

Molecular features of sexual steroids on cartilage and bone

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SUMMARY

In Brazil, the increase in the reported cases of degenerative diseases of articular cartilage is 20% per year, meaning that 200,000 Brazilians develop degenerative joint diseases every year, which have a negative impact on bone mass. This study shows evidence that hormone production of sexual steroids (estrogens, progesterones, and androgens) have an influence on cartilage quality, as well as on bone mass. Therefore, this review aimed to analyze literature data on the molecular and genetic action of sexual steroids on hyaline cartilage and bone physiology, as well as osteoarthritis interference on the quality of these structures.

Keywords: Sexual steroids; bone; hyaline cartilage; osteoarthritis.

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BONE FORMATION

Bone formation occurs mainly during the embryo development and postnatal growth, being important in adults for bone remodeling and to maintain calcium homeostasis due to adaptation to physical forces. Bone formation requires recruitment, proliferation, and differentiation of osteoprogenitor cells¹.

Bone tissue is formed in two ways: one is an intramembranous process, and the other is endochondral. The process termed intramembranous occurs within a connective tissue membrane, and the endochondral process occurs on a hyaline cartilage model, which is gradually destroyed and substituted for bone tissue formed from the differentiation of cells in the adjacent connective tissue. Both in intramembranous and endochondral ossification, the first bone tissue formed is the primary type, which is slowly replaced with secondary, more resistant, bone tissue².

Long bone formation is endochondral, a more complex process than that occurring in other bones. From a cartilage model similar to the coming bone, a narrow middle part and wider ends are seen, corresponding, respectively, to the shaft and epiphyses of the mature bone. The first bone tissue found in a long bone is formed by intramembranous ossification of the cartilage model periphery at the perichondrium covering the mid-shaft, forming a cylinder, the bone collar. While the bone collar is formed, cartilage cells involved in the collar enlarge and die from apoptosis, leading to cartilage matrix mineralization. Blood vessels from the periosteum go through the bone cylinder and penetrate the calcified cartilage, taking along osteoprogenitor cells from the periosteum, which proliferate and differentiate into osteoblasts. These osteoblasts form a continuous cell layer on the surface of calcified cartilage partitions and initiate the synthesis of the bone matrix, that is soon mineralized. Primary bone tissue is thus formed on the remains of calcified cartilage³.

The ossification center described above is seen at the mid-shaft, and it is called a primary center. Its longitudinal growth is fast and ends up occupying the whole shaft (diaphysis), that is then formed by bone tissue. This primary center spread is followed by the growth of the bone cylinder formed from the perichondrium, which also grows toward the epiphyses. Later on, secondary ossification centers are formed, one at each epiphysis, but not simultaneously, and radial growth takes place in these centers².

After epiphysis and diaphysis ossification, the remaining cartilages in the cartilage model of long bones are the articular cartilage and the epiphyseal plate. The articular cartilage will persist throughout life, and the epiphyseal plate cartilage, consisting of a cartilage disk that was not substituted for the expanding bone, will be responsible for the longitudinal bone growth and will disappear over

time. This cartilage is found between the epiphysis and diaphysis bone tissue. It disappears approximately at the age of 20, when the longitudinal growth of long bones is arrested, thus causing arrest of growth^{2,3}.

Bone tissue is a specialized connective tissue presenting blood vessels, nerves, and cells (osteoblasts, osteocytes, and osteoclasts) which synthesize, reabsorb, and maintain the bone matrix (BM); these activities are under hormone influence^{3,4}. Bone tissue formation involves, in addition to osteoprogenitor cells, a complex process, such as the apoptosis of cells present in the cartilage tissue (chondrocytes), which are replaced for bone tissue cells (osteoblasts and osteocytes). Bone remodeling takes place by sequential synthesis and breakdown of the bone matrix during its growth, which is performed by special cells (osteoblasts and osteoclasts, respectively)².

