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PERSPECTIVES

The Gut Feeling of Bone Remodeling

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Maintenance and repair of the skeleton involve regulation of both mechanical integrity and calcium metabolism. Both of these processes influence skeletal health, and alterations in the balance of skeletal maintenance and repair may result in metabolic bone disease. Bone remodeling is believed to consist of bone resorption coupled with formation processes that are necessary for skeletal growth and maintenance of bone structure. The processes of skeletal maintenance, growth and repair, as well as calcium homeostasis, have been suggested to involve both site-dependent and site-independent bone remodeling (1;2). Furthermore, these forms of bone remodeling might be independently controlled, resulting in several types of bone remodeling pathways with different cause-and-effect relationships. Thus, preventing site-independent bone remodeling without affecting bone repair and adaptation may offer alternative therapeutic intervention prospects for the treatment of metabolic bone diseases. The following will consider pathways involved in skeletal homeostasis, with an emphasis on the emerging understanding of the roles played by gastrointestinal hormones.

Calcium Homeostasis and Bone Remodeling

Calcium homeostasis is a tightly regulated process involving the coordinated efforts of the bone remodeling processes, kidney, parathyroid glands and gastrointestinal tract. Bone resorption in this respect is stimulated by parathyroid hormone and by the bioactive metabolite 1,25-dihydroxyvitamin D (calcitriol). The renal tubular capacity to reabsorb calcium is regulated by parathyroid hormone (PTH), and intestinal absorption of calcium is increased by calcitriol. In turn, several systems serve to monitor and balance calcium homeostasis (3;4). Changes in the extracellular calcium concentration trigger a series of hormonal reactions that maintain calcium homeostasis and reduce serum calcium perturbations. A decrease in the serum ionized calcium concentration increases synthesis and secretion of PTH and increases serum levels of calcitriol. Similarly, reduced serum calcium also decreases the production and release of calcitonin from parafollicular cells of the thyroid. Calcitonin has been shown to inhibit bone resorption in pharmacological doses, but the exact physiological role of calcitonin in calcium homeostasis and bone metabolism has not been established in humans (5;6). Disturbances at any level in this intricate regulatory network will result in a host of compensatory changes that may lead to metabolic disease. In addition to the physiological responsiveness of the above systems, signal pathways linked to the circadian rhythm and food intake act as rapid continual regulators of skeletal homeostasis.

Circadian Pattern of Bone Remodeling

Biochemical assessments of bone remodeling processes show a circadian pattern, with reduced bone resorption during daytime followed by a nocturnal increase. Bone formation follows the same pattern, but the magnitude of the variation is less pronounced (7;8). The circadian pattern of the bone remodeling processes is independent of age and of the same magnitude in men and women (9). Mechanical stress does not seem to affect the circadian pattern, as bone remodeling processes were unaffected by skeletal unloading following a 5-day bed rest or weightlessness during space flight (10;11). Several hormones, including PTH and cortisol, exhibit circadian rhythms and could therefore be candidates for mediating the circadian pattern in bone remodeling. The

circadian variation in serum cortisol may be partly responsible for the pattern of bone formation, since it has been demonstrated that infusion of cortisol in the morning depresses osteocalcin production during the day (12). A similar effect was not observed for bone resorption markers, and elimination of the morning peak of cortisol with metyrapone had no effect on the circadian pattern of bone resorption (13). Likewise, abolition of the circadian variation of serum PTH by continuous infusion of calcium had no effect on the circadian pattern of bone resorption (14). Melatonin has also been implicated as a possible mediator of the circadian pattern of bone remodeling (15). The plasma concentration of melatonin at night is 10-50 times higher than during the daytime (16) and this coincides with increased nocturnal bone resorption. On the other hand, blind individuals, who have no circadian melatonin variation (17), have a normal circadian pattern of bone resorption (10), which makes it unlikely that melatonin is involved in the regulation of the circadian osteoclast activity.

Postprandial Effects on Bone Remodeling

Key elements controlling the circadian pattern of bone resorption became evident through the discovery that bone resorption is significantly attenuated in fasting individuals. It therefore became apparent that food intake, not circadian pattern, was the major cause of variation observed in biochemical markers of bone resorption over a 24-hour period (18-21). The postprandial effect on bone resorption is independent of gender, age and menopausal status. In contrast to bone resorption, the markers of bone formation are seemingly unaffected by food intake (18-21). Thus, biochemical assessment of these processes demonstrates that food intake results in dissociation of bone formation and bone resorption. Moreover, the effects of food intake leading to inhibition of bone resorption would be expected to be fast, cascade-like and non-transcriptional, to account for the rapid decrease in bone resorption observed.

The nocturnal increase in bone resorption could be a consequence of a dwindling supply of nutrients and minerals to maintain the plasma calcium homeostasis and proliferative processes like hematopoiesis and epithelial renewal. In this situation, the mobilization of skeletal stores of nutrients and minerals can be brought about by stimulation of osteoclastic bone resorption. Conversely, postprandial availability of these nutrients and minerals would abolish the need to harvest from the skeletal stores, resulting in an acute reduction of bone resorption.

