

NEWS

New Studies Implicate Molecular Clock Genes, as Well as Pancreatic and Enteric Hormones, in Bone Homeostasis

Neil A. Andrews

Two new studies—one an examination of the role of molecular clock genes in bone formation (1), the other a review of evidence in support of pancreatic and enteric hormone involvement in the acute nutrient-induced regulation of bone homeostasis (2)—argue strongly for a widening of perspective amongst researchers who study the genetic and hormonal factors important to the bone formation process.

In the first study (1), entitled “The Molecular Clock Mediates Leptin-Regulated Bone Formation” and published in the September 9th issue of *Cell*, Gerard Karsenty and colleagues at Baylor College of Medicine in Houston, the Wellcome Trust Sanger Institute in Cambridge, U.K. and the Research Institute of Molecular Pathology in Vienna studied bone remodeling in mice genetically engineered to exhibit defects in *Period (Per)* or *Cryptochrome (Cry)*, two key genetic components of the molecular clock involved in circadian regulation. “The idea is that bone remodeling is a homeostatic function, and since most homeostatic functions occur in a circadian manner, it makes sense that the molecular clock could play a role in bone remodeling,” Karsenty, Professor of Molecular and Human Genetics at Baylor, told BoneKEy-Osteovision. Karsenty and his colleagues found that mice lacking both *Per1* and the *Per2* PAS domain; or both *Per1* and *Per2*; or both *Cry1* and *Cry2* all exhibited significant increases in bone mass in their vertebrae and distal femora. Furthermore, the investigators discovered that these mutants showed increases in osteoblast numbers and in mineral apposition and bone-formation rates.

Further experiments designed to account for these findings allowed Karsenty and his fellow researchers to show the precise role of the molecular clock genes in the

regulation of bone formation and to trace the molecular pathway via which the genes exert their effects. Most unexpected to the investigators was the particular location in the pathway from which the molecular clock genes operate. “We were surprised,” Karsenty said, “because we thought that the molecular clock would function upstream, not downstream, of sympathetic signaling.” Indeed, in response to sympathetic signaling produced by leptin’s action in the hypothalamus, the researchers found that the clock genes inhibit the proliferation of osteoblasts by inhibiting *c-myc*, which results in decreased levels of G1 *cyclins*, particularly *cyclin D1*. However, as all of this happens, an antagonistic pathway is also activated. In this other arm of the pathway mapped by the investigators, AP-1 genes activated by leptin-dependent sympathetic regulation upregulate *c-myc*, which leads to increased levels of cyclin D1 and the promotion of osteoblast proliferation. Because the clock gene pathway is dominant, Karsenty explained, in part because of its inhibition of AP-1 gene expression, wild-type mice exhibit decreases in bone-formation parameters and bone mass in response to long-term leptin i.c.v. infusion.

While these original findings regarding the molecular clock help to make sense of the diurnal variation in bone turnover, a new review article suggests that researchers should also look to the pancreatic and enteric hormones to further understand the factors important in that variation. Preliminary findings supported this contention. For instance, eating a meal results in a marked inhibition of bone resorption, accounting for much of the diurnal variation in bone turnover; oral glucose inhibits bone resorption considerably more than intravenous glucose, suggesting that enteric hormones

known as incretins may be responsible for much of the effect that the ingestion of meals produces. In addition, a residual diurnal variation of approximately 10% in both bone formation and bone resorption markers is found in subjects who fast throughout a 24 hour period. For reasons like these, the traditional ways of thinking about bone metabolism may stand in need of further expansion to include consideration of the enteric and pancreatic hormones.

"We hope to raise awareness of the potential for research in this area and to move beyond the established dogma that calcium is the primary nutritional factor, and PTH and vitamin D the key hormonal regulators, involved in bone metabolism," said Jackie Clowes, Assistant Professor of Medicine at Mayo Clinic College of Medicine in Rochester, Minnesota and co-author of the review (2). In fact, in "Perspective: Potential Role of Pancreatic and Enteric Hormones in Regulating Bone Turnover," published in Volume 20, Number 9 of the *Journal of Bone and Mineral Research* (2), Clowes, along with colleagues at Mayo Clinic and at the University of Sheffield in the U.K., review the evidence demonstrating the involvement of GIP, GLP 1 and GLP 2, amylin, leptin, and calcitonin in the acute nutrient-induced regulation of bone homeostasis.

However, while the evidence to support the idea of pancreatic and enteric hormonal involvement in bone turnover continues to mount, precisely how these hormones may regulate bone metabolism, particularly bone resorption, *in vivo* at physiological concentrations remains unclear, according to Clowes. Only additional studies, which Clowes hopes the review will encourage, will allow investigators to reach an understanding of the physiological role these hormones may play in bone metabolism. Several studies funded by the Arthritis Research Campaign in the United Kingdom are currently underway in this regard. Early evidence does suggest, however, that the hormones may directly modulate the function, as well as the maturation and differentiation, of osteoblasts and osteoclasts.

Interestingly, the investigators speculate that an evolutionary perspective may best explain the phenomenon of acute nutrient-induced changes in bone turnover and the role of the pancreatic and enteric hormones in this phenomenon. In fact, they hypothesize that the ability to regulate bone homeostasis in response to changes in diet represents a physiological adaptation on the part of the organism to deal with fluctuations in energy and nutrient intake. Specifically, mechanisms may be in place to guarantee net bone formation during periods of excess energy and nutrients, and net bone resorption during periods when energy and nutrients are lacking.

References

1. Fu L, Patel MS, Bradley A, Wagner EF, Karsenty G. The molecular clock mediates leptin-regulated bone formation. *Cell*. 2005 Sep 9;122(5):803-15.
2. Clowes JA, Khosla S, Eastell R. Potential role of pancreatic and enteric hormones in regulating bone turnover. *J Bone Miner Res*. 2005 Sep;20(9):1497-506.