Gastrointestinal peptides and bone health

Iris P.L. Wong[a], Paul A. Baldock[a] and Herbert Herzog[b]

[a]Osteoporosis and Bone Biology Program and [b]Neuroscience Research Program, Garvan Institute of Medical Research, St. Vincent's Hospital, Sydney, New South Wales, Australia

Correspondence to Herbert Herzog, PhD, Neuroscience Research Program, Garvan Institute of Medical Research, St. Vincent's Hospital, 384 Victoria Street, Darlinghurst, Sydney, NSW 2010, Australia
Tel: +61 2 9295 8296; fax: +61 2 9295 8281; e-mail: h.herzog@garvan.org.au

Current Opinion in Endocrinology, Diabetes & Obesity 2010, 17:44–50

Purpose of review

To outline recent developments in research surrounding gastrointestinal peptides and their role in skeletal regulation.

Recent findings

Bone remodeling is influenced by many regulatory systems, which interact to ensure that the complex demands upon mineralized tissue are met without undue compromise. These include local actions such as mechanical factors, but are dominated by systemic endocrine factors. Although the involvement of hypothalamo-pituitary actions on bone homeostasis is well defined, growing evidence suggests that peripheral tissues and the circulating factors they produce represent an important regulatory axis in bone. Given the critical role of diet in mineral homeostasis, the gastrointestinal tract is a rich source of circulating factors capable of regulating bone homeostasis. After a review of manuscripts on known mechanisms and effects of gastrointestinal peptide on bone, these were summarized.

Summary

Although clearly an exciting and emergent field of research, more studies are required to define the specific actions of gastrointestinal regulator in bone, in particular, the relative contribution of systemic and local effects, to aid interpretation of their potential impact on human health and disease. Nonetheless, this exciting research will further our understanding on bone physiology and provide novel approaches to therapy in a wide range of skeletal conditions.

Keywords

anorexia, bone, diabetes, gastrointestinal peptide, obesity

Curr Opin Endocrinol Diabetes Obes 17:44–50

1752-296X

Introduction

In recent years, appreciation of the influence of gastrointestinal peptides on bone mass has increased. It represents one aspect of the broadening of contemporary skeletal research and, with it, our understanding of the complexity of interactions that govern the skeletal system. Indeed, gastrointestinal regulation of bone encompasses interactions with a number of homeostatic systems, including energy homeostasis and appetite, the neural system and factors associated with higher functions such as mood and depression. Thus, an appreciation of the gastrointestinal/bone interface is vital for an up-to-date understanding of skeletal regulation and its application to a number of aspects of human health.

Ghrelin

Ghrelin, a growth hormone (GH)-releasing peptide synthesized mainly in the stomach [1], is released preprandial, acting as an appetite stimulant [2,3] and suppressed through postgastric feedback after a meal [2,4]. Ghrelin's stimulatory effects on GH secretion via ghrelin receptor (GHS-R) indicate a possible involvement in bone metabolism via the well known GH–insulin-like growth factor (IGF) axis [5]. However, ghrelin infusion into GH-deficient rats increased bone mineral density (BMD) compared with isotonic saline control, similar to what was observed in rats with intact GH signaling, demonstrating a GH-independent effect of ghrelin on bone metabolism [6]. In fact, there is emerging evidence indicating a direct involvement of ghrelin in bone metabolism through actions on osteoblasts.

It has been demonstrated that GHS-R and ghrelin are expressed by osteoblasts *in vitro* [6–8] as well as *in vivo* [9]. Osteoblasts were found to secrete [9] and respond to ghrelin by increased proliferation and differentiation [6,7,9,10•]. The proliferative effects of ghrelin were shown to act via the mitogen-activated protein kinase (MAPK)/phosphoinositide 3-kinase (PI3K) pathways [9] and, more recently, via the intracellular nitric oxide/cyclic GMP (cGMP) signaling pathway [10•]. Together, these findings suggest a direct stimulatory effect of ghrelin on osteoblasts. However, at present, the relative contributions of gastric

and osteoblastic ghrelin to the control of bone mass are yet to be defined.

Interestingly, in contrast to the increased BMD observed in ghrelin-infused rats [6], ghrelin knockout mice have unaltered BMD and bone mineral content (BMC) as well as other metabolic effects of ghrelin, indicating that compensatory pathways exist to counter ghrelin deficiency [11]. Similar effects were observed in GHS-R knockout mice with no significant changes in BMD and BMC, despite having suppressed IGF-1 serum levels [12]. The ability of these compensatory pathways to act in postdevelopmental, conditional models of ghrelin deficiency is unknown.

Ghrelin secretion is downregulated in obesity and is upregulated under conditions of negative energy balance such as anorexia nervosa [13,14], and its actions may be influenced by prevailing metabolic tone. The inverse association between ghrelin and bone mass in anorexia nervosa, however, is in contrast to in-vitro as well as animal studies demonstrating a positive effect of ghrelin on osteoblast activity and bone density [6,7]. It is reasoned that this inverse association may be a consequence of ghrelin-stimulated increases in adrenocorticotropic hormone (ACTH) and therefore cortisol, with deleterious effects on bone, whereas ghrelin-stimulated increases in GH are not associated with bone anabolic effects because of GH resistance in anorexia nervosa [15••].

