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Review Article

Osteofertility: Nexus between bone health and fertility

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ABSTRACT

Conventionally, bone was considered a static organ that only serves the protective and locomotive functions. However, our understanding of bone has evolved significantly during the past few decades. From an uninteresting scaffold, bone has become a fascinating organ that regulates various local and systemic functions. Recent research suggests that the skeleton is an important endocrine organ, with osteocalcin being the most prominent hormone produced by bone-forming osteoblast cells. Osteocalcin controls several physiological functions, such as glucose homeostasis, energy expenditure, and brain development. Another intriguing role of osteocalcin is that it regulates male fertility by enhancing testosterone biosynthesis independently of the hypothalamus-pituitary axis. This demonstrates that bone is an endocrine regulator of reproduction and expands the functional repertoire of osteocalcin. Furthermore, osteocalcin interconnects the bone, energy metabolism, and reproduction to form the novel pancreas-bone-testis axis. This review summarizes the current knowledge of osteocalcin-mediated regulation of different biological functions with special reference to its role in regulating male reproduction. This will provide deep insights into the underlying mechanisms involved in male fertility and improve our understanding of the relationship between bone and reproduction.

Keywords: Bone, Glucose, Insulin, Male fertility, Osteocalcin, Reproduction

INTRODUCTION

Bone is a dynamic connective tissue that provides various important functions, mainly mechanical support, protection, mineral balance, and hematopoiesis. The adult's skeleton is composed of 80% cortical bone and 20% trabecular bone, though the ratio differs by bones and regions. The vertebrae have a cortical-to-trabecular bone ratio of 25:75, the femoral head has a 50:50 ratio. Cortical bone is compact and surrounds the marrow space, and the cancellous bone has a honeycomb structure with the marrow section inside. Bone includes the majority of the body's connective tissue mass. Different from other connective tissue matrices, bone matrix is biomineralized and is distinctive in that it is continuously renewed over a lifetime due to bone remodeling. Bones contain the cartilaginous joints, the calcified cartilages in evolving individuals, the bone marrow cavity, and the cortical and cancellous mineralized structures. Bone as a tissue comprises the mineralized and non-mineralized (osteoid) parts of the cortical and cancellous sections of elongated and planar bones. There are three cell categories in bone tissue: (1) the bone-forming osteoblasts, which, when engulfed in minerals, become (2) osteocytes, and (3) the bone-destroying osteoclasts. Each of these cells interacts among themselves through any direct cellular interaction or through signaling molecules, and they communicate with each other.[1-3]

Apart from the role of bone in protection and locomotion, studies from the past decade have extensively delineated the endocrine role of bone, which maintains functions such as glucose

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homeostasis, neuronal development, and energy expenditure. The molecule that is responsible for these endocrine functions of the bone is the osteoblast-secreted molecule osteocalcin. Further intriguing role of osteocalcin in the maintenance of testicular function has been revealed, making the bone an endocrine regulator of reproduction. Osteocalcin induces the production of testosterone from the testis, thereby regulating male fertility. In this review, we have discussed in detail the role of osteocalcin in male fertility to provide deep insights into the relationship between bone and reproduction.

