Effect of the physical activity on normal bone and on the osteoporosis prevention and treatment*

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ABSTRACT

Osteoporosis has been increasingly diagnosed in women and men worldwide. Although the sexual steroids are important in the genesis of human osteoporosis, it is believed that the lack of physical activity constitutes a risk factor. Physical activity acts on the bone by direct effect via mechanical force, or indirect effect through hormonal factors. However, the mechanism through which physical activity improves the bone mass is not completely known. Sports practice has been increasingly recommended for prevention and even treatment of osteoporosis based on the results that have demonstrated the beneficial effects of physical activity on the bone tissue. The goal of this review is to describe the effects of physical activity in the normal bone tissue and on the osteoporosis prevention and treatment.

Keywords: Osteoporosis. Exercise. Bone.

INTRODUCTION

Osteoporosis is an increasingly diagnosed disease in women and men worldwide with the life expectancy[1]. Much has been said about the role of the sexual steroids deficiency in the osteoporosis genesis, mainly of menopause, but it is also known that the lack of physical activity is an important risk factor[2].

Although physical activity shows powerful and complex effect over the bone tissue directly or indirectly, research results are contradictory yet and dependent on the frequency, duration and exercise intensity[2]. The effect of exercising over some bones, evaluated through densitometry or bone biopsy, is insufficient to provide any conclusion about the whole skeleton response to physical stimulus. Thus, the histological and morphometrical studies of different bones and with distinct metabolisms are essential to a better understanding of the benefits of physical exercise over the whole skeleton, once the bones have differentiated metabolism and can present distinct response to many different stimuli, no matter if nutritional, hormonal or physical.

The aim of this review is to describe the effects of physical activity in the normal bone tissue and in the prevention and treatment of osteoporosis.

THE NORMAL BONE TISSUE

The bone is a multifunctional tissue consisted of three cell types: the osteoblasts, the osteocyte and the osteoclasts. The first cell type derives from the osteoprogenitor cells of the bone marrow and is located on the surface of the trabeculae; in the Havers channel of the osteonic bone tissue and in the periosteum. Its main function is synthetizing not mineralized bone marrow matrix (bone apposition)[3] consisted of collagen type I, of non-collagenic proteins (fibronectin, tenascin and osteopontin), of λ-carboxilated proteins (osteocalcin and Gla protein) and of proteoglicanes (condroitine sulphate), among others[4]. An average of 70% of the non-mineralized bone marrow matrix is mineralized right after its synthesis and the residues suffer gradual mineralization[5].

As soon as the bone matrix is synthetized, the osteoblasts are envolved by it and are called osteocyte. Such cells have the function to keep the bone tissue viability[6] and reabsorb the matrix and the bone minerals through the osteolysis, deep reabsorption mechanism, essential to keep the extracellular calcium levels stable[7]. The osteocyte place themselves in columns inside the mineralized bone tissue and communicate with other osteocyte and osteoblasts through interchannel projections, the gap junctions[6]. Such junctions are intramembranous channels consisted of proteins known as connexins (Cx) and that promote the communication between the cytoplasm of two neighbor cells allowing the passage of metabolites, ions and intracellular signal molecules, such as calcium and AMPc[8].

The osteoclasts are multinuclear cells derived from the fusion of the precursors of the hematopoietic mononuclear cells with differentiation dependent on factors liberated by the osteoblastic lineage cells.

The osteoclasts are on the surface of the trabeculae and the Havers channels and in the periosteum, placed in the Howship columns. Whenever they are activated, their main function is to promote the bone reabsorption by osteoclasia[9].

The bone is responsive to a series of stimuli due to its multifunctional tissue constitution, among them we can mention biological, biochemical and biomechanical ones.

THE BONE TISSUE AND OSTEOPOROSIS

The bone is a metabolically dynamic tissue and its hardness depends on the balance between the anabolic (apposition) and catabolic (reabsorption) processes. It is known that the genetic constitution, the diet and the physical stimuli are factors that influence the bone metabolism, but the effective bone apposition and reabsorption is mediated by hormones, cell products and by the bone matrix.