The two distinct isoforms of ghrelin may influence BMD in a different manner in obese and healthy individuals. In healthy children, acylated ghrelin was a negative predictor of whole body BMD. In contrast, a positive association was observed between des-acyl ghrelin and whole body BMD in obese children [16]. In boys, ghrelin concentration decreased during puberty, while regular physical activity maintained ghrelin levels. Thus, in addition to actions associated with obesity, ghrelin appears to be an important hormonal predictor for BMD in physically active boys, whereas BMD is mostly determined by IGF-1 in physically inactive boys [17]. Menopause stages and the underlying follicle-stimulating hormone (FSH) changes are associated with notable changes in levels of adipocytokines and ghrelin. Ghrelin levels were higher in the perimenopause in both obese and nonobese women. The association between the difference in ghrelin in the perimenopause and bone loss around the final menstrual period awaits further investigation [18].

Peptide YY and pancreatic polypeptide

Peptide YY (PYY) and pancreatic polypeptide are members of the neuropeptide Y (NPY) family. They are released in response to feeding primarily by L cells in the ileum and colon and PP cells in the pancreas, respectively [19]. There are two major forms of circulating PYY, the full length PYY_{1-36} and the most abundant form PYY_{3-36}. Both of these forms bind to the NPY Y2 receptor with high affinity.

In PYY knockout mice, a reduction in total BMD and BMC was detected accompanied by a functional deficit in bone strength [20]. Analysis of the lumbar vertebrae showed a reduction in cancellous bone mass and volume. In contrast, mice deficient in Y2 receptor, the receptor for both PYY_{1-36} and PYY_{3-36}, have a two-fold increase in cancellous bone volume and cortical bone mass [21]. The skeletal effects of Y2 receptor deficiency were isolated to hypothalamic receptors, suggesting that PYY may act in an alternate region to affect bone.

In humans, several disorders associated with altered PYY levels also have altered bone metabolism. Obesity, as measured by greater BMI, is associated with lower levels of PYY than controls [22] and greater BMD [23]. Anorexia nervosa patients associated with a deliberate reduction in food intake and low bone density have greater PYY levels compared with normal-weight controls [14], although no significant difference was observed in a smaller study [24]. In anorexia nervosa, this elevated PYY may contribute to the decrease in bone turnover, as measured by serum and urinary markers [14], and the diminished BMD, particularly at the spine [25]. Interestingly, baseline PYY levels are inversely associated with subsequent changes in whole body bone mass in anorexia nervosa patients [15••], consistent with the antiosteogenic actions of the Y2 receptor, described above [21]. Amenorrheic athletes who display hypogonadism and lower BMD compared with eumenorrheic athletes also have higher PYY levels [26•]. PYY was found to be a negative predictor of aminoterminal propeptide of type 1 collagen (PINP), a bone formation serum marker, and lumbar bone mineral apparent density *Z*-scores in this model. Together, these disorders seem to suggest a negative correlation between PYY levels and BMD; however, whether these indicate an effect of PYY on bone metabolism or, more likely, a result of numerous effects of the response to altered energy metabolism remains to be determined.

Pancreatic polypeptide and its receptor, the NPY Y4 receptor, may play a role in osteoblast differentiation. Both pancreatic polypeptide and Y4 receptor are found in MC3T3-E1 cells, a murine transformed osteoblastic cell line, and pancreatic polypeptide treatment stimulates differentiation of MC3T3-E1, suggesting a role of pancreatic polypeptide in bone [27]. This is supported by findings from Y4 receptor knockout mice, in which osteoblast number is decreased [28]. However, this role in osteoblast differentiation does not seem to translate into a

change in bone mass in mouse models. Y4 receptor knockout mice, pancreatic polypeptide transgenic mice [28] and pancreatic polypeptide knockout mice [20] all have unaltered bone mass. In addition, pancreatic polypeptide overexpression does not alter bone turnover, including osteoblast surface or number [28]. Interestingly, $Y2^{-/-}$ $Y4^{-/-}$ double knockout mice result in greater increases in trabecular number and cancellous bone volume than in mice with deficiency of either the Y2 or Y4 receptor alone [28]. Again, lineage-specific effects of pancreatic polypeptide on bone are yet to be investigated.

Serotonin

One of the most important discoveries in recent years in skeletal research involved the elucidation of the role of Wnt signaling in the regulation of bone mass. Wnt signaling has emerged as a critical regulator of bone modeling and remodeling, acting through the low-density lipoprotein-related receptors 5 and 6 (Lrp5/6), which serve as coreceptors for the frizzled family of Wnt receptors.

