The Effects of Aging and Treadmill Running on Soleus and Gastrocnemius Muscle Morphology in the Senescence-Accelerated Mouse (SAMP1)

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We investigated the effects of aging on the soleus and gastrocnemius muscles in male SAMP1 (senescence-accelerated mouse prone 1). Body mass, muscle wet weight, fiber size, and the percent of type II fibers declined from 50 weeks of age. Voluntary motor behavior also significantly declined with age. Furthermore, we examined the effects of high (twice daily) and low (once daily) frequency treadmill running, for 6 weeks at 5 days per week, beginning when the mice were 50 weeks old. Muscle fiber size for the high frequency running significantly increased. Pathological fiber alterations in these mice were increased by running, especially by high frequency running. This suggests that age-related muscle morphological changes in SAMP1 occur from 50 weeks of age, and that the decline in voluntary motor behavior is an important factor in aging muscle atrophy. In addition, high frequency running is more beneficial for aged muscle hypertrophy. This model is useful for studying the acceleration of the aging process in skeletal muscle of the SAM.

As we age, our skeletal muscle becomes smaller and weaker (1), and decreased skeletal muscle mass is one of the most striking features of aging. In old muscle, the number and size of fibers, especially type II (fast-twitch) fibers, decrease (2–4). However, it was reported that fiber numbers and size decrease in fast and slow muscles in rats (5,6). Type I and II muscle fiber atrophies are different in weight-bearing and non-weight-bearing muscles in humans (7). Aged skeletal muscles are prone to overwork weakness associated with muscle damage (8). A common complaint of older adults is a decline in motor function, which can lead to falls and injuries (9).

As a senescence-accelerated animal model, the senescence-accelerated mouse (SAM), has been developed at the Department of Senescence Biology, Chest Disease Research Institute, Kyoto University, Japan (10,11), and includes nine accelerated-senescence-prone mice strains (P strains) (10). The SAMP strains are characterized by normal development during growth after birth. Afterward, the rapid progression of senescence occurs, which is associated with amyloid deposition, decreased activity, hair loss, cataract formation, enhanced lordosis, and death as early as 12–15 months after birth (10,12). At present, nine SAMP varieties have been established through selective cross-breeding (10). These strains provide a unique model system for studying senescence or the aging process (10). SAMP mice, a group of related inbred strains, express the phenotypes of age-associated diseases and are widely used in aging research (11). For example, P strains show senile amyloidosis (P1, P2, P7, and P11), degenerative arthrosis of temporomandibular joint (P3), senile osteoporosis (P6), thymoma (P7), deficits in learning and memory with brain atrophy (P8, P10) and cataract (P9) (10,11). Among them, SAM prone 1 (SAMP1) exhibits the most pronounced acceleration of senescence and short-life (13). Reports exist on the aging research using SAM such as motoneuron, intervertebral disk, age-associated DNA damage, and bone density (4,12–14), but few have examined the changes that occur in SAM skeletal muscle. It is unknown whether age-associated morphological changes in SAM skeletal muscle occur from an early stage. Therefore, we examined the onset of age-associated alterations in SAMP1 leg skeletal muscle using morphology and histochemistry. In addition, we investigated the effects of different exercise programs (free cage activity, single and double treadmill running sessions each day) on SAMP1 skeletal muscle. This research will contribute to the studies of age-related skeletal muscle change.

METHODS

Animals and Experimental Protocols

Male SAMP1 and Institute of Cancer Research (ICR) mice aged 24, 40, 50, and 56 weeks (n = 5 for each group) were used. We reproduced these mice by aseptically raising them and cross-breeding them at 80 weeks in our experimental laboratory, after which only males were used for this study. ICR mice were prepared as normal senescence models.

