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REVIEW

The hunger games of skeletal metabolism

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Gastrointestinal peptides and adipokines are critical signalling molecules involved in controlling whole-body energy homeostasis. These circulating hormones regulate a variety of biological responses such as hunger, satiety and glucose uptake. *In vivo* experiments have established that these hormones also regulate bone metabolism, while associations between these hormones and bone mass have been observed in human clinical studies. With a focus on recent research, this review aims to describe the roles that gastrointestinal peptides (ghrelin, peptide YY, glucose-dependent insulinotropic polypeptide, glucagon-like peptide 1 and glucagon-like peptide 2) and adipokines (leptin and adiponectin) have in bone metabolism and to examine their effects on bone in situations of altered metabolism, such as obesity. As the prevalence of obesity continues to increase, there is a growing interest in understanding the interactions between nutritional regulators from the gut and adipose tissue and their influence on bone mass.

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Introduction

Energy homeostasis is a complex state involving numerous organ systems, including circulating hormones arising from the gastrointestinal tract and from adipose tissue. These hormonal peptides are known to affect a range of factors such as hunger, satiety, metabolic rate, glucose homeostasis and bone metabolism. Alterations in biochemical markers of bone turnover are observed following food intake, demonstrating interactions between feeding and the skeleton.[1] Gastrointestinal hormones that regulate or respond to feeding include peptide YY (PYY), ghrelin, glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide 1 (GLP-1) and glucagon-like peptide 2 (GLP-2). In addition, adipocytes secrete adipokines such as leptin and adiponectin, which respond to changes in nutritional status, obesity, inflammation and also regulate bone metabolism.[2–5] This review will outline the involvement of the gastrointestinal peptides PYY, ghrelin, GIP, GLP-1 and GLP-2, and the adipokines leptin and adiponectin, in bone homeostasis.

Gastrointestinal Peptides: PYY

PYY is a gastrointestinal hormone and a member of the neuropeptide Y (NPY) family that is secreted in response to a meal, to suppress appetite, and limit meal size and overall calorie intake.[6] PYY exists in two forms, PYY1-36 and PYY3-36, which are produced when PYY1-36 is cleaved by DPP-4.[6] Affinities for Y receptors differ between the two forms, with PYY1-36 having a strong affinity for Y1 and Y2 receptors, whereas PYY3-36 has a strong affinity for Y2 only. Experiments in rodents and clinical studies have determined that increased PYY levels are detrimental to bone mass.

The effects of PYY on bone mass in rodents have been controversial as it is difficult to produce mice with genetically altered levels of PYY, because PYY overexpression is lethal in embryonic development.[7] An initial study observed a decrease in BMD, BMC and cancellous bone mass in PYY −/− mice.[8] However, Wong *et al.*[9] demonstrated that PYY −/− mice have increased bone mass, coincident with an increase in osteoblast activity.[9] The reason for this divergent phenotype is unclear; however, it is emerging that NPY/PYY effects can be context dependent, with stressors, such as cold or restraint, producing opposing effects, linked to stimulation of noradrenaline circuits.[10,11] However, the later study, demonstrating an increase in bone anabolism with PYY deletion, is consistent with a reduction in osteoblast activity and an increase in osteoclastic surface, as evident in adult-onset PYY-overexpressing mice,[9] and with previous studies showing similar responses to loss of NPY/PYY receptors.[12,13] Treatment of osteoblasts in culture with PYY increased ERK signalling via the Y1 receptor; this response was diminished in the presence of an Y1 antagonist.[9] Thus, taken together, the inhibitory nature of PYY signalling on bone mass is consistent with a number of studies examining the role of the NPY system and its receptors in the regulation of bone mass (reviewed in Khor *et al.*[14]). Thus, recent murine studies indicate that PYY activity has a negative influence on bone.