Osteoporosis pseudoglioma (OPPG) is a rare syndrome associated with a dramatic reduction in skeletal mass, an increase in fracture and progressive blindness. Inactivating mutations in Lrp5 were identified as the causative genetic basis for OPPG [29]. Consistent with the role of Lrp5 in bone, a point mutation in Lrp5 (G171V) was present in affected individuals of families showing an autosomally dominant high bone mass trait. So marked are the Lrp5 effects on bone, that these two genetically independent families have BMD 5 SD above that of unaffected family members and the general population [30,31]. Lrp5 is expressed by cells in the osteoblast lineage, and much of the subsequent work was focused on this cell type as the central mediator of its actions on bone. Deletion of Lrp5 in mice leads to low bone mass phenotype [32], whereas deletion of b-catenin, a key downstream mediator of the canonical Wnt pathway, in differentiated osteoblasts caused severe osteopenia [33,34].

However, Lrp5 is widely expressed, and the Wnt proteins have wide tissue distributions. Indeed, the importance to bone mass of Lrp5-mediated signaling within osteoblasts now requires reconsideration based on a recent study examining the role of gastrointestinal processes on the regulation of Lrp5 activity. In particular, findings by Yadav *et al.* [35••] challenge the osteoblastic model of Lrp5 action. Targeted deletion of Lrp5 in the duodenal enterochromaffin cells of mice resulted in high circulating levels of serotonin caused by upregulation of the rate-limiting enzyme for serotonin synthesis, tryptophan hydroxylase 1 (Tph1). Dietary restriction of tryptophan reduced circulating serotonin in $Lrp5^{-/-}$ mice and, importantly, normalized the suppressed bone formation parameters in these animals. This result suggested that the increase in serotonin production might be responsible for this effect. Consistent with this idea, restricted expression of Lrp5 G171V in the duodenum resulted in a high bone mass phenotype. Moreover, selective deletion of the serotonin receptor Htr1b in osteoblasts resulted in a high bone mass phenotype, indicating that serotonin mediates its actions in osteoblasts via this receptor. These data reveal that serotonin may play an important role in bone metabolism via a novel endocrine loop from the gastrointestinal tract to the bone.

Clinical studies have confirmed a role for serotonin signaling in the regulation of bone mass. Serotonin is known to play a role in the pathophysiology of depression, and many antidepressant medications function by inhibiting the serotonin transporter, known as selective serotonin reuptake inhibitors, SSRIs. Several studies have indicated increased bone loss [36], reduced bone mass [37] and an increased risk of fracture in patients taking SSRIs [38]. Consistent with these clinical findings, mice with genetic disruption of the 5-hydroxy-tryptamine (5-HTT) display a skeletal phenotype of altered architecture, reduced mass and mechanical properties, whereas bone mineral accrual was impaired in growing mice treated with an SSRI [39].

Amylin, adrenomedullin and preptin

Amylin and adrenomedullin are members of the calcitonin family of peptide hormones that share structural similarities with calcitonin. Whereas amylin and preptin are cosecreted with insulin from the pancreatic β-cell, adrenomedullin is colocalized with pancreatic polypeptide and secreted from the F cells of the pancreatic islets to inhibit insulin secretion [40].

Amylin can act as an osteoblast mitogen [41] and as an inhibitor of osteoclast activity and differentiation *in vitro* [42]. Systemic administration of amylin resulted in an anabolic effect on bone [43–45]. In addition, amylin treatment in hens increased bone calcium levels while decreased serum calcium levels, suggesting that amylin may improve bone quality by increasing calcium uptake from the bloodstream [46]. Mice with amylin inactivation show a low bone mass phenotype, with increased biochemical and histological indices of bone resorption, but no apparent defect in osteoblast differentiation or function [47]. Further studies demonstrated sex-dependent effects of amylin deficiency on bone during growth [48].

In humans, low levels of amylin have been associated with a range of conditions with reduced BMD. Type I diabetes mellitus (T1DM) is associated with total or near total absence of amylin [49]. Fasting plasma levels of

amylin were lower in patients with osteoporosis than in patients with type II diabetes mellitus and healthy controls [50]. Aging has also been shown to be associated with impairment of amylin release [51]. However, treatment of T1DM without osteopenia for 1 year with the synthetic analogue of amylin, pramlintide, had no effect on BMD or bone markers [52]. Nonetheless, further studies are needed to evaluate whether pramlintide may improve bone mass in patients with reduced BMD.

Adrenomedullin is also anabolic to bone, stimulating osteoblast proliferation *in vitro* and increasing indices of bone formation *in vivo* [53]. Adrenomedullin-deficient mice are not viable [54]; however, the presence of adrenomedullin and adrenomedullin receptors on cultured osteoblasts implies that it may be a local regulator of osteoblast proliferation in an autocrine/paracrine manner via the cyclic AMP (cAMP) pathway [55,56]. The proliferative effect of adrenomedullin and amylin is dependent on the presence of the IGF-1 receptor [57], suggesting an overlapping mechanism of action by which amylin, adrenomedullin and IGF-1 induce osteoblasts proliferation. Apart from its effect on osteoblast proliferation, adrenomedullin has also been shown to inhibit osteoblast apoptosis [58]. However, this antiapoptotic action of adrenomedullin on osteoblasts is mediated via extracellular signal-regulated kinase (ERK) signaling through calcitonin gene-related peptide (CGRP1) receptor and not through adrenomedullin receptor [58]. In contrast to amylin, adrenomedullin does not seem to have an inhibitory effect on osteoclasts [53].