Male SAMP1 and ICR mice aged 50 weeks underwent two different treadmill running programs (n = 5 for each running program). In the high-frequency running program (HRP), the mice exercised twice a day (at least 1 hour apart between the sessions). In the low-frequency running program (LRP), the mice exercised once per day. A motorized
treadmill (Rat runner, RR-1200; AKK, Shimane, Japan) forced the mice to run, whereby an electric stimulation system installed on the rear floor was used to administer typical treadmill running 5 days per week for 6 weeks.

In treadmill running protocols, the treadmill speed was 10 m/min with an inclination of 10°, and the running speed was increased from 10 to 16 m/min over the training period. The program was progressive so that the running time increased from 15 min/session in the first week to 20 min/session over 6 weeks. All mice were individually housed in similar plastic cages, and food and water were provided ad libitum.

The following experimental protocol was approved by the ethical board of the Institute of Laboratory Animal Sciences of Kagoshima University. Attention was paid to changes in muscle wet weight, muscle fiber cross-sectional area (fiber size), fiber type composition, and pathological fiber alteration in SAMP1 aged leg muscles.

**Sample Preparation**

The soleus and gastrocnemius muscles in both legs were dissected, cleaned of fat and connective tissue, weighed, and quickly frozen in isopentane chilled with liquid nitrogen. The muscles were then stored at −80°C until analysis, and the muscle from both sides was analyzed for all animals. In this study, soleus and gastrocnemius muscles were used because these muscles have different fiber-type distributions (15,16). Samples for microscopy of slow- and fast-twitch muscle fibers were taken from the middle part of the muscles of both limbs.

**Histological and Histochemical Analysis**

Soleus and gastrocnemius muscles were vertically mounted on cork plates in tragakanth gum jelly of the appropriate softness (17) to obtain cross-sections. Transverse serial sections 10 µm thick were cut with a cryostat microtome at −20°C and stained with hematoxylin and eosin for general observation. Sections were also stained for myosin adenosine triphosphatase (ATPase, pH 10.5, 4.3) reaction according to Guth and Samaha (18) with some modifications, and an additional section was stained by the nicotinamide adenine dinucleotide (NADH)-reductase reaction. Sections stained for ATPase activity were used to classify fibers as type I or II, and sections stained by the NADH-reductase reaction were used for the semiquantitative evaluation of oxidative capacity to confirm the fiber type (19).

Transverse sections from the soleus and gastrocnemius muscles were used for morphometric study. A whole cross-section of each soleus and gastrocnemius muscle was photographed at 20× magnification for fiber-type composition. Two regions of both muscles were photographed at 50× magnification without visual field overlap, and all the muscle fibers delineated by entire fiber boundaries were measured for cross-sectional area. The cross-sectional area of the gastrocnemius muscle was measured at part of the deep layer. A random sample of 150 fibers from each muscle (1500 fibers total) was analyzed.

The number (%) of fibers by pathological, morphological, and histochemical alterations was determined with hematoxylin and eosin staining, ATPase preparation, and NADH-reductase reaction. Pathological alterations were determined for 300–350 consecutive adjacent fibers in each control and experimental muscle, mainly type I fibers from the soleus muscle and type II fibers from the gastrocnemius muscle. According to their characteristic histological and histochemical features, the alterations were classified as: moth-eaten fibers (referring to a spiral-type deformation and destruction of the myofibrillar network of the fiber, the term being derived from the microscopic moth-eaten appearance of the fiber); centrally placed nuclei (referring to centronuclear fibers that appeared as regenerated fibers); central core formation within the fiber (referring to abnormally increased oxidative enzyme activity and the normal aggregation of myofibrils in the central area of the fiber); fiber splitting; shell-like fiber (referring to shell-like degradation and degeneration of the myofibrillar network of the fiber, the term being derived from the microscopic shell-like appearance of the fiber); and other alterations (necrosis fibers) (20). The total percentage of fibers with pathological alterations was calculated for SAMP1 and ICR mice. The results of the SAMP1 treadmill running groups were compared with those of ICR mice aged 56 weeks. These methods of analysis were adopted because it was possible to quantify the ratio of type II fibers and the rate of fibers with pathological alteration in the soleus and gastrocnemius muscles.