It has been speculated that feeding causes the release of a factor that exerts two concurrent effects: strong inhibition of bone resorption as well as a partial inhibition of bone formation (21). Alternatively, the postprandial effect could be mediated by a primary factor that can rapidly inhibit bone resorption. This inhibition of bone resorption might in turn cause a putative secondary factor to elicit a subsequent signal, maintaining the level of bone formation. By linking bone resorption to formation, this secondary factor could act to ensure that the total amount of bone resorbed will equal the total amount formed over a 24-hour period. Hence, a reduction of overnight bone resorption would have a favorable effect on bone homeostasis.

Intestinal Hormones and Bone Remodeling

Several hormones are released in response to the ingestion of nutrients, including insulin. However, insulin appears to be unable to influence the bone resorption process. Bone resorption is reduced by 40-50% from baseline after ingestion of fat or protein with little or no effect on insulin secretion (20;22), excluding insulin as a key factor in the postprandial regulation of bone resorption. Furthermore, in experiments involving an insulin clamp technique, induction of hyperinsulinemia gave no significant change in bone remodeling from baseline in either bone resorption or bone formation levels when euglycemia was maintained (22). This suggests that insulin does not play an important role in mediating

the acute effect of feeding on bone resorption.

The signals regulating the acute postprandial reduction in bone resorption seem to arise from the gastrointestinal tract. This notion stems from studies of postprandial stimulus in patients after gastrectomy, patients with short bowel syndrome (SBS) and healthy controls. In these studies, healthy controls respond normally to a meal with a reduction in bone resorption of more than 50% from baseline. A similar response is observed in patients after gastrectomy, suggesting that gastric hormones do not play a role in the postprandial reduction of bone resorption. On the other hand, patients with short bowel syndrome, where a large proportion of the small intestine has been excised, but the duodenum and colon remain functional, have an intermediate response. Moreover, SBS patients who have little small intestine and no colon lack the postprandial response in bone resorption completely (23). The serum levels of the gastric hormone, ghrelin, produced by endocrine cells in the stomach, are increased during fasting and significantly elevated overnight, paralleling to the nocturnal increase in bone resorption. However, as already mentioned, patients after gastrectomy express a normal postprandial reduction in bone resorption and therefore this effect is likely not mediated by ghrelin.

In a recent study, the effect on bone resorption was substantially greater after oral glucose than after intravenous glucose (20), suggesting a possible role for the incretin hormones, glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1). The secretion of GIP and GLP-1 is only evident after oral glucose and not after intravenous glucose.

GIP is a 42-amino acid peptide synthesized and secreted from endocrine K-cells in the duodenum (24;25). The role of GIP in coupling nutrient intake and insulin secretion, the so-called incretin effect, is well known. GIP receptor messenger RNA and protein are reported to be present in normal bone and osteoblast-like cell lines, and the presence of high affinity receptors for GIP has been demonstrated in [^{125}I]GIP binding studies (26). GIP administration prevents the bone loss associated with ovariectomy, and GIP may therefore play a role in signaling nutrient availability to the bone. Hence, gut hormones may play a key role in directing absorbed nutrients to the bone, suggesting the concept of an 'entero-osseous axis' (27).

Nutrient ingestion causes enteroendocrine L-cells to secrete the gastrointestinal hormone peptide YY and the two proglucagon-derived glucagon-like peptides, GLP-1 and GLP-2. These peptides are thought to serve in the regulation of absorption and disposal of ingested nutrients (28;29).

Peptide YY is released from the intestine postprandially in proportion to the energy content of a meal. Intraperitoneal administration of exogenous peptide YY elicits an anorectic effect in mice that could make this peptide an interesting candidate for postprandial regulation of calcium homeostasis. However, targeted disruption of the peptide YY gene in mice does not perturb the control of food intake and energy homeostasis. Peptide YY knock-out mice display normal growth, food intake, energy expenditure, and responsiveness to peptide YY (30). Further investigation is needed to determine whether peptide YY plays a role in calcium homeostasis and bone remodeling.

GLP-1 enhances glucose-stimulated insulin secretion and inhibits glucagon secretion, gastric emptying and feeding. Furthermore, GLP-1 has proliferative, neogenic and antiapoptotic effects on pancreatic beta cells. Recent studies illustrate a potential protective role for GLP-1 in the cardiovascular and central nervous systems (31).