The catabolic process or bone reabsorption, has the vital function of keeping stable the extracellular calcium levels. The bone apposition (the bone matrix synthesis and mineralization) has two main objectives that are to re-put the bone tissue lost by the catabolic process and fulfill the organ needs in adapting to the functional conditions. Imbalances between these two processes have been accumulated throughout one's lifetime, especially due to isocalcemia[12]. As a consequence of the supremacy of the catabolic process, we have the bone loss, especially if inhibiting factors of the bone neoformation – as the ones inherent to the senescence- are associated. Thus, changes in metabolism, in the calcium absorption and in the hormonal profile – specially in women after menopause – associated to physical inactivity, contribute to an

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imbalance to the bone remodelation[13], having osteoporosis as the main consequence[10,11].

Osteoporosis is a generalized metabolic disease, characterized by lower bone apposition due to osteoblastic insufficiency[5], with bone mass reduction by volume unit and by multifactor etiology[13]. Concerning the sexual hormones deficiency in the menopause osteoporosis genesis, a lot of attention is given, once after the sexual hormones production ceases, the feminine bone mass decreases rapidly in the first 10 years and slowly in the following years[15]. Moreover, a smaller quantity of bone is found and a greater quantity of it is reabsorbed in each remodellation cycle[14,15]. Estrogen and progesterone act in the bone remodellation[14], however, by mechanisms not totally clear yet[10]. The presence of receptors to strogen in osteoblasts and osteocytes suggest a direct effect of this hormone over the bone tissue[16]. Concerning the osteoblasts, estrogen increases these cells differentiation and stimulates the synthesis and the bone matrix mineralization, regulating the expression of genes that decode the type I collagen and the non-collagenic proteins such as osteopontin, osteocalcin, osteonectin, and so forth[11]. Besides that, estrogen indirectly inhibits the bone reabsorption when it regulates the synthesis and citocines, prostaglandines and growing factors liberation[14,15]. Progesterone also participates in the bone metabolism, especially in the bone matrix synthesis[11,17]. This hormone stimulates the osteoprogenitor cells proliferation and differentiation[15] and works in the osteoblasts regulating the secretion of growing factors and stimulating the bone apposition and mineralization[11]. Thus, the osteopenia observed in the progesterone deficiency seems to be derived from the bone apposition decrease[11,18].

The bone mass reduction derived from the hypogonadism, is also related to the intestinal calcium absorption decrease. Estrogen has a direct action over the intestinal mucous membrane and indirect mediated by vitamin D[19]. Thus, in the estrogen deficiency a decrease in the number of receptors for vitamin D in the intestine[20] and smaller renal conversion of inactive vitamin D in its active manner is expected. Consequently, in the hypogonadism, smaller formation of the linking calcium protein decreasing its intestinal absorption is observed[21].

The T3 and T4 thyroid hormones control the bone metabolism and the mineral homeosthasis of adults[22] and the thyroid-osteoporosis relation has been studied for many years. The thyroid hormones and the sexual hormones control the gene expression in the osteoblats, increasing the alcaline phosphatasis activity and the type I collagen and the non-collagenic proteins production[11,17] and growing factors similar to insulin, increasing the bone matrix synthesis and mineralization after that[23]. In the thyroid hypofunctions, general metabolism is reduced, which directly affects the recruiting, the differentiation, the maturation and the metabolism of cells responsible for bone apposition, mineralization and reabsorption[24] with osteoporosis as a consequence[12]. In the hypothyroidism, the bone matrix mineralization decrease seems to be due to the calcium and phosphorus plasmatic values decrease[25], once the thyroid hormones (T3 e T4) are important in the intestinal transportation of calcium and phosphorus mediated by $1,25(OH)_2D_3$[26].

Hormonal factors are not the only ones involved in the osteoporosis genesis. It is believed that the lack of physical activity is a risk factor, once extensive evidence has been connecting physical inactivity to bone loss. It was demonstrated in humans a deduction of 0,9%/week of the trabecular bone volume associated to the 1,3%/day decrease of the muscular contraction force after extended imobilization[27]. Physical activity has been adopted as a strategy to prevent mineral bone loss and to keep the skeleton integrity, since there is a close relation between physical activity, mineral bone density and bone mass[28]. However, some mechanisms through which physical activity works in the bone tissue and through which the physical inactivity promotes bone loss, have not been clarified.