Preptin, a 34-amino acid peptide product of the pancreatic β-cell [59], has also emerged as a regulatory element in bone metabolism. Preptin treatment stimulates osteoblast proliferation and differentiation *in vitro* [60], mediated by ERK/connective tissue growth factor [61]. Daily subcutaneous injections of preptin over the right hemicalvaria increased bone area and mineralizing surface in mice [60]. As preptin is cosecreted with insulin and amylin from the pancreatic β-cell, it may also contribute to the preservation of bone mass observed in hyperinsulinemic states such as obesity. The anabolic activity of preptin on bone is likely to contribute to the development of osteosclerosis in some patients with hepatitis C. Immunoactivity of pro-IGF-2, which contains the preptin sequence, is increased in this condition, whereas excess of other forms of pro-IGF-2 that do not contain the preptin sequence is not associated with increased bone mass [62].

Glucose-dependent insulinotropic polypeptide

Glucose-dependent insulinotropic peptide (GIP) is a 42-amino acid peptide synthesized and secreted from duodenal endocrine K cells after absorption of glucose or fat. GIP receptors (GIPRs) were present on osteoblasts, osteocytes and osteoclasts as demonstrated in normal bone and cell lines [63,64]. Pharmacological doses of GIP have been shown to regulate bone turnover *in vitro* and *in vivo*. GIP treatment stimulates cAMP and increases intracellular calcium, resulting in an increase in bone formation marker and alkaline phosphatase activity *in vitro* [63,65]. In addition, GIP also inhibits osteoclastic differentiation and activity using both cell and organ culture systems [64]. In-vivo studies showed that daily GIP administration prevents ovariectomy-induced bone loss in rats [66].

Effects evident in genetically modified mouse models support an anabolic effect of GIP on bone. GIP overexpression resulted in a significant increase in bone mass accompanied by elevated bone formation and reduced bone resorption [67]. In contrast, mice lacking GIPR ($GIPR^{-/-}$) had a significant decrease in bone mass accompanied by reduced bone formation markers and no change in bone resorption marker [68]. Consistent with previous findings, bone histomorphometric analyses revealed that $GIPR^{-/-}$ mice have decreased osteoblastic mineral apposition rate and increased osteoclast number [69]. Interestingly, $GIPR^{-/-}$ mice exhibited an increased plasma calcium concentration after meal ingestion, suggesting that GIP may promote the efficient storage of ingested calcium into bone, linking calcium contained in meal to calcium deposition on bone [69]. In addition, expression of GIPR decreases in an age-dependent manner, and age-related loss of bone mass and bone strength is prevented in GIP transgenic mice [65]. Therefore, it is proposed that decreases in GIPR expression may play a pathophysiological role in age-related bone loss, and that GIP may be an effective countermeasure.

Human studies of GIP are potentially confounded by experimental design, particularly the absence of placebo groups. However, it was reported that pharmacological doses of glucagon-like peptide (GLP)-2, but not GIP or GLP-1, acutely reduced markers of bone resorption in humans [70].

Glucagon-like peptide

GLP-1 and GLP-2, two peptides with 50% homology to glucagon, are synthesised by cleavage of proglucagon. GLP-1 and GLP-2 are cosecreted in response to nutrient ingestion from the intestinal L cells that are abundant in the distal jejunum, ileum and colon. However, only GLP-1 is capable of stimulating insulin secretion, whereas GLP-2 acts in the intestine to stimulate mucosal growth and nutrient absorption.

The effect of GLP-2 on bone has been investigated predominantly in humans. In postmenopausal women,

subcutaneous injection of GLP-2 produces a dose-dependent decrease in bone resorption markers [70,71], whereas bone formation appears to be unaffected. Similar effects were observed with repeated parental administration of GLP-2 for 14 days and up to 4 months [72,73], changing the bone remodeling balance in favor of bone formation.

Consistent with a role of GLP-2 on postprandial inhibition of bone resorption, patients with short bowel syndrome (SBS) and no colon, who did not secrete GLP-2 in response to meal ingestion, showed no postprandial reduction in bone resorption markers [74]. However, this antiresorption response of GLP-2 seems to require an intact small intestine, as GLP-2 treatment for 56 days reduces serum C-terminal telopeptide region of type I collagen (s-CTX) only in colectomized patients with distal ileostomy and not in colectomized patients with jejunostomy and SBS [75]. The mechanism(s) underlying the GLP-2-mediated modulation of bone turnover remain unclear, although suppression of parathyroid hormone (PTH) secretion has been proposed. Given this inhibitory effect of GLP-2 on bone resorption markers, BMD, however, did not change in a 2-year longitudinal study [76••] on a small group of SBS patients.

