

PERSPECTIVES

The Bone-Fat Mass Relationship: Laboratory Studies

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Abstract

Over the past 20 years, a large number of clinical studies have shown a positive correlation between bone density and soft tissue mass. Furthermore, studies of fracture epidemiology have shown that low body weight is a risk factor for fractures. The greater mechanical load exerted on the skeleton by additional weight can only provide a partial explanation for the correlation between adipose tissue and the skeleton and a number of other physiological mechanisms operate in maintaining this correlation. Adipose tissue itself secretes factors known as "adipokines" that circulate and affect other target tissues. Levels of circulating adipokines vary with changes in food intake and weight, and there is evidence that numerous adipokines can regulate bone metabolism through direct and indirect mechanisms of action. Another source of hormones that regulate energy metabolism are β -pancreatic cells, which in obese states hypersecrete hormones with direct and indirect effects on bone. Hormones secreted from the gut are also likely to act as mediators of the bone-fat mass relationship, and in addition there is evidence for direct effects of ingested nutrients such as glucose and fatty acids on bone turnover. This review focuses mainly on results from laboratory studies investigating possible mechanisms involved in the positive relationship between bone mass and fat mass. *IBMS BoneKEy*. 2009 September;6(9):311-322.
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Body weight influences both bone turnover and bone density, such that low body weight is a risk factor for osteoporotic fracture (1). The effects of body weight on skeletal health may be attributable to independent effects of fat mass and lean mass, but in postmenopausal women fat mass appears to be more important. Total body fat mass is positively related to bone density and inversely related to fracture risk, and changes in fat mass are positively related to changes in bone density (1). These clinical findings were the impetus for our group and others to investigate the possible mechanisms involved in the correlation of fat mass to bone mass. Many studies describe the bone effects of hormones, secreted by different tissues and cell types, which are responsive to changes in food intake and body mass, and are therefore likely to contribute to the relationship between adipose tissue and bone. This review will summarize some of the findings primarily from laboratory studies in the field.

Adipokines

Whereas in the past the adipocyte was not considered to play a significant regulatory role, it is now well-accepted that it is an important source of circulating factors, or "adipokines", which act as regulators of metabolism. Several adipokines have been studied extensively and their effects in bone and other tissues are well-described, while a list of additional adipokines is steadily growing and their roles in bone biology remain to be elucidated.

Leptin

Leptin was cloned more than a decade ago and was originally described as a hormone, secreted by adipocytes, whose primary target was within the central nervous system, where it regulates appetite and energy expenditure (2). Consequent studies showed a wide distribution of leptin receptors outside the central nervous system, and the expression of the signaling form of the leptin receptor in osteoblasts and

chondrocytes suggested the skeleton as a potential target (3;4). Leptin directly increases proliferation and differentiation of osteoblasts and inhibits osteoclastogenesis through reducing the expression of RANK and increasing osteoprotegerin levels (3;5). Systemic administration of leptin in intact or leptin-deficient animals results in increases in bone formation and skeletal mass and reduced bone fragility (3;4;6). Indirect effects of leptin on bone were discovered by infusion of leptin into the third ventricle of the brain (7). With central administration, leptin caused bone loss in leptin-deficient and wild-type mice through inhibition of bone formation and stimulation of bone resorption. Blockade of the sympathetic nervous system abrogates these effects, which appear to be mediated by β -adrenoreceptors on

osteoblasts (8). There are alternative explanations for the bone loss caused by the central administration of leptin, suggesting indirect effects through changes in body weight. Leptin, acting via the hypothalamus, produces a dramatic reduction in appetite leading to profound weight loss, adipocyte shrinkage, reduced serum levels of both leptin and insulin, and increased ghrelin levels (9). The potential effects of leptin on bone and their interactions are depicted in Fig. 1. Under normal physiological conditions, leptin is a systemic hormone produced outside the central nervous system and its central effects are balanced by its direct effects on bone cells, which are likely to be dominant, as attested to by the consistent positive relationship between fat mass and bone density.

