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Original Article

Hylonome

Effects of aging and exercise on density and cross-sectional area of femur in senescence-accelerated mouse prone 6

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Abstract

The densities and cross-sectional areas (CSAs) of the femur in 40- and 60-week-old senescence-accelerated mice prone 6 (SAMP6) were determined using peripheral quantitative computed tomography (pQCT) and compared with those in age-matched control mice (ICR). In addition, the effects of 20 weeks of voluntary running exercise with increasing loads on femur density and CSA were examined in SAMP6. There were no differences in the cortical and trabecular densities or CSAs between the 40- and 60-week-old ICR. The cortical and trabecular densities of the 60-week-old control SAMP6 were lower than those of the 40-week-old control SAMP6. There was no difference in the cortical CSA between the 40- and 60-week-old control SAMP6, while the trabecular CSA of the 60-week-old control SAMP6 was higher than that of the 40-week-old control SAMP6. It was concluded that an age-related decline in femur cortical and trabecular densities occurs at an earlier age in control SAMP6, indicating that SAMP6 show accelerated progression of age-associated osteoporosis. There were no differences in the cortical density between the 40-week-old control and 60-week-old exercised SAMP6. These data indicate that running exercise with increasing loads prevents age-associated osteoporosis in this senescence-accelerated mouse strain.

Keywords: Aging, Bone Density, Exercise with Increasing Loads, Peripheral Quantitative Computed Tomography, Senescence-Accelerated Mouse

Introduction

An experimental animal model for accelerated senescence, the senescence-accelerated mouse (SAM) has been developed in the Department of Senescence Biology, Institute for Frontier Medical Sciences (former Chest Disease Research Institute), Kyoto University, Japan[1,2]. Nine SAM prone (SAMP) strains have been maintained by sister-brother mating. The SAMP strains have a short life span and strain-specific pathological phenotypes compared with control mouse strains including ICR and ddY[1,2]. The SAMP strains provide a unique model system for study of the aging process because they show a marked acceleration in the appearance of several indicators of aging, e.g., senile amyloidosis (P1, P2, P7, and P11), degenerative arthrosis of the temporomandibular joint (P3), senile osteopenia (P6), atrophy and decrease in oxidative capacity of muscle fibers and their spinal motoneurons (P6), thymona (P7), deficits in learning and memory with brain atrophy (P8 and P10), and cataract (P9)[1-6].

Osteoporosis is one of the metabolic diseases in which reduction of bone mass is caused by the resorption of bone in excess over a long period. It has been reported that SAMP6 is a murine model of senile osteoporosis[1,2,4]. However, bone mineral density was measured using single- or dual-energy photon, X-ray absorptiometry, or conventional radiography[1,2]. Therefore, the values obtained from these measurements do not reflect a volumetric density. The peripheral quantitative computed tomography (pQCT) techniques used in the present study were developed to provide simultaneous information on geometric properties and volumetric density of appendicular bone[7]. In addition, pQCT has been used to separately assess cortical and trabecular bone compartments[8]. Osteopenia in SAMP6 may be expected to occur from 40 to 60 weeks of age, because the neuromuscular system of SAMP6 is affected dramatically during this period[6]. For example, the hind limb muscles atrophy and, presumably, the associated bones experience

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lower forces (strains) than normal during this period.

The purpose of the present study was therefore to investigate whether mice showing accelerated-senescent features of the neuromuscular system also have an early onset of age-related changes in the skeletal system. Our hypothesis was that osteoporotic changes in SAMP6 would occur in parallel to the previously observed changes in the neuromuscular system. We also determined the potential of voluntary running exercise with increasing loads to prevent these age-related changes. The overall rationale for the study was to establish SAMP6 as a model for the study of age-related osteopenia and its prevention by running exercise with increasing loads.

Materials and methods

Experimental animals and treatment

Forty-week-old male ICR (n = 10) and SAMP6 (n = 14) were used in the present study. Mice were killed at 40 and 60 weeks of age (n = 5 for each strain and age) to serve as baseline controls. Four 40-week-old SAMP6 were subjected to voluntary running exercise in running wheels for 20 weeks. All mice were housed individually in similar plastic cages, except that there was no running wheel in the cages for the control groups. The mice were kept in a controlled environment with a fixed 12:12 light:dark cycle (lights off 19:00-07:00) with the room temperature maintained at 22 ± 2˚C. Food and water were provided ad libitum. All experiments were approved by the Institutional Animal Care Committee at the University and conducted under the Guide for the Care and Use of Laboratory Animals published by the Office of Science and Health Reports of the USA National Institutes of Health, Bethesda, Maryland.

