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Mechanisms of Osteoporosis Development during Prolonged Restriction of Motor Activity in Dog

Y. G. Zorbas, K. A. Naexu and Y. F. Federenko*

European Institute of Environmental Cybernetics
Athens (Greece)

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The pattern in the mechanisms of osteoporosis development during prolonged motor activity restriction (hypokinesia) in animals has been studied. Twenty-four male dogs with initial body weights of 6.8 to 8.9 kg were divided into two equal groups: the 1st group were placed under ordinary vivarium conditions and served as control and the 2nd group were subjected to pure hypokinesia (HK) without any form of physical exercise and served as experimental animals. They were kept for 364 days in small individual wooden cages which restricted their movements without hindering food and water intake. Animals of each group were decapitated on the 120th, 240th and 360th day of the hypokinetic period and bones were x-rayed, histological specimens were examined, mineralization of organic bone was measured, microroentgenographic analysis was performed and calculation was made of calcium, phosphorus, potassium and sodium. By the 120th and 240th day of the hypokinetic period bone resorption increased significantly, while by the 360th day it decreased significantly. The mature bone microstructures manifested a higher degree of mineralization, whereas the young bone microstructures exhibited a lower degree of mineralization. In bone, calcium content decreased, that of potassium increased, while sodium and phosphorus content remained unchanged. It was concluded that the development of osteoporosis in osseous tissues during prolonged restriction of motor activity of animals is associated not only to quantitative changes, wich consist of a reduction of bone mass, but qualitative changes as well.

Key words: Osteopenia, Hypokinesia, Dogs.

Research on the problems of bone demineralization in the area of hypokinetic physiology, has so expanded that there arises a need to study the results achieved and to analyze trends in the field of development of osteopenia during prolonged motor activity restriction.

* To whom all correspondence should be addressed: Hypokinetic Physiology Lab., European Institute of Environmental Cybernetics, 4 Kerasundos Road, GR-162 32 Athens (Greece)

The results obtained from morphological and histological examinations revealed that hypokinesia does not elicit specific changes, previously unknown in the field of clinical pathology and hypokinetic physiology, and that exposure to prolonged restriction of motor activity for up to 364 days is not associated with development of severe or irreversible structural changes. In the changes developed during prolonged muscular activity restriction, a large share is referrable to processes of functional and structural reorganization of the skeletomuscular system. Such changes, besides being important *per se*, play a certain role in the genesis of other changes that also develop during prolonged muscular activity restriction. Hypokinetic conditions inevitable lead to hypofunction of the skeleto muscular system and, as a consequence, to development of atrophic and osteoporotic changes.

Dystrophic changes in bone tissue, which were classified as osteoporotic changes, have been repeatedly demonstrated during prolonged motor activity restriction (1, 7, 8, 11, 12). Development of osteoporosis or osteopenia is attributable to activation of bone tissue resorption with concurrent loss of minerals, and organic substances. The objective of this investigation was to determine the patterns of changes in the mechanism of osteoporosis development in dogs during prolonged motor activity restriction.

Materials and Methods

Experiments were performed on 24-mongrel dogs which had been delivered to our Institute from 2.9 to 4.0 years of age and weighed 6.8 to 7.6 kg. The dogs were daily screened and only the clinically healthy ones were selected for this investigation. The studies were approved by the Committee on Use and Care of Animals of the University.

The experimental studies, were preceded by a series of clinical and physiological examinations, training, testing and adapting of animals to new type of laboratory conditions. In this period the animals were placed under ordinary vivarium conditions (air temperature; 22±2 °C, relative humidity, 79±2 %; 12-h dark/ light periods with fluorescent lighting of approximately 100 ft candle intensity) in stationary individual wooden cages.

Adaptation process of the animals to laboratory conditions lasted from about 560 to 690 h. Twenty-four truly comparable animals were selected out of thirty, initially used for the pre-experimental studies; 12 of them were subjected to pure hypokinesia (experimental animals) and the other 12 were placed under ordinary vivarium conditions (control).

For the simulation of the hypokinetic effect the experimental group of animals were kept in small individual cages (65 x 45 x 25 cm) which restricted their movements in all directions without hindering food and water intake. When necessary, the dimentions of the individual cages could be reduced by using special wood inserts. The cages were constructed in such a way that their size could change in accordance with the size of each animal so that the degree of rectriction of movements could be maintained at a relatively constant level. The animals could assume a postural position and groom different parts of their body and were able to bear weight on their limbs.

The animals of both groups were fed Purine Certified Canine Diet and had access to water both *ad libitum*. During 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.