HORMONES ACTING ON CARTILAGE AND BONE FORMATION

Several factors act on bone cells during their differentiation, such as circulating molecules, hormones (parathyroid hormone – PTH, growth hormone – GH, progestogens, and androgens) or non-hormonal molecules (1,25 dihydrocholecalciferol, insulin-like growth factors types 1 and 2 – IGF 1 and 2), locally produced molecules with autocrine/paracrine action (IGF1 and 2, bone morphogenetic protein – BMP, prostaglandin E2 – PGE2, interleukin 1 – IL1, tumor necrosis factor α – TNF- α , granulocyte macrophage colony-stimulating factor – GM-CSF, transforming growth factor β – TGF β , basic fibroblast growth factor 2 – BFGF2), and molecules present in the bone extracellular matrix (FGF2, TGF β , GM-CSF, IGF1 and 2); they are inactive when bound to bone extracellular matrix (BEM) constituent molecules, but active on bone cells when BEM breakdown takes place. Quiescent osteoblasts regulate the osteoclast access, but under the action of bone-reabsorbing factors (PTH, dihydrocholecalciferol, and PGE2), osteoblasts retract and give place to the osteoclasts, which can adhere to the extracellular matrix. Vitamin D and PTH stimulate osteoclast activity, whereas calcitonin inhibits it. Oncogenes *c-fos* and *c-myc* are expressed in osteoblast proliferation^{5,6}.

Cartilage growth regulation is complex and is under hormone action – growth hormone, IGF1 and 2, estrogens, and androgens, but also a number of locally produced factors (FGF2, TGF β , epidermal growth factor [EGF], platelet-derived growth factor [PDGF])⁵.

Estrogens act by hastening chondrocyte proliferation, and the androgen action on cartilage is ensured by the activation of estrogen receptors, as the androgens synthesized by gonads penetrate the chondrocytes, where they are transformed into estrogens by the enzyme aromatase^{7,8}.

The peptide related to the parathyroid hormone (PTHrP) is synthesized in a number of bone cells in the epiphysis, in contrast with its receptor, present only in the epiphyseal plate in the transition zone among proliferating and hypertrophic chondrocytes. The Indian hedgehog protein and its receptor play a role in regulating growth and in the differentiation of the epiphyseal plate. Indian hedgehog is identified in prehypertrophic chondrocytes and acts on perichondrium cells expressing its receptor. Indeed, the receptor activation determines an increase in PTHrP secretion by perichondrium cells. There is a regulation loop: chondrocytes at the resting region (preproliferation) cause PTHrP synthesis via Indian Hedgehog, acting on epiphyseal plate chondrocytes and allowing their proliferation⁸.

Thyroid parafollicular cells secrete, among many hormones, calcitonin, which acts on calcium blood level regulation and calcium storage in bones. The parathyroid gland secretes PTH, which acts on bones, kidneys, and intestines in order to maintain the interstitial fluid calcium level balanced. In the bone, PTH is bound to receptors in the osteoblasts, signaling for an increased secretion of osteoclast stimulating factor by the cells⁹.

SEXUAL STEROIDS

Sexual hormones are steroids interacting with androgen and estrogen receptors in vertebrates. Natural sexual steroids are produced by gonads (ovaries and testicles), by adrenals, or by conversion from other sexual steroids. Sexual steroids play important roles by inducing bodily changes known as primary sexual characteristics and secondary sexual characters¹⁰.

PROGESTOGENS

Progestogens are female sexual steroids produced by the menstrual corpus luteum or up to an eight-week pregnancy, when their synthesis is taken on by the placenta. They are parent hormones of the sexual steroids estrogens and androgens and of the cortisone synthesis by the adrenal cortex. At the first stage, the cholesterol molecule is converted into pregnenolone (P5). P5 and other members of the progestogenic steroid class serve as parent hormones for all other steroids, including estrogens, androgens, mineralocorticoids, and glucocorticoids. Progesterone is the hormone that prepares women for breastfeeding, and affects women physically and emotionally, preparing her for a pregnancy. Many women who are progesterone-deficient can have amenorrhoea and recurring miscarriages¹¹.

Progestogens further have many functions, such as the endometrium preparation for the zygote reception and implantation, milk production over breast-feeding,

endometrial proliferation blocking, and estrogen predominance balance, with a key role in preventing most common symptoms in the premenstrual syndrome (PMS). In bone tissue, progestogens stimulate osteoblast proliferation and differentiation by stimulating bone formation, thus avoiding bone loss¹¹⁻¹³.

ANDROGENS

Androgens are male hormones produced by the testicles and found in small amounts in women. They have many functions similar to estrogens, since they are estrogen parent hormones: they increase osteoblast activity, inhibit calcium removal from the body by decreasing osteoclast formation and activity, and stimulate long bone longitudinal growth at puberty, as well as epiphyseal plate ossification. When this sexual hormone secretion is reduced, the osteoclastic activity becomes higher than osteoblastic activity, and bone formation is potentially reduced^{6,7}.

The main types of androgens are testosterone and androsterone. Testosterone is the main male hormone, produced under the influence of the luteinizing hormone from the pituitary gland, and stimulates sperm production and male sexual characteristics at puberty^{14,15}.

