD14+ cells, bone marrow macrophage with adiponectin | Micro-CT, EIA (NTx), Histological examination (TRAP+), real-time (ALP), Alizarin Red S staining |
| **In vitro:** | ↓ osteoclast differentiation | Bone marrow cell (from adult mouse long bones, neonatal calvarial osteoblasts, osteoclast precursor M-CSF-dependent bone marrow macrophage, ST2 cells) | Histological analysis, osteoclast formation assay, immunoprecipitation and immunoblotting | [9] |
| **In vivo:** | No difference between overexpressing adiponectin and WT | Ad−/− mice, Ad-Tg mice | | |
| **In vitro:** | ↓ osteogenesis in Ad−/− mice bone marrow cell 1. Treat adiponectin recombinant protein inhibit osteogenesis | AdKO mice (C57BL/6j) Rat and human primary osteoblast, osteoclast | Micro-CT, primary osteoblast culture | [51] |
| **In vivo:** | ↑ trabecular bone volume and trabecular number in 14 week Ad KO mice. | | | |
| **In vitro:** | ↓ osteoclastogenesis in rat and human osteoclast | ADN-KO mice RAW264.7 | Histological examination, micro CT | [52] |

ApN and its receptors are expressed in bone and bone stromal cells which suggest that they play critical roles in bone metabolism. The present review summarizes effects of ApN on bone metabolism, both in physiological states and in Ob or Oc culture in vitro. There are still several controversial results on the correlation of ApN and bone mineral density or other bone parameters. The critical role of ApN in the bone remodeling process and bone metabolism is an indisputable fact. Herein, we propose some possible future research directions.

1) In the circulatory system, ApN exists multi-mer complexes, such as trimers, hexamers and HMW forms [16]. Several researches suggest that different forms of ApN may give rise to different activities and have different roles in insulin sensitivity [62] [63]. In both mice and human diabetic patients, an increased ratio of HMW ApN to total ApN in the plasma correlates with improvement in insulin sensitivity during treatment with an insulin-sensitizing drug, TZD; there is no correlation with total amount of ApN [63]. Only one research group indicates that HMW ApN affects bone metabolism in both male and female hemodialysis patients [64]. We speculate that different multi-mer forms of ApN cause different influences on bone formation, but more molecular functions and signaling pathways need to be elucidated in future studies.

6. Conclusions and Future Perspectives

ApN and its receptors are expressed in bone and bone stromal cells which suggest that they play critical roles in bone metabolism. The present review summarizes effects of ApN on bone metabolism, both in physiological states and in Ob or Oc culture in vitro. There are still several controversial results on the correlation of ApN and bone mineral density or other bone parameters. The critical role of ApN in the bone remodeling process and bone metabolism is an indisputable fact. Herein, we propose some possible future research directions.

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level observations are needed to confirm this speculation.

2) Energy equilibrium in mammals is controlled by the actions of circulatory hormones that coordinate fuel production and utilization in metabolically active tissues. Bone remodeling can be affected by metabolic related hormones, implying that bones are involved in the control of energy homeostasis [65] [66]. Osteocalcin, the most abundant non-collagenous protein of the bone extracellular matrix regulates β-cell proliferation, insulin secretion and glucose homeostasis [66] [67]. Furthermore, there are several consistent results showing the insulin-osteocalcin endocrine loop in humans [68] [69] were decreased in adipocyte and pancreatic islets respectively co-culture with osteocalcin knockout osteoblasts [65]. Whether osteocalcin has any interaction with ApN in bone metabolism or as a feed-back loop via ApN needs further investigation.

3) Mao et al. [70] used the cytoplasmic domain of AdipoR1 as bait to screen a yeast two-hybrid cDNA library derived from human brain. They found that APPL1 (adaptor protein containing pleckstrin homology domain, phosphotyrosine binding domain (PTB) and leucine zipper motif) interacted with ApN receptors in mammalian cells and the interaction was stimulated by ApN. It has been suggested that APPL1 mediates the effect of ApN on inhibition of osteoclastogenesis and bone resorption [52]. Although this observation is an indirect evidence, we speculate that APPL1 may have functions to mediate ApN effects on bone development. APPL1 not only has an AdipoR1 binding domain in the PTB domain, but also has a FSH receptor binding domain [71]. The existence of FSH receptor domain in APPL1 increases the possibility of APPL1 mediating the regulation of ApN on bone metabolism [71] [72]. In order to investigate this possible regulator, we have to further investigate the role of APPL1 by culture system or loss-of-function and gain-of-function model.

4) Epigenic regulation—MicroRNAs (miRNAs) are small endogenous RNA fragments (19 - 25 nt) that regulate gene expression by targeting mRNA in post-transcriptional stage [73]. Controlling the differentiation of the mesenchymal stem cells into osteogenic or adipogenic lineage is important for maintaining healthy bone and is necessary for prevention of bone related disease. Recently, there are some evidences showing that the differentiation between Ob and adipocytes is tightly controlled by miRNAs [74] [75]. We speculate that miRNA may have the effects on regulating expression of ApN and therefore affect bone formation or bone resorption.

5) AdipoR2 is expressed in bone-forming cells [10]. So far, there is no evidence showing the effect of ApN through AdipoR2 in bone formation or bone resorption. The role of AdipoR2 in bone metabolism must be investigated.

6) Bone marrow microenvironment and its endocrine or paracrine regulatory systems are actively studied. Osteoporosis models such as ovariectomy and senescence have been used extensively in rodents or other mammals [76] [77]. Ovariectomy mimics postmenopausal women whose bone loss is caused from acute ovary hormonal deficiency. Ovariectomy destroys the overall hormonal balance, but is a difficult model to investigate changes in bone microenvironments. More appropriate models are needed to investigate regulatory mechanisms.

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