Running wheel apparatus

A running wheel apparatus in which the load and running distance can be controlled and monitored electronically was used[9]. This apparatus includes a standard plastic cage and a running wheel (width 5.0 cm, diameter 25.5 cm) attached vertically to a freely rotating shaft inserted into a metal controller box that is supported on a metal base. The running wheel rotates on the shaft whenever the mouse runs in either direction in the running wheel, and the number of revolutions of the running wheel is recorded continuously. A transducer in the controller box connected to the running wheel produces an electric signal for each revolution of the running wheel. This signal is then sent to, and subsequently stored by, a computer that is equipped to continuously monitor the number of signals from several running wheels simultaneously. The time interval for data collection is set by a time-mark generator (from 3 seconds to 24 hours). The load attached to the wheel can be controlled magnetically and changed arbitrarily. The mice were exercised with no load for the first 5 weeks, and then the load was progressively increased: the 6th week at 5 g (17% of mean body weight of the exercised group), the 7th week at 10 g (34%), the 8th and 9th weeks at 15 g (52%), the 10th and 11th weeks at 20 g (69%), and the last 9 weeks at 25 g (87%). Work was calculated by the following formula: work (mg/day) = running distance (m/day) x (body weight (g) + additional load (g)).

Tissue preparation

The mice were killed by an overdose of sodium pentobarbital (85 mg/kg body weight, i.p.). The left tibialis anterior (TA), plantaris (PL), and soleus (SOL) muscles, and left femur were dissected and cleaned of all connective and soft tissues and then wet weighed. The femur lengths were measured and then the bones were stored in 70% ethanol. After pQCT measurement (see below), the bones were dried in an oven overnight at 80˚C and the dry weights determined.

pQCT measurements

All left femurs were analyzed using pQCT (XCT Research SA+, Stratec Medizintechnik GmbH, Pforzheim, Germany), i.e., scanned from the cross-sectional images with 80 x 80 x 460 μm voxel size (ELK Corporation, Tokyo, Japan). The middle and distal sites were chosen for analysis of cortical and trabecular densities (mg/cm^3) and cross-sectional areas (CSAs, mm^2) because of the considerable quantities of cortical and trabecular bones, respectively. The scan lines were adjusted to the diaphysis for the middle site and the metaphysis, 1 mm proximal from the distal growth plate, for the distal site using the scout view of the pQCT system. The middle and distal sites were defined by peel mode 2 using thresholds of 690 and 395 mg/cm^3, respectively. All analyses were performed with pQCT program Rev. 5.40 (ELK Corporation, Tokyo, Japan).

Statistics

Means, standard deviations, and correlation coefficients were calculated from individual values using standard procedures. A two-way analysis of variance (ANOVA) was used to evaluate the influence of age (40 vs. 60 weeks) and strain (ICR vs. SAMP6 strains at the same age) in control groups. When the main effects or interactions between age and strain were significant based on the ANOVA analyses, comparisons between individual group means were made using Scheffé's post hoc tests. The unpaired Student's t-test was used to determine any differences in the means between 40-week-old control and 60-week-old exercised SAMP6 and between 60-week-old control and 60-week-old exercised SAMP6. Statistical significance was set at $p<0.05$ for all analyses.

Results

Running distances

As the load was increased, the average running distance of the exercised SAMP6 group decreased gradually (Figure 1).