PHYSICAL ACTIVITY EFFECT OVER THE BONE TISSUE

The majority of researchers agree that physical activity presents powerful and complex effect over the bone. Yet, the research results are still contradictory. No alteration, increase and even bone mass reduction have been described in female rats submitted to physical activity[29]. There is also evidence that in humans, physical exercises developed in the growing and development phases, determine a 7 to 8% of bone mass gain in adults, reducing substantially the breaking risks in advanced age[30]. It is obvious that depending on the kind and exercise intensity, the effects over the bone tissue vary, and can be deleterious[31]. Intense sports activity may lead to osteoporosis, to damage of the gonadotropic hormone pulsing (GnRH) and to gonodal disfunction in young adults[30,32] and not protect women against bone loss in menopause[13]. Pre-puberty gymnasts submitted to intense exercising present growing delay and higher frequency of locomotory disorders, besides the reduction of the growth factor similar to the insulin-1 (IGF-1) and the thyroid hormones and blood cortisol increase[33]. Similar results were observed in female rats in the growing phase, submitted to moderate impact physical activity (treadmill running). Such animals presented osteoporosis in the nasal bones and chest vertebrae, probably caused by the hypothalamus-hypophysis-ovary axis suppression[34].

There is evidence that physical activity minimizes the osteopenia derived from age advance and the sexual steroids decline. However, not every kind of exercise promotes benefits over post-menopause women's skeleton. Some studies demonstrate that moderate load exercises such as walks and cooper, promote increase in the bone minerals of those women. On the other hand, it was observed that in post-menopause women submitted to low load physical exercises, such as swimming, that these are beneficial to the cardiovascular conditioning, but do not promote change in the skeleton mineral content[13]. The relation of physical activity with the sexual steroids in determining osteoporosis has been widely studied. In castrated female rats with osteoporosis daily submitted to controlled and systematic treadmill running, physical exercise promoted not only bone mass gain in the whole skeleton when compared to the bone tissue quantity before the exercise, but also made the bone tissue quantity equal in some sites of the skeleton, or even greater than in the normal groups[35].

Moreover, it is also proved that physical exercise with moderate load, help in the maintenance or bone mass gain in post-menopause women[36]. In that same phase, physical activity almost always causes synergic anabolic effect when associated to a conventional kind of osteoporosis treatment[2].

Physical activity promotes changes in the bone metabolism through direct effect, via mechanical force, or indirect, promoted by hormonal factors[30,37]. The mechanical force when applied over the bone tissue forms endogenous signs that interfere in the processes of bone remodelling. Such signs are captured by a mechanosensoric system in which the osteocyte is the main cell responsible for translating the mechanical force into biochemical signs that regulate the bone turnover[8,38]. It is believed that the cell deformation caused by direct force over the cell, the intracanalicular pressure increase caused by dynamic force and the increase of the interstitial fluid flow speed, are factors that directly affect the osteocyte, being the last one, the higher stimulus to the osteocyte in response to mechanical load. The interstitial fluid flow through the canaliculum around the osteocyte, seems to be responsible for the extracellular matrix deformation and for changes in the cellular membranes[8]. Physical activity also promotes increase in the canaliculus branching connections of the osteocytes, increasing the bone matrix viability[35].

Concerning the effect of the mechanical force over the osteocytes, a lot is speculated about the autocrine function of the E2 prostaglandine (PGE2) in the gap junctions regulation and in the

Cx43 expression in the osteocytes membrane. The conversion of the aracdonic acid into E2 prostaglandine is observed in the osteocyte when mechanical force is applied. Its liberation would act as an autocrine[8] factor, once increase in prostaglandine liberated in the bone immediately after a mechanical force imposed by weight lifting, has been described in humans[39]. A PGE2 connects to its receptor expressed in the osteocyte membrane increasing the AMPc concentrations and stimulating the Cx43 protein formation and new gap junctions[8]. However, the prostaglandine effect over the skeleton is controversial. It usually acts as an anabolic agent, since that the prostaglandine administration in osteoblasts culture increases the number and activity of those cells, with increase in the bone matrix synthesis as a consequence[40]. Its catabolic effect stimulating the formation and osteoclasts activity has been already described, though[10]. It is believed that the distinct effects of the PGE2 over the bone are due to their many subtypes of receptors, such as EP1, EP2, EP3 and the EP4, connected to the G specific proteins. The exact function of these receptors over the bone cell, especially over the osteocyte, is unknown yet. It has been demonstrated that the EP2 receptor expression increases in response to the intersticial fluid flow[8]. Although it has been already described that the prostaglandine *in vivo,* activates the bone remodeling, in intact and oophorectomized animals[41], it is unknown what would be the prostaglandine participation as intermediary of physical activity effects in the skeleton with osteoporosis.

