Bone Mineralization in Senescence-Accelerated OXYS Rats

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We compared age-related changes in the mineral composition of bone tissues in Wistar and senescence-accelerated OXYS rats. The mass concentrations of calcium, phosphorus, and magnesium were measured by atomic emission spectrometry. OXYS rats are characterized by early mineralization of the bone tissue, which peaked by the 6th month of life. Mineralization defects in 12-month-old OXYS rats included accumulation of iron and phosphorus, decrease in the Ca/P ratio, and increase in ash weight. These changes reflected early development and accelerated aging of these animals. Studies of the bone tissue in OXYS rats indicate that these animals can be used for evaluation of the mechanisms of involutional osteoporosis.

Key Words: *accelerated aging; osteoporosis; bone mineral composition; OXYS rats*

The composition of the bone tissue in humans and animals undergoes similar age-related changes that underlie the pathogenesis of osteoporosis (most prevalent disease of elderly people) [12]. The age, at which this disease develops, significantly differs between various individuals. Studies of the cause and prevention of early osteoporosis are urgent medical problems. Senescence-accelerated OXYS rats (W/SSM rats) were obtained by selection and inbreeding of Wistar rats sensitive to the cataractogenic effect of galactose at the Institute of Cytology and Genetics [3,9]. Here we evaluated whether these animals can be used to study osteoporosis. Early cataract formation and short life span are typical of OXYS rats. The maximum life span of these animals is 28% lower compared to Wistar rats (24.6±4.0 months, $p<0.001$). OXYS rats are characterized by high incidence of malignant tumors, early involutional changes in internal organs (*e. g.*, cardiomyopathies [2]), and disturbances in cognitive functions [1]. Involution of the bone tissue in OXYS rats manifested in deformations of the spinal column is roentgenologically similar to hereditary scoliosis in humans with sphenoid defects in vertebral bodies [11]. However, visible changes in the spinal column rarely occur in OXYS rats. Age-related changes in the mineral composition of bone tissues in OXYS rats, which results from and contributes to the development of osteoporosis [7], remain unclear.

MATERIALS AND METHODS

Experiments were performed on 42 male OXYS and Wistar rats aging 2, 6, and 12 months and obtained from the Laboratory of Animal Breeding (Institute of Cytology and Genetics). Each group consisted of 7 rats. The animals were decapitated. Samples of the alveolar process were taken from the region of maxillary central incisors (without teeth). Ash weight was measured after wet combustion of 100-300 mg sample in nitric or sulfuric acid [3,5]. The mineral composition was studied in

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70-250 mg bone tissue. The mass concentrations of Ca, P, Mg, Na, and Fe were measured by atomic emission spectrometry and high-frequency induction plasma discharge on a Dy-38 VHR spectrometer (Joben Ivon). Induction plasma is stimulated with a DURR Jy radio-frequency generator operating at 56 mHz (1.5-2.2 kW) and equipped with a water-cooled six-turn inductor. We used a Fussel plasma burner (diameter 23 mm). The plasma-forming, cooling, and spraying gas was argon. The device was equipped with a Silex computer system that controlled the measurements, processed analytical data, and outputted results on a display or printer. Spectral lines that were most sensitive and did not produce interpositions served as analytical lines for test elements: Ca, 422.67 nm (I); P, 253.56 nm (I); Fe, 259,94 nm (II); Mg, 279.55 nm (II); and Na, 589.59 nm (I). The relative accuracy of measurements was not less than 7% (confidence coefficient 95%).

RESULTS

Calcium, the component of hydroxyapatite crystals is the major element responsible for bone mineralization. Bone mass rapidly increases in young individuals, peaks by the end of the first third of life, remains unchanged, and then progressively decreases due to age-related disturbances in bone formation [10].

Calcium content in the bone tissue was similar in 2-month-old OXYS and Wistar rats. Its amount increased by the 6th month of life, particularly, in OXYS rats (34% higher compared to Wistar rats). These differences were not observed in animals aging 12 months (Fig. 1, *a*). Therefore, the peak concentration of calcium in the bone tissue is similar in OXYS and Wistar rats. However, in OXYS rats calcium content reaches its maximum at an earlier age (6 month).