In contrast to information of GLP-2 on bone formation and resorption, which is derived mainly from human studies, the physiological role of GLP-1 on bone was only investigated recently in rodents. GLP-1 receptor (GLP-1R) knockout mice have cortical osteopenia and bone fragility by bone densitometry as well as increased osteoclastic numbers and bone resorption activity by bone histomorphometry [77]. However, GLP-1R has not been identified on osteoblast or osteoclast. Moreover, GLP-1 has no direct effect on osteoblasts and osteoclasts in culture [77], suggesting an indirect role of GLP-1 on bone. Endogenous GLP-1R signaling in the control of bone resorption is likely to act through a calcitonin-dependent pathway as supported by several following observations. First, GLP-1R is expressed in thyroid C cells that synthesize calcitonin, a potent inhibitor of osteoclastic bone resorption. Second, GLP-1 and GLP-1R agonist, exendin-4 (Ex-4), increased calcitonin mRNA in the thyroid and directly stimulates the secretion of calcitonin [77–79]. Third, GLP-1R knockout mice have reduced levels of calcitonin mRNA in the thyroid whereas calcitonin treatment effectively suppressed elevation of urinary bone resorption marker [77]. Rats treated with GLP-1 or Ex-4 for 3 days via subcutaneously implanted osmotic pump displayed elevated expression of osteoblastic genes in bone tissue of wild-type as well as rat models with glucose intolerance (streptozotocin-induced type 2 diabetic and fructose-induced insulin-resistant), without any change in plasma glucose and insulin after treatment [80,81].

Cholecystokinin

Cholecystokinin (CCK) is synthesized by I-cells in the mucosal epithelium of the small intestine, secreted in the duodenum and causes the release of digestive enzymes and bile from the pancreas and gallbladder, respectively, to stimulate the digestion of fat and protein. It also acts as a hunger suppressant. CCK binds to two specific receptors, CCK1R and CCK2R, which belong to the G-protein-coupled receptor superfamily [82].

To date, no study directly links CCK to bone regulation. However, there are indications from cancer studies that CCK may play a role in Ewing tumors, a group of highly malignant tumors arising mainly in the bone. CCK is upregulated in Ewing tumor cells, and knockdown of CCK using specific small interference RNA impaired cell proliferation and tumor growth *in vivo*, suggesting that CCK acts as an autocrine growth factor in Ewing tumor cells [83]. Further studies showed that devazepide, a nonpeptide antagonist of CCK1R, but not L365260, a nonpeptide antagonist of CCK2R, inhibits growth of Ewing tumor cells both *in vitro* and *in vivo* by inducing apoptosis [84].

Conclusion

The field of skeletal biology is undergoing a period of notable expansion at present, with a growing appreciation of the breadth and complexity of factors that interact to govern the production and maintenance of bone mass. Once considered a tissue dominated by pituitary hormones, mechanical strain and mineral balance, bone is now recognized as being closely tied to a number of additional regulatory systems, including metabolic and gastrointestinal processes. This review outlines a number of gastrointestinal regulators that have been implicated in the control of bone homeostasis. Although clearly an exciting and emergent field of research, more studies are required to define their specific actions in bone. In particular, the relative contribution of the systemic and local production of many of these compounds is yet to be defined. Such information will be critical to interpretation of their potential impact in human health and disease. However, it appears certain that this exciting research will make important contributions to our knowledge of bone physiology and provide novel approaches to therapy in a wide range of skeletal conditions.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 99).

1 Kojima M, Hosoda H, Date Y, *et al.* Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 1999; 402:656–660.

2 Ariyasu H, Takaya K, Tagami T, *et al.* Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. J Clinical Endocrinol Metab 2001; 86:4753–4758.

3 Cummings DE, Purnell JQ, Frayo RS, *et al.* A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 2001; 50:1714–1719.

4 Williams DL, Cummings DE, Grill HJ, Kaplan JM. Meal-related ghrelin suppression requires postgastric feedback. Endocrinology 2003; 144:2765–2767.

5 Ohlsson C, Bengtsson BA, Isaksson OG, *et al.* Growth hormone and bone. Endocr Rev 1998; 19:55–79.

6 Fukushima N, Hanada R, Teranishi H, *et al.* Ghrelin directly regulates bone formation. J Bone Miner Res 2005; 20:790–798.

7 Maccarinelli G, Sibilia V, Torsello A, *et al.* Ghrelin regulates proliferation and differentiation of osteoblastic cells. J Endocrinol 2005; 184:249–256.

8 Kim SW, Her SJ, Park SJ, *et al.* Ghrelin stimulates proliferation and differentiation and inhibits apoptosis in osteoblastic MC3T3-E1 cells. Bone 2005; 37:359–369.

9 Delhanty PJ, van der Eerden BC, van der Velde M, *et al.* Ghrelin and unacylated ghrelin stimulate human osteoblast growth via mitogen-activated protein kinase (MAPK)/phosphoinositide 3-kinase (PI3K) pathways in the absence of GHS-R1a. J Endocrinol 2006; 188:37–47.

10 • Wang DH, Hu YS, Du JJ, *et al.* Ghrelin stimulates proliferation of human osteoblastic TE85 cells via NO/cGMP signaling pathway. Endocrine 2009; 35:112–117.
Novel signaling pathway of ghrelin on osteoblast.

