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Comparative Analysis Of Bone Density Of Rats With Experimental Hypoparathyroidism During Calcium And Magnesium Intake.

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ABSTRACT

Osteoporosis is a systematic skeletal disease, characterized by reduction in bone mass and disruption of the microarchitectural structure of bone tissue, resulting in increase of brittleness of the bones and high risk of fracture. Mineral metabolism can be corrected by calcium and magnesium intake. Bone neogenesis is adequately displayed in CT scan partitions.

Keywords: Hypoparathyroidism, osteoporosis, bone density, correction of mineral metabolism.

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INTRODUCTION

Hypoparathyroidism leads to the mineral metabolic imbalance, calcium metabolic imbalance in particular. Parathormone plays an important role in the regulation of this ion. As the level of parathormone decreases, bone tissue resorption leads to hypercalcaemia. Mineral bone density changes, which results in the development of osteoporosis. Osteoporosis is a systematic skeletal disease characterized by reduction in bone mass per volume unit and disruption of its microarchitectural structure leading to higher brittleness of the bones and high risk of fracture. According to WHO, osteoporosis takes the forth place among noncommunicable diseases (first three places are distributed as follows: cardio-vascular, oncological diseases and diabetes mellitus) as a cause of death rate and disability of the population. Parathormone therapy of hypoparathyroidism (replacement therapy) has not been widely used. Long-term application of parathormone leads to side effects and anaphylactic reactions as well to its resistance. There is a risk of its overdose among patients with the renal form of primary hypoparathyroidism who underwent the parathyroid adenoma resection as such overdose can cause irreversible changes in renal system at any moment [6].

The disruption of parathyroid gland functions results in mineral metabolic imbalance, for example, lower levels of ionized calcium in the blood cause nervous and muscular irritability, which leads to tonic spasms as a specific symptom of hypoparathyroidism. However, it should be taken into account that nervous and muscular irritability depends on the blood pH, calcium ionization degree, phosphates, sodium, potassium and magnesium levels. Vitamin D deficiency, which in its turn affects bone density, can provoke the development of osteoporosis. According to up-to-date sources, osteoporosis therapy of hypoparathyroidism patients includes thyroxin (supported by practical evidence of increasing rate of the bone tissue loss). After a 1-year period of medication treatment (replacement therapy) the following was noticed: 1) higher rate of the bone loss in the spine bone and femoral neck; 2) at the same time, there is an evidence of the absence of the bone tissue loss in the radius bone after a 3-year period of thyroid hormone treatment; 3) the length of treatment is of importance as well [9]. That is why the research of nonhormonal medication which will both effectively restore the bone tissue and regulate mineral metabolism is of primary importance these days.

Bone tissue is an active dynamic system characterized by the constant processes of the old bone tissue resorption and the formation of the new one. These processes represent a bone remodeling cycle which begins before birth and continues until death [7,9]. Parathyroid hormone (PTH) contributes most into the bone remodeling regulation; bones, intestine, renal system are its target organs. PTH primary effect is calcium homeostasis maintenance: the decrease in Ca^{2+} concentration by 1 – 2 % results in the immediate increase in PTH level by 40 – 50 % (feedback mechanism), which is accompanied by fast enhansion of bone resorption, higher calcium absorption in intestine (due to stimulation of renal calcitriol production) as well as renal calcium reabsorption [14,15].

Hypoparathyroidism can be caused by either insufficient PTH secretion or the disruption of the hormone structure or its receptors. Hypoparathyroidism can be diagnosed even in neonatal period: transitional, sporadic, inherited forms of the disease. At pseudohypoparathyroidism the PTH concentration in the blood is normal but the target cells are resistant to it due to G-protein mutation. It is accompanied by hypocalcemia and hyperphosphaturia. Such patients are characterized by physical and mental developmental delay, early cataract and subcutaneous ossificates. Skeletal changes are also typical, manifestations of which can be notable in the blunting of the 4^{th} and 5^{th} metacarpal and metatarsal foot and hand bones. These skeletal changes are easily identified during patient examination: if the patient is asked to clench his fist, the knuckles will be visibly out of level. Apart from the skeletal pathology, there are other hypocalcemia-related symptoms at any type of hypoparathyroidism: tonic spasms of the skeletal muscles, mostly of the extensor muscles of the upper limbs, preceded by the signal symptoms (shivers, paraesthesia, etc.); facial muscles spasm (risus sardonicus); main d'accoucheur spasm; intestinal smooth muscles spasm manifesting itself as the abdominal pain; breathing muscles spasm (laryngospasm). Sometimes spasms of internal smooth muscles simulate clinical picture of other diseases such as appendicitis, cholecystitis, gastric ulcer, asthma, migraine, etc. Long-lasting laryngospasm can be fatal due to the threat of asphyxia.