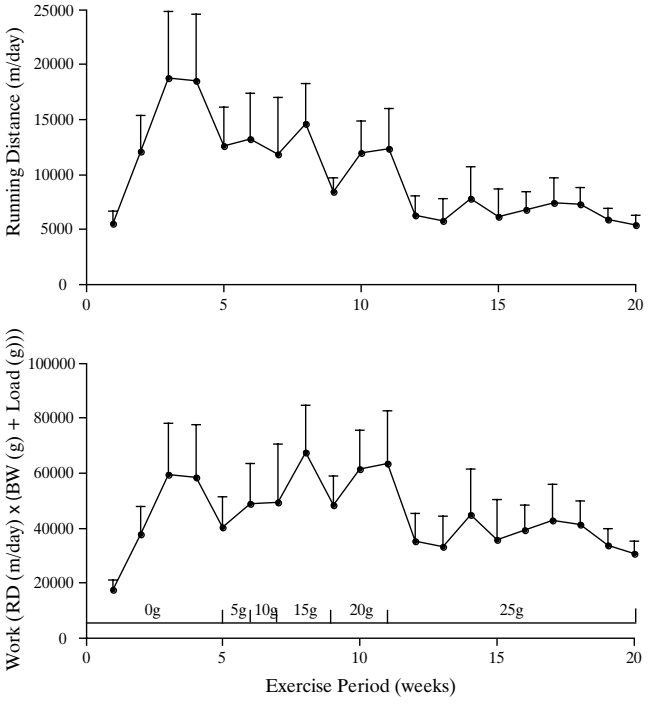

Figure 1. Voluntary running distances (top) and work (bottom) of the exercised SAMP6 group. Bars, standard deviations. Work is calculated as running distance (m/day) x (body weight (g) + additional load (g)). Values of the load added for the running wheel are inserted above the horizontal line. RD, running distance; BW, body weight.

The work was not increasing gradually in parallel with the increment in load. The work really showed a plateau between the last 2 days at 0 g and the last day at 20 g and then decreased to a new plateau with 25 g.

Body weights

The mean body weight of the 60-week-old ICR group was significantly higher than that of the 40-week-old ICR group, whereas there was no significant difference in the mean body weight between the 40- and 60-week-old control SAMP6 groups (Figure 2). The mean body weights of the 40- and 60-week-old control SAMP6 groups were significantly lower than those of the age-matched ICR groups.

The mean body weight of the 60-week-old exercised SAMP6 group was significantly lower than that of the 40-week-old control SAMP6 group, whereas there was no significant difference in the mean body weight between the 60-week-old control SAMP6 and 60-week-old exercised SAMP6 groups (Figure 2).

Muscle weights

The mean absolute weight of the TA of the 60-week-old ICR group was significantly higher than that of the 40-week-old ICR group, whereas there were no significant differences in the mean absolute weight of the PL or the SOL between the 40- and 60-week-old ICR groups (Figure 3). The mean absolute weights of the TA, PL, and SOL of the 60-week-old control SAMP6 group were significantly lower than those of the 40-week-old control SAMP6 group. The mean absolute weights of the TA, PL, and SOL of the 40- and 60-week-old control SAMP6 groups were significantly lower than those of the age-matched ICR groups.

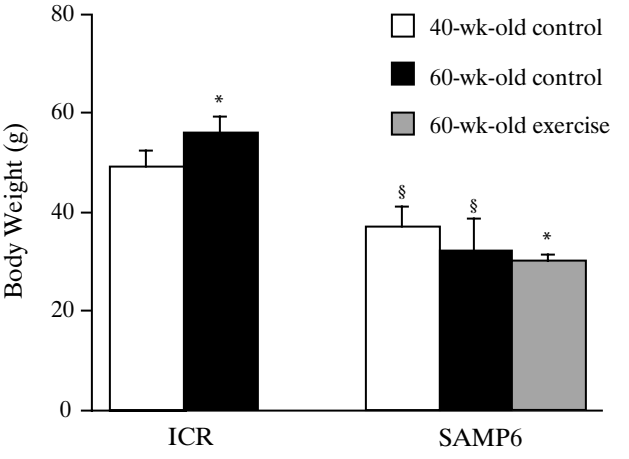

Figure 2. Body weights of the ICR and SAMP6 groups. Bars, standard deviations. * and §, significantly different from the corresponding 40-week-old control and the age-matched ICR groups, respectively, at $p<0.05$.

The mean absolute weight of the PL of the 60-week-old exercised SAMP6 group was significantly lower than that of the 40-week-old control SAMP6 group (Figure 3).

There were no significant differences in the mean relative weight of the TA, PL, or SOL between the 40- and 60-week-old ICR groups, whereas the mean relative weights of the TA, PL, or SOL of the 60-week-old control SAMP6 group were significantly lower than those of the 40-week-old control SAMP6 group (Figure 3). There were no significant differences in the mean relative weight of the TA, PL, or SOL between the 40-week-old ICR and 40-week-old control SAMP6 groups and between the 60-week-old ICR and 60-week-old control SAMP6 groups, except that the mean relative weight of the SOL of the 60-week-old control SAMP6 group was significantly lower than that of the 60-week-old ICR group.

The mean relative weights of the TA, PL, and SOL of the 60-week-old exercised SAMP6 group were significantly higher than those of the 60-week-old control SAMP6 group (Figure 3).