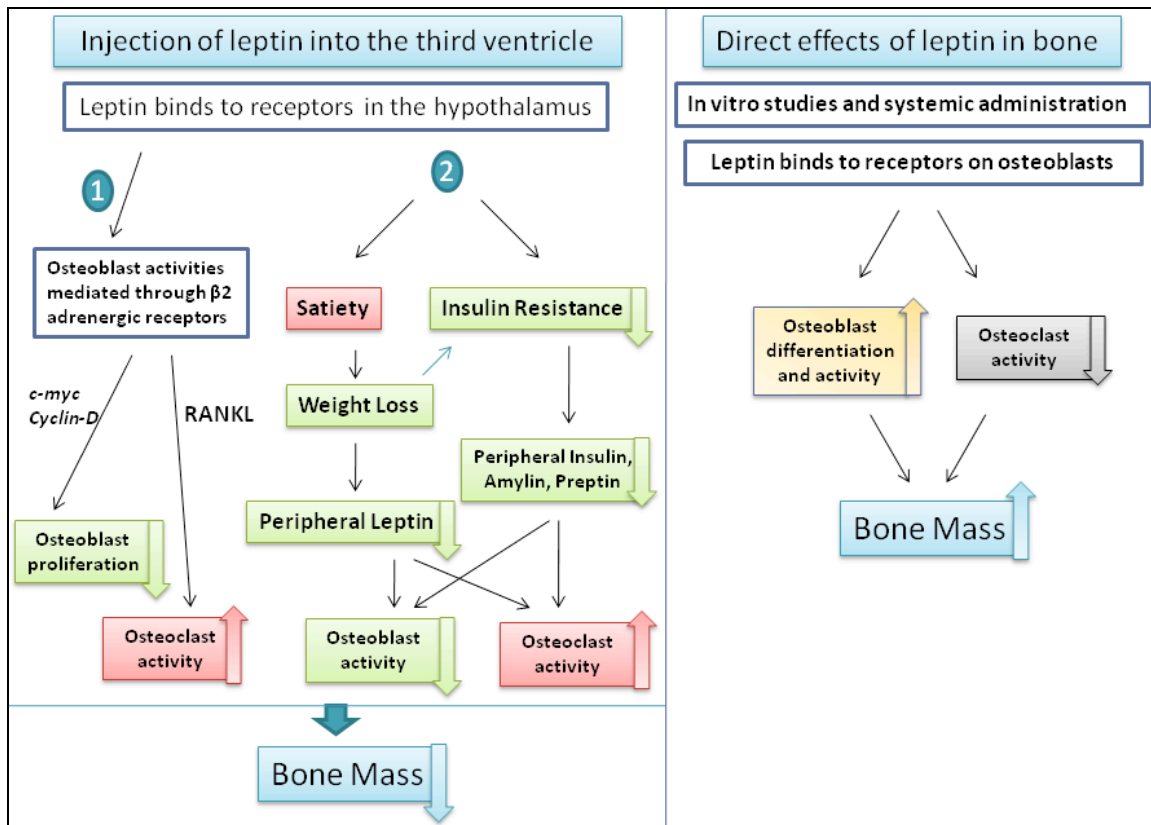


Fig. 1. Direct and indirect effects of leptin in bone. The left panel summarizes changes observed when leptin is injected into the third ventricle of the brain. (1) The direct effect of leptin via the sympathetic nervous system on β -adrenergic receptors expressed on osteoblasts. (2) An alternative mechanism that explains the reduction in bone mass, where leptin acts via the sympathetic nervous system to increase insulin sensitivity and to induce satiety. The right panel summarizes studies of peripheral administration of leptin. © IR Reid and Dorit Naot, used with permission.

Adiponectin

Adiponectin, secreted almost exclusively by adipocytes, is a 28-kD protein that circulates in humans as trimers, hexamers and high-molecular weight oligomers, in total concentrations of 0.5–30 µg/mL (10-12). Plasma concentrations of adiponectin are inversely related to visceral fat mass and body mass index (12). This relationship, the inverse of leptin, is yet to be fully explained but may be mediated by inhibition of adiponectin secretion by cytokines and hormones that are increased in obesity, by adipose tissue hypoxia, or by direct inhibition of its own production. Adiponectin regulates energy homeostasis, glucose and lipid metabolism and inflammatory pathways (13).