Four animals from each group were sacrificed under anaesthesia (3-4 ml 10 % hexenal solution, i.v.) on the 120th, 240th and 360th day of the hypokinetic period and, both femurs were cleared from soft

tissue and stored in 1 % neutral formalin. The bones were first x-rayed in anteroposterior and lateral projections, then with the use of an MBS-2 microscope with a metric scale the outside width, thickness of control layer and width of medullary cavity strictly in the center of the diaphysis were determined (margin of error ± 0.03 mm). For further examinations, circular fragments in the middle third of the diaphysis were cut and histological specimens were prepared from bone decalcified in 7 % nitric acid. Sections 8 to 10 μm^2 in thickness, were stained with hematoxylin and eosin, picrofuchsin according to Schmorl. The area of vascular canal lumen on histological preparations (by means of morphological grid (A) attached to the microscope ocular (7x), with 90x objective was measured. The determined vascular canals were ranked according to cross sections: up to 50, 103, 156, 203, 257, 309, 412 and up to 617 μm^2. The results obtained were expressed as percentage of all canals counted in the preparations.

For quantitative evaluation of porosity of cortical bone and distal epyphysis, the density (in g/cm^3) and volume of mineral (mineralization, g/cm^3) were determined (9). Mineralization of organic bone was measured from ash content (ratio of mineral mass to mass of dry, defatted bone fragment). Polished sections of the diaphyses of the animals were subjected to microreontgenographic analysis by using available methods (4).

The microhardness of bone tissue in polished sections, which were defatted and desiccated at 20-22 °C, imbedded in "protacryl" plastic, was determined (5). Twenty identations on each sample with the diamons pyramid-shaped indenter of a PMT-3 instrument at a pressure of 100 g/cm^2 were made.

Determinations. — Calcium (atomic absorptiometer), phosphorus (spectrophotometer using sodium molybdate) and potassium and sodium (flame photometer).

Statistical Analysis. — The results obtained were compared by using the Student's *t* test. Statistical significance was accepted when $p<0.05$.

Results

During the experimental period all of the examined dogs had good appetite, and were in good health; however, as the duration of the experimental period increased, the hypokinetic animals manifested typical symptoms observed in the course of prolonged motor activity restriction (10). At the later stages of the experimental period the hypokinetic animals gradually adapted to their situation as shown by the fact that the animals no longer left over any amount of food, as they did during the initial stages, and by their willingness to return to their cages after these had been cleaned. During the experimental period no serious disorders or deaths were reported.

Table I. *Changes in Body Weight (b.w.) of Dogs during Prolonged Restriction of Motor Activity.* Mean ± S.E.M., n = 12 per group.

Group	Initial b.w. (kg)	Duration of Hypokinesia in days		
		120th	240th	360th
Control	6.8 ± 5.2	9.4 ± 13.7	10.8 ± 12.9	13.9 ± 7.4
Experim.	7.6 ± 4.5	5.4 ± 9.5*	6.5 ± 8.3*	9.8 ± 7.5*

*P<0.05 as compared to the control group.

Table II. *Cross Sectional Changes of Femoral Diaphyses of Dogs during different Hypokinetic Periods.* Mean ± S.E.M., n = 12 per group.

Days	Group	Thickness of Cortical Layers	Outside width of Bone	Width of Marrow Cavity	Thickness of Cortical Layer Outside Width of Bone Ratio
			Anteroposterior Projections		
120	Control	4.13 ± 0.10	9.77 ± 0.38	5.43 ± 0.11	0.41 ± 0.016
	Experim.	3.70 ± 0.19	9.55 ± 0.15	5.97 ± 0.21	0.30 ± 0.014
240	Control	4.04 ± 0.16	9.90 ± 0.11	5.94 ± 0.18	0.42 ± 0.019
	Experim.	3.26 ± 0.12*	9.64 ± 0.14	6.86 ± 0.20*	0.27 ± 0.013*
360	Control	3.99 ± 0.25	10.95 ± 0.26	6.97 ± 0.47	0.38 ± 0.052
	Experim.	2.80 ± 0.21*	10.09 ± 0.48	7.91 ± 0.34*	0.13 ± 0.033*
			Lateral Projections		
120	Control	3.88 ± 0.08	8.90 ± 0.27	5.03 ± 0.30	0.40 ± 0.017
	Experim.	3.52 ± 0.17	8.75 ± 0.33	5.32 ± 0.41	0.28 ± 0.025*
240	Control	3.67 ± 0.19	9.93 ± 0.38	5.17 ± 0.36	0.45 ± 0.020
	Experim.	3.20 ± 0.23	9.57 ± 0.35	5.60 ± 0.43	0.19 ± 0.027*
360	Control	4.15 ± 0.12	11.55 ± 0.66	7.15 ± 0.48	0.41 ± 0.015
	Experim.	2.70 ± 0.16*	11.10 ± 0.45	8.43 ± 0.67*	0.12 ± 0.024*

*P<0.05 as compared to the control group of dogs.