In an evaluation of the physical activity effect in the whole skeleton in the osteoporosis treatment, it was observed that after three months of physical activity, the thickining of the nasal bone of female rats with osteoporosis submitted to daily physical activity, was significantly higher in relation to sedentary animals with osteoporosis and even in relation to normal animals. However, the nasal bone does not suffer any kind of impact during exercising, suggesting that the beneficial effect caused by physical activity is not only mediated by the mechanical force, but also by hormones and growing factors[35].

The indirect effect of the physical activity over the bone put into action by hormonal factors, comprehends the citocines production and the liberation of growing factors by osteocyte, consequently, with the increase of the osteoblastic activity[30]. Besides that, the physical activity triggers a series of physiological responses involving the hypothalamus-hypophysis-adrenal and hypothalamus-hypophysis-gonades[32]. The physical activity stimulates the secretion of the growing hormone (GH)[31,37] that has direct or indirect anabolic effect, via growing factor similar to the 1 insulin (IGF-1)[37]. The IGF-1 is a citocine that stimulates the DNA synthesis and consequently promotes the collagen synthesis by the osteogenic cells, increasing the *in vivo* bone matrix formation[38]. Studies report that six hours after exercise there is an increase of the RNAm levels of IGF-1. However, the liberation of IGF-1 in the physical activity is controversial[31,37]. It is claimed that in humans, even without plasmatic increase, the IGF-1 local liberation is the responsible for the exercise anabolic effect over the bone tissue[37].

The circulant thyroid hormones and the cortisol secretion[33] also increase during physical activity. However, no mention is done about their participation as intermediary of the physical activity effect in the bone metabolism. Similar comment is made about the paratormonioum (PTH), whose concentrations suffer no change or increase during or after physical acitivity[37]. Differently, the sexual steroids can have their secretion inhibited by physical activity, with delay of sexual maturity and[42], if the activity is conducted with inadequate intensity, may cause hypostrogenism or hypoprogenism[30,32].

Physical activity affects the bone not only as tissue, but also as an organ for its action in the growth cartilages. The mechanical stress regulates the cartilage momeostasia not only during the endocondrial growth, but also during the fractures repairing[43]. The cartilage growing process basically involves three stages: the first consists of the chondrocytes activation in rest and the type II collagen production and the agrecan by the cells. The second stage is marked by the cease of the cellular proliferation, with the chondrocytes maturation and increase of the cartilage matrix synthesis. The last growing stage is characterized by the chondrocytes hypertrophy and type X collagen synthesis. During this stage yet, the chondrocytes go into apoptosis, the bone matrix is synthetized over the cartilage skeleton which is later removed[45]. The mechanism through which the mechanical force regulates the cartilage growth, changing the chondrocytes proliferation, maturation, hypertrophy and apoptosis has not been totally elucidated yet. It has been reported that the mechanical force promotes matrix deformations in the cartilage, eletrocinetic effects, hydrostatic pressure changes and increase in the intersticial fluid flow[44]. According to these researchers, all these factors would be important in the mechanotrasduction process of the mechanical sign. It has also been reported that ionic channels, especially the calcium ones, would be responsible for transmitting the mechanical sign to the cell, stimulating then the chondrocytes proliferation, maturation and hypertrophy. It is also mentioned that, the opening and closing control of these ionic channels would be done by the collagen matrix deformation caused by the mechanical stress[45].

