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Effect of Fluid and Salt Supplementation in Preventing Osteopenia in Rats after Exposure to Hypokinesia

Y. G. Zorbas, Y. F. Federenko and M. N. Togawa

European Institute of Environmental Cybernetics, Athens (Greece)

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The objective of this investigation was to evaluate the efficacy of fluid and salt supplementation in preventing the development of osteopenia in 150 Wistar male rats (370 - 390 g) after exposure to 90-days of hypokinesia. They were divided into three equal groups: 1st placed under ordinary vivarium conditions (vivarium control animals); 2nd subjected to hypokinesia (unsupplemented hypokinetic animals, HK); and 3rd submitted again to HK and daily supplemented with water (5 ml/100 g b. w.) and NaCl at 0.9 g % (3 ml/100 g b. w.) orally administered (supplemented hypokinetic animals). The hypokinetic effect was carried out by keeping the rats in small individual wood cages which restricted all their movements without hindering feed and water intake. Determination was made of weight and volume of their entire bone, head and distal epiphysis, as well as density, ash and mineral content. Thickness of the cortical layer and width of the bone marrow canal were measured on frontal and lateral x-ray projections. Histological transverse sections of the femoral diaphysis were prepared from the femoral bone fragments. The concentrations of calcium, phosphorus and creatinine in serum were also measured. The results obtained indicate that the daily administration of fluid and salt supplementation inhibited the progressive development of osteopenia in rats subjected to prolonged restriction of motor activity.

Key words: Osteopenia, Hypokinesia, Hyperhydration, Rat.

Different causes of osteoporosis are presently known. Experimental studies on animals and men indicate that a restriction of motor activity elicits impairment of the normal process of bone formation and leads to development of osteopenia (5).

Actual and simulated hypokinesia in different animals leads to inhibition of bone growth, development

Correspondence to Y. F. Federenko: Hypokinetic Physiology Lab., European Institute of Environmental Cybernetics, GR-162 32 Athens (Greece).

of osteoporosis and reduction in bone strength (4, 5).

In previous initial studies in animals (8) and men (9), the effect of fluid and salt supplementation in counteracting the development of osteopenia in bones following exposure to long-term restriction of motor activity has been studied.

The objective of this investigation was to evaluate the efficacy of a daily administration of fluid and salt supplementation in counteracting regional osteopenia in extremities of rats after exposure to prolonged restriction of motor activity.

Materials and Methods

Experiments were performed on 168 white male Wistar rats delivered at our laboratory at the age of 64 to 66 days and weighed 285 to 287 g. After screening only 150 clinically healthy animals, 370-390 g b. w. and about 97 days old, were selected.

The experiments were conducted according to the WHO Helsinki medical considerations and were accepted by the ethical committee of the Institute.

The experimental studies were preceded by clinical and physiological examinations, screening, training, testing and conditioning of rats to the new type of laboratory conditions.

During the preexperimental period, the animals were placed under ordinary vivarium conditions in stationary cages made of wood which had a size of 490 × 380 × 165 mm. Air temperature was about 22 ± 2 °C, and relative humidity was about 79 ± 3 %, with 12-hours of day light throughout the entire experimental period.

The animals were switched from dry food (40 g/rat) to homogenized laboratory food, which was given once a day, 15 days prior to the start of the experimental studies. All rats were pair-fed.

Adaptation process to laboratory conditions lasted about 540 to 680 h and 150 truly comparable animals were selected, 100 of which were subjected to hypokinesia and 50 were placed under ordinary vivarium conditions (vivarium control). The vivarium control animals were housed in the same cages as those used during the preexperimental period (4 to 5 animals/cage).

The experimental animals (unsupplemented hypokinetic and supplemented hypokinetic animals) were kept in small individual cages of 145 × 50 × 65 mm, and allowed for restricting movements in all directions without hindering food and water intake. The experimental animals could assume a postural position and groom different parts of their body. When necessary, the dimentions of the individual cages could be reduced using special wood inserts. The cages were constructed in such a way so that their size could change in accordance with the size of each animal so that the degree of restriction of movements could be maintained at a relatively constant level. The cages were placed on aluminium trays that were 540 × 220 × 30 mm in size. The presence of wood shavings on the trays provided for good heat insulation, convenience and simplicity of animal care. The biologically active substances contained in the sawdust provided an adequate microclimate in the cage. When it was necessary to collect excrements, the cages were placed on a perforated tray, which permitted separated collection of feces and urine.

All animals were kept on a normal diet with 0.4 % NaCl, 0.6 % calcium and 0.6 % phosphorus and they were given vitamin D3 in a synthetic form by mouth in a dosage of 1.25 μg in a 0.1 % propylene glycol solution. At the initial 15 days of the preexperimental period, the animals

adapted to their diet; in the next 15 days baseline data were collected, and control values of the examined parameters were measured. Water intake was not limited, but we kept a strict record of fluid intake (the sum of water content of foods and additional water consumed by the animals) during the preexperimental period of 30 days and during the experimental period of 90 days.