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In obese patients, PYY levels are commonly found to be reduced in comparison with non-obese patients. This leads to a decrease in satiety and an increase in food intake. An inverse correlation between reduced PYY levels and increased BMD in obesity was identified in these patients. Conversely, increased PYY levels and decreased BMDs are observed in anorexic adolescents.[15] Importantly, in lean premenopausal women, circulating PYY levels were found to be significantly and negatively correlated with total body and hip bone mass, with PYY contributing to 9% of the variance in BMD of the hip.[16] However, a recent study by Amini *et al.*[17] did not identify an association between PYY and BMD in the Newfoundland population.[17] The relationship between PYY and bone clearly requires greater investigation; the above studies examined associations in either small subsets of the population (that is, anorexic or obese individuals, menopausal status) or cross-sectional population studies; the differences in subject numbers and the characteristics of subjects may contribute to the presence/absence of associations between PYY and BMD. Initial human studies also suggest that the effects of PYY on bone regulation may be context-dependent, such as following bariatric surgery, where PYY levels are markedly altered.[18]

Ghrelin

The appetite stimulant, ghrelin, is secreted from the endocrine cells of the stomach.[19] Ghrelin levels increase during fasting and reach their highest peak prior to the ingestion of a meal. Shortly after a meal, post-gastric feedback inhibits ghrelin secretion.[20] In addition to ghrelin being a growth hormone stimulant, increased ghrelin levels have a positive effect on bone mass.

Ghrelin-null mice and ghrelin-receptor-deficient mice have no differences in BMD and BMC when compared with WT mice.[21,22] However, Fukushima *et al.*[23] demonstrated that rats infused with ghrelin had higher BMDs. *Ex vivo* cultures demonstrated that ghrelin inhibits osteoclastogenesis[24,25] and prevents apoptosis in osteoblastic cells.[26] Similarly, deficiency of ghrelin or ghrelin signalling in culture led to increased osteoclastic differentiation and fusion.[25]

van der Velde *et al.*[25] determined that ghrelin-receptor-deficient mice had a reduction in cancellous bone volume at 6 months of age in comparison with younger (3 month old) mice. These age-dependent effects of ghrelin were found to be entwined with the skeletal actions of leptin. These late-onset leptin-dependent changes in bone resorption in *Ghrs* $-/-$ mice suggested the possible involvement of cocaine amphetamine regulated transcript (CART), a hypothalamic neuropeptide known to stimulate bone resorption in leptin-deficient mice[27] and the regulation of bone mass in humans.[28]

Recently, Choi *et al.*[29] examined the effects of chronic intracerebroventricular injection of ghrelin on bone metabolism in rats. This study demonstrated that chronic central administration of ghrelin had an anabolic effect on bone mass.[29] Although the mechanism for central ghrelin action was not determined, it was postulated that increased ghrelin activity within the brain was suppressing the sympathetic nervous system (SNS), which is ordinarily regulated by leptin levels, and thus reducing bone mass.[29] Therefore, central signalling pathways are implicated in regulating the actions of ghrelin on bone.

The positive contribution of ghrelin to bone mass is also observed in humans, and these actions are largely dependent on other metabolic factors such as hormonal status. Ghrelin was also established to positively correlate to estrogen and BMD in post-menopausal women.[30] Whether the actions of ghrelin were dependent or independent of those of estrogen was not examined.[30] The combination of low ghrelin, low estrogen and low BMD does implicate ghrelin as a component in post-menopausal osteoporosis. Amini *et al.*[17] identified a positive correlation between ghrelin and bone mineral density in women in pre-menopausal and post-menopausal groups. However, they found no association between ghrelin and bone in men,[17] thus suggesting that hormonal status can dictate the influence ghrelin has on bone.

An investigation into obese and non-obese children and adolescents established a negative correlation between ghrelin and bone mass in the normal BMI individuals.[31] Interestingly, no correlations were found in the obese cohort.[31] This suggests that there is diminution in the action of ghrelin under an altered metabolic state such as obesity (that is, increased leptin levels). Therefore, ghrelin supplementation may have a positive influence on bone; however, under altered metabolic conditions the effects of ghrelin may be attenuated.