11 Sun Y, Ahmed S, Smith RG. Deletion of ghrelin impairs neither growth nor appetite. Mol Cell Biol 2003; 23:7973–7981.

12 Sun Y, Wang P, Zheng H, Smith RG. Ghrelin stimulation of growth hormone release and appetite is mediated through the growth hormone secretagogue receptor. Proc Natl Acad Sci U S A 2004; 101:4679–4684.

13 Shiiya T, Nakazato M, Mizuta M, *et al.* Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. J Clin Endocrinol Metab 2002; 87:240–244.

14 Misra M, Miller KK, Tsai P, *et al.* Elevated peptide YY levels in adolescent girls with anorexia nervosa. J Clin Endocrinol Metab 2006; 91:1027–1033.

15 •• Misra M, Prabhakaran R, Miller KK, *et al.* Prognostic indicators of changes in bone density measures in adolescent girls with anorexia nervosa-II. J Clin Endocrinol Metab 2008; 93:1292–1297.
This study reveals PYY as one of the predictors of changes in BMD in anorexia nervosa patients.

16 Pacifico L, Anania C, Poggiogalle E, *et al.* Relationships of acylated and desacyl ghrelin levels to bone mineralization in obese children and adolescents. Bone 2009; 45:274–279.

17 Jurimae J, Cicchella A, Tillmann V, *et al.* Effect of pubertal development and physical activity on plasma ghrelin concentration in boys. J Endocrinol Invest 2009; 32:18–22.

18 Sowers MR, Wildman RP, Mancuso P, *et al.* Change in adipocytokines and ghrelin with menopause. Maturitas 2008; 59:149–157.

19 Hazelwood RL. The pancreatic polypeptide (PP-fold) family: gastrointestinal, vascular, and feeding behavioral implications. Proc Soc Exp Biol Med 1993; 202:44–63.

20 Wortley KE, Garcia K, Okamoto H, *et al.* Peptide YY regulates bone turnover in rodents. Gastroenterology 2007; 133:1534–1543.

21 Baldock PA, Sainsbury A, Couzens M, *et al.* Hypothalamic Y2 receptors regulate bone formation. J Clin Invest 2002; 109:915–921.

22 Batterham RL, Cohen MA, Ellis SM, *et al.* Inhibition of food intake in obese subjects by peptide YY3-36. N Engl J Med 2003; 349:941–948.

23 Reid IR. Relationships among body mass, its components, and bone. Bone 2002; 31:547–555.

24 Stock S, Leichner P, Wong AC, *et al.* Ghrelin, peptide YY, glucose-dependent insulinotropic polypeptide, and hunger responses to a mixed meal in anorexic, obese, and control female adolescents. J Clin Endocrinol Metab 2005; 90:2161–2168.

25 Utz AL, Lawson EA, Misra M, *et al.* Peptide YY (PYY) levels and bone mineral density (BMD) in women with anorexia nervosa. Bone 2008; 43:135–139.

26 • Russell M, Stark J, Nayak S, *et al.* Peptide YY in adolescent athletes with amenorrhea, eumenorrheic athletes and nonathletic controls. Bone 2009; 45:104–109.
This study reveals an association between PYY and amenorrheic athletes.

27 Hosaka H, Nagata A, Yoshida T, *et al.* Pancreatic polypeptide is secreted from and controls differentiation through its specific receptors in osteoblastic MC3T3-E1 cells. Peptides 2008; 29:1390–1395.

28 Sainsbury A, Baldock PA, Schwarzer C, *et al.* Synergistic effects of Y2 and Y4 receptors on adiposity and bone mass revealed in double knockout mice. Mol Cell Biol 2003; 23:5225–5233.

29 Gong Y, Slee RB, Fukai N, *et al.* LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell 2001; 107:513–523.

30 Boyden LM, Mao J, Belsky J, *et al.* High bone density due to a mutation in LDL-receptor-related protein 5. N Engl J Med 2002; 346:1513–1521.

31 Little RD, Carulli JP, Del Mastro RG, *et al.* A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone-mass trait. Am J Hum Genet 2002; 70:11–19.

32 Kato M, Patel MS, Levasseur R, *et al.* Cbfa1-independent decrease in osteoblast proliferation, osteopenia, and persistent embryonic eye vascularization in mice deficient in Lrp5, a Wnt coreceptor. J Cell Biol 2002; 157:303–314.

33 Glass DA 2nd, Bialek P, Ahn JD, *et al.* Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. Dev Cell 2005; 8:751–764.

34 Holmen SL, Zylstra CR, Mukherjee A, *et al.* Essential role of beta-catenin in postnatal bone acquisition. J Biol Chem 2005; 280:21162–21168.

35 •• Yadav VK, Ryu JH, Suda N, *et al.* Lrp5 controls bone formation by inhibiting serotonin synthesis in the duodenum. Cell 2008; 135:825–837.
A novel endocrine loop from the gastrointestinal tract to the bone.

36 Diem SJ, Blackwell TL, Stone KL, *et al.* Use of antidepressants and rates of hip bone loss in older women: the study of osteoporotic fractures. Arch Intern Med 2007; 167:1240–1245.

37 Richards JB, Papaioannou A, Adachi JD, *et al.* Effect of selective serotonin reuptake inhibitors on the risk of fracture. Arch Intern Med 2007; 167:188–194.

