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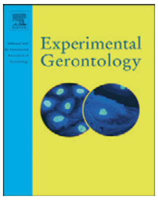

Short-term exercise-induced improvements in bone properties are for the most part not maintained during aging in hamsters

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ABSTRACT

Physical exercise during growth affects composition, structure and mechanical properties of bone. In this study we investigated whether the beneficial effects of exercise during the early growth phase have long-lasting effects or not.

Female Syrian golden hamsters (total $n = 152$) were used in this study. Half of the hamsters had access to running wheels during their rapid growth phase (from 1 to 3 months of age). The hamsters were sacrificed at the ages of 1, 3, 12, and 15 months. The diaphysis of the mineralized humerus was analyzed with microCT and subjected to three-point-bending mechanical testing. The trabecular bone in the tibial metaphysis was also analyzed with microCT. The collagen matrix of the humerus bone was studied by tensile testing after decalcification. The weight of the hamsters as well as the length of the bone and the volumetric bone mineral density ($\mathrm{BMD_{vol}}$) of the humerus was higher in the running group at the early age (3 months). Moreover, the mineralized bone showed improved mechanical properties in humerus and had greater trabecular thickness in the subchondral bone of tibia in the runners. However, by the age of 12 and 15 months, these differences were equalized with the sedentary group. The tensile strength and Young's modulus of decalcified humerus were higher in the runners at early stage, indicating a stronger collagen network. In tibial metaphysis, trabecular thickness was significantly higher for the runners in the old age groups (12 and 15 months).

Our study demonstrates that physical exercise during growth improves either directly or indirectly through weight gain bone properties of the hamsters. However, the beneficial effects were for the most part not maintained during aging.

1. Introduction

It is generally accepted that physical exercise improves health in many ways resulting in improved function of, e.g., the cardiovascular system, the musculoskeletal tissues and balance. Bone's ability to respond to mechanical stimuli is well known; however, the clinical importance is still being debated. During aging, some bone\ loss is inevitable, which in many cases finally leads to osteoporosis. Previous studies have shown a decrease in bone volume, architecture and mineral content (Beck et al., 1993; Karlsson et al., 2005) as well as changes in the relative composition of mineral and organic matrix during aging (Boskey and Mendelsohn, 2005; McCreadie et al., 2006). It has also been reported that the bone collagen matrix is most likely affected by aging (Bailey and Knott, 1999; Viguet-Carrin et al., 2006). At the same time, the bone's ability to respond to mechanical loading decreases with age (Turner et al., 1995). In fact, the skeletal benefit of a lifetime of exercise seems to occur mainly during the years of skeletal development (Kannus et al., 1995; MacKelvie et al., 2002; Warden et al., 2007).

Several studies using animal models have shown that both the organic matrix and the mineral component of the bone change their composition and structural organization during growth (Danielsen et al., 1986; Holopainen et al., 2008; Isaksson et al., 2009a, 2009b, 2010; Puustjarvi et al., 1999; Ramasamy and Akkus, 2007). We also demonstrated that BMD and the Young's modulus of the mineralized bone in rabbits increase until the age of 6 months, to thereafter be maintained during adult age (Isaksson et al., 2010). With regard to the

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collagen network we have earlier reported that the mechanical strength increases during early maturation in mice (Isaksson et al., 2009b), whereas during adult age the stiffness of the collagen matrix declines (Isaksson et al., 2009a).

Mechanical loading has been shown to have positive effects on long bones of rats in terms of their length (Steinberg and Trueta, 1981), shape (Rubin, 1984), mass (McDonald et al., 1986; Raab et al., 1991), cross-sectional area (Bennell et al., 2002), and strength (Jarvinen et al., 2003). Moreover, total immobilization causes atrophic changes in bone (Turner and Bell, 1986). Continuous physical exercise throughout life can reduce the loss of bone mineral content during aging (Bailey and Brooke-Wavell, 2008), and thus, exercise has widely been considered as a suitable means to prevent age-related osteopenia or osteoporosis. The effects of short-term exercise on bone mineral content, mass, structure and strength have been widely investigated. The positive effects of exercise have been demonstrated using young, aged or ovariectomized rats (Barengolts et al., 1993; Iwamoto et al., 1999; Jarvinen et al., 2003; Kodama et al., 2000; Plochocki et al., 2008; Raab et al., 1990; Sogaard et al., 1994). Specifically, 60 weeks of voluntary exercise of female mice was demonstrated to increase bone density and breaking force in the diaphysis of the femur (Hoshi et al., 1998). In female rats, increased loading at an early age resulted in increased bone size, strength and fatigue resistance at older age (Warden et al., 2007). However, some clinical studies suggest that exercise-induced changes in the skeleton during growth may not be maintained after exercise cessation (Gustavsson et al., 2003; Karlsson et al., 2000; Nordstrom et al., 2005).

Thus, timing and duration of the exercise seem to be critical. However, it is still unknown at what age, with what intensity and for how long exercise should be carried out to reach the optimal response of bone microstructure. Comparison between studies is often difficult due to, e.g., the species differences of experimental animals, the type of bone and the biophysical analysis methods used to investigate the mineral and the organic component.