GIP

The post-prandial peptide GIP has a positive effect on bone. Previous studies utilising *GIPR* $-/-$ mice and GIP-over-expressing models have identified that GIP promotes bone formation and inhibits bone resorption; therefore, GIP is considered to be beneficial to the skeleton.[32–34]

Recently and in agreement with prior studies, Mieczkowska *et al.*[35] published that deficiency of GIPR is detrimental to cortical bone mass and strength.[35] However, Gaudin-Audrain *et al.*[36] observed increased cancellous bone mass in this model, thus suggesting that GIP may be deleterious to cancellous bone. These recent studies used a different *GIPR* $-/-$ model that was accompanied by alterations in leptin and adiponectin levels.[36] Reduced leptin levels affect cortical and cancellous bone differently;[37] therefore, altered adipokine levels in this model may explain the loss of cancellous bone.[36] Furthermore, the change in adipokine levels in this model emphasises the interconnected nature/complexity between gastrointestinal peptides, adipokines and bone metabolism *in vivo*.

GLP-1 and GLP-2

GLP-1 and GLP-2 are post-prandial peptides that regulate both energy and bone homeostasis.[6] Administration of GLP-1 in WT mice had no effect on bone structure; however, in rodent models of type 2 diabetes and insulin resistance, GLP-1 has a positive effect on bone mass.[38] Similarly, the administration of the non-mammalian peptide Exendin-4, a GLP-1 receptor agonist with a longer half-life compared with GLP-1, reduced fat mass and has an anabolic effect on bone.[39,40] Ma *et al.*[41] determined that Exendin-4 increased bone mass in aged ovariectomised rats; thus, Exendin-4 is a potential therapeutic intervention in post-menopausal women.

Like GLP-1, GLP-2 has a positive contribution to bone mass. Subcutaneous injections of GLP-2 reduced circulating bone resorption markers but did not affect bone formation in rodents.

Similarly, administration of GLP-2 in healthy post-menopausal women increased hip and trochanter BMD with a reduction in serum CTX.[42,43] Thus, interest in GLP-2 and bone has turned towards therapeutics that enhances the action of GLP-2. Active GLP-2 has a short half-life and is cleaved by DPP-4; therefore, DPP-4 inhibitors may be used to increase the half-life and effects of GLP-2.[44] Askov-Hansen *et al.*[44] determined that administration of the DPP-4 inhibitor, sitagliptin, prior to subcutaneous GLP-2 injection was the most effective method at increasing the half-life of circulating GLP-2, and thus increasing the exposure time of circulating GLP-2 and suppressing bone resorption marker CTX. However, as noted above, DPP-4 also cleaves PYY1-36 to PYY 3-16; thus, inhibition of DDP-4 activity will increase the signalling through the suppressive Y1, NPY receptor, expressed by osteoblasts,[45] highlighting the complexity involved in modulation of the interrelated aspects of energy homeostasis.

Overall, GLP-1 and GLP-2 contribute positively to bone mass; however, their effects are short-lived; therefore, other pharmacological interventions such as GLP-1R agonists and DPP-4 inhibitors in conjunction with GLP-2 injection are emerging as better candidates for preventing bone resorption.

Adipokines: Leptin

Leptin is a circulating peptide released from adipocytes in proportion to fat mass and acts as a marker of body fuel storage levels. It is positively associated with obesity and acts powerfully to stimulate calorie accrual when leptin levels are reduced. Circulating leptin passes through the blood–brain barrier to bind to its receptor in the hypothalamus. Rodent models with deficiency of leptin or leptin signalling represent a model of constant central starvation, with an obese phenotype and increased levels of the starvation signals, NPY and AgRP (agouti-related peptide), and reduced levels of the satiety signals POMC (pro-opiomelanocortin) and CART.[46] As leptin is secreted from adipocytes from adipose tissue and bone marrow, leptin has both central and peripheral actions on bone.