By the 6th and 12th months of life bone mineralization in OXYS rats was more pronounced than

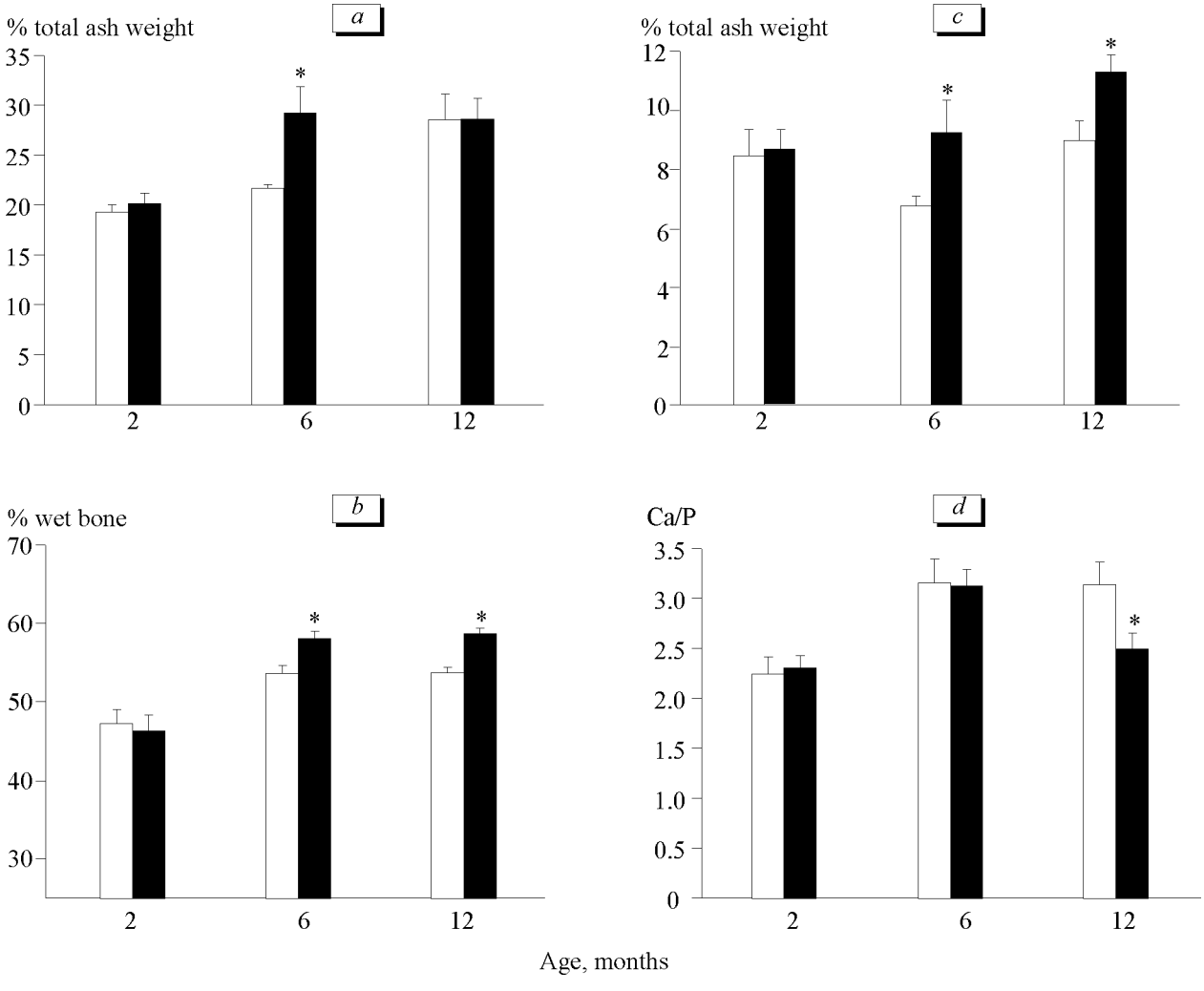

Fig. 1. Age-related changes in the amount of calcium (*a*), ash (*b*), and phosphorus (*c*) and calcium-phosphorus ratio (*d*) in the alveolar bone in Wistar (light bars) and OXYS rats (dark bars). Here and in Fig. 2: *significant differences compared to Wistar rats.