PHYSICAL ACTIVITY EFFECT OVER THE BONE MARROW

The bone as an organ consists of many mesenchemical cells, among them are the osteoblasts, the chondrocytes, the myoblasts and the marrow stroma cells, including the adipocytes[46]. It is believed that all these cell lineages are originated from ordinary progenitor cells called bone marrow stroma stem-cells (MSCs). The bone tissue is constantly renewing during ones's lifetime and, such process demands the recruiting and proliferation of stem-cells able to differentiate into osteoblasts which will synthetize and mineralize the bone matrix. The MSCs offer osteoprogenitor cells and evidence in literature is found reporting that bone marrow cells transplant in the epiphysis of mice long bones promoted repopulation of cells able to differentiate into functional osteoblasts[47], which reinforces the importance of the marrow stroma cells in the osteoblastic differentiation.

The main factors that stimulate the marrow cells differentiation into osteoprogenitor cells have been subject to research. Although the factors that stimulate the bone marrow in the supply of osteocyte are not completely reported, it is known that the E2 prostaglandine[28], the growth hormone, the IGF1 and the bone morphogenetic proteins (BMPs) are important factors to the marrow osteoprogenitor cells recruiting[46]. Besides that, recent studies have shown that bone marrow stroma cell cultures *in vitro,* submitted to mechanical stimulation present greater differentiation to cells able to produce bone matrix[49]. A similar result *in vivo* was observed, in which physical activity promoted osteoblastic hyperplasia and increase of the red bone marrow/yellow bone marrow ratio in the osteopenic skeleton[35]. Physical activity increases the intracannicular fluid speed inside the osteocyte and consequently increases the E prostaglandine levels[39] that present autocrine action in the bone, stimulating the marrow stroma cell differentiation in osteoprogenitor cells[8].

FINAL CONSIDERATIONS

Although some results are contradictory, literature is clear about the beneficial effects of physical activity over the bone tissue in normal individuals and in osteoporosis prevention and treatment as well. Yet, the mechanisms through which the physical activity stimulates the osteoblastic differentiation from bone marrow stroma cells should be better elucidated. Such field is a promising one, especially with the expectation increase concerning the stem-cells for diseases treatment. Once the factors and mechanisms through which physical activity increases the bone mass in osteopenic in-

dividuals are elucidated, more suitable treatments can be developed to individuals with osteoporosis, especially to the ones who have restrictions to physical activity or post-menopause women, once the restriction to hormonal reposition has been gradually increasing due to vast side effects.