The 1st group of rats were placed under ordinary vivarium conditions (vivarium control rats) and took fluid and salt supplements; the 2nd group of rats were kept under hypokinetic conditions supplementation (unsupplemented hypokinetic rats); and the 3rd group of rats were subjected to combined hypokinesia and consume daily an additional amount of fluid and salt supplementation (supplemented hypokinetic rats). This supplement consisted in 5 ml water/100 g b. w. and 3 ml NaCl at 0.9 g %/100 g b. w. and administered orally with a stomach tube. After the 90 day experimental period, the animals weight and length of the tail were measured and then sacrificed under ether anesthesia. Then, the femurs of the examined animals were isolated, fixed in 0.5 % neutral formalin and stored under refrigeration conditions until used. Determination was made of weight and volume of the entire bone, head and distal epiphysis, as well as density, ash and mineral content. Thickness of the cortical layer and width of the bone marrow canal were measured on frontal and lateral x-ray projections (6). Histological transverse sections of the femoral diaphysis were prepared from the femoral bone fragments. The preparations were stained with hematoxylin, and eosin according to van Gieson. Mineralization of bone microstructures was determined by the method of quantitative contact microroentgenography of cross-sections, determining mineralization of 20-30 points on each microroentgenogram. Cross-sections of the femoral diaphysis were used for microroentgenography, and mineralization of microstructures was measured over the entire width of the cortical layer of the examined animals. Each pair of microroentgenograms of cross-sections of both femurs from the same animal was measured according to mean mineralization of microstructures in the experimental and control groups of animals.

Serum calcium and phosphorus concentrations and creatinine content were measured. All measurements were performed in duplicate. Total calcium was determined using atomic absorption spectrophotometry, inorganic phosphorus measured using flame photometry and creatinine was determined by a Technicon Autoanalyzer.

Statistical analysis.– Analysis of variance was used followed by an ANOVA, and the Tukey-Kramer test for post-hoc comparisons. $P < 0.05$ were considered statistically significant.

Results

By the end of the experimental period the body weight, length of tail and length of tibia of the unsupplemented hypokinetic animals (2nd group) decreased significantly (table I). A significant reduction in thickness of the cortical layer of the femoral diaphysis in the limb of the 2nd group of animals was also observed, as compared to the 1st and the 3rd group of animals, more so in the frontal projection than lateral, without any noticeable increase in width of the medullary canal (table II). As a result, density of the spongiosa of the femur of the unsupplemeted hypokinetic limb of rats, head and distal epiphysis, was considerably lower by the

Table I. *Effect of fluid and salt supplementation on body weight, length of tail and tibia of rats after exposure to 90-days of hypokinesia.*
(Mean ± SD; n = 50).

Groups	Initial Body Weight (g)	End of the Hypokinetic Period		
		Body Weight (g)	Length of Tail (mm)	Length of Tibia (mm)
1st	387 ± 12.3	570 ± 10.7	169.0 ± 3.5	40.2 ± 0.8
2nd	383 ± 11.6	249 ± 9.5*	154.2 ± 2.7*	34.6 ± 0.8*
3rd	386 ± 10.4	598 ± 14.2+	173.6 ± 2.6	45.7 ± 0.2+

* $p < 0.05$ as compared to the ambulatory control animals (1st group).
+ $p < 0.05$ as compared to the unsupplemented hypokinetic animals (2nd group).

Table II. *Changes in transverse dimensions (mm) of femoral diaphysis of the limb of rats after exposure to pure hypokinesia and combined hypokinesia with fluid and salt supplementation.*
(Mean ± SEM; n = 50).

Parameters	Groups of Animals		
	1st	2nd	3rd
Thickness of Cortical Layer	1.20 ± 0.04	0.94 ± 0.02*	1.05 ± 0.07+
Diaphysis Width	3.56 ± 0.03	3.22 ± 0.06*	3.38 ± 0.05+
Width of Medullary Canal	2.53 ± 0.05	2.24 ± 0.08*	2.30 ± 0.06+

* $p < 0.05$ as compared to the 1st group. + $p < 0.05$ as compared to the 2nd group.

90th day of the experimental period than the other rats (table III). The reduction in bone mineralization reflected development of osteopenia and a smalller reduction in mineralization of organic matter; ash content of bone tissue and the changes in the head were less pronounced.