Body Weight Changes. — The body weight of the control group increased progressively during the experimental period (table I) while it decreased signficantly in the experimental group by the 120th day, increased somewhat by the 240th day and stabilized by the 360th day just above the initial level. Between the control and the experimental groups of dogs differences with regard to the body weight were significant in the course of the experimental period.

Bone Mass and Bone Density Changes. — Prolonged motor activity restriction elicited a significant reduction in bone mass of the experimental group, as compared to the control, as indicated by thinning of the cortical layer on the side of the marrow cavity, without changes in outside width of the epiphysis. The observed alterations in bone were particularly evident by the 360th day of the hypokinetic period (table II). There was also an increase in width of the medullary canal corresponding approximately to the reduction in thickness of the cortical layer, due to intensified reabsorption of diaphyseal bone tissue, mainly on the side of the endosteum. By the 120th day of the hypokinetic period the diaphysis preparations manifested an increase in lacunal reabsorption of bone tissue with involvement of polynuclear osteoclasts. The considerable area of rearbsorption zones deep in the cortical layer shows that there was a reduction in cortical bone density (table III). The cortical layer thickness of femur diaphysis decreased along the hypokinetic period. At the same time limb density differed from that of the control animals, as confirmed by histological examinations, which were characterized by an absence of reabsorption zones deep in the bone of the experimental animals during the hypokinetic period.

Table III. *Changes in Density, Mineralization and Ash content of Compact and Spongy Substances of Femurs of Dogs During Different Hypokinetic Periods.*
Mean ± S.E.M., n = 12 per group.

Days	Group	Bone Density (g/cm³)	Bone Mineralization (g/cm³)	Ash Content (%)
		Diaphyseal Compact Bones		
120	Control	1,85 ± 0.05	1.16 ± 0.03	65.11 ± 0.32
	Experim.	1.74 ± 0.02	1.10 ± 0.057	63.44 ± 0.61
240	Control	1.888 ± 0.04	1.19 ± 0.04	60.50 ± 0.43
	Experim.	1.638 ± 0.05**	1.09 ± 0.02**	54.75 ± 0.52**
360	Control	1.89 ± 0.03	1.22 ± 0.02	56.82 ± 0.46
	Experim.	1.57 ± 0.04**	1.02 ± 0.04**	40.32 ± 0.28**
		Distal Epiphyseal Spongy Bones		
120	Control	0.44 ± 0.01	0.26 ± 0.01	60.86 ± 0.33
	Experim.	0.29 ± 0.02	0.18 ± 0.00	49.22± 0.77
240	Control	0.43 ± 0.05	0.25 ± 0.02	50.16 ± 0.50
	Experim.	0.25 ± 0.03**	0.15 ± 0.01**	38.91 ± 0.87**
360	Control	0.42 ± 0.02	0.24 ± 0.02	40.08 ± 0.29
	Experim.	0.22 ± 0.02**	0.12 ± 0.01**	26.25 ± 0.42**

*P<0.01 as compared to the control group of dogs.

Morphological examinations of histological sections revealed a reduction of reorganization of bone tissue of experimental animals. Asymmetrical distribution of canals according to area was established in the lower extremity bones of the control animals. About 80 % of all vascular canals had an area of less than 156 μm^2, and only about 20 % of canals had large areas, while the share of canals with an area of 627 μm^2, or more constitued only 3.9 %, which shows that there was a rather impaired reorganization of osseous tissues of the control group of animals as they increased in age.

By the 120th and 240th day of the experimental period a 16 % increase was shown in the number of the smallest, i.e. less than 50 μm^2, vascular canals in the bone of animals whose motor activity was restricted over a period of 364 days, while in the group of animals control there was a 6 % increase in the number of the largest canals during the experimental period. An 8 % increase in the number of the largest canals was also observed in the experimental animals by the 360th day of the hypokinetic period.

Microroentgenographic examinations of osseous tissues revealed some quantitative changes in microstructural minerals. By the 120th and 240th day of the hypokinetic period, signs of osteoporotic processes in the experimental group of animals are visualized. At the same time, none of the microroentgenograms demonstrated *de novo* formation of osteons with low mineral density, which indicates the presence of the osteogenesis drastic, depression during hypokinesia.