Femur wet and dry weights and lengths

There were no significant differences in the mean wet or dry weight between the 40- and 60-week-old ICR groups and between the 40- and 60-week-old control SAMP6 groups (Figure 4). The mean wet and dry weights of the 40- and 60-

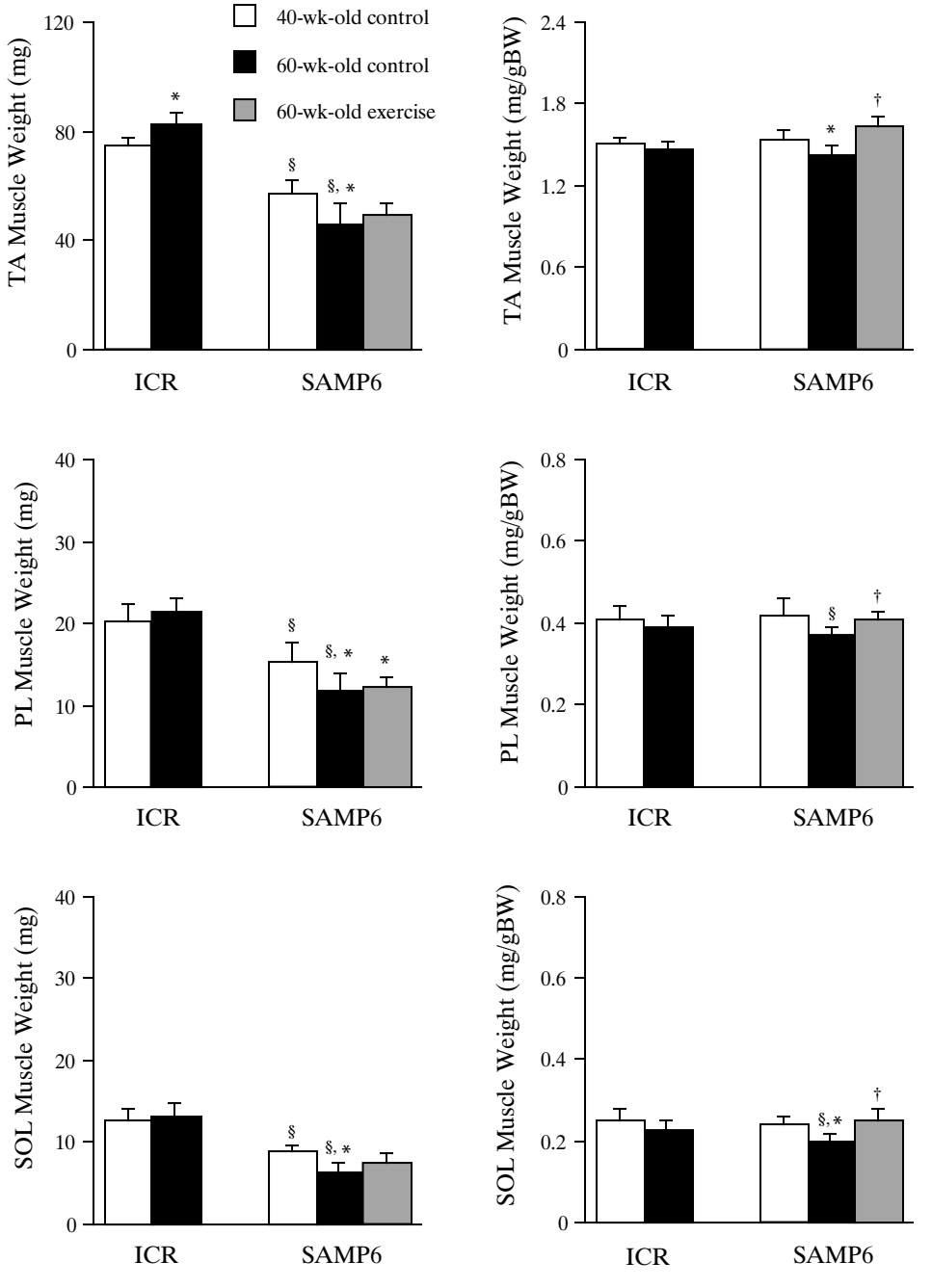

Figure 3. Absolute (left) and relative (right) weights of the tibialis anterior (TA), plantaris (PL), and soleus (SOL) muscles of the ICR and SAMP6 groups. Bars, standard deviations. BW, body weight. ∗, §, and †, significantly different from the corresponding 40-week-old control, the age-matched ICR, and the 60-week-old control SAMP6 groups, respectively, at $p<0.05$.

week-old control SAMP6 groups were significantly lower than those of the age-matched ICR groups.

There were no significant differences in the mean wet or dry weight between the 40-week-old control SAMP6 and 60-week-old exercised SAMP6 groups and between the 60-week-old control SAMP6 and 60-week-old exercised SAMP6 groups (Figure 4).