**Voluntary Motor Behavior Analysis**

We examined the voluntary motor behavior for over 1 hour in SAMP1 and ICR mice. The animals were placed in a plastic cage (24.0 × 17.0 × 12.0 cm) enclosed by wire gauze and were allowed to engage in free activity. A red line in the middle portion (12.0 cm) of the cage was drawn, and we counted the number of times (times/hour) the mice crossed it for 1 hour. A count was recorded when both hindlimbs crossed the red line. Measurements were carried out in the daytime (between 1:00 PM and 3:00 PM) by 7 people. The crossing frequency for ICR mice did not exhibit significant differences at four time points studied. Therefore, these data was pooled and treated as a normal control.

**Data Analysis**

Statistical analyses were performed using StatView version 4.5 software (StatView, SAS Institute, Inc., Cary, NC). Values for body weight, muscle wet weight, fiber cross-sectional area, percent of type II fibers, and voluntary motor behavior were compared using a one-way analysis of variance (ANOVA). If significance was achieved (p < .05), post hoc Fisher’s protected least significant differences (PLSD) test was performed to determine where significant differences existed. For variable pathological fiber alterations, the groups were compared using the chi-square test. Unpaired Student’s t tests were also used to determine differences between SAMP1 and ICR mice. Significance was set at p < .05.

**RESULTS**

**Changes in Body Weight, Muscle Wet Weight, and Cross-Sectional Area of Each Fiber Type**

The mean body weights for the SAMP1 were 36.0 ± 2.2 g (mean ± standard deviation [SD]) at 24 weeks of age,
The mean body weights of the ICR mice were 57.3 ± 6.2 g at 24 weeks, 53.1 ± 6.7 g at 50 weeks, and 29.9 ± 2.3 g at 56 weeks. The mean body weights significantly decreased at 56 weeks compared with 40 weeks (p < .01). The mean body weights of the ICR mice were 57.3 ± 4.9 g at 24 weeks, 53.1 ± 2.3 g at 40 weeks, 53.5 ± 4.2 g at 50 weeks, and 57.0 ± 3.5 g at 56 weeks. There were no significant differences in the mean body weights of the ICR mice at any time. The mean body weight for the SAMP1 mice was significantly higher than that of the ICR mice at each time point (p < .01).

Changes in the soleus muscle weight and mean fiber cross-sectional areas of the SAMP1 and ICR mice from 24 to 56 weeks of age are presented in Table 1. In the SAMP1, the muscle wet weights decreased at 56 weeks, and significantly decreased after 56 weeks compared with those at 40 or 50 weeks (p < .05). Changes in mean type I and II fiber cross-sectional areas were similar to those for the muscle wet weights. The fiber cross-sectional areas of type I and II fibers significantly decreased at 56 weeks compared with those at 40 weeks for SAMP1 mice (p < .01). In ICR mice, the soleus muscle wet weights and type I or II fiber cross-sectional areas were not significantly different at any time (Table 1). The fiber cross-sectional area of the type I fibers for the SAMP1 and ICR mice was significantly smaller than that of ICR mice at each time point (p < .01).

Changes in the gastrocnemius muscle wet weights and mean fiber cross-sectional areas of SAMP1 and ICR mice from 24 to 56 weeks of age are presented in Table 2. In SAMP1, the muscle wet weights significantly decreased at 50 and 56 weeks compared with those at 40 weeks (p < .01). The change in the mean cross-sectional area of type I fibers was similar to that for the muscle wet weight, and significantly decreased at 50 and 56 weeks compared with that at 40 weeks (p < .05). However, no significant differences were observed in the type II fiber area at 50 and 56 weeks. In the ICR mice, the gastrocnemius muscle wet weights and type I and II fiber cross-sectional areas were not significantly different at any time (Table 2).