The role of leptin in bone was established using the leptin-deficient model *ob*/*ob*.[2] These mice had a complex phenotype with increases in cancellous bone mass and decreases in cortical bone mass.[2,4,37] Ducy *et al.*[2] demonstrated that a central pathway was involved in regulating bone mass as the reintroduction of leptin into the brain by intracerebroventricular (icv) injection rescued the bone phenotype of the *ob*/*ob* mice. Conversely, increased central leptin levels reduced the cancellous bone mass in WT mice.[2] Therefore, hypothalamic leptin and its downstream signalling have an important role in regulation of bone metabolism.

Two central (fat–brain–bone) pathways are implicated in regulating bone metabolism, the SNS and the leptin-regulated downstream effector, NPY. Takeda *et al.*[3] demonstrated that central leptin influences cancellous bone mass via the SNS, independently of signals regulating body fat and weight, through actions in the ventromedial hypothalamus. Functional β2-adrenergic receptors were identified on murine and human osteoblasts[3] and the administration of a β2-blocker to WT mice resulted in increased cancellous bone mass.[27,47] No change in cancellous bone mass was found when β2-adrenergic receptor-deficient mice were given an intracerebroventricular (i.c.v.) infusion of leptin.[27] These results prove that autonomic nerves are required for leptin-sensitive hypothalamic neurons to affect bone remodelling. WT osteoblasts treated with a β2-adrenergic agonist and cocultured with WT bone marrow monocytes increased osteoclastogenesis through the modulation of RANKL expression.[27,47] Therefore, leptin acts via the brain and SNS to regulate changes in osteoblastic RANKL expression and subsequently increases osteoclastogenesis and cancellous bone resorption.

Recently, Zhang *et al.*[48] re-examined the effect of propranolol, a β-adrenergic antagonist, on bone metabolism. Ovariectomised (OVX) rats treated with propranolol, a non-selective β1/β2 blocker, had increases in vertebral and femoral BMD,[48] confirming previous studies. Interestingly, these OVX rats also had increases in serum leptin and increased in hypothalamic leptin receptor expression,[48] changes that would be associated with a stimulation of anabolism by central pathways (see below). The regulation of bone mass by adrenergic signalling is more complex, with β1 deletion reversing the high bone mass and greater bone anabolic activity of β2 knockout mice.[47] The β1 response, in contrast to β2, was associated with mechanical stimulation, and thus may relate in part to the envelope-specific phenotype evident in *ob*/*ob* mice.

Within the hypothalamus, the arcuate nucleus is primarily responsible for regulating feeding behaviour. The neurons of the arcuate nucleus have a reduced blood–brain barrier and respond to changes in circulating leptin levels to regulate the transcription of a number of genes, such as NPY. Wong *et al.*[5] recently demonstrated that NPY was an important determinant of cortical bone mass using NPY and leptin-deficient (NPY −/− *ob*/*ob*) mice. These mice had no change in body weight but marked increases in whole-body BMD.[5] Increased cortical bone volume and bone formation indices were present in the femora and vertebrae of NPY −/− *ob*/*ob* mice in comparison with *ob*/*ob* mice.[5] Thus, NPY is a powerful modulator of cortical bone independent of weight-bearing effects. Cancellous bone mass was not altered in the NPY −/− *ob*/*ob* model, indicating that the leptin–NPY pathway is capable of functioning independently of the leptin-β2/SNS adrenergic pathway. However, it is clear that stress-mediated NPY pathways do trigger noradrenergic pathways, with important effects on bone,[11] indicating that context-dependent effects determine the coordination of the NPY/β2 pathways to bone.