The results of laboratory studies investigating adiponectin's effects on bone are not always congruent. The receptors for adiponectin, AdipoR1 and AdipoR2, have been identified on both osteoblasts and osteoclasts (14;15), implying a potential direct influence of this hormone on bone. Adiponectin has been shown to increase osteoblast proliferation and differentiation while inhibiting osteoclastogenesis *in vitro* (16;17), and transient overexpression of adiponectin in mice increased trabecular bone mass and reduced osteoclast number and bone resorption (16). However, in another study adiponectin was found to reduce osteoblast growth, and mice either transgenically overexpressing or deficient in the adiponectin gene showed no abnormality in bone phenotype at 8 weeks of age (15).

We have studied adiponectin effects on bone *in vitro* and in knockout mice (18) and have found that adiponectin is dose-dependently mitogenic to primary osteoblasts, and markedly inhibits osteoclastogenesis. It has no effect on bone resorption in isolated mature osteoclast assays. Micro-computed tomography of bones of 14-week-old adiponectin knockout mice showed that trabecular bone volume is increased by 30% and trabecular number by 38% in these animals (18). These data indicate that adiponectin acts directly on

bone cells, but the activities measured *in vitro* do not explain the bone phenotype of the knockout animals. Thus, adiponectin may also exert indirect effects on bone, possibly through modulating insulin sensitivity (19) or growth factor binding (20).

In summary, adiponectin has direct actions on bone cells, and adiponectin knockout mice have increased bone mass. Taken together with the clinical data, this suggests that the final sum of direct and indirect actions of adiponectin *in vivo* is a negative impact on bone mass. Moreover, epidemiologic evidence suggests that the relationship between adiponectin and bone mineral density (BMD) is distinct from other potential sources of influence arising from fat, such as body weight and leptin levels. Thus, adiponectin appears to be an adipokine that independently impacts bone mass.

Resistin

Resistin is an adipokine that appears to be downregulated by agonists of PPAR γ . Both osteoblasts and osteoclasts express resistin, and its expression is upregulated during osteoclast differentiation (21). We have demonstrated that resistin modestly increases the proliferation of osteoblasts in both cell and organ culture systems (22). It also increases the formation of osteoclasts in bone marrow culture and their activity in organ culture. These data suggest that elevated levels of resistin may contribute to higher levels of bone turnover, consistent with an inverse correlation shown between serum resistin levels in humans and lumbar BMD (23).

Estrogens

The adipocyte has long been recognized as an estrogen-producing cell, particularly in postmenopausal women. We have demonstrated a relationship between circulating estrone levels and bone density, but this has been shown to be both independent of the effects of fat mass and substantially weaker (24). This implies that estrogen is not a major pathway by which fat influences bone density, a suggestion

supported by the finding of a bone-fat relationship in premenopausal women, in whom the adipocyte is a relatively minor source of estrogens.

β -Pancreatic Cell Hormones

In obese states, insulin resistance leads to hypersecretion of insulin from β -pancreatic cells. Amylin and preptin, two hormones co-secreted with insulin, also circulate in increased levels in obesity. As these three β -pancreatic hormones directly affect bone cells they are likely to contribute to the bone-fat mass correlation.

Insulin

In obesity, hyperinsulinemia arises from resistance to the hypoglycemic effects of insulin. Circulating insulin concentrations are related to BMD, height, body weight, fat mass and in some studies insulin concentrations have been found to be the principal determinant of bone density (25; 26). Osteoblasts express insulin and IGF1 receptors and *in vitro*, insulin directly stimulates osteoblast proliferation (27;28). When administered locally over the calvariae of adult male mice, insulin produces two- to three-fold increases in histomorphometric indices of bone formation (29).