38 Liu B, Anderson G, Mittmann N, *et al.* Use of selective serotonin-reuptake inhibitors of tricyclic antidepressants and risk of hip fractures in elderly people. Lancet 1998; 351:1303–1307.

39 Warden SJ, Robling AG, Sanders MS, *et al.* Inhibition of the serotonin (5-hydroxytryptamine) transporter reduces bone accrual during growth. Endocrinology 2005; 146:685–693.

40 Martinez A, Weaver C, Lopez J, *et al.* Regulation of insulin secretion and blood glucose metabolism by adrenomedullin. Endocrinology 1996; 137:2626–2632.

41 Cornish J, Callon KE, Cooper GJ, Reid IR. Amylin stimulates osteoblast proliferation and increases mineralized bone volume in adult mice. Biochem Biophys Res Commun 1995; 207:133–139.

42 Cornish J, Callon KE, Bava U, *et al.* Effects of calcitonin, amylin, and calcitonin gene-related peptide on osteoclast development. Bone 2001; 29:162–168.

43 Cornish J, Callon KE, King AR, *et al.* Systemic administration of amylin increases bone mass, linear growth, and adiposity in adult male mice. Am J Physiol 1998; 275 (4 Pt 1):E694–E699.

44 Horcajada-Molteni MN, Davicco MJ, Lebecque P, *et al.* Amylin inhibits ovariectomy-induced bone loss in rats. J Endocrinol 2000; 165:663–668.

45 Horcajada-Molteni MN, Chanteranne B, Lebecque P, *et al.* Amylin and bone metabolism in streptozotocin-induced diabetic rats. J Bone Miner Res 2001; 16:958–965.

46 Guzel S, Gunes N, Yildiz H, Yilmaz B. Effects of amylin on bone development and egg production in hens. Poult Sci 2009; 88:1719–1724.

47 Dacquin R, Davey RA, Laplace C, *et al.* Amylin inhibits bone resorption while the calcitonin receptor controls bone formation in vivo. J Cell Biol 2004; 164:509–514.

48 Davey RA, Moore AJ, Chiu MW, *et al.* Effects of amylin deficiency on trabecular bone in young mice are sex-dependent. Calcif Tissue Int 2006; 78:398–403.

49 Schwartz AV. Diabetes mellitus: does it affect bone? Calcif Tissue Int 2003; 73:515–519.

50 Bronsky J, Prusa R. Amylin fasting plasma levels are decreased in patients with osteoporosis. Osteoporos Int 2004; 15:243–247.

51 Dechenes CJ, Verchere CB, Andrikopoulos S, Kahn SE. Human aging is associated with parallel reductions in insulin and amylin release. Am J Physiol 1998; 275 (5 Pt 1):E785–E791.

52 Borm AK, Klevesath MS, Borcea V, *et al.* The effect of pramlintide (amylin analogue) treatment on bone metabolism and bone density in patients with type 1 diabetes mellitus. Horm Metab Res 1999; 31:472–475.

53 Cornish J, Callon KE, Coy DH, *et al.* Adrenomedullin is a potent stimulator of osteoblastic activity in vitro and in vivo. Am J Physiol 1997; 273 (6 Pt 1): E1113–E1120.

54 Caron KM, Smithies O. Extreme hydrops fetalis and cardiovascular abnormalities in mice lacking a functional adrenomedullin gene. Proc Natl Acad Sci U S A 2001; 98:615–619.

55 Naot D, Callon KE, Grey A, *et al.* A potential role for adrenomedullin as a local regulator of bone growth. Endocrinology 2001; 142:1849–1857.

56 Hamada H, Kitamura K, Chosa E, *et al.* Adrenomedullin stimulates the growth of cultured normal human osteoblasts as an autocrine/paracine regulator. Peptides 2002; 23:2163–2168.

57 Cornish J, Grey A, Callon KE, *et al.* Shared pathways of osteoblast mitogenesis induced by amylin, adrenomedullin, and IGF-1. Biochem Biophys Res Commun 2004; 318:240–246.

58 Uzan B, Villemin A, Garel JM, Cressent M. Adrenomedullin is antiapoptotic in osteoblasts through CGRP1 receptors and MEK-ERK pathway. J Cell Physiol 2008; 215:122–128.

59 Buchanan CM, Phillips AR, Cooper GJ. Preptin derived from proinsulin-like growth factor II (proIGF-II) is secreted from pancreatic islet beta-cells and enhances insulin secretion. Biochem J 2001; 360 (Pt 2):431–439.

60 Cornish J, Callon KE, Bava U, *et al.* Preptin, another peptide product of the pancreatic beta-cell, is osteogenic in vitro and in vivo. Am J Physiol 2007; 292:E117–E122.

61 Liu YS, Lu Y, Liu W, *et al.* Connective tissue growth factor is a downstream mediator for preptin-induced proliferation and differentiation in human osteoblasts. Amino Acids 2009. [Epub ahead of print]

62 Khosla S, Ballard FJ, Conover CA. Use of site-specific antibodies to characterize the circulating form of big insulin-like growth factor II in patients with hepatitis C-associated osteosclerosis. J Clin Endocrinol Metab 2002; 87:3867–3870.