By the 360th day of the hypokinetic period, quite a few osteons with diminished mineralization were observed on bone sections from limbs, as compared to interstitial lamellae. These osteons were encountered in groups, in some parts of the transverse diaphyseal section, particularly on the side of endosteum. In osseous

tissue from the extremity of the control group of animals, the osteons essentially remained at a high degree of mineralization during the experimental period. By the 120th and 240th day of the hypokinetic period average mineralization of mature osteons and interstitial lamellae constituting most of the bone in the limb of the experimental animals was insignificantly greater by 0.02 g/cm^3, than in the control group. This difference increased to 0.06 g/cm^3 by the 360th day. This tendency toward increase in mineral microstructures in the bone of the experimental animals can be attributed to a passive mechanism with microcirculatory changes. Where mineralization of osteons differed from that of interstitial lamellae, average mineralization on all hypokinetic periods was virtually the same in the limbs of both groups (fig. 1).

Analysis of the obtained data revealed that there is a reduction in average microhardness in bone tissue of the femoral diaphysics of the limbs of all the experimental animals. These changes were due to the fact that several microsegments with diminished microhardness on the bone section of the extremities of the experimental animals were determined, as compared to other segments. In reflected light at a magnification of 480x, these segments appear as clear points, which apparently represent osseous microstructures with low mineralization during prolonged motor activity restriction.

The reduction in microhardness was also observed in the monosteonic spongy bone tissue of the epiphysis of the extremities of the hypokinetic animals, as compared to the control group.

Bone Mineral Changes. — Consistent changes in mineral composition of bone associated with prolonged hypokinesia are observed (table IV). The calcium con-

Table IV: *Changes in Electrolyte Composition of Spongy and Compact Substances of Femurs of Dogs During Different Hypokinetic Periods*
(Mean ± S.E.M., n = 12) per group

Days	Group	Electrolyte Content/100 g Ash			
		Calcium	Potassium	Sodium	Phosphorus
		Diaphysis			
120	Control	48.04 ± 1.23	49.16 ± 1.47	466.7 ± 15.7	14.16 ± 0.14
	Experim.	40.59 ± 0.47	72.25 ± 4.89*	484.2 ± 22.3	12.67 ± 0.53
240	Control	41.25 ± 0.89	50.33 ± 2.27	477.6 ± 29.0	14.23 ± 0.28
	Experim.	28.60 ± 0.66*	85.82 ± 8.16**	449.1 ± 24.8	11.80 ± 0.17
360	Control	43.00 ± 0.29	56.28 ± 7.21	496.4 ± 24.5	15.34 ± 0.69
	Experim.	12.25 ± 0.53*	90.13 ± 12.19	418.8 ± 21.1*	10.93 ± 0.33
		Epiphysis			
120	Control	47.18 ± 0.98	35.10 ± 4.54	599.2 ± 36.5	14.89 ± 0.21
	Experim.	39.03 ± 1.09*	50.86 ± 3.35*	558.6 ± 29.4	13.08 ± 0.30
240	Control	44.28 ± 0.77	36.21 ± 2.44	564.8 ± 30.7	14.96 ± 0.63
	Experim.	30.86 ± 0.68*	65.79 ± 2.58**	520.3 ± 41.0	12.14 ± 0.34*
360	Control	41.02 ± 0.64	56.24 ± 14.58	440.4 ± 30.2	14.77 ± 0.40
	Experim.	20.30 ± 1.25*	79.41 ± 5.66**	491.2 ± 19.4	11.39 ± 0.56*

*P<0.05 and **P<0.01 as compared to the control group of dogs.

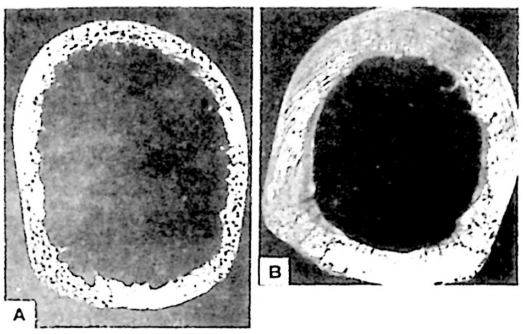

Fig. 1. *Microroentgenographs of Sections of Femoral Diaphyses of Dogs After Exposure to Prolonged Restriction of Motor Activity (magnification x 3.5).*
A) Hypokinetic animals; B) Control animals.

tent decreases in both compact and spongy bone tissue and the potassium content increases respect to the control animals. Because the phosphorus content did not change, there was an alteration in calcium and phosphorus ratio in the experimental animals.