In the long run of hypoparathyroidism children have growth delay, disrupted odontogenesis and caries. As a result of low skeletal calcium mobilization its level in the blood serum remains low. X-ray diagnostics and estimation of bone mineral density reveals the decreased level of calcium in bone tissue.

One of the efficient combination medications containing calcium and vitamin D is "Calcium-D_3 Nycomed". Currently, there are enough data on the results of various controlled trials which confirm the efficiency and safety of the calcium and vitamin D combination therapy [10,12,13,14].

However, this ion cannot be examined without its interaction with magnesium. Magnesium is also a part of the bone tissue – more than 50 %, it maintains electrical irritation of nerve and muscle fibres, participates in biochemical power supply reactions in the cells, is responsible for the production and release of mediators in the nerve cells as well as affects potassium, sodium, calcium, phosphorus and vitamin metabolism. In addition, calcium channels inside the cells are activated only with magnesium ions, although when being absorbed in the intestinal tract calcium and magnesium are antagonists.

Having regard to the above said, the aim of our research is the study of the comparative bone density of rats with experimental hypoparathyroidism under conditions of calcium and magnesium intake.

MATERIALS AND METHODS

The research was conducted on adult white feminine Vistar line rats (96 animals in total) which were divided into eight groups (12 in each group): 1 – (intact rats) control group; 2 – intact rats that were given "Calcium-D_3 Nycomed" at a dose of 100 mg of Ca^{2+} and 80ME of D_3 per 100 gr of live weight; 3 – intact rats that were given "Magnelis B_6 Forte" at a dose of 10 mg of Mg^{2+} per 100 gr of live weight; 4 – intact rats that were given Ca^{2+} and Mg^{2+} at the same time according to the scheme (Ca^{2+} at 10 a.m., Mg^{2+} at 4 p.m. daily over a 30-day period); 5 – rats with hypoparathyroidism; 6 – rats with hypoparathyroidism that were given 100 mg of Ca^{2+} and 80ME of D_3 per 100 gr of live weight; 7 – rats with hypoparathyroidism that were given10 mg of Mg^{2+} per rat; 8 – rats with hypoparathyroidism that were given at the same time according to the scheme. Medications were included into the dietary supplement – cottage cheese balls.

Hypoparathyroidism was created by the electrocoagulation of the pair of parathyroid glands according to the best practice. We conformed to the works by Khudaverdian D.N. (1978) [8], Fugii T., Gamomoto H., Morimoto Sh. (1988) [11] when modeling deficiency and excess of PTH. These methods of parathormone level change have been used in our laboratory since 1985.

Estimation of bone mineral density

Estimation of bone mineral density (BMD) of animals was conducted *ex vivo* on femoral bones selected during postmortem dissection and put into 70% ethyl alcohol. The research was carried out using SkyScan 1176 X-ray microtomograph (Bruker-microCT, Belgium). Scanning was performed in accordance with the manufacturer's recommendations. Polymer disks (with BMD values of 0.25 and 0.75 g/cm^3 calciumhydroxyapatite CaHA) were used as reference standards for mineral density determination and were scanned at the same parameters as the rats' femoral bones.

Scanning parameters: Skyscan 1176 control program (10.0.0.0, Bruker-microCT, Belgium): X-ray voltage 80 kV, X-ray current 300 µA, filter Cu+Al, image pixel size 17.74 µm, rotation step 0.3, frame averaging 3.

The scanned objects were reconstructed using program Nrecon (1.7.4.2, Bruker-microCT, Belgium) with the following reconstruction parameters: ring reduction (15), beam hardening (41).

Orientation in space (x, y, z) and selection of reconstructed material areas were performed using program DataViewer (1.5.6.2, Bruker-microCT, Belgium) in the same manner for all bones. Visualization and 3D analysis of data were conducted in program CT-analyser (1.18.4.0, Bruker-microCT, Belgium). 3D analysis of the trabecular part of femoral bones in all the experimental animal models was carried out in automatic mode, with an area of the same location being selected [2].

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Statistics

Statistical processing of data was conducted using parametric analysis methods and Microsoft Excel software package. The accuracy of different averages was determined using Student t-test (t). As for the variation coefficient, the p-level chosen was not less than 0.05.

During the laboratory experiment we fully complied with the international rules of European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasburg, 1987) [2], Declaration on ethical treatment of animals used for scientific purposes set out in contemporary recommendations for European independent ethics committees (Brussels, 1995,1997) [1], Operational Guidelines for Ethics Committees that Review Biomedical Research (Geneva, 2000) [4], Principles of Good Laboratory Practice and Order of Ministry of Public Health and Social Development of the Russian Federation dated August 23, 2010 N 708n Moscow "On approval of regulations of laboratory practice" [3].