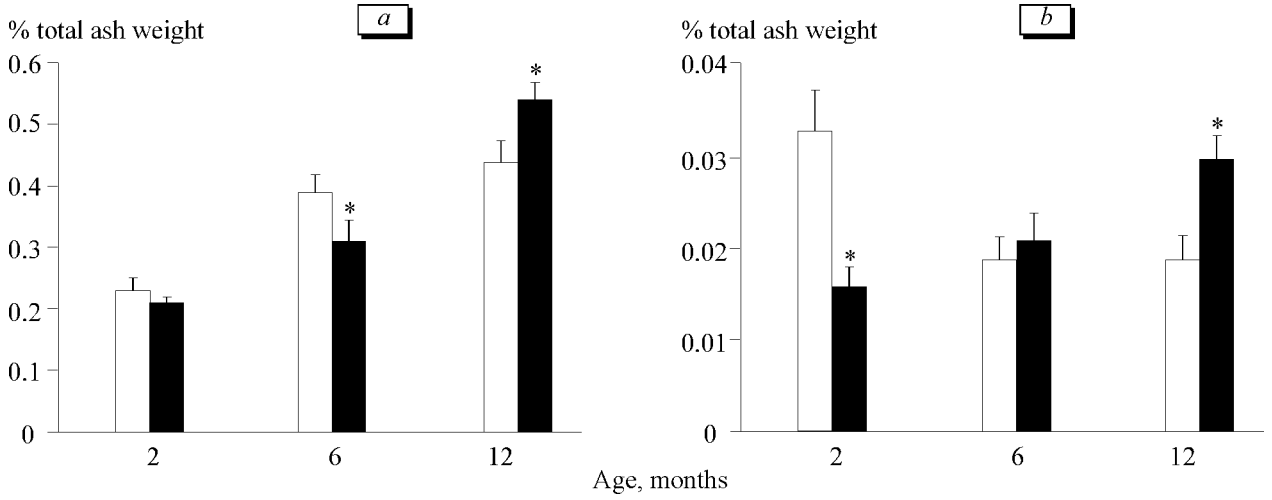

Fig. 2. Age-related changes in the contents of magnesium (*a*) and iron (*b*) in the alveolar bone in Wistar (light bars) and OXYS rats (dark bars).

in Wistar rats. Therefore, the relative content of organic substances in the bone tissue in OXYS rats was lower than in Wistar rats. Ash weight in the bone tissue increased in OXYS rats aging 6 and 12 months ($p<0.01$, Fig. 1, *b*). These changes increase brittleness of bones, which is typical of aging organism [5].

Phosphorus is the major element determining high content of inorganic substances in the bone tissue in OXYS rats. In 6 and 12 month-old OXYS rats phosphorus concentration was 36 and 25% higher than in Wistar rats, respectively (Fig. 1, *c*). These changes were followed by a shift in the Ca/P ratio, which reflects the state of bone tissues. The Ca/P ratio differed only between 1-year-old OXYS and Wistar rats. The decrease in this ratio associated with the increase in phosphorus content at normal calcium concentration indicates disturbances in bone mineralization (Fig. 1, *d*).

Magnesium content in the bone tissue increased in aging OXYS and Wistar rats (Fig. 2, *a*). The concentration of this element in the bone tissue in 6-month-old OXYS rats was 21% lower than in Wistar rats. However, by the 12th month of life bone magnesium content in OXYS rats was 20% higher than in Wistar rats. Magnesium is the most common element in the body, which is involved in general metabolic processes. Magnesium deficiency leads to genomic instability, causes structural and functional disturbances in biological membranes, increases cell sensitivity to oxidative stress, and accelerates aging [8].

An excessive increase in iron content is accompanied by similar changes. Accumulation of iron in tissues is typical of aging organisms [6]. Bone iron concentration linearly increased only in OXYS rats. The content of iron in Wistar rats was maximum by the 2nd month of life and 2-fold surpassed that in OXYS rats ($p<0.001$, Fig. 2, *b*). The mass concentration of iron increased in aging OXYS rats, but decreased in Wistar rats. Interstrain differences in iron content were not found in 6-month-old animals. By the 12th month of life bone iron concentration in OXYS rats was 1.5 times higher than in Wistar rats ($p<0.001$). Previous studies showed that age-related changes in the content of iron in various tissues are similar and correlate with accumulation of lipid peroxidation products [6]. It should be emphasized that in OXYS and Wistar rats age-related changes in bone iron content coincided with variations in the amount of protein oxidation products in liver cells. Their concentration in liver mitochondria in young OXYS rats was lower than in Wistar rats. These interstrain differences were not found in 6-month-old animals. However, by the 12th month of life the content of protein oxidation products in OXYS rats surpassed that in Wistar rats (data not shown).

Mitochondria of somatic cells are the major source of superoxide anions that are involved in the Fenton's reaction in the presence of iron. This reaction results in the formation of $OH^{\bullet}$ (most potent reactive oxygen radical). Published data show that progressive functional disturbances in mitochondria underlie accelerated aging of OXYS rats [5]. Energy deficiency and high iron content in tissues in 12-month-old OXYS rats markedly increase the risk for activation of free radical processes and oxidative damages to macromolecules. In 6-month-old rats magnesium deficiency also contributes to the development of oxidative stress [9].

Our results show that the bone tissue in OXYS rats is characterized by early mineralization, which peaks by the 6th month of life. Mineralization defects in 12-month-old OXYS rats include accumula-

tion of iron and phosphorus, decrease in the Ca/P ratio, and increase in ash weight and reflect the early development and accelerated aging of these animals. Studies of the bone tissue in OXYS rats indicate that these animals can be used to evaluate the mechanisms of involutional osteoporosis.

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