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REFERENCES

1. Notomi T, Okimoto N, Okazaki Y, Nakamura T, Suzuki M. Tower climbing exercise started 3 months after ovariectomy recovers bone strength of the femur and lumbar vertebrae in aged osteopenic rats. J Bone Miner Res 2003;18:140-9.
2. Mosekilde L, Thomsen JS, Orhii PB, McCarter RJ, Meijia W, Kalu DN. Additive effect of voluntary exercise and growth hormone treatment on bone strength assessed at four different skeletal sites in an aged rat model. Bone 1999;24:71-80.
3. Mackie EJ. Osteoblast: novel roles in orchestration of skeletal architecture. Int J Biochem Cell Biol 2003;35:1301-5.
4. Robey PG. The biochemistry of bone. Biol Metab Clin North Am 1989;18:859-902.
5. Nunes IJ, Nunes VA. Doenças metabólicas do osso. Cad Tec Esc Vet UFMG 1988;3:1-66.
6. Tate MK, Adamson JR, Tami AE, Bauer TW. The osteocytes. Int J Biochem Cell Biol 2004;36:1-8.
7. Cilliname DM. The role of osteocytes in bone regulation: mineral homeostasis versus mechanoreception. J Musculoskelet Neuronal Interact 2002;2:242-4.
8. Cherian PP, Cheng B, Gu S, Sprague E, Bonewald LF, Jiang JX. Effects of mechanical strain on the function of gap junctions in osteocytes are mediated through the prostaglandin EP2 receptor. J Biol Chem 2003;278:43146-56.
9. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. Nature 2003;423:337-42.
10. Raisz LG. Physiology and pathophysiology of bone remodeling. Clin Chem 1999; 45:1353-8.
11. Bland R. Steroid hormone receptor expression and action in bone. Clin Sci 2000; 98:217-40.
12. Ribeiro AFC, Serakides R, Ocarino NM, Nunes VA. Efeito da associação hipotireoidismo-castração no osso e nas paratireóides de ratas adultas. Arq Bras Endocrinol Metab 2004;48:525-34.
13. Sinaki M. Exercise and osteoporosis. Arch Phys Med Rehabil 1989;70:220-9.
14. Hillard TC, Stevenson JC. Role of oestrogen in the development of osteoporosis. Calcif Tissue Int 1991;49:S55-9.
15. Ishida Y, Tertinegg I, Heersche JNM. Progesterone and dexamethasone stimulate proliferation and differentiation of osteoprogenitors and progenitors for adipocytes and macrophages in cell populations derived from adult rat vertebrae. J Bone Miner Res 1996;11:921-30.
16. Tomkinson A, Gevers EF, Wit JM, Reeve J, Noble BS. The role of estrogen in the control of rat osteocyte apoptosis. J Bone Miner Res 1998;13:1243-50.
17. Bilezikian JP, Raizs LG, Rodan GA. Principles of bone biology. San Diego: Academic Press, 1996;1398p.
18. Serakides R. Relação tireóide, gônadas e metabolismo ósseo em ratas adultas hipertireóideas e eutireóideas (Tese, Doutorado em Ciência Animal). Belo Horizonte, Escola de Veterinária, UFMG, 2001;90p.
19. Colin EM, Van Den Bemd GH, Van Aken M, Christakos S, De Jonge HR, Deluca HF, et al. Evidence for involved of 17 beta-estradiol in intestinal calcium absorption independent of 1,25-dihydroxyvitamin D3 level in the rat. J Bone Miner Res 1999;14:57-64.
20. Liel Y, Shany S, Smirnoff P, Schwartz B. Estrogen increases 1,25-dihydroxyvitamin D receptors expression and bioresponse in the rat duodenal mucosa. Endocrinology 1999;140:280-5.
21. Schwartz B, Smirnoff P, Shany S, Sliel Y. Estrogen controls expression and bioresponse of 1,25-dihydroxyvitamin D receptors in the rat colon. Mol Cell Biochem 2000;203:87-93.
22. Williams GR, Robson H, Shalet SM. Thyroid hormone actions on cartilage and bone: interactions with other hormones at the epiphyseal plate and effects on linear growth. J Endocrinol 1998;157:391-403.
23. Serakides R, Nunes VA, Ocarino NM, Nascimento EF. Efeito da associação hipertireoidismo-castração no osso de ratas adultas. Arq Bras Endocrinol Metab 2004; 48:875-84.
24. Banovac K, Koren E. Triiodothyronine stimulates the release of membrane-bound alkaline phosphatase in osteoblastic cells. Calcif Tissue Int 2000;67:460-5.
25. Cross HS, Pölzleitner D, Peterlik M. Intestinal phosphate and calcium absorption: joint regulation by thyroid hormones and 1,25-dihydroxyvitamin D3. Acta Endocrinol (Copenh) 1986;113:96-103.
26. Cross HS, Peterlik M. Differentiation-dependent expression of calcitriol actions of absorptive processes in cultured chick intestine: modulation by triiodothyronine. Acta Endocrinol (Copenh) 1991;124:679-84.