The aim of the present study was to assess the immediate and long-lasting effects of physical exercise during early age on the mechanical, compositional and structural properties of hamster cortical and trabecular bone and its collagen matrix. We hypothesized that voluntary physical exercise in a running wheel at young age would improve the tissue properties, and strengthen the collagen network and the trabecular structure. We further hypothesized that the beneficial effects of physical exercise would be maintained during aging.

2. Materials and methods

2.1. Experimental conditions

Female Syrian golden hamsters ($n = 152$, Harlan, The Netherlands) were kept in individual cages (365 mm in length, 207 mm in width and 140 mm in height). The humerus bone and proximal tibia were examined at the ages of 1, 3, 12 and 15 months from sedentary hamsters and from hamsters which had access to running wheels from 1 to 3 months of age (Fig. 1). Hence, the immediate effect of exercise was examined at 3 months of age; and the prolonged effects of early exercise were investigated at 12 and 15 months of age. Running activity of the exercising animals on the running wheel was recorded with infrared sensors connected to a computer (Lapvetelainen et al., 1997). Sedentary animals did not have access to running wheels; however they were allowed to move freely in their cages. All animals were weighed monthly and at sacrifice. The hamsters were anesthetized with carbon dioxide inhalation and then killed by cervical dislocation. After euthanasia, the tibia and humerus bones were carefully dissected free from soft-tissues and stored in air tight specimen tubes containing PBS (phosphate buffered saline, pH 7.4) at −20 °C. The distances between the proximal and the distal articulating surfaces of the bones were measured with a digital caliper. The number of animals used in the present study for each characterization method of bone is given in Table 1. The sample set of the whole animal experiment was divided into different sub-studies. One sub-set was devoted to a study that included mechanical testing and analysis of the composition of the tibial articular cartilage (Julkunen et al., 2010), whereas the second sub-set is presented in the present study. Permission to conduct the experiments was obtained from the Animal Care and Use Committee of the University of Kuopio, Kuopio, Finland.

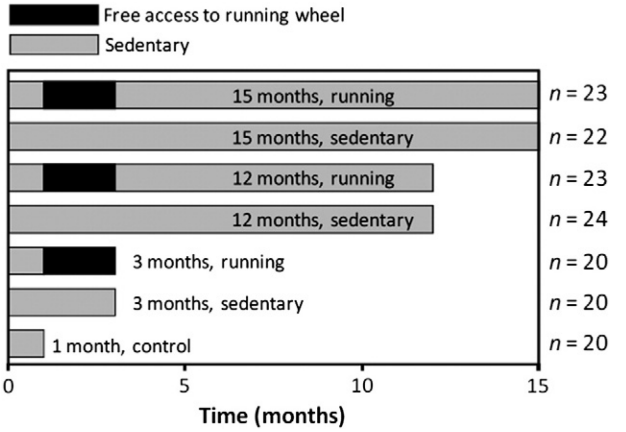

Fig. 1. Study setup. Duration of the physical exercise and the sedentary life-style in the hamsters. Control hamster groups followed a sedentary life-style. This is indicated with a gray bar. The exercising hamsters had access to running wheels between 1 and 3 months of age. This is indicated with a black bar. The number of animals in each group is presented next to the bars.

2.2. MicroCT of humerus

The mid-diaphysis of the left humerus was imaged with an isotropic voxel size of 15 μm (Skyscan 1172, v. 1.5, Skyscan, Aarteselar, Belgium). The microCT scanner acquired topographic images of the bone at the energy settings of 100 kV and 100 μA, using aluminum filter of 0.5 mm, and 10 repeated scans. The images were reconstructed using NRecon (Skyscan, v 1.5.1.4, Aarteselar, Belgium), and corrected for ring artifacts and beam hardening. Calibration of the volumetric bone mineral density (BMD_{vol}) was carried out according to the system manufacturer's protocol, by scanning a water phantom, and two hydroxyapatite phantoms of known density (0.25 and 0.75 g/cm^3). Mineralized bone tissue was assumed to have a mineral density above 0.70 g/cm^3. The mid-diaphyseal section was selected and analyzed. In addition to measuring the BMD_{vol}, the cross-sectional area, and the polar moment of inertia were calculated using CTAn (v.1.9.1.0 Skyscan, Aarteselar Belgium). Finally, the bone diaphysis was used for the mechanical testing.

2.3. Mechanical testing of mineralized humerus bone

Following the microCT measurements, the left humerus bones were subjected to three-point-bending test (Table 1) (Isaksson et al., 2010). Prior to the bending tests, the specimens were thawed at room temperature, and thereafter kept on an ice bath until the temperature was stabilized before testing. An electromechanical material testing device (Lloyd LFPlus, Lloyd Instruments Ltd., Fareham, UK) equipped with a load cell of 0.5% accuracy was used. The span length was set individually to 70% of the total length of the bone, to ensure comparable testing for each specimen in different age groups. The loading head and the two support stands were rounded (4–6 mm diameter) to avoid shear load and cutting. The bone was centered on the supports and positioned with the deltoid tuberosity downwards and the loading force was directed vertically onto the bone.

After preconditioning to a load of 0.2 N, each bone was compressed until failure using constant speed of 10 mm/min. Time, force and