Discussion

The observed behavioral and physiological reactions of experimental animals in the course of prolonged motor activity restriction are typical of those changes and reactions that develop in the presence of hypokinetic conditions (2, 3, 10). The body weight reduction in the experimental group demonstrated during different periods of muscular activity restriction, was apparently attributable to impairment of metabolic processes in the direction of prevalence of catabolic processes or anabolic ones, dissimilation, fat mobilization from the fat depots (2, 3) and body dehydration due to intensification of urine excretion during prolonged motor activity restriction (10).

The results obtained show that the mean mineralization of the bone section segment was lower on the limbs of the experimental animals than the control. According to the available data (6), the resorption process of bone tissue is always associated with its *de novo* formation. However, in the instance of loss of function of an osseous organ, the rate of bone tissue maturation can drop significantly during prolonged motor activity restriction.

Osteoporosis development is characterized not only by quantitative changes in osseous tissue, which consists of bone mass reduction, but qualitative changes as well. This is manifested by a change in the proportion of osteons differing in degree of mineralization. The higher degree of mineralization of mature osteons could be attributed to the fact that these microstructures existed before the exposure to hypokinetic conditions, they persisted at the end of the experimental period and, perhaps were subject to additional levels of mineralization during prolonged motor activity restriction.

Other distinctive features of the bone tissue of limbs of experimental animals were an increase in number of microstructures with low mineralization and decrease in their mean density in some bone segments during prolonged motor activity restriction. Perhaps these microstructures, which developed during that restriction in the experimental animals, have a slower capacity for mineralization. The proportion of different osteons in distinct segments of bone sections changes in the direction of immature microstructures, which is manifested by a reduction in mean mineralization of bone tissue and microhardness in experimental animals during prolonged motor activity restriction.

The relative increase in number of smallest vascular canals (120th and 240th day) could be attributable to intensification of bone resorption in the subendothelial region, as a result of which mainly the osteon systems with largest vascular canals localized near the medullary cavity disappeared. However, by the 360th day, the distribution of vascular the canals changed, there being for example a considerable re-

duction in the relative number of the smallest vascular canals due to an increase in the number of the medium and largest ones, which shows that there is an intensification in he rate of bone tissue resorption.

The foregoing results made it possible to demonstrate the existence of serveral new patterns in the mechanisms of development of osteopenia in animals during prolonged motor activity restriction. The higher level of resorption activity of bone tissue, which was manifested during the initial 240 days, gradually diminished and, by the 360th day of the hypokinetic period exhibited a tendency toward reaching a level of maintenance, which neither the motor activity required nor a physical load needed. However, additional studies must be performed for further substantiation of the foregoing assumption as well as the mechanisms of osteopenia development during prolonged restriction of motor activity of animals.

Y. G. ZORBAS, K. A. NAEXU e Y. F. FEDERENKO. *Mecanismos del desarrollo de la osteoporosis durante restricción prolongada de la actividad motora en perro.* Rev. esp. Fisiol. (J. Physiol. Biochem.), 50 (1), 47-54, 1994.

Se estudia el desarrollo de la osteoporosis durante la restricción prolongada de la actividad motora (hipocinesia). Los experimentos se llevan a cabo en 24 perros machos de un peso inicial de 6,8 a 8,9 kg. Un grupo de 12 animales en condiciones ordinarias sirve de control; los 12 restantes se someten a hipocinesia pura (HK) sin ningún tipo de ejercicio físico (grupo experimental). Para la simulación del efecto hipocinésico se mantienen los animales experimentales, durante 364 días, en pequeñas jaulas de madera individuales, que restringen sus movimientos en todas las direcciones sin impedir la ingesta de comida y bebida. Tras decapitar los animales de cada grupo los días 120, 240 y 360 del período hipocinésico, se realiza un examen radiográfico e histológico de los huesos, se mide la mineralización ósea, llevándose a cabo un análisis microroentgenográfico con cálculo del calcio, fósforo, potasio y sodio. Los días 120 y 240 del período hipocinésico la reabsorción del hueso aumenta considerablemente, disminuyendo significativamente el día 360. Las microestructuras del hueso maduro manifiestan un grado de mineralización más alto, mientras que las del hueso joven, que predominan en el fémur de los animales hipocinésicos, lo tienen más bajo. En hueso, el contenido de calcio disminuye, el de potasio aumenta, mientras que el de sodio y fósforo permanecen inalterados. Se concluye que el desarrollo de la osteoporosis durante la restricción prolongada de la actividad motora no está asociada solamente a cambios cuantitativos, con reducción de la masa ósea, sino también a cambios cualitativos.

Palabras clave: Osteopenia, Hipocinesia, Perro.

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