Amylin

Amylin is a 37-amino acid peptide that belongs to the calcitonin family and is evolutionarily related to insulin. *In vitro*, amylin directly stimulates osteoblast proliferation (30) and inhibits osteoclast differentiation and activity, although with lower potency than calcitonin (31). Systemic administration of amylin substantially increases bone volume in mice (32) and rats (33;34). Amylin-deficient mice have a typical osteoporotic phenotype, showing decreased bone density of long bones, low bone mass and a decrease in trabecular bone volume (35). Amylin-deficient mice have an increased number of osteoclasts and an increase in degradation products of collagen in the urine, suggestive of accelerated bone

resorption, whereas the number of osteoblasts and the bone formation rate are similar to wild-type controls.

Preptin

Preptin, a peptide that corresponds to Asp⁶⁹ - Leu¹⁰² of pro-insulin-like growth factor-2 (pro-IGF2), has been isolated from the same secretory vesicles that contain insulin and amylin, and was found to increase glucose-mediated insulin secretion (36). *In vitro*, preptin is anabolic to osteoblasts in cell and organ culture, but does not influence osteoclast activity (37). *In vivo*, its local administration increases bone formation and bone area in adult male mice (37). The anabolic activity of preptin on bone is likely to contribute to the development of osteosclerosis in some patients with hepatitis C, since immunoactivity of pro-IGF2-(89-101) is increased in this condition (38). Recently, a potential role of preptin in the pathogenesis of type 2 diabetes mellitus was demonstrated when preptin was found to circulate at higher levels in diabetic patients compared to patients with impaired glucose tolerance and controls (39).

Thus, the β -pancreatic cell exerts a cluster of anabolic effects on bone, all of which are accentuated in individuals with high fat mass (Fig. 2).

Gut Hormones

Bone turnover is acutely responsive to food intake, with bone resorption increased during fasting and suppressed during feeding. Specific macronutrients such as glucose and fatty acids can directly affect bone turnover. Additionally, gastric surgeries affect bone health in both animal models and humans with notable trends toward osteopenia following gastrectomy. Here, we review studies of several gut-derived hormones and their impact on bone metabolism.