There were no significant differences in the femur length among all groups studied (Figure 4).

Relationships between muscle and bone weights

There was a strong correlation between muscle wet weight and femur wet weight across all groups studied for each muscle (Figure 5).

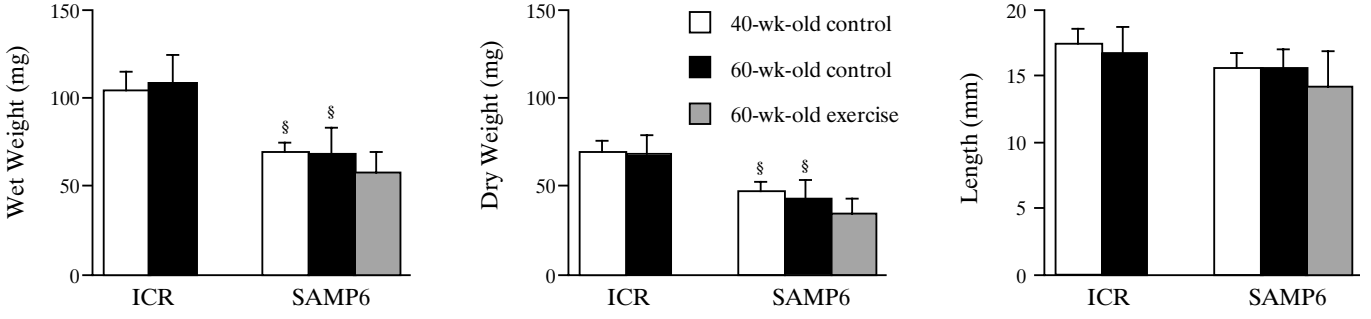

Figure 4. Wet (left) and dry (middle) weights and lengths (right) of the femur in the ICR and SAMP6 groups. Bars, standard deviations. §, significantly different from the age-matched ICR group at $p<0.05$.

Femur densities

There were no significant differences in the mean cortical or trabecular density between the 40- and 60-week-old ICR groups (Figure 6). The mean cortical and trabecular densities of the 60-week-old control SAMP6 group were significantly lower than those of the 40-week-old control SAMP6 group. The mean cortical densities of the 40- and 60-week-old SAMP6 groups were significantly lower than those of the age-matched ICR groups. The mean trabecular density of the 40-week-old control SAMP6 group was significantly higher than that of the 40-week-old ICR group, whereas there was no significant difference in the mean trabecular density between the 60-week-old ICR and 60-week-old control SAMP6 groups.

There was no significant difference in the mean cortical or trabecular density between the 40-week-old control SAMP6 and 60-week-old exercised SAMP6 groups and between the 60-week-old control SAMP6 and 60-week-old exercised SAMP6 groups (Figure 6).

Femur CSAs

There were no significant differences in the mean cortical or trabecular CSA between the 40- and 60-week-old ICR groups (Figure 7). There was no significant difference in the mean cortical CSA between the 40- and 60-week-old control SAMP6 groups, whereas the mean trabecular CSA of the 60-week-old control SAMP6 group was significantly higher than that of the 40-week-old control SAMP6 group. The mean cortical and trabecular CSAs of the 40- and 60-week-old control SAMP6 groups were significantly lower than those of the age-matched ICR groups.

The mean cortical CSA of the 60-week-old exercised SAMP6 group was significantly lower than that of the 40-week-old control SAMP6 group, whereas the mean trabecular CSA of the 60-week-old exercised SAMP6 group was significantly lower than that of the 60-week-old control SAMP6 group (Figure 7).