ESTROGENS

Estrogens are female steroids producing the female phenotype, such as physical appearance and sexual and emotional characteristics. They are mainly produced by ovarian follicles and are found in small amounts in men. The fact that estrogens are produced by ovaries, with androgens as parent hormones, make women produce male hormones first and subsequently turn them into female hormones. Estradiol (E2), as other sexual steroids, is obtained from cholesterol. After side chain cleavage, a fraction of androstenedione is converted into testosterone, which, in turn, is converted into estradiol by an enzyme called aromatase. Alternatively, androstenedione is "aromatized" to estrone and further to estradiol^{10,15}.

These sexual steroids have several functions in bone tissue, such as: increasing osteoblast activity, inhibiting calcium removal from the body by interfering and reducing osteoclast formation and activity, and stimulating long bone growth after puberty. They further promote rapid bone calcification, causing it to reduce the proliferation activity until it ceases^{4,10}.

In adults, bone maintenance is due to estrogens because of their antireabsorptive and anabolic effects. In females, osteoclasts have estrogen alpha receptors (ERa), thus these hormones act by reducing osteoclast reabsorption activity; in males, however, bone formation stimulus is mediated by ERa present in osteoblasts¹⁶.

The term “estrogen receptor” refers to a receptor group activated by the hormone 17 β -estradiol. There are two types of estrogen receptors – ER α and ER β –, members of an intracellular receptor nuclear family, with G protein coupled to GPR30 receptor – GPER. Estrogen and the expression of its receptor ER- α are present in the nucleus and cytoplasm of articular cartilage cells and in subchondral bone, directly affecting bone metabolism during the individual’s life. Hormone therapy with estrogens (estradiol and diethylstilbestrol) has been used to inhibit bone reabsorption and prevent bone loss in postmenopausal women^{9,11,17}.

OSTEOARTHRITIS

In Brazil, reported cases of articular cartilage degenerative diseases increase 20% every year, meaning that over 200,000 Brazilians develop joint and bone degenerative diseases yearly. There is an incidence of 35% of cases affecting the knees from the age of 30, rising dramatically with age, reaching 80% of people older than 50 years. In osteoarthritis (OA), the bone mineral density (BMD) is reduced, bone microarchitecture is broken, and the amount of noncollagenous proteins in bone is changed. Bone matrix synthesis dysfunction may occur from excess PTH production and from decreased sexual steroid secretion, such as estrogen, progesterone, and androgen, following menopause; from enzyme action on cartilage tissue degradation, or from vitamin A deficiency, as this vitamin balances the activity between osteoblasts and osteoclasts^{3,18}.

The cartilage matrix, containing collagen fibers and proteoglycans, undergoes constant remodeling by chondrocytes, which are sources of both catabolic and anabolic activities in the cartilage. The catabolic process is mediated by metalloproteinases, such as gelatinase, stromelysin, and collagenase. Metalloprotease activity can be blocked by tissue inhibitors – TIMPs –, also produced by chondrocytes. The chondrocytes, in turn, are subject to biochemical mediator influence, with two groups mainly found, one of them predominantly related to interleukin-1 (IL-1) and tumor necrosis factor α (TNF- α) activity, whereas the other comprises growth factors (fibroblast growth factor [FGF], platelet-derived growth factor [PDGF], and insulin-like growth factor [IGF]). The initial osteoarthritis mechanism is still controversial, but an initial increased IL-1 and TNF- α production by synoviocytes is known, with IL-1 and TNF- α acting on chondrocytes and stimulating the catabolic pathway. As long as the chondrocyte can replace the catabolized material through a compensatory increase in anabolic activities, the disease is under control. However, when their repair capacity is exhausted, clinical

disease supervenes. Therefore, OA can be understood as an imbalance in the physiological remodeling process of the articular cartilage^{18,19}.

When the increase in matrix degeneration by chondrocytic enzymes exceeds the increase in new synthesized matrix, cartilages will naturally degenerate (excess chondrocytes create substances that are useful for cartilage replacement, but they also create enzymes that destroy their components). If this process continues, there will be a decrease in the osteoblast number and an increase in osteoclasts that degenerate the joint, causing cartilage loss and changes in the subchondral bone. The outcome is joint pain, deformity, and limited range of movement²⁰.

CONCLUSION

Sexual steroids (estrogens, progesterone, and androgens) are related to cartilage and bone tissue maintenance, thus in postmenopausal women or in those with osteoarthritis, hormone administration should be evaluated.

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