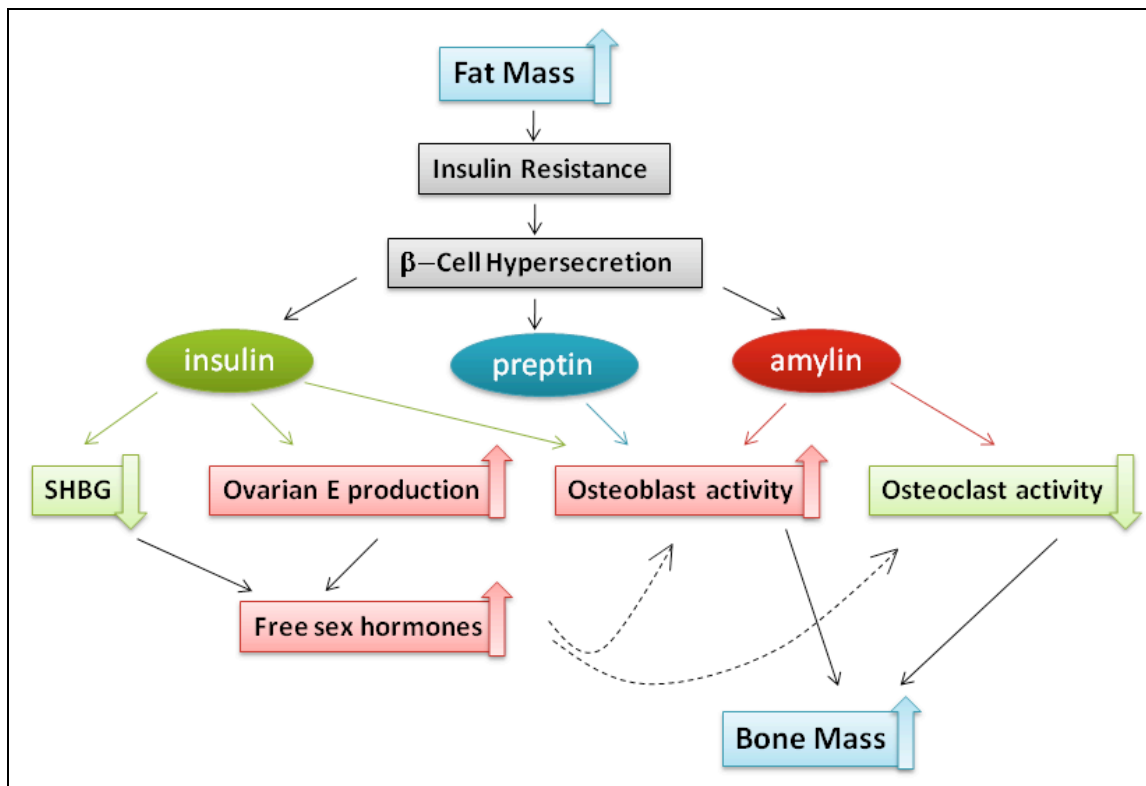


Fig. 2. Summary of the principal mechanisms by which insulin resistance associated with obesity contributes to increased bone mass. SHBG = sex hormone-binding globulin. © IR Reid and Dorit Naot, used with permission.

Glucose-dependent polypeptide

Glucose-dependent polypeptide or gastric inhibitory polypeptide (GIP) is a 42-amino acid protein secreted from K-cells of the duodenum of the small intestine. GIP induces both insulin and glucagon secretion from the pancreas, and was shown to have direct anabolic effects on bone cells (40) while *in vivo* administration of GIP reduces bone loss in the ovariectomized mouse (41). GIP also inhibits osteoclast activity *in vitro* (42) and has a role in bone metabolism changes that occur directly after food intake (43). Transgenic mice overexpressing GIP have increased bone formation, decreased bone resorption and increased bone mass (44). The GIP receptor is expressed on chondrocytes, osteoblasts, osteocytes and osteoclasts, further confirming GIP's direct effect on bone metabolism (40;42). Mice lacking the GIP receptor have increased

insulinotropic

numbers of mature osteoclasts and reduced bone mass (45).

Glucagon-like peptides

Glucagon-like peptide-1 (GLP-1) and GLP-2 are hormones derived by proteolytic cleavage of the preproglucagon precursor. These hormones are expressed in intestinal L-cells and their levels rise rapidly after food intake, whereupon they induce insulin secretion. Although GLP-1 had no direct effect on osteoclasts and osteoblasts, mice deficient in the GLP-1 receptor have cortical osteopenia and increased osteoclast numbers and bone resorption activity (46). In contrast, GLP-2 can act directly on bone cells, as GLP-2 receptors are expressed on osteoclasts and GLP-2 has been shown to decrease bone resorption *in vitro* (47). In several human studies, administration of exogenous GLP-2 reduced bone resorption, as measured by a reduction in circulating bone turnover markers (48;49).

Ghrelin

Ghrelin is a 28-amino acid peptide hormone synthesized primarily by A-like cells of the stomach and released in response to fasting, such that circulating levels are maximal prior to meals and fall upon re-feeding (50;51). *In vivo*, ghrelin is an orexigenic factor, increasing food intake and inducing release of growth hormone from the pituitary via the hypothalamus.

The ghrelin receptor is expressed on osteoblastic cells, but we have found that ghrelin only weakly activated cell proliferation in cultures of primary rat and human osteoblasts (52). In contrast, other groups have shown that ghrelin increases proliferation and differentiation in cultures of animal and human osteoblastic cells (53). Loss of ghrelin by genetic deletion produces no change in either bone mineral content or BMD (54). Transgenic mice overexpressing ghrelin in the brain and stomach showed increased food intake and impaired glucose tolerance but otherwise had normal body size (55). No analysis of bone metabolism was carried out in this study. Systemic ghrelin administration increases BMD in rats (53), while in human studies of ghrelin infusion no short-term changes in bone metabolism were observed (56).