Histological examinations of the femoral diaphysis of all groups of animals revealed a significant increase in porosity of bone only in the unsupplemented hypokinetic animals, which were characterized by an increase in diameter of haversian canals, narrowing of cortical layer, discontinuity and unevenness of the layer of external general plates. The appearance of a smaller width in the cortical bone plate was indicative of depression of osteogenetic processes on the limb of rats in the absence or reduction of motor activity as a result of exposure to prolonged hypokinetic conditions.

By the end of the experimental period mean levels of mineralization of bone microstructure in the unsupplemented hypokinetic animals did not change significantly as compared to the ambulatory control and supplemeted hypokinetic animals.

In the unsupplemented hypokinetic animals serum concentrations of calcium, phosphorus and creatinine increased significantly as compared to the ambulatory control and supplemented hypokinetic animals by the 90th day of the experimental period (table IV).

Table III. *Changes in mineralization, density and ash content of fragments of rat femur, after exposure to pure hypokinesia and combined with fluid and salt supplements.* (Mean ± SEM; n = 50).

Parameters	Femoral Fragments	Groups of Animals		
		1st	2nd	3rd
Mineralization (g/cm^3)	Head	0.595 ± 0.02	0.433 ± 0.07*	0.569 ± 0.02+
	Distal Epiphysis	0.515 ± 0.04	0.294 ± 0.01*	0.493 ± 0.03+
Density (g/cm^3)	Head	1.023 ± 0.02	0.702 ± 0.03*	1.016 ± 0.06+
	Distal Epiphysis	0.780 ± 0.01	0.603 ± 0.01*	0.741 ± 0.08+
Ash Content (%)	Head	0.607 ± 0.02	0.555 ± 0.02*	0.580 ± 0.01+
	Distal Epiphysis	0.558 ± 0.03*	0.474 ± 0.01*	0.532 ± 0.04+

* $p < 0.05$ as compared to the 1st group.
+ $p < 0.05$ as compared to the 2nd group.

Table IV. *Blood serum concentrations of creatinine, calcium and phosphorus in rats after exposure to pure hypokinesia and combined with fluid and salt supplementation.* (Mean ± SEM; n = 50).

Parameters	Groups of Animals		
	1st	2nd	3rd
Creatinine (mg/dl)	0.6 ± 0.03	1.7 ± 0.06*	0.7 ± 0.03+
Calcium (mEq/l)	4.3 ± 0.04	5.5 ± 0.5*	4.8 ± 0.4+
Phosphorus (mg/dl)	3.2 ± 0.03	3.9 ± 1.3*	3.6 ± 1.3+

* $p < 0.05$ as compared to the 1st group.
+ $p < 0.05$ as compared to the 2nd group.

Discussion

The demonstrated losses of body weight in the unsupplemeted hypokinetic animals after exposure to hypokinesia is a typical reaction that developed during prolonged restriction of motor activity (1, 2). The slower growth of the unsupplemented hypokinetic animals, after exposure to hypokinesia was apparently attributable to impaired metabolic processes in the direction of prevalence of dissimilation, mobilization of fat from fat depots (1) and dehydration of the body (7) due to intensification of excretion of fluid during prolonged restriction of motor activity.

The results obtained made it possible to demonstrate that prolonged restriction of motor activity is an adequate experimental model for the development of osteopenia that occurs under actual and simulated hypokinetic conditions (5). It must be assumed that there were systemic changes in neurohumoral regulation of phosphorus and calcium metabolism, while osteopenia developed by the introduction of experimental conditions, i. e. hypokinesia and hypodynamia, which led to local changes in trophic conditions and subsequently, to regional structural impairment of bone tissue. It is also possible that, in the absence of motor activity for the

body, there was a change in reactivity bone tissue to the most important regulators of bone metabolism.

The increased requirements of bone tissue for greater circulating blood volume stimulators could be attributed to a set of factors, including reduction of osteocytes to adequate regulators of their functions, in the presence of decreased microcirculatory processes that are always associated with prolonged restriction of motor activity.

From these studies two principal conclusions can be drawn: 1) when animals are subjected to prolonged restriction of motor activity osteopenic processes developed in bone tissue, and 2) when animals are submitted to hypokinesia and consume daily a fluid and salt supplementation the process of progressive development of osteopenia is inhibited. There are also data available indicative of the direct effect of chronic hyperhydration on bone tissue (8, 9). Results obtaining from experiments involving long term restriction of motor activity of animals (8) and humans (9) revealed that chronic hyperhydration may lead to the inhibition of development of osteopenia.

Previous experirmental studies (3) have drawn the assumption that the inhibition of precursor cells is one of the pathogenetic mechanisms of hypokinetic induced osteopenia. This is indicated by the low weight of heterotropic bone and its thinning, absence of osteoblasts and bone marrow in such bone, and significant decrease in number of stromal precursor cells. The beneficial effect of fluid and salt supplementation, which diminishes the degree of development of hypokinetic induced osteopenia, consists of stimulating osteogenesis, precursor cells, which is associated subsequently with restoration of activity of the bone remodeling and metabolic unit, the function of which had been inhibited due to prolonged restriction of motor activity (8).