ENDOCRINE ASPECT OF BONE

Till now, bone has always been defined in terms of physical strength. However, bone has a lot more function than just a support system. Studies have evidenced the endocrine role of bone, and the most important player responsible for this endocrine function is osteocalcin. Among non-collagenous proteins, osteocalcin is found to be the most abundant. Human osteocalcin, which comprises 49 amino acids, is encoded by the bone gamma-carboxyglutamate protein (BGLAP) gene. Using gamma-glutamyl carboxylase and vitamin K as a cofactor, the molecule goes through several changes, the most important of which is the enzymatic carboxylation of the glutamine residues at positions 17, 21, and 24. Due to these residues, osteocalcin has a strong affinity for hydroxyapatite and can be readily integrated into bone tissue's extracellular matrix (ECM). This is why when osteocalcin was first identified, it was assumed that it would be required to initiate or regulate the mineralization of the bone ECM. However, gain or loss of function mutations have failed to detect any meaningful association between osteocalcin and ECM mineralization.[4] Carboxyl groups on osteocalcin are eliminated when osteoclasts reabsorb bone due to the acidic pH in the resorption lacuna, resulting in the formation of uncarboxylated osteocalcin, which is released into the systemic circulation. Thus, the rate of bone turnover, or remodeling, determines the amount of uncarboxylated osteocalcin in the blood. Serum concentrations of total osteocalcin, carboxylated osteocalcin, and uncarboxylated osteocalcin can generally rise with increased bone production, while uncarboxylated osteocalcin levels can rise in tandem with increased bone resorption. The serum total osteocalcin level (<50 ng/mL in mice and <30 ng/mL in humans) is regarded as a bone turnover marker in clinical conditions and reflects the bone formation ability of osteoblasts. Uncarboxylated osteocalcin generally makes up about one-third of total osteocalcin.[5] Uncarboxylated osteocalcin is considered the active form and G-protein coupled receptor 61 (Gprc61) is the receptor for the uncarboxylated osteocalcin.[5] Numerous functions of osteocalcin have been identified thus far, and recent reports – which primarily rely on *in vitro* and mouse research – indicate that the uncarboxylated form of osteocalcin regulates physiological pathways in an endocrine way.[6]

Regulation of glucose metabolism and energy expenditure are crucial endocrine functions of bone. Decreased osteocalcin level is associated with type 2 diabetes and obesity.[7,8] Osteocalcin-deficient mice exhibit decreased β-cell proliferation, glucose intolerance, and insulin resistance. Osteocalcin enhanced β-cell proliferation by inducing the expression of *CyclinD1*. Osteocalcin promotes the expression of *insulin* in β-cells and *Adiponectin* in adipocytes in a Gprc61 receptor-mediated manner.[9-11] Insulin further regulates the osteocalcin expression by suppressing runt-related transcription factor 2 (Runx2) inhibitor twist family basic helix-loop-helix transcription factor 2 (Twist2) through insulin receptor signaling in osteoblasts. Mice lacking insulin receptor signaling in osteoblasts have reduced levels of uncarboxylated osteocalcin, which is accompanied by hyperglycemia, glucose intolerance, and insulin resistance.[12] Daily injections of osteocalcin can partially restore insulin sensitivity, glucose tolerance, and lipid metabolism in mice fed with a high-fat diet.[13,14]

Osteocalcin and cognitive performance are positively associated.[15] Lower levels of osteocalcin in serum are associated with changes in brain microarchitecture and worse cognitive performance.[16] Osteocalcin favors postnatal neurogenesis, memory, and learning and prevents anxiety and depression. Osteocalcin crosses the blood-brain barrier, enhancing monoamine neurotransmitters' synthesis and suppressing gamma-aminobutyric acid (GABA) synthesis by binding to the neurons of the brainstem, midbrain, and hippocampus. In embryos before osteocalcin synthesis, maternal osteocalcin crosses the placenta to prevent neuronal apoptosis, contributing to fetal brain development.[17] Exogenous osteocalcin reduced anxiety-like symptoms and improved hippocampal-dependent memory in aging mice.[18] Furthermore, a positive association between depression and low bone mineral density was observed, indicating that individuals diagnosed with depression are at a high risk of having low bone mineral density.[19,20]

Osteocalcin improves muscle function during exercise by favoring the uptake and utilization of glucose and fatty acids [Figure 1]. Osteocalcin also promotes the secretion of interleukin (IL)-6 in myofibers, which has a role in maintaining glucose homeostasis during prolonged exercise.[21,22] IL-6 also promotes adaptation to exercise partly by driving the induction of bioactive osteocalcin.[22] Osteocalcin treatment enhances muscle mass in older mice and, thus, prevents age-related loss in muscle mass.[23] Another key function of osteocalcin is in regulating male fertility which is discussed in a later section.