The best documented physiological effect of GLP-2 is the stimulation of intestinal epithelial growth in mice and rats (32). Both GLP-1 and GLP-2 are probably involved in the "ileal brake" mechanism (33), an endocrine mechanism that is activated by the presence of nutrients in the ileal lumen, and which serves to inhibit gastric motility and secretion. It has been speculated that

the hormones of the ileal brake may also participate in the regulation of energy intake in humans (34;35). GLP-2 induces a pronounced decrease in the apoptosis rate of the cells of the intestinal epithelia (36) and also regulates intestinal glucose transport, food intake and gastric acid secretion and emptying, and improves intestinal barrier function. With these potential effects in mind, GLP-2 has been examined as a potential therapeutic treatment for patients with short bowel syndrome (37).

Thus, GLP-1 and GLP-2 exhibit a diverse array of metabolic, proliferative and cytoprotective actions with important clinical implications for the treatment of diabetes and gastrointestinal disease, respectively.
While GIP, GLP-1 and GLP-2 secretion in humans after oral glucose, fat or protein were qualitatively similar, fructose did not result in GIP secretion. Bone resorption was significantly reduced after ingestion of all macronutrients including fructose, eliminating GIP as a key mediator of the effect on bone resorption (38).

GLP-2 and Bone Remodeling

Of the gastrointestinal peptides GIP, GLP-1 and GLP-2, only GLP-2 displays a direct effect on bone remodeling. Biochemical markers for bone remodeling were not affected by either subcutaneous injection of GLP-1, or intravenous injection of GIP (38). However, a subcutaneous injection of GLP-2 resulted in a significant and acute reduction of bone resorption in a dose-dependent manner and with no apparent effect on bone formation as assessed by osteocalcin. Thus, part of the circadian variation of bone resorption might result from the nutrient-induced release of GLP-2 during the non-fasting phase of the day.

In a recently published study of SBS patients, it was found that treatment with GLP-2 for 5 weeks resulted in a moderate increase in bone density of the spine (1.1%, $P<0.05$) and the hip (1.9%, $P=0.06$) (39). The beneficial effects of GLP-2 on bone mass remain unexplained, although the investigators speculated that the effect on BMD was related to an increased mineralization of bone matrix resulting from improved intestinal calcium absorption. However, the intestinal calcium absorption increase of 2.7% ($P=0.87$) was insufficient to explain the BMD increases.

The physiological significance of these findings remains to be clarified. However, the gastrointestinal hormone, GLP-2, could be involved in the proposed entero-osseous axis, which may coordinate bone resorption in response to nutrient intake.

Possible Transmitters of GLP-2 Effects on Bone Remodeling

The surprisingly acute effect of GLP-2 on bone resorption could indicate a direct action of the hormone on osteoclasts and osteoblasts or an induction of local growth or differentiation factors, which in turn may inhibit osteoclast function. Alternatively, the recent localization of the GLP-2 receptor in the intestine to the myenteric ganglia could indicate that afferent nerve fibres might play a role in GLP-2 signaling in the gastrointestinal system (40). Furthermore, GLP-2 is synthesised in the central nervous system in the caudal brainstem and hypothalamus, and intra-cerebroventricular infusion of GLP-2 in rats diminishes food intake (41). Thus, activation of afferent nerve fibres via the receptors in the myenteric plexus could provide a neuronal signal to the bone remodeling processes and perhaps explain the rapidity of the onset of the effect. In this context, the hormone leptin has been suggested as a hypothalamic regulator of bone remodeling homeostatic function to maintain a constant bone mass (42;43). Leptin determines the extent of bone formation by modulating osteoblast proliferation through two pathways, one of which involves a molecular clock (44). On the other hand, the postprandial increase in leptin has a delayed onset of several hours, which indicates that leptin is not a mediator of the acute postprandial reduction of bone resorption that is already fully expressed after 1-2 hours (45).

Pharmacological Effect of GLP-2 on Bone Remodeling

Human studies with exogenous GLP-2 have shown a dose-response curve for bone resorption over the range of 100 to 1600 µg GLP-2 (given as a subcutaneous injection) and the reduction in bone resorption was similar when GLP-2 was given in the morning or at night (38;46). Examination of bone remodeling in these studies indicates that the effect of GLP-2 injections on bone formation resembles that of food intake, modulating bone resorption and leaving bone formation unaffected. Moreover, the nocturnal rise in urine calcium was reduced and approximately normalized to the level at injection time of GLP-2, which would also be the case if the output of calcium resulting from the bone resorption process was reduced after administration of GLP-2.

GLP-2 apparently affects the bone remodeling balance by disassociating bone resorption and bone formation. Shifting this balance in favour of bone formation may ultimately increase bone mass, and possibly, bone strength. Since the nocturnal increase in bone resorption reflects the highest activity level of the osteoclasts, a reduction of bone resorption during the night should have an overall positive influence on bone calcium balance. Furthermore, treatment modalities that reduce bone resorption but do not affect bone formation may have a more positive impact on skeletal health than therapies that reduce both bone resorption and formation.

These findings provide a new understanding of bone remodeling regulation and will possibly lead to new therapeutic intervention for the management of bone metabolic diseases.

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