However, whether this mechanism affects the entire skeleton or prevails only in bones that carry a static load is not clear yet, i. e. we are referring to the fact that if the entire population of osteogenesis precursor cells "buffers", osteogenesis may spread entirely over the whole bone system, regardless of the supporting function of different parts of the skeleton. It is also still unclear if there is an analogous pattern of changes in histogenesis (senile, postclimacteric, dietetic, glucocorticoid, idiopathic and hypokinetic induced osteopenia). Investigation of all these clinical and hypokinetic conditions is a promising direction for research in order to elaborate tactical ways and means of prevention and treatment of osteopenia of different etiology. Meanwhile, previous experimental studies (8, 9) have demonstrated that a stimulus generated from increased body hydration level could change the local electrolyte composition and the activity of bone cells. However, the physiological and biochemical mechanisms of increased body hydration level or other factors influencing bone cell activity during prolonged restriction of motor activity it is still unknown.

One posibility could be that with reduced muscle mass (10) during HK an endogenous potassium "load" into extracellular fluid develops; this might be more readily excreted during FSS and could conceivably affects mineral fluxes into bone electrolytes.

Y. G. ZORBAS, Y. F. FEDERENKO y M. N. TOGAWA. *Efecto de la suplementación de líquido y sales en la prevención de la osteopenia en rata, tras hipocinesia.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 50 (4), 245-252, 1994.

Se evalúa la eficacia de la suplementación de líquidos y sales en la prevención del desarrollo

de la osteopenia en 150 ratas macho Wistar (370-390 g), tras exposición a hipocinesia durante 90 días. Se forman tres grupos iguales de animales: 1° condiciones normales de vivario (animales control de vivario); 2° sometidos a hipocinesia (animales hipocinéticos no suplementados); y 3° sometidos de nuevo a hipocinesia y diariamente suplementados v.o. con 5 ml/100 g peso corporal de agua y 3 ml/100 g peso de ClNa al 0,9 % (animales hipocinéticos suplementados). El efecto hipocinético se efectúa manteniendo las ratas en pequeñas jaulas individuales de madera, que restringen todos sus movimientos sin impedir la ingesta de comida y agua. Al final del experimento se determina el peso y el volumen del hueso entero, de la cabeza y de la epífisis distal, así como la densidad y el contenido de ceniza y minerales. Se mide el grosor de la capa cortical y la anchura del canal de la médula ósea, sobre proyecciones radiográficas frontales y laterales. Los cortes transversales histológicos de la diáfisis femoral se preparan a partir de fragmentos del hueso femoral. También se miden, en suero, las concentraciones de calcio, fósforo y creatinina. Los resultados obtenidos indican que la suplementación diaria de líquido y sal inhibe el desarrollo progresivo de la osteopenia en ratas sometidas a una restricción prolongada de la actividad motora.

Palabras clave: Osteopenia, Hipocinesia, Hiperhidratación, Rata.

References

1. Fedorov, I. V. (1982): In "Metabolism Under Hypokinetic Conditions", Izdatel'stvo Nauka, Moscow. p 319.
2. Kovalenko, Ye. A. and Gurovskiy, N. N. (1980): In "Hypokinesian Meditsina", Moscow. p. 254.
3. Shvets, V. N., Burkovskaya, T. Ye., Vnukova, Z. Ye. *et al.* (1985): *Kosmicheskaya Biol.*, 19, 48-53.
4. Stupakov, G. P. and Korolev, V. V. (1979): In "Effects of Cosmicflight Factors on Animals" Izdatel'stvo Nauka, Moscow. p. 354.
5. Volozhin, A. I. (1977): Interkosmos. Council, Academy of Sciences USSR and Directorate of Cosmic Biology and Medicine. Ministry of Health, USSR, Moscow. pp 123-132
6. Volozhin, A. I., Stupakov, G. P., Pavlova, M. N. *et al.* (1972): *Pat. Fiziol.*, 2, 30-33.
7. Zorbas, Y. G., Alexeyev, N. B. and Nikolescu, Z. A. (1990): *Mat. Med. Polona*, 4, 267-275.
8. Zorbas, Y. G., Federenko, Y. F. and Togawa, M. N. (1991): *Acta Astronaut.*, 25, 111-116.
9. Zorbas, Y. G., Federenko, Y. F. and Naexu, K. A. (1991): *Acta Astronaut.*, 25, 134-139.
10. Zorbas, Y. G., Bobylev, V. R., Market, A. N. and Federenko, Y. F. (1993): *Sports Med. Training Rehab.*, 4, 167-176.