OSTEOCALCIN AND REPRODUCTION

Reproductive organs, by producing different hormones, deeply influence bone development. For instance, estrogen

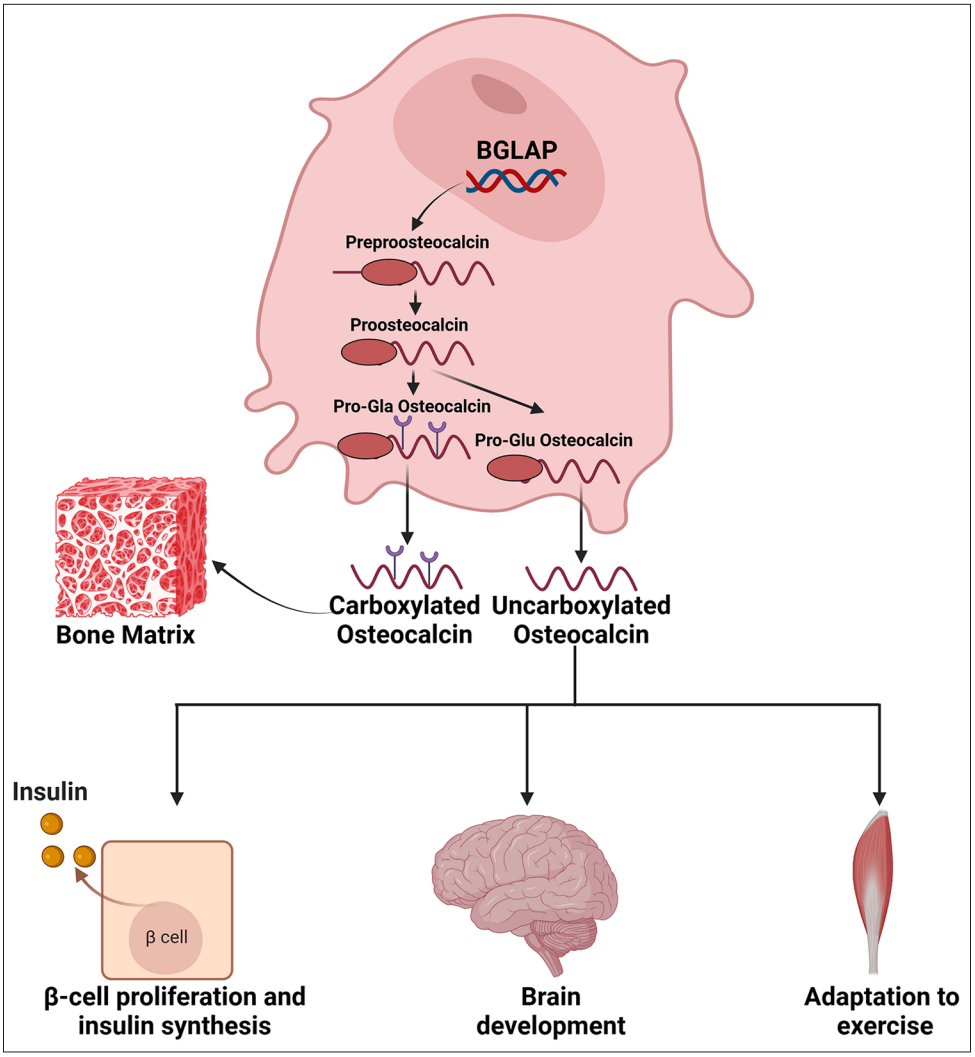

Figure 1: Synthesis and functions of carboxylated and uncarboxylated osteocalcin. Human osteocalcin is encoded by the bone gamma-carboxyglutamate protein (BGLAP) gene. After transcription preproosteocalcin peptide undergoes proteolysis, yielding pro osteocalcin peptide which undergoes enzymatic carboxylation of the glutamine residues at positions 17, 21, and 24, resulting in the formation of gamma-carboxyglutamic acid (Gla) residues. The Gla and glutamic acid (Glu) pro-osteocalcin peptides result in the formation of carboxylated and undercarboxylated osteocalcins after subjecting to a final proteolytic process. Both forms of osteocalcin are released from osteoblasts. While carboxylated osteocalcin integrates into the bone matrix due to its high affinity for hydroxyapatite, undercarboxylated osteocalcin is released into the circulation. Uncarboxylated osteocalcin has a role in the regulation of glucose metabolism, brain development, and improving muscle function during exercise. The Figure is created with the help of Biorender.