27. Krolner B, Toft B. Vertebral bone loss: unheeded side effect therapeutic bed rest. Clin Sci 1983;64:537-40.
28. Mo A, Yao W, Li C, Tian X, Su M, Ling Y, et al. Bipedal stance exercise and prostaglandin E2 (PGE2) and its synergistic effect in increased bone mass and in lowering the PGE2 dose required to prevent ovariectomized-induced cancellous bone loss in aged rats. Bone 2002;31:402-6.
29. Bourrin S, Genty C, Palle S, Gharib C, Alexandre C. Adverse effects of strenuous exercise: a densitometric and histomorphometric study in the rat. J Appl Physiol 1994;76:1999-2005.
30. Henderson NK, White CP, Eisman JA. The roles of exercise and fall risk reduction in the prevention of osteoporosis. Endocrinol Metab Clin North Am 1998; 27:369-87.
31. Wallace J D, Cuneo RS, Lundberg PA, Rosen T, Jorgensen JOL, Longobardi S, et al. Responses of markers of bone and collagen turnover to exercise, growth hormone (GH) administration, and GH withdrawal in trained adult male. J Clin Endocrinol Metab 2000;85:124-33.
32. Warren MP, Perlroth NE. The effects of intense exercise on the female reproductive system. J Endocrinol 2001;170:3-11.
33. Jahreis G, Kauf E, Frohner G, Schmidt HE. Influence of intensive exercise on insulin-like growth factor 1, thyroid and steroid hormones in female gymnasts. Growth Regul 1991;1:95-9.
34. Guimaraes CV, Borges LS, Rocha CC, Ocarino NM, Cardoso TGS, Marubayashi U, Serakides R. Atividade física em ratas durante o crescimento causa osteoporose em ossos não submetidos ao impacto mecânico direto. Arq Bras Med Vet Zootec 2005;57(Supl 1):127.
35. Ocarino NM. Atividade física no tratamento da osteoporose: histomorfometria do esqueleto axial e apendicular de ratas (Dissertação, Mestrado em Medicina Veterinária). Belo Horizonte, Escola de Veterinária, UFMG, 2004;78p.
36. Berard A, Bravo G, Gauthier P. Meta-analysis of the effectiveness of physical activity for the prevention of bone loss in postmenopausal women. Osteoporos Int 1997;7:331-7.
37. Brahm H, Piehl-Aulin K, Ljunghall S. Net fluxes over working thigh of hormones, growth factors and biomarkers of bone metabolism during short lasting dynamic exercise. Calcif Tissue Int 1997;60:175-80.
38. Nomura S, Yamamoto TT. Molecular event caused by mechanical stress in bone. Matrix Biol 2000;19:91-6.
39. Thorsen K, Kristoffersson AO, Lerner H, Lorentzon RP. In situ microdialysis in bone tissue- stimulation of prostaglandin E2 release by weight-bearing mechanical loading. J Clin Invest 1996;98:2446-9.
40. Baylink TM, Mohan S, Fitzsimmons RJ, Baylink DJ. Evaluation of signal transduction mechanisms for the mitogenic effects of prostaglandin E2 in normal human bone cells in vitro. J Bone Miner Res 1996;10:1413-8.
41. Juliani MH, Souza GM, Klein C. cAMP stimulation of Dictyostelium discoideum destabilizes the mRNA for 117 antigen. J Biol Chem 1990;265:9077-82.
42. Pellerin-Massicotte J, Brisson GR, St-Pierre C, Rioux P, Rajotte D. Effect of exercise on the onset of puberty, gonadotropins, and ovarian inhibin. J Appl Physiol 1987;63:1165-73.
43. Kim YJ, Sah RL, Grodzinsky AJ, Plaas AH, Sandy JD, et al. Mechanical regulation of cartilage biosynthetic behavior: physical stimuli. Arch Biochem Biophys 1994; 31:1-12.
44. Sah RL, Grodzinsky AJ, Plaas AH, Sandy JD. Effects of tissue compression on the hyaluronate-binding properties of newly synthesized proteoglycans in cartilage explants. Biochem J 1990;267:803-8.
45. Wu QQ, Chen Q. Mechanoregulation of chondrocyte proliferation, maturation and hypertrophy: ion-channel dependent transduction of matrix deformation signals. Exp Cell Res 2000;256:383-91.
46. Yamaguchi A, Komori T, Suda T. Regulation of osteoblast differentiation mediated by bone morphogenetic proteins, hedgehogs, and Cbfa1. Endocr Rev 2000;21:393-411.
47. Olmsted-Davis EA, Gugala Z, Camargo F, Gannon FH, Jackson K, Kienstra KA, et al. Primitive adult hematopoietic stem cells can function as osteoblast precursors. Medical Sci 2003;100:15877-82.
48. Mauney JR, Sjostorm S, Blumberg J, Horan R, O'Leary JP, Vunjak-Novakovic G**,** et al. Mechanical stimulation promotes osteogenic differentiation of human bone marrow stromal cells on 3-D partially demineralized bone scaffolds *in vitro*. Calcif Tissue Int 2004;74:458-68.