Serotonin

Serotonin (5-hydroxytryptamine) is a monoamine signaling molecule that is largely produced in enterochromaffin cells of the gut, while a small amount of the body's serotonin is produced and acts as a neurotransmitter in the brain. Serotonin is released locally in the gut and stimulates intestinal peristalsis, and from the gut it also enters the circulation where it is taken up by platelets.

A number of studies have shown that serotonin acts via serotonin receptors and transporters expressed on osteoblasts and osteoclasts. Serotonin stimulated proliferation of osteoblast precursor cells *in vitro* and long-term serotonin administration leads to high BMD in rats (57;58). It has also been shown that the serotonin 5-HT_{2B}

receptor controls bone mass via osteoblast recruitment and proliferation and that mice deficient in this receptor show marked osteopenia (59). In contrast, there is evidence for a negative effect of serotonin on bone and a correlation was found between circulating serotonin levels and bone density (60). Further studies are needed to clarify the relationship between serotonin and bone metabolism.

Nutrition

Apart from hormonal signals that can coordinate food intake and weight with bone metabolism, there is a further possibility that ingested nutrients themselves might act directly on bone. Feeding experiments have shown that bone resorption is reduced by food intake, an effect that could be mediated by a combination of indirect and direct mechanisms. There is experimental evidence to support the theory that some dietary components, and specifically fatty acids, might influence bone cell function and skeletal health.

Direct actions of fatty acids on bone

A number of G protein-coupled receptors (GPRs) have been identified as fatty acid receptors, binding fatty acids of different carbon chain length and degree of saturation with different affinities. We have recently reported the expression of GPRs 40, 41, 43 and 120 in osteoclastic cells, with the level of expression of GPR120 being approximately 100-fold higher than that of the others (61). GPR120 is also expressed in osteoblastic cells. *In vitro* studies of the direct effects of fatty acids on osteoblasts showed that high concentrations of palmitic acid (100-250 μ M) induced apoptosis in human fetal osteoblasts (62) and that docosahexanoic acid and arachidonic acid inhibit osteoblast proliferation (63). Palmitic and stearic acids were shown to inhibit osteoblast differentiation and survival (64), whereas in our experiment we found a weak proliferative effect of these fatty acids on osteoblasts (61). In osteoclasts, a detailed evaluation of the effects of individual fatty acids on osteoclastogenesis *in vitro*

revealed that saturated fatty acids of carbon chain length 14-18 reduced osteoclast development (61). A study of the effects of polyunsaturated fatty acids on osteoclast development found inhibitory actions of linoleic acid and conjugated linoleic acid in bone marrow cultures and RAW264.7 cells (65). In a subsequent study, docosahexanoic acid, but not eicosapentanoic acid substantially decreased osteoclast development in RANKL-treated RAW264.7 cells (66), whereas other investigators reported inhibitory actions of both docosahexanoic acid and eicosapentanoic acid on osteoclastogenesis in bone marrow cultures (67). *In vivo* studies of the skeletal effects of fatty acids have largely focused on feeding experiments with polyunsaturated fatty acids. The complex designs of these studies and the diverse endpoints measured make it difficult to draw an overall conclusion, but the available data suggest that n-3 polyunsaturated fatty acids might exert beneficial skeletal effects.

Conclusion

The key clinical finding in the area of the bone-fat mass relationship is that low body weight is a major risk factor for most fractures. Although the observation that fat mass correlates with bone mass and bone density seems simple and straightforward, studies of this correlation clearly show that the underlying mechanisms are very complex and include many direct and indirect pathways. The association between adipose tissue and bone is mediated by a combination of hormonal regulation, neuronal inputs and direct actions of ingested nutrients on bone metabolism. Some of these pathways are better understood than others, and in some areas the experimental results appear to be contradictory and further studies are required. Future investigations are likely to attempt to dissociate the fat and bone effects of specific factors, such that the positive bone impact is retained without weight gain.

Conflict of Interest: None reported.

Peer Review: This article has been peer-reviewed.

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