deficiency during post-menopausal conditions results in bone loss and osteoporosis. However, intriguing studies in the past decade have shown a reciprocal relationship in which the skeleton regulates male fertility by producing osteocalcin. Osteocalcin regulates the testosterone biosynthetic pathway; thus, a decrease in osteocalcin level is positively associated with a reduction in testosterone and further spermatogenesis [Figure 2]. Oury *et al.* for the 1st time, have demonstrated that osteoblasts enhanced the testosterone biosynthesis from the testis through osteocalcin, which binds to the G protein-coupled receptor expressed on Leydig cells and induces the expression of enzymes related to testosterone synthesis in cyclic adenosine monophosphate response element-binding protein (CREB)-dependent manner.[24] They observed that the supernatant of osteoblast cultures enhanced testosterone production by acting on Leydig cells, but did not influence the estrogen or progesterone synthesis from the ovaries. After several experiments, it was detected that osteoblast

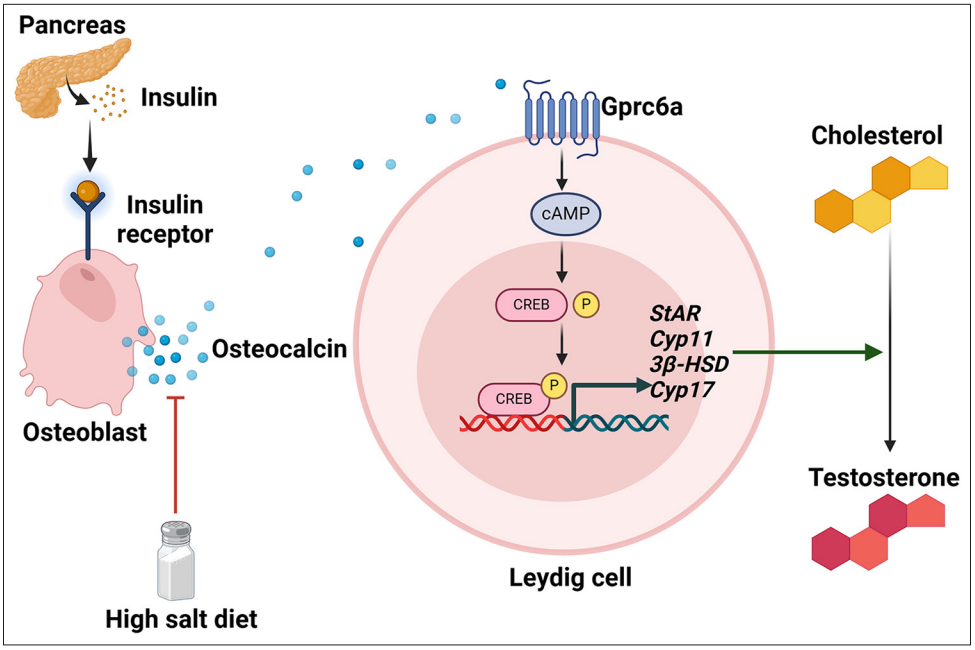

Figure 2: Pancreas-Bone-Testis axis: Insulin produced by the pancreas stimulates the osteocalcin production from osteoblasts. Osteocalcin further acts on the Leydig cells through G protein-coupled receptor family C group 6 subtype A (Gprc6a) and induces testosterone biosynthesis. A high salt diet can reduce male fertility by decreasing the osteocalcin synthesis from osteoblasts. cAMP: Cyclic Adenosine Monophosphate, CREB:Cyclic-AMP Response Element Binding Protein, HSD: Hydroxysteroid Dehydrogenase. The figure is created with the help of Biorender.