RESULTS

According to the results of the given research, trabecular bone density in rats with experimental hyperparathyroidism is half as high as in control group (Group 5 – 0.078±0.007 g/cm^3, Group 1 – 0.158±0.014 g/cm^3), which is a compensatory response for maintaining calcium homeostasis in blood. However, during calcium intake the density definitely increases and exceeds the stated values, Group 6 – 0.256±0.038 g/cm^3. In case of pure magnesium intake the density also increases significantly compared to PTE Group (parathyroidectomy) – Group 7 – 0.220±0.025 g/cm^3. During simultaneous calcium and magnesium intake in rats with experimental hyperparathyroidism (Group 8), the trabecular bone density increases up to intermediate values – 0.231±0.034 g/cm^3 between Group 6 (pure calcium) – 0.256±0.038 g/cm^3 and Group 7 (pure magnesium) – 0.220±0.025 g/cm^3.

In intact rats that were given calcium and magnesium at the same time, the bone density twice exceeds the stated values: Group 4 – 0.324±0.028 g/cm^3 compared to Group 1 – 0.158±0.014 g/cm^3.

Table 1: Mineral density of trabecular bone in experimental animal models (BMD g/cm^3).

№	Groups	BMDg/cm^3
1.	Intact (control).	0.158±0.014
2.	Intact rats that were given Ca^{2+}.	0.220±0.057
3.	Intact rats that were given Mg^{2+}.	0.180±0.042
4.	Intact rats that were given Ca^{2+} and Mg^{2+} at the same time.	0.324±0.028
5.	Rats with experimental hyperparathyroidism.	0.078±0.007
6.	Rats with experimental hyperparathyroidism that were given Ca^{2+}.	0.256±0.038
7.	Rats with experimental hyperparathyroidism that were given Mg^{2+}.	0.220±0.025
8.	Rats with experimental hyperparathyroidism that were given Ca^{2+} and Mg^{2+} at the same time.	0.231±0.034

Based on the obtained X-ray partitions of rat femoral bones it can be seen that there is an increase in the number of trabecules, which results in bone density increase during calcium and magnesium intake in a comparatively short period of time – 1 month (Figures 1). BMD change is especially noticeable in experimental hyperparathyroidism and its correction (Figures 2). Notably, as can be seen in Figures 1 b, c and Figures 1 c, e the trabecule structure during magnesium intake is richer in contrast compared to that during calcium intake.

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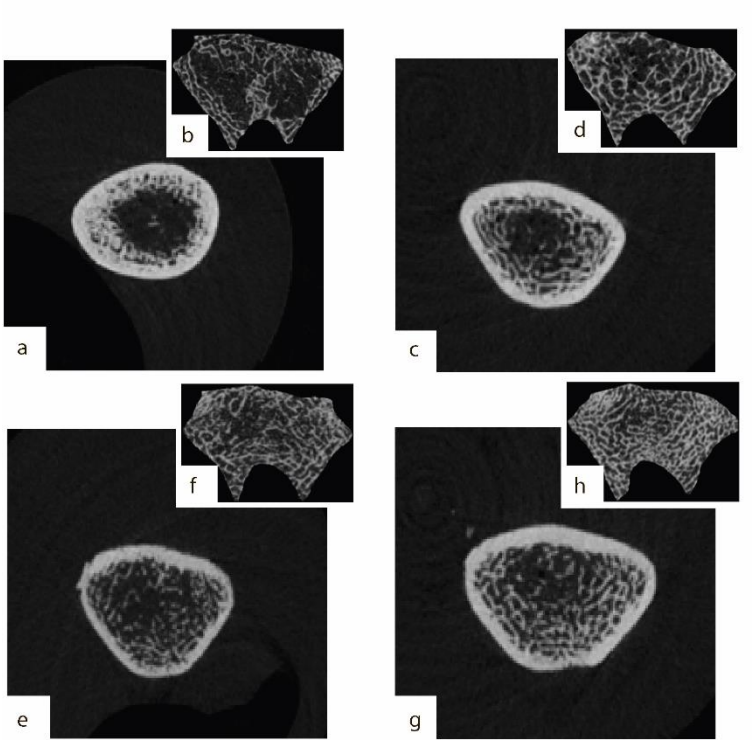

Figure 1:. a,b - X-ray partition of the intact rat's femoral bone; c,d - X-ray partition of the femoral bone of the intact rat that was given calcium; e, f - X-ray partition of the femoral bone of the intact rat that was given magnesium; g, h - X-ray partition of the femoral bone of the intact rat that was given calcium and magnesium at the same time.