**Changes in the Muscle Fiber Composition**

Changes in the percent of type II fiber in the soleus muscle are shown in Table 1. In SAMP1, the percent of fiber that were type II for the soleus muscle at 24 weeks was similar to that at 40 weeks, but decreased from 50 weeks. The percent of fiber that were type II was significantly decreased at 56 weeks compared with that at 40 weeks (p < .05, Figure 1). In ICR mice, the percent of type II fibers for the soleus muscle was not significantly different at 40, 50, and 56 weeks (Table 1).

Changes in the percent of type II fiber in the gastrocnemius muscle are shown in Table 2. The percent for SAMP1 was significantly decreased at 50 weeks compared with 40 weeks (p < .05). However, there was no significant difference at 56 weeks. In ICR mice, the percent of type II fiber for the gastrocnemius muscle was not significantly different at 40, 50, and 56 weeks (Table 2).

**Changes in Muscle Morphology (Pathological Fiber Alteration)**

The occurrence of abnormal fibers in the soleus and gastrocnemius muscles in SAMP1 and age-matched ICR mice is shown in Table 3 (soleus) and Table 4 (gastrocnemius). We observed muscle fibers with pathological alterations, such as moss-eaten fibers, central core formation within fibers, fiber splitting, centrally placed nuclei, and necrotic fibers (Figure 2). In the muscles of 24, 40, 50, and...
56 week-old ICR mice and SAMP1, few fibers with pathological alterations were observed.

In the soleus muscle, the number of pathological fibers in SAMP1 was increased by 5.5% at age 56 weeks. However, these results were not significantly different at 24, 40, 50, and 56 weeks. The number of pathological fibers in ICR mice was not significantly changed at 24, 40, 50, and 56 weeks. The total number of pathological fibers was not significantly different between SAMP1 and ICR mice at 24, 40, 50, and 56 weeks.

In the gastrocnemius muscle, the percentage of pathological fibers for SAMP1 was not different from that between 24 to 56 weeks. The number of pathological fibers in the gastrocnemius muscle in SAMP1 was not significantly different from ICR mice at 24, 40, 50, and 56 weeks.

Effects of the Different Frequency Treadmill Running Programs

We examined the effects of different frequency treadmill running (HRP or LRP) on aged muscle morphology for SAMP1 and age-matched ICR mice. The mean body weights of 32.1 ± 1.1 g for the HRP group and 27.1 ± 2.7 g for the LRP group SAMP1 were not significantly different from that of the no-treadmill running group at 56 weeks. The mean body weights of 53.0 ± 6.0 g for the HRP group and 54.0 ± 2.0 g for the LRP group ICR mice were not significantly different from that of the nonrunning group.

In the muscle wet weight of the soleus of SAMP1, there was no significant difference for the HRP and LRP groups (Table 1). The type I and II fiber cross-sectional areas for the HRP group were significantly increased compared with...
those of the nonrunning group \( (p < .05, \text{Table 1}) \). The percent of fibers that were type II significantly increased from 39% to 50% with LRP \( (p < .05, \text{Table 1}) \).

In the gastrocnemius muscle of SAMP1, muscle wet weight for the HRP group was significantly increased compared with that of the nonrunning group \( (p < .05, \text{Table 2}) \). The type I fiber cross-sectional area for the HRP group was significantly increased compared with that of the nonrunning group \( (p < .05, \text{Table 2}) \). In HRP and LRP group ICR mice, there was no significant difference in all soleus and gastrocnemius muscle parameters (Tables 1 and 2).