Consistent with leptin/NPY signalling in the arcuate, NPY-induced changes in cortical bone mass occur in a centralised manner. The administration of a recombinant adenovirus-associated virus producing NPY (AAV-NPY) in the arcuate nucleus of WT mice led to increases in body weight and adiposity, but with significant reductions in cortical bone formation,[49] as evident in *ob*/*ob* mice.[5,37] A reversal (albeit incomplete) of the bone phenotype was observed when the AAV-NPY was injected into NPY-deficient (NPY −/−) mice.[49] At the level of the osteoblast, NPY inhibits bone formation via Y1 receptors. Osteoblastic Y1 deletion results in increased bone mass.[45] These peripheral Y1 receptors can be blocked by an Y1 antagonist BIBO3304. Administration of BIBO3304 has been demonstrated to increase cancellous bone mass and increase periosteal mineral apposition rate.[50] Therefore, in addition to NPY acting centrally and being regulated by leptin, peripheral intervention at the osteoblast can alter the bone phenotype.

In addition to central actions, leptin can be directly secreted into the bone microenvironment from bone marrow adipocytes.

Treatment of osteoblastic cultures with leptin resulted in increased cell proliferation,[51] increased differentiation via GSK-3β and the WNT pathway,[52] increased mineralisation[53] and a reduction in the *Bax-α/Bcl-2* mRNA ratio, which is indicative of an anti-apoptotic effect.[53] Exposure of osteoblast cultures to leptin increases the RANKL:OPG ratio, thus promoting osteoclastogenesis.[53] Interestingly, leptin's direct action on osteoblasts promotes bone formation, which opposes the central actions of leptin. This enigma of the central and peripheral actions of leptin with opposing actions has been rather controversial.

Our understanding of leptin and its signalling has predominantly been determined using rodent and cell culture models. The role of leptin on bone in humans is not well defined. *Ex vivo* comparisons of human and rodent osteoblasts consistently show that leptin directly promotes bone formation. However, clinical studies examining associations between leptin and bone have produced mixed results, suggesting that leptin can have either a positive association or no association with bone.[54–58] Some of these in consistencies may relate to the well-documented development of leptin resistance in obesity.[59] Interestingly, however, congenitally leptin-deficient children do not display the short stature that has been observed in *ob/ob* mice.[60] Thus, differences in leptin signalling may be present between rodents and humans. Altogether, leptin is a dynamic regulator of bone metabolism acting through multiple pathways.

Adiponectin

Adiponectin is secreted from adipose tissue, and, in contrast to leptin, is negatively correlated with fat mass such that obese individuals have reduced adiponectin levels. Adiponectin and its receptors have been identified in osteoblasts and osteoclasts.[61] However, the effects of adiponectin on bone mass are controversial. Initial reports indicated the potential for opposing direct and indirect effects, with mitogenic effects upon osteoblasts and inhibitory effects on osteoclasts in culture, but greater bone mass in knockout mice.[62]

Consistent with the earlier report, Kajimura *et al.*[61] found that young adiponectin-deficient mice had increased cancellous bone mass. Consistent with these results, overexpression of circulating adiponectin using the *p-Liv-Adiponectin* mouse model demonstrated that adiponectin is detrimental to cancellous bone mass by inhibiting proliferation and promoting apoptosis of osteoblasts.[61] Interestingly, no bone phenotype was present at 24 weeks of age and a reduction in cancellous bone mass was present at 36 weeks of age.[61] Thus, adiponectin was suggested to also mediate an opposing role in central bone homeostasis by reducing SNS activity in older mice.[61]

Wu *et al.*[63] examined the central role of adiponectin in bone homeostasis, by examining the response of WT and adiponectin-deficient mice to i.c.v. infusions of adiponectin beginning at 5 weeks of age and collecting the bones 28 days post cannulation. In this system, centrally administered adiponectin increased the commitment of BMSCs to differentiate into osteoblasts, increased cancellous bone mass and inhibited osteoclast differentiation.[63] In addition, a decrease in sympathetic activity was also observed. The emergence of these recent studies revealing that adiponectin can also centrally regulate bone mass has demonstrated that the relationship between adiponectin and bone is complex and contentious.