mediates its effect on testosterone production by secreting osteocalcin. Osteocalcin$^{-/-}$ osteoblast cultures could not induce testosterone production in Leydig cells, and treatment of Leydig cells with uncarboxylated osteocalcin increased testosterone secretion in a dose-dependent manner. Moreover, osteocalcin injections to the wild-type mice enhanced the testosterone level in circulation. Testosterone synthesis was markedly increased in the osteotesticular protein tyrosine phosphatase (ESP)$^{-/-}$ mice, which is the gain of the function mouse model of osteocalcin. Osteoblasts express *Esp,* which inhibits the endocrine function of osteocalcin by promoting its carboxylation. Osteocalcin deficiency significantly decreases the number of spermatocytes, round spermatids, and the size of the epithelium in testis tubules and increases germ cell apoptosis, further supporting the notion that osteocalcin is responsible for testosterone biosynthesis. Osteocalcin enhances testosterone levels by inducing the expression of enzymes such as *StAR*, *Cyp11a*, *Cyp17*, and *3β-HSD*, essential for testosterone biosynthesis. Osteocalcin acts on Leydig cells with the help of the G protein-coupled receptor family C group 6 subtype A (Gprc6a) receptor and testosterone biosynthesis was decreased in the case of Gprc6a$_{Leydig}^{-/-}$ male mice. Interaction of Gprc6a and osteocalcin favors CREB phosphorylation, indicating CREB acts downstream of Gprc6a and further could bind to the promoter regions of *Cyp11a, 3β-HSD,* and *StAR,* promoting testosterone synthesis.[24] Furthermore, it was reported that osteocalcin deficiency is associated with increased levels of luteinizing hormone, indicating that the reproductive role of osteocalcin is luteinizing hormone-mediated. However, it was observed that osteocalcin promotes testosterone biosynthesis independently of the luteinizing hormone. Uncarboxylated osteocalcin level in the circulation depends on bone resorption, and therefore, bone resorption is the biological determinant of the osteocalcin-mediated regulation of male fertility. Male fertility is further observed to be connected with the metabolism as insulin signaling in osteoblasts was observed to enhance testosterone synthesis via inducing osteocalcin activity indicating the presence of the pancreas-bone-testis axis.[17] This is further evidenced by a study showing that in type 2 diabetes patients, the levels of undercarboxylated osteocalcin are positively associated with free testosterone.[25] Further, results suggested that Gprc6a may be a new susceptibility gene for primary testicular failure in humans, extending the biological relevance of osteocalcin from mice to humans. These results, therefore indicate that osteocalcin provides a synergistic route to upsurge the level of testosterone in circulation previously thought to be regulated mainly by the pituitary gonadotropins. Furthermore, dietary compounds can influence male fertility by regulating osteocalcin synthesis. A high salt diet was observed to impair male fertility by reducing osteocalcin production

from bone.[26] This study thus provides the association between diet and fertility and reveals how dietary habits can be leveraged to control male fertility-related issues.

CONCLUSION

Previously bone was considered as the relatively static tissue that fulfills only protective and mechanical functions and was documented as an uninteresting scaffold. However, exciting results from studies in the past decade recognized bone as a fascinating organ and changed our perception of the bone. Gerald Karsenty's laboratory has added another twist to bone biology, showing that skeletal in an endocrine organ and the osteocalcin secreted by osteoblasts is one of the prominent hormones. Karsenty's group has revealed that osteocalcin regulates various functions, such as glucose metabolism, energy expenditure, and cognitive functions. The same group has further extended the function of osteocalcin in testosterone production. They uncovered the unknown feed-forward loop between the skeletal and the testis and thus answer to the existing sexual dimorphism of the bone due to which male bones are bigger than females. Osteocalcin enhances testosterone synthesis in males but not females, favoring gender discrimination and thus increasing the bone size in the males. Further, metabolism has been linked to the bone-testis axis, signifying the existence of the pancreas-bone-testis axis, which regulates testosterone synthesis independently of the parallel hypothalamus-pituitary-testis axis. Thus, the discovery of osteocalcin as a regulator of male fertility advances our understanding of the homeostatic mechanisms that regulate testosterone production in the testis. Strikingly, additional results proposed that Gprc6a is the new locus for primary testicular failure in humans. Therefore, Gprc6A can be a prominent pharmacological target, and research in this field could lead to treatments for male infertility and other hormone-dependent illnesses. To enhance the clinical relevance of the bone-testis axis, our laboratory has demonstrated how diet can modulate this axis. Our results indicate that a high salt diet can reduce male fertility by decreasing osteocalcin synthesis from the osteoblasts. This specifies that dietary habits can be modified to prevent impaired male fertility through modulating osteocalcin synthesis.

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