The effects of different treadmill running programs for SAMP1 on the occurrence of soleus and gastrocnemius muscle fibers with pathological alterations are shown in Tables 3 and 4. HRP group SAMP1 exhibited a marked change in pathological alterations, and the histological appearance of pathological alterations in fibers were observed for the soleus and gastrocnemius muscles compared with that of the nonrunning group. Muscle fibers of the SAMP1 frequently exhibited morphological alterations such as centrally placed nuclei and moth-eaten fibers (Figure 2). In the HRP group, the total number of pathological fibers in soleus and gastrocnemius muscle SAMP1 was higher than that of ICR mice \( (p < .05) \). In the NADH-reductase reaction, the muscle fibers in LRP were increased in oxidative capacity compared with that of the nonrunning group in SAMP1 and ICR mice. The number of pathological fibers was not significantly changed by treadmill running for the soleus and gastrocnemius muscles of ICR-exercised mice compared with that of the nonrunning group.

**Voluntary Motor Behavior**

Voluntary motor behavior in SAMP1 is shown in Figure 3. The frequency (times/hr) at which the red line was crossed for normal control mice (ICR mice) ranged from 160 to 272. ICR mice constantly moved inside the cage during the measurement period. However, the SAMP1 moved often inside of the cage during the first 20 minutes, but their movement gradually decreased thereafter. The crossing frequency for SAMP1 was significantly decreased compared with that of ICR mice \( (p < .01) \); the crossing frequency for SAMP1 was significantly decreased at 56 weeks compared with that at 24 or 40 weeks \( (p < .01) \).

**DISCUSSION**

Skeletal muscle atrophy, a decrease in the number of muscle fibers, and morphological changes in neuromuscular junctions in old mice and rats have been examined \((2,4,21,23)\). Changes in muscle fiber size serve as an important morphologic parameter of age-related changes in mammalian skeletal muscle. The mass of many weight-bearing muscles declines in old rats, secondary to the atrophy of fibers and in particular fast-twitch fibers \((\text{type IIb}) (3,6)\). The present results showed age-associated morphological changes associated with the soleus and gastrocnemius muscles of SAMP1 mice. Body mass, muscle wet weight, the mean cross-sectional areas of type I and II fibers, and the percent of fibers that were type II decreased at 50 weeks of age, and decreased significantly at 56 weeks. These morphological changes in skeletal muscle suggest that muscle atrophy, a decrease in fiber cross-sectional area, and the percent of fibers that were type II are related to aging.
In male SAMP6, the mean cross-sectional areas of the tibialis anterior muscle fibers is smaller at 60 weeks of age (4).

Age-related muscle atrophy is related not only to histological and histochemical changes in muscle fiber but also to decreased motor behavior. Age-related changes in the spontaneous motor rhythm of SAMP8 occurs as early as 7 months of age (24). Our results showed a decrease in voluntary motor activity for aged SAMP1. This suggests that age-related muscle atrophy in SAMP1 mice is related to declined voluntary motor activity.

The muscle fiber morphological findings (pathological fiber alteration) were not different at any time for non-running SAMP1 mice. Histologically, pathological changes in fibers bear resemblance to those seen in mice with muscular dystrophy, neurogenic atrophy, and after strenuous muscle activity, all of which can be classified as degenerative (20). Our result suggests that the pathological fibers studied in healthy old mice were not age-related morphological fiber changes.

This study was undertaken to observe the response of skeletal muscle to different frequencies of treadmill running in SAMP1 and age-matched ICR mice, and to evaluate and compare the morphological findings of exercise and non-running groups. Increased muscle strength is the desired outcome of many rehabilitation therapies. Strength gain is often a result of muscle hypertrophy, and progressive resistance exercise is the primary mode used to induce muscle hypertrophy in rehabilitation. Exercise has been implicated in the modulation of muscle fiber behavior (19), and it is known that motor activity causes muscle fiber damage in both human and animals (25). Endurance training, regular exercise, weight-lifting, and resistance training have often been used to increase muscle mass and strength (6, 21, 26, 27). Endurance training is generally considered to increase muscle oxidative capacity with little or no change in muscle volume (27). Resistance training leads to increased muscle mass and strength in old rats (6); while weight-lifting by old rats results in significant muscle atrophy and lower exercise capacity than young rats (8). The effects of different intensity or frequency training programs on muscle structure and mechanics at various ages is not well known. It is important to determine optimal exercise programs for aged people by varying the type, intensity, and frequency of the exercise.