Recently, Wang *et al.*[64] demonstrated that mice deficient in adiponectin were protected against ovariectomy-induced vertebral bone loss and that no difference was present between the two genotypes that underwent the sham surgery at 6–8 weeks of age. These ovariectomised, adiponectin-deficient mice had increased alkaline phosphatase expression and protein levels, indicative of increased osteoblast activity, in comparison with ovariectomised WT mice,[64] thus suggesting that adiponectin may have a negative role in post-menopausal osteoporosis.

Meanwhile, a clinical study of 989 elderly men from Sweden demonstrated that serum adiponectin is a risk factor for fracture; however, the predictive value of adiponectin was found to be attenuated with age.[65] The dynamic range of changes to bone mass observed in a range of adiponectin-deficient, adiponectin-overexpressing and adiponectin receptor-deficient rodent models and humans clearly demonstrates that the relationship between whole-body homeostasis and bone metabolism is complex and dependent on the age of the subjects.

Discussion— Interplay between the Factors *In Vivo*

Using transgenic and knockout mouse models, the contribution of each gastrointestinal peptide or adipokine to bone homeostasis has been individually evaluated. However, translating and utilising this research in humans with altered metabolic states such as obesity has been difficult because of the interconnected nature between these gastrointestinal peptides and adipokines and their potential for context-dependent actions.

Although obesity involves an increase in adiposity with elevated circulating leptin levels and reduced adiponectin levels, there are a range of other metabolic disturbances occurring in support of/response to the gains in adipose mass. The relationship between obesity and bone mass has been controversial, with evidence towards obesity being beneficial because of the weight-loading effects; however, the detrimental effects of obesity on bone are now emerging as a greater concern, likely associated with greater incidence of extreme obesity and obesity occurring from early in life.

Several studies have examined the effects of diet-induced obesity on bone in rodents to report that cancellous bone mass in femurs and tibiae is reduced following 10–14 weeks on a high-fat diet.[66–68] The effects of diet-induced obesity are greater in immature mice than in older mice, as examined by Inzana *et al.*,[69] who demonstrated that switching mice to a chow diet following a period of high-fat diet was able to restore the bone phenotype of older mice butnot immature mice. This suggests that high-fat diets cause greater damage in growing bones, which is concerning considering the current increases in the rate of childhood obesity in Western countries.

The relationship between obesity and bone homeostasis is also likely confounded by the complexity of leptin's actions in obesity, which range from increased leptin signalling to the presence of varying degrees of leptin resistance in the periphery and in the hypothalamus.[59] As a result, reduced leptin signalling, a powerful starvation signal, and one associated with reduced cortical and greater cancellous bone mass, may be in effect, despite the elevated leptin exposure created by obesity. Although leptin resistance has been well described in hypothalamic nuclei, the potential for bone cells to become leptin resistant has not been clearly examined.

Another area of active research is the response of gastrointestinal peptides to bariatric surgery. These responses have great interest in terms of obesity and glycaemic control; however, they are also likely to shed light upon their influence upon bone mass (reviewed in Brzozowska *et al.*[70]). Given the increase in prevalence of such surgical procedures world-wide, and moreover their increasing use in adolescents, the effects upon bone and the mechanisms involved are an important issue.

Conclusion

Overall, the relationships between gastrointestinal peptides, adipokines and bone metabolism clearly highlight the complexity involved in maintaining whole-body homeostasis. Recently, our knowledge of their roles has broadened and has better helped in understanding their context-specific effects. Although the effect or mechanism of some hormones may be unresolved with respect to their influence on bone, several potential therapeutic agents have been identified for further exploration. Thus, the relationship between feeding and bone metabolism is an area of continued research, which will further extend our understanding of metabolic disorders and their subsequent influence on bone homeostasis.

Conflict of Interest

The authors declare no conflict of interest.

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