

Nutritional hormones and the entero-osseous axis

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Keywords: GIP, GLP-2, Incretin, Osteoblast, Osteoclast

Enteric hormones represent the mechanism by which ingested nutrients are distributed to the various tissues in the body, so as to maximize their utilization. These hormones play a key role in regulating the energy balance. Nutritional hormones are also known to be important in bone turnover as evidenced by the fact that as soon as a meal is ingested, bone breakdown is suppressed¹⁻³. Many nutrition-related hormones have been shown to have effects on bone turnover through *in vitro* or *in vivo* studies including: (A) *Intestinal Hormones*: (1) Glucose-dependent insulinotropic peptide (GIP); (2) Ghrelin and (3) Glucagon-like peptide (GLP) 2; (B) *Pancreatic Hormones*: (1) Insulin (2) Amylin (3) Adrenomedullin and (4) Preptin (C) *Adipocyte-secreted Hormones*: (1) Leptin (2) Adiponectin and (3) Resistin, as recently reviewed by Clowes et al.⁴ and Reid et al.⁵.

As a group however, the incretin hormones (GLP-1; GLP-2 and GIP) have the strongest evidence for affecting post-prandial bone turnover. GLP-1 is a 37-amino-acid peptide secreted from L-cells in the small intestine in response to nutrients and potentiates insulin secretion. We have previously demonstrated that GLP-1 receptors are not present in either osteoblasts or osteoclasts. This finding has been confirmed by others; although recently, GLP-1 receptor knock-out mice have been shown to have increased bone breakdown suggesting that GLP-1 can also indirectly modulate bone turnover⁶.

GLP-2 is a 33 amino acid peptide expressed mainly in the L-cells of the small intestine. GLP-2 is secreted in response to nutrient ingestion and its physiologic function appears to be to regulate intestinal motility and stimulate intestinal cell growth, and it is also anti-apoptotic⁷. GLP-2 receptors are

expressed in osteoclasts and administration of GLP-2 to human subjects inhibits bone resorption and increases bone mass^{3,8,9}. GIP was first identified in the 1970s as a hormone secreted by cells in the enteric endocrine system. Because this 42-amino-acid peptide was found to inhibit gastric acid secretion, it was initially named gastric-inhibitory peptide (GIP)¹⁰. Subsequently, GIP was shown in the presence of glucose to stimulate insulin secretion from pancreatic β cells¹¹. Because of GIP's role in the regulation of insulin secretion, its name was changed to glucose-dependent insulinotropic peptide (GIP)¹²⁻¹⁶. GIP is secreted from endocrine cells (K cells) in the proximal small intestine¹⁷. GIP secretion from the enteroendocrine cells appears to be regulated both by enteric neurons in the small intestine (bombesin) and direct stimulation by nutrients¹⁸.

Since multiple enteric hormones have been shown to affect bone turnover, we first screened osteoblast and osteoclast cell lines for the presence of all the members of the seven transmembrane domain G-protein coupled family of receptors (GPCR).

An osteoblastic cell lines (MC3T3, a mouse osteoblast cell line) and an osteoclastic cell line (RAW 264.7, a mouse macrophage cell line) were screened for expression of all 15 members of this receptor family including those for: (a) corticotropin-releasing hormone 1 and 2, (b) pituitary adenylate cyclase-activating polypeptide, (c) glucagon-like peptide 1 and 2, (d) vasoactive intestinal peptide 1 and 2, (e) calcitonin, (f) glucose-dependent insulinotropic peptide, (g) parathyroid hormone receptor 1 and 2, (h) secretin, (i) glucagon and (j) growth hormone-releasing hormone.

We found that osteoblasts expressed receptors for seven members of this family: (a) corticotropin-releasing hormone 2, (b) vasoactive intestinal peptide receptor 2, (c) calcitonin receptor 1, (d) glucose-dependent insulinotropic peptide, (e) parathyroid hormone 1 and (f) secretin. In contrast, osteoclasts expressed receptors for only five members of this family: (a) vasoactive intestinal peptide 2, (b) calcitonin receptor 1, (c) parathyroid hormone 1, (d) glucose-dependent insulinotropic peptide and (e) glucagon like peptide 2.

Thus, of the hormones that rise after a meal (i.e., VIP and

The authors have no conflict of interest.

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Accepted 11 August 2008

PTH do not rise after a meal) and act within the time period observed for suppression of the markers of bone breakdown (i.e., calcitonin rises only in response to calcium in diet), only the receptors for GIP and GLP-2 are expressed in osteoclasts. These data would suggest that GLP-2 acts mainly as an antiresorptive hormone while GIP can act both as an antiresorptive and anabolic hormone.

Data from our laboratory has shown that GIP is in fact involved in normal bone turnover. Specifically, our GIP data *in vitro* demonstrates: (1) GIP receptors are present in both osteoblasts and osteoclasts¹⁹; (2) in osteoblasts, GIP increases collagen type I synthesis and increases alkaline phosphatase activity¹⁹; (3) in osteoblasts, GIP stimulates proliferation and functions as an anti-apoptotic agent²⁰; and (4) in osteoclasts, GIP inhibits PTH-induced long bone resorption and decreases osteoclastic resorption pit depth²¹. In *in vivo* studies, (5) GIP receptor knockout mice have a lower bone mass, decreased serum markers of bone formation and increased markers of bone breakdown²² and (6) GIP-overexpressing transgenic mice have increased bone mass²³.

Taken together, data from our studies and those of other investigators suggest that the intestine (and the hormones produced there) is a major regulator/co-ordinator of nutrient delivery to the bone, and this gut-bone cross-talk (which we have called the "entero-osseous axis"²⁴) is an important determinant, and is a reflection of, the body's energy balance.

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