63 Bollag RJ, Zhong Q, Phillips P, *et al.* Osteoblast-derived cells express functional glucose-dependent insulinotropic peptide receptors. Endocrinology 2000; 141:1228–1235.

64 Zhong Q, Itokawa T, Sridhar S, *et al.* Effects of glucose-dependent insulinotropic peptide on osteoclast function. Am J Physiol 2007; 292:E543–E548.

65 Ding KH, Shi XM, Zhong Q, *et al.* Impact of glucose-dependent insulinotropic peptide on age-induced bone loss. J Bone Miner Res 2008; 23: 536–543.

66 Bollag RJ, Zhong Q, Ding KH, *et al.* Glucose-dependent insulinotropic peptide is an integrative hormone with osteotropic effects. Mol Cell Endocrinol 2001; 177:35–41.

67 Xie D, Zhong Q, Ding KH, *et al.* Glucose-dependent insulinotropic peptide-overexpressing transgenic mice have increased bone mass. Bone 2007; 40:1352–1360.

68 Xie D, Cheng H, Hamrick M, *et al.* Glucose-dependent insulinotropic polypeptide receptor knockout mice have altered bone turnover. Bone 2005; 37:759–769.

69 Tsukiyama K, Yamada Y, Yamada C, *et al.* Gastric inhibitory polypeptide as an endogenous factor promoting new bone formation after food ingestion. Mol Endocrinol 2006; 20:1644–1651.

70 Henriksen DB, Alexandersen P, Bjarnason NH, *et al.* Role of gastrointestinal hormones in postprandial reduction of bone resorption. J Bone Miner Res 2003; 18:2180–2189.

71 Henriksen DB, Alexandersen P, Byrjalsen I, *et al.* Reduction of nocturnal rise in bone resorption by subcutaneous GLP-2. Bone 2004; 34:140–147.

72 Henriksen DB, Alexandersen P, Hartmann B, *et al.* Disassociation of bone resorption and formation by GLP-2: a 14-day study in healthy postmenopausal women. Bone 2007; 40:723–729.

73 Henriksen DB, Alexandersen P, Hartmann B, *et al.* Four-month treatment with GLP-2 significantly increases hip BMD: a randomized, placebo-controlled, dose-ranging study in postmenopausal women with low BMD. Bone 2009; 45:833–842.

74 Holst JJ, Hartmann B, Gottschalck IB, *et al.* Bone resorption is decreased postprandially by intestinal factors and glucagon-like peptide-2 is a possible candidate. Scand J Gastroenterol 2007; 42:814–820.

75 Gottschalck IB, Jeppesen PB, Hartmann B, *et al.* Effects of treatment with glucagon-like peptide-2 on bone resorption in colectomized patients with distal ileostomy or jejunostomy and short-bowel syndrome. Scand J Gastroenterol 2008; 43:1304–1310.

76 •• Jeppesen PB, Lund P, Gottschalck IB, *et al.* Short bowel patients treated for two years with glucagon-like peptide 2: effects on intestinal morphology and absorption, renal function, bone and body composition, and muscle function. Gastroenterol Res Pract 2009; 2009:616054.
A preliminary study of the long-term effects of GLP-2 treatment in SBS patients.

77 Yamada C, Yamada Y, Tsukiyama K, *et al.* The murine glucagon-like peptide-1 receptor is essential for control of bone resorption. Endocrinology 2008; 149:574–579.

78 Crespel A, De Boisvilliers F, Gros L, Kervran A. Effects of glucagon and glucagon-like peptide-1-(7-36) amide on C cells from rat thyroid and medullary thyroid carcinoma CA-77 cell line. Endocrinology 1996; 137:3674–3680.

79 Lamari Y, Boissard C, Moukhtar MS, *et al.* Expression of glucagon-like peptide 1 receptor in a murine C cell line: regulation of calcitonin gene by glucagon-like peptide 1. FEBS Lett 1996; 393:248–252.

80 Nuche-Berenguer B, Moreno P, Esbrit P, *et al.* Effect of GLP-1 treatment on bone turnover in normal, type 2 diabetic, and insulin-resistant states. Calcif Tissue Int 2009; 84:453–461.

81 Nuche-Berenguer B, Moreno P, Portal-Nunez S, *et al.* Exendin-4 exerts osteogenic actions in insulin-resistant and type 2 diabetic states. Regul Pept 2009. [Epub ahead of print]

82 Wank SA. Cholecystokinin receptors. Am J Physiol 1995; 269 (5 Pt 1): G628–G646.

83 Carrillo J, Garcia-Aragoncillo E, Azorin D, *et al.* Cholecystokinin down-regulation by RNA interference impairs Ewing tumor growth. Clin Cancer Res 2007; 13:2429–2440.

84 Carrillo J, Agra N, Fernandez N, *et al.* Devazepide, a nonpeptide antagonist of CCK receptors, induces apoptosis and inhibits Ewing tumor growth. Anticancer Drugs 2009; 20:527–533.