Treadmill exercise is a form of endurance training and affects muscular tissue differently than other forms of physical exercise such as weight-lifting or stretching (28). This exercise is beneficial as a therapeutic intervention in osteoporosis, stroke, aging, cardiovascular disease, and muscular disease (22, 28, 29). Skeletal muscle has the ability to adapt to altered functional demands under experimental as well as physiological conditions such as physical training. Regular exercise training programs in clinical and experimental assays can alter the distribution of different muscle fiber types (19, 30), and appropriate exercise programs produce skeletal muscle hypertrophy (20). Low-intensity exercise increases strength and function in old rats (31). Our results indicate that the muscle fiber cross-sectional area of SAMP1 in the HRP group was significantly increased compared with that of non-running groups. The LRP group of SAMP1 did not exhibit significant changes in fiber size, but a decrease in type II fiber ratio was suppressed by running. Endurance training led to a significant increase in maximal oxygen consumption, accompanied by an increase in mitochondrial volume density, but not in the muscle cross-sectional area of a human trained leg muscle (27, 32). In this study, the muscle fibers in LRP were increased in oxidative capacity compared with that of the non-running group in NADH-reductase reaction. This data suggests that appropriate physical exercise for aging skeletal muscle leads to muscle hypertrophy. In addition, this shows that a high frequency running program is more beneficial than a low frequency one for muscle hypertrophy, and treadmill running affects muscle fiber composition according to the aging process. However, in clinical practice, time is needed for aging muscle hypertrophy, because many elderly patients have neuromuscular diseases such as osteoarthritis, osteoporosis, and lumbago, and compliance and motivation must also be considered.

Both running programs had little effect on ICR mice. We noted that SAMP1 did not move inside the cage at 50 or 56 weeks of age, while ICR mice moved often. These results show that the voluntary motor behavior of SAMP1 was significantly decreased compared with that of ICR mice. Therefore, the effect of treadmill exercise on increasing muscle mass and mean fiber cross-sectional area in ICR mice was not strong because of spontaneous daily movement. On the other hand, exercise for SAMP1 may be effective for increasing muscle mass and mean cross-sectional area because of the lack of daily movement. In male SAMP6 mice, the number of tibialis anterior motoneurons was small at 60 weeks (4). However, the number of spinal anterior horn motoneurons at 60 weeks was slightly decreased in comparison with that at 20 weeks (4), suggesting that the decrease in motor neurons had little effect on muscular atrophy. Our results suggest an age-related decrease in muscle wet weight and fiber size related to disuse muscle atrophy in SAMP1 mice.

Old muscle is susceptible to contraction-induced damage (23). Therefore, we examined the effect of treadmill running on pathological fiber alteration. Pathological fiber alterations in SAMP1 were increased by treadmill running, especially high frequency running. In young animals, skeletal muscles possess the inherent ability to adapt to exercise (33). The skeletal muscle of SAMP1 does not appear to be able to cope with such strain. In atrophied muscles, exercise training induces muscle degeneration, which results in the synthesis of new fibers by satellite cell activation (20). Pathological fiber alteration is necessary for the synthesis of new fibers by satellite cell activation, when muscles become hypertrophied. Muscle alteration exhibited by the HRP group SAMP1 may be a process by which hypertrophy and the synthesis of new fibers induced by running occur.

Conclusion
We demonstrated that age-associated morphological changes in the leg muscles of SAMP1 occurs earlier than for normal mice. This provides direct evidence that there is an acceleration of the aging process in the skeletal muscle of this strain of mouse, indicating that this model is useful in studying the aging process. Our results also suggested that high frequency running is more beneficial than low frequency
running for muscle hypertrophy during the aging process. However, physical exercise aimed at reducing skeletal muscle weakness in the aged should be carried out only after the evaluation of exercise intensity, time, and frequency.

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