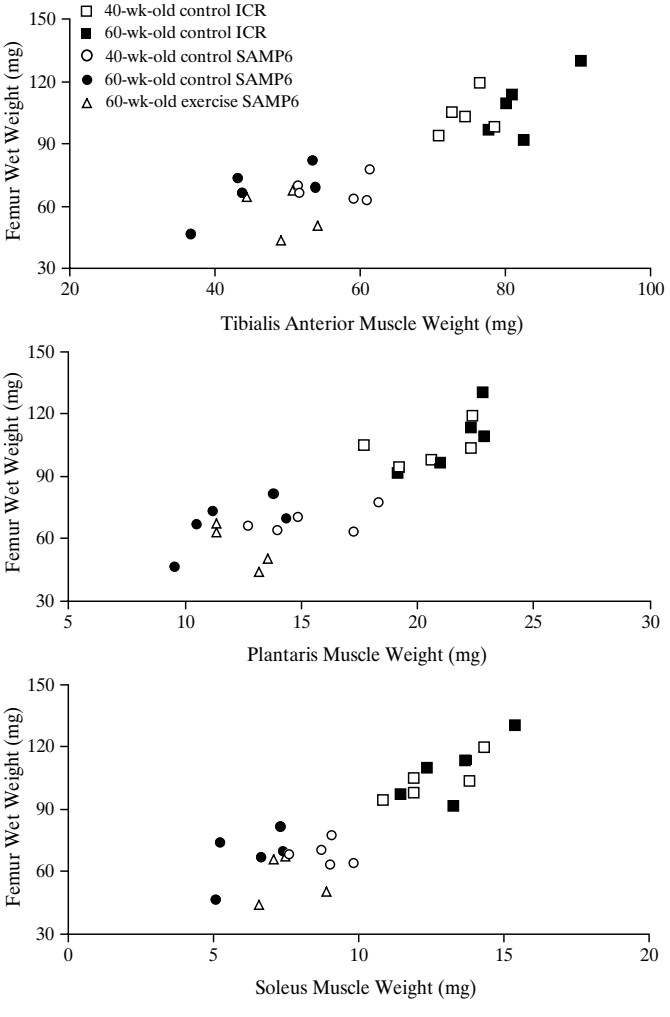

Figure 5. Relationships between the tibialis anterior (top), plantaris (middle), or soleus (bottom) muscle weight and the femur wet weight. Tibialis anterior, $r=0.88$, $n=24$, $p<0.05$; Plantaris, $r=0.87$, $n=24$, $p<0.05$; Soleus, $r=0.88$, $n=24$, $p<0.05$.

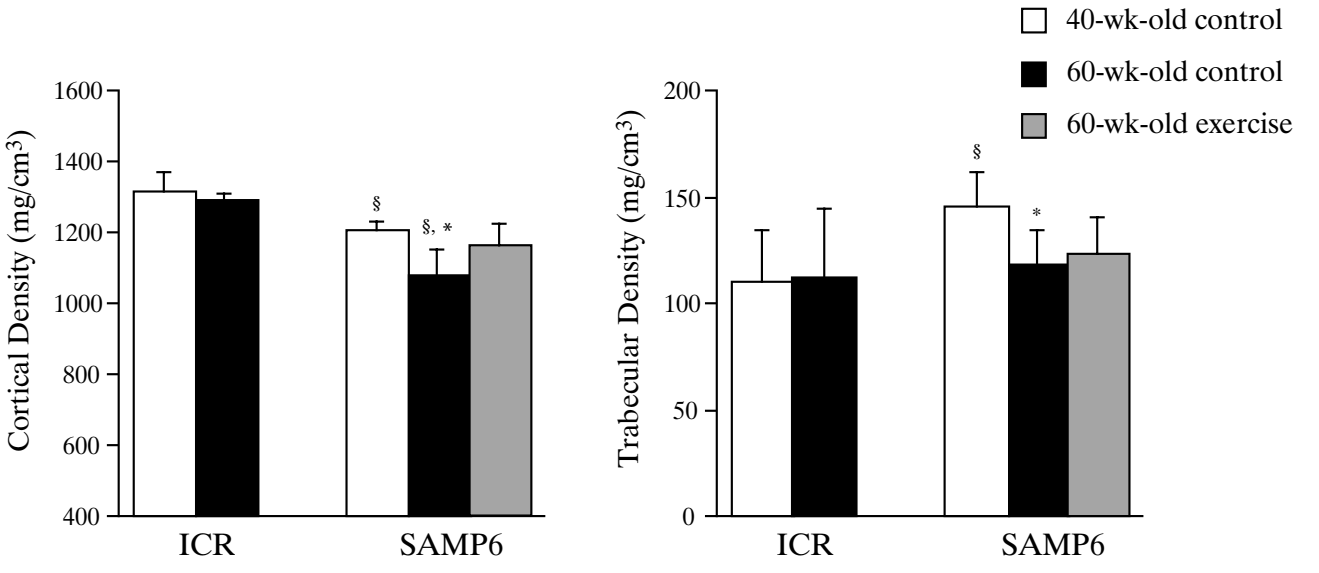

Figure 6. Cortical (left) and trabecular (right) densities of the femur in the ICR and SAMP6 groups. Cortical and trabecular densities were analyzed at the middle and distal sites of the femur, respectively. Bars, standard deviations. * and §, significantly different from the corresponding 40-week-old control and the age-matched ICR groups, respectively, at $p<0.05$.

Discussion

Bone mineral density has been measured using single- or dual-energy photon absorptiometry, dual-energy X-ray absorptiometry, or conventional radiography. The values obtained from these measurements, however, do not reflect the volumetric density. Rather than an actual bone density, a quotient of bone mineral content to a two-dimensional projected corresponding area is calculated. A pQCT technique was developed to provide simultaneous information on the geometric properties and volumetric density of appendicular bone[7]. In addition, pQCT procedures have been used to separately assess cortical and trabecular bone compartments[8]. In the present study, therefore, the morphometric profiles of the mouse femur were determined using pQCT.

Male SAMP6 housed in a laboratory animal colony have a median survival time of ~64 weeks[6], a much shorter life span than other strains of mice[1,2]. We have previously reported that the TA muscle weight and the CSAs and oxidative enzyme activities of its fibers are lower in the 60-week-old SAMP6 than in the 40-week-old SAMP6, but not for the same comparisons in age-matched control rats (SAMR1)[6]. In the present study, we observed that the TA, PL, and SOL weights were lower in the 60- than 40-week-old SAMP6 groups, whereas these muscle weights were similar in the 40- and 60-week-old ICR groups (Figure 3). These data clearly indicate that SAMP6 have an early onset of age-related decrease in muscle mass. In addition, the oxidative enzyme activities of spinal motoneurons innervating the TA muscle have been shown to be lower in the 60- than 40-week-old SAMP6, but similar at these two time points in control rats (SAMR1)[6]. Similar neuromuscular adaptations have been observed in aging rats[10,11] and these changes may have been associated with senile osteopenia. Therefore, we hypothesized that senile osteopenia may occur in SAMP6 from 40 to 60 weeks of age, i.e., during the period that the neuromuscular system is affected dramatically. In the present study, significant decreases in the cortical and trabecular density of the femur in SAMP6 were observed between 40 and 60 weeks of age (Figure 6), verifying that SAMP6 exhibits senile osteoporosis at an earlier age relative to other strains of mice. It should also be noted that the cortical density in the 40-week-old SAMP6 group was lower than that in the age-matched ICR group, indicating that osteopenia may begin at an even earlier stage in SAMP6, i.e., prior to 40 weeks of age.

Exercise results in a large number of adaptations in the contractile, morphological, and metabolic properties of skeletal muscle that are specific to the type and amount of exercise. In general, skeletal muscles exposed to relatively low-intensity and long-duration exercise show endurance-related adaptations including an increased percentage of oxidative fibers, higher oxidative capacities, higher myoglobin and mitochondrial levels, and increased capillarization[12,13]. In contrast, strength or power type (relatively high-intensity and short-duration) exercise results in fewer apparent metabolic adaptations, but elicits muscle fiber hypertrophy[12,14].

A marked effect of mechanical loading on bone density has been well established. It has been demonstrated that mechanical loading, e.g., running or jumping exercises, swimming, or functionally overloading a muscle via synergist removal, can increase bone mass and strength in young and adult animals[15-21]. Aged animals, however, present additional considerations. For example, bones in aged animals respond less efficiently to mechanical stimulation than those in young animals[22-26]. Therefore, a longer duration of exercise or increased amount of loading (total work) may be necessary to increase bone for-

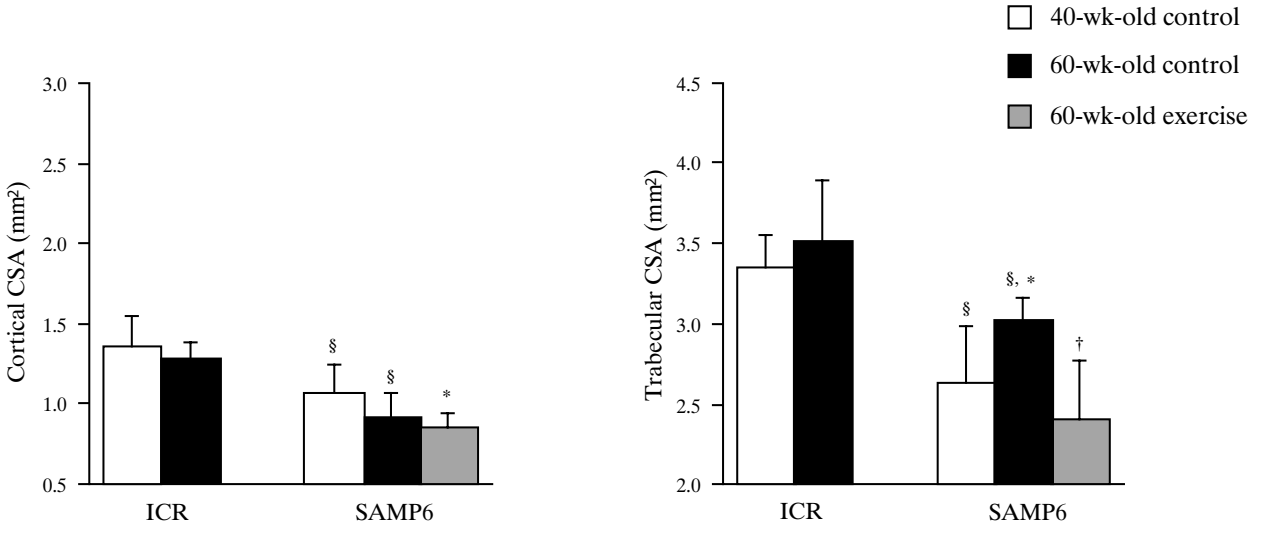

Figure 7. Cortical (left) and trabecular (right) cross-sectional areas of the femur in the ICR and SAMP6 groups. Cortical and trabecular cross-sectional areas were analyzed at the middle and distal sites of the femur, respectively. Bars, standard deviations. CSA, cross-sectional area. *, §, and †, significantly different from the corresponding 40-week-old control, the age-matched ICR, and the 60-week-old control SAMP6 groups, respectively, at $p<0.05$.

mation and/or inhibit bone loss in aged animals.

Another problem is that there is a large individual variability in the response to exercise among aged animals, thus making it difficult to determine differences between control and exercised groups. Consequently, exercise-related investigations using senescent animals have been equivocal. For example, some previous studies[27,28] reported that increased loading using treadmill exercise or jump training increased bone CSA in aged rats to the same extent as in young animals. In contrast, a recent study[29] reported that resistance exercise, i.e., 50 squat-like hind limb extensions of the ankle performed three times per week while wearing a weighted backpack that is gradually increased to 65% of body weight, did not elicit any beneficial effects on the bones of aged rats. Therefore, it appears that the responsiveness to changes in the loading environment may be altered as a function of age.

The SAM strains provide a unique model system to study senescence or the aging process because they show a marked acceleration in the appearance of several indicators of aging[1,2]. In the present study, therefore, 40-week-old SAMP6, which show acceleration of the aging process in the neuromuscular system from 40 to 60 weeks of age[6], exercised for 20 weeks to investigate the effect of intermittent loading on bone density. Previous studies[30-32] have shown that the adaptation to mechanical loading is influenced by the magnitude and duration of the applied load, e.g., the lower activity of aged rats places their bones in a state of decreased use contributing to a decrease in bone mass with aging. Therefore, in the present study we used voluntary running exercise with progressively increasing loads in an attempt to ameliorate these detrimental effects. The mean work increased gradually in parallel to the increase in load from the 1st to 11th weeks and then plateaued for the last 9 weeks of the exercise period when the load was held constant (Figure 1). The cortical densities of the 60-week-old exercised SAMP6 group were similar to those of the 40-week-old control SAMP6 group (Figure 6), indicating that voluntary running with some level of loading may be a beneficial therapeutic intervention for senile osteopenia. An unexpected finding was that the exercised SAMP6 group had smaller cortical and trabecular CSAs than the 40- and 60-week-old control SAMP6 groups, although there were no differences in the bone wet and dry weights and lengths (Figure 4). The exercised SAMP6 had decreased cortical CSAs, but maintained cortical density, suggesting periosteal resorption in this model by running exercise. At this time, we have no explanation for these observations. Recent studies[33,34] reported that age-associated bone loss may be due to reductions in the availability and/or synthesis of bone TGF-β which results in fewer, less osteogenic marrow osteoprogenitor cells and lower levels of bone formation. In fact, it appears that the age-associated reduction in bone formation can be reversed by introducing exogenous TGF-β[34]. Together, the results of these studies suggest that a combination of exercise and exogenous TGF-β administration may be very effective countermeasures for age-associated bone loss.

Conclusions

Age-related declines in the cortical density of the femur in SAMP6 were observed at a relatively early age, and voluntary running exercise with increasing loads ameliorated these changes. These results indicate that this senescence-accelerated mouse model may be very useful for studying 1) age-related osteoporosis in a shorter time span than is possible with other mouse strains; and 2) the interaction between aging and exercise on the development of osteoporosis associated with aging in this strain of mice.

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