Lack of skeletal muscle hypertrophy in very aged male Fischer 344 × Brown Norway rats

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Blough, E. R., and J. K. Linderman. Lack of skeletal muscle hypertrophy in very aged male Fischer 344 × Brown Norway rats. J Appl Physiol 88: 1265–1270, 2000.—To examine the effect of extreme old age on muscle plasticity, 6- (adult) and 36-mo-old (old) male Fischer 344 × Brown Norway hybrid rats underwent bilateral surgical ablation of the gastrocnemius muscle to functionally overload (OV) the fast-twitch plantaris muscle for 8 wk. Plantaris muscle wet weight, muscle cross-sectional area (CSA), and average fiber CSA decreased by 44, 42, and 40%, respectively, in old compared with adult rats, and peak isometric tetanic tension decreased by 83%. Compared with muscles in age-matched controls, plantaris muscle mass increased by 53% and type I, IIA, and IIX/IIB CSA increased by 91, 76, and 103%, respectively, in adult-OV rats, but neither wet mass nor fiber CSA increased in old-OV rats. OV decreased type I, IIA, and IIX/IIB mean fiber CSA by 31, 35, and 30%, respectively, in old-OV rats. Collectively, our data indicate that in extreme old age the plantaris muscle undergoes significant loss of mass, fiber CSA, and contractile function, as well as its capacity to undergo hypertrophy in response to a chronic increase in mechanical load.

sarcopenia; myosin

IT IS WELL ESTABLISHED that the frail elderly have by definition deficits in functional capacity that may make them dependent on others for daily care. In comparison to young adults, maximal voluntary strength is decreased by over 40% in persons in their sixth to seventh decade of life, and loss of strength is accelerated during the eighth decade of life (19). Muscle weakness has been found to be a common feature of elderly individuals who have fallen (27); however, the etiology and reversibility of this weakness and its impact on gait and balance remain unknown.

In a study that used 100 frail nursing home residents, Fiatarone et al. (7) reported a 113% increase in muscle strength and, more importantly, a 28% increase in stair-dimming power in response to a 10 wk program of progressive resistance training of the lower extremity. Thigh muscle cross-sectional area (CSA) increased by <3%, and because these investigators did not report any changes in muscle fiber CSA, it remains unclear whether age-associated decreases in muscle CSA can be reversed with resistance training.

Because of many difficulties associated with human aging studies, e.g., cross-sectional design and an inability to control for lifetime activity patterns, a vast amount of aging research has been performed by using the aging rat (3, 5, 9). Several studies have reported that skeletal muscle from aged rats retains the ability to hypertrophy in response to an overload stimulus provided by isotonic exercise (2) or surgical ablation of synergistic muscles (15, 28). However, previous studies designed to study muscle hypertrophy in aged rats observed neither whole muscle atrophy (15, 28) nor a decrease in the average muscle fiber CSA in aged control animals (2). The absence of such changes is important because in humans both muscle mass and fiber CSA decrease with aging (4). In previous gerontological studies utilizing rats, the lack of age-associated muscle atrophy may have been due to premature death in highly inbred rat genotypes such as Wistar and Fischer 344 rats (14, 20).

In gerontological work, it is critical to use rat strains that are not prone to high incidences of disease, such as kidney disease (14). Incidences of tumor susceptibility and renal disease are reportedly lower in the Fischer 344 × Brown Norway hybrid rats than in the Fischer 344 rat, which has been used extensively in the past. To date, only one investigation has examined age-associated changes in skeletal muscle structure and function in the Fischer 344 × Brown Norway rat (3). Unlike other aging rat strains that show very little muscle atrophy (2, 5, 9, 20, 28), muscle mass, average fiber CSA, and peak isometric tetanic tension (P0) were all found to decrease in Fischer 344 × Brown Norway rats between 28 and 36 mo of age, suggesting perhaps that this hybrid better models the age-associated changes seen in aging human skeletal muscle.

The present investigation was designed to determine whether in extreme old age skeletal muscle has an attenuated hypertrophic response compared with that of muscles from adult animals. We hypothesized that the capacity for skeletal muscle hypertrophy in extremely old male Fischer 344 × Brown Norway rats, manifesting significant skeletal muscle atrophy, will be attenuated in comparison to muscles in adult animals.

METHODS

Animal care and use. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory
Animals as approved by the Council of the American Physiological Society and the Animal Use Review Board of The Ohio State University (no. 95A0191). Twelