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NEWS AND VIEWS

An "endocrine function of" bone to pick: starting with males

Allan Z. Zhao ✉, **Dongming Su**

The Center of Metabolic Disease Research, Nanjing Medical University, Nanjing 210029, Jiangsu Province, China
✉ Correspondence: azzhao@njmu.edu.cn

A stereotypical presentation of a hero in a Hollywood action movie would lavishly brag about not only strong muscle but also a large statue (a.k.a. bone structure), ostensibly for an attractive image. Unknown to these movie producers is that the bone tissue does send a "sex-related" message, an endocrine message to be exact, to the male reproductive system. Maybe we shouldn't blame Hollywood for their naiveté after all; even in the scientific community, there is a long-held traditional belief that regards skeletal bone as merely a structural and supportive tissue. However, this view has been challenged by a series of recent studies.

We now begin to realize that bone can influence adiposity, glucose homeostasis, insulin secretion, and male fertility via an osteoblast-derived bone matrix protein, called osteocalcin. It is a small protein of 100 amino acids, and is secreted solely by osteoblasts. Functionally, osteocalcin is pro-osteoblastic and bone building by influencing mineralization (Lee et al, 2007). Osteocalcin can be modified post-translationally by carboxylation through the activity of a vitamin-K dependent carboxylase. Accordingly, when residing in the bone matrix, osteocalcin is fully carboxylated and inactive. Upon decarboxylation, osteocalcin becomes active, and it is this active form that exists in circulation. The endocrine actions of bone were initially postulated in the studies of the mutant mice with a targeted deletion of osteocalcin gene. Relative to their wild-type siblings, the mutant mice displayed insulin-resistance, increased adiposity, hyperglycemia, impaired glucose stimulated insulin secretion, and low blood concentrations of insulin. Further corroborating these conclusions are the clinical observations that low blood concentrations of osteocalcin are associated with increased incidence of hyperglycemia. These data have given rise to a theory that bone can exert endocrine actions via circulating osteocalcin on insulin target tissues and on pancreatic β-cells.

As striking as these metabolic phenotypes are, the purported endocrine actions of osteocalcin have been controversial due to the lack of any knowledge about osteocalcin's receptors in peripheral tissues. The possibility of osteocalcin acting on an intermediate step to influence glucose metabolism and insulin secretion has not been excluded. In a recent edition of *Cell* (Oury et al, 2011), a collaborative effort led by Karsenty's group in the Medical Center of Columbia University unequivocally addressed these issues and assigned an unexpected attribute to skeletal bone—an endocrine regulation of male fertility.

A number of key observations were made in this latest study. The osteocalcin knockout mice had low fertility rate, displayed smaller testes, lower sperm counts, and lower testosterone levels relative to the wild-type mice. Consistent with these observations, in *ex vivo* experiments, treatment of testis explants as well as isolated Leydig cells, the major site for testosterone synthesis, with recombinant osteocalcin sharply elevated testosterone production. The decreased testosterone synthesis as a result of osteocalcin deficiency also led to defect in spermatogenesis due to increased germ cell apoptosis. Perhaps this should not come as a total surprise because testosterone is known to have a protective effect on germ cells. Interestingly, osteocalcin failed to stimulate estrodial synthesis in ovary explants, suggesting a sexual dichotomy in response to the endocrine effect of the bone on gonads. What made these results striking was that blood concentration of leuteinizing hormone (LH), a well-recognized hormone responsible for stimulating testosterone synthesis in Leydig cells (Walker, 2009), was elevated in the mutant mice, suggesting that the role of osteocalcin was so powerful that it was even dominant over the effect of LH on Leydig cells. To further solidify their conclusion that the observed effects of osteocalcin on testis were endocrine rather than paracrine, the authors ruled out the possibility of local production of osteocalcin in testis. In addition to immunocytochemistry method, an elegant knock-in approach was used to create a fluorescently tagged osteocalcin protein

as a way of following its *in vivo* expression. In such assays, the expression of osteocalcin was clearly from the bone, not from the testis.

The growing number of claims about osteocalcin's endocrine actions demands the identification of its receptor. In this regard, the ability of osteocalcin to increase cAMP production and cAMP responsive element binding protein (CREB) activity in Leydig cells and the dichotomy of osteocalcin's effects between male and female gonads led the authors to investigate Gprc6a, a C-type G-protein-coupled receptor (GPCR) family, as a candidate for the osteocalcin receptor. Indeed, Gprc6a is almost exclusively expressed in the Leydig cells of human and mouse testes, and its expression level peaks at the time of reaching adult when testosterone level also peaks. Gprc6a is *not* expressed in follicular cells of ovaries, further providing a molecular basis for the sexual dichotomy of osteocalcin effects. Importantly, Leydig cell-specific targeting of Gprc6a completely abolished the effects of osteocalcin on testosterone synthesis. All reproduction-related phenotypes of Leydig-specific Gprc6a mutant mice were similar to those osteocalcin knockout mice, such as small testis weight, low sperm counts, increased germ cell apoptosis, and low fertility. Combined together, these data presented arguably the first convincing case about the endocrine effects of the bone—in the scheme of regulation of male fertility.

The newly discovered endocrine regulation of male fertility by skeletal bone raises some profound biological (or even philosophical) questions, perhaps more than what has already been answered. Foremost, despite the strong genetic and biochemical evidence in mice as well as the demonstrated expression of Gprc6a in the Leydig cells in humans, it has yet been proven that bone-initiated endocrine control of male fertility exists in humans. Of all the published genetic screenings, no null mutation has been identified for osteocalcin, let alone its association with human male fertility. The absence of such identification may potentially signal an even more critical role of osteocalcin in controlling fertility for humans than for mice. Thus, secondly, the observations made by Karsenty's group suggest a possible (and previously unknown) selective pressure during the course of evolution. Osteocalcin is secreted from osteoblasts, the appearance of which is indicative of terminally well-differentiated bone. Any genetic trait rendering defect in bone differentiation and formation, ostensibly a disadvantage for survival, will by inference cause a drop in circulating osteocalcin as well as in male fertility. Predictably, such genetic trait will be phased out from a genetic pool as a result of low fertility. Thirdly, is Gprc6a the only receptor for osteocalcin? What type of receptors mediates the purported endocrine effects of osteocalcin on glucose homeostasis and insulin secretion? Fourthly, a lingering and unavoidable question: what about the females? The lack of a parallel impact of osteocalcin on estrodiol synthesis, and hence bone-initiated endocrine regulation of female reproductive system, is at minimum perplexing, especially considering that the counter regulation of bone formation by estrogen is paramount. Although it is tempting to speculate another unidentified (bone-derived or not) factor acting in a fashion similar to that of osteocalcin, researchers should bear in mind that females may not even need such regulation of estrogen synthesis because their fat tissue, another critical endocrine organ, is also an important and non-trivial source of estrogen (via aromatization of testosterone). As always, such speculation needs to be tested or refuted experimentally in the future.

REFERENCES

Lee, N.K., Sowa, H., Hinoi, E., Ferron, M., Ahn, J.D., Confavreux, C., Dacquin, R., Mee, P.J., McKee, M.D., Jung, D.Y., *et al.* (2007). Endocrine regulation of energy metabolism by the skeleton. Cell 130, 456–469.

Oury, F., Sumara, G., Sumara, O., Ferron, M., Chang, H., Smith, C.E., Hermo, L., Suarez, S., Roth, B.L., Ducy, P., *et al.* (2011). Endocrine regulation of male fertility by the skeleton. Cell 144, 796–809.

Walker, W.H. (2009). Molecular mechanisms of testosterone action in spermatogenesis. Steroids 74, 602–607.