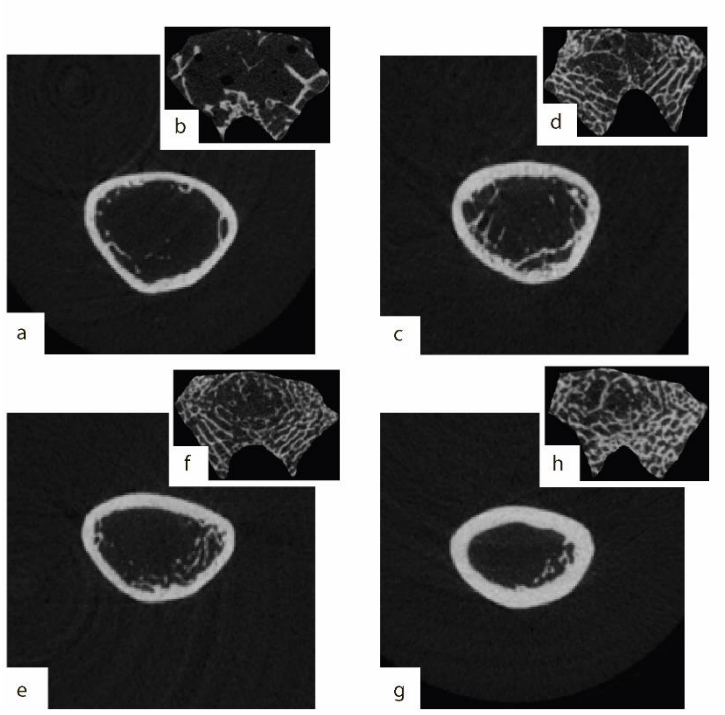

Figure 2: a, b - X-ray partition of the femoral bone of the rat with experimental hyperparathyroidism; c,d - X-ray partition of the femoral bone of the rat with experimental hyperparathyroidism that was given calcium; e, f - X-ray partition of the femoral bone of the rat with experimental hyperparathyroidism that was given magnesium; g, h - X-ray partition of the femoral bone of the rat with experimental hyperparathyroidism that was given calcium and magnesium at the same time.

DISCUSSION

Thus, trabecular bone density definitely increases during hyperparathyroidism correction by simultaneous calcium and magnesium intake, which has a favorable effect on the ions relationship.

At the same time, as far as a relatively healthy constitution is concerned, simultaneous calcium and magnesium intake results in the twofold increase in trabecular bone density, which can evidence alimentary hypercalcaemia and hupermagnesemia. This, in turn, can lead to the risk of mineral metabolic imbalance, and in the worst case, calcificate formation.

REFERENCES

[1] The declaration on the humane treatment of animals used for scientific purposes, set forth in the current recommendations of the European Independent Ethics Committees. Brussels. 1995 –1997.

[2] European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. Strasbourg, 1987.

[3] The Ministry of Health and Social Development of the Russian Federation of August 23, 2010 N 708n Moscow "On approval of the Laboratory Practice Rules".

[4] Recommendations by the Ethics Committee conducting an examination of biomedical research. Geneva. –2000.

[5] Bouxsein M. L., Boyd S. K., Christiansen B. A., Guldberg R. E., Jepsen K. J., Muller R. (2010) :Guidelines for assessment of bone microstructure in rodents using micro–computed tomography. J. of Bone and Mineral Res. 25 (7), 1468-1486. DOI:10.1002/jbmr.141.

[6] Rational pharmacotherapy of diseases of the endocrine system and metabolic disorders: a Guide for practitioners / Ed. ed. I.I. Dedova, G.A. Melnichenko. Moscow2006.

[7] Riggs B.L., Melton L.D. (2000): Osteoporosis. Etiology, diagnosis, treatment. Moscow

[8] Khudaverdyan D.N. (1978): Features of reflex reactions of the spinal cord in experimental hypoparathyroidism // Bull. expert. biol. and med. 86 (12), 659-662.

[9] Schwartz G.Ya. (2002) Pharmacotherapy of osteoporosis. Moscow

[10] Heaney RP, Weaver CM. (2003):Calcium and vitamin D.Endocrin Metab Clin NA; 32(1): 181–94.

[11] Fugii, T., Gamomoto H., Morimoto Sh. (1988): Hypercalcitoninemia in the offepring of parathyroid transplanted rats// Proc. Jap. Acad. 64 (10), 315-318.

[12] Painter S.E, Kleerekoper M, Camacho P.M.2006: Secondary osteoporosis: a review of the recent evidence. Endocr Pract; 12(4): 436–45.

[13] Peacock M. (2010): Calcium metabolism in health and disease. CJASN; 5: S 23-S30.

[14] Schacht E, Richy F, Reginster J-Y. : The therapeutic effects of alfacalcidol on bone strength, muscle metabolism and prevention of falls and fractures. J Musculoskelet Neuronal Interact 2005; 5 (3): 273-84.

[15] Steingrimsdottir L, Gunnarsson O, Indridason OS, et al. (2005): Relationship between serum parathyroid hormone levels, vitamin D sufficiency, and calcium intake. JAMA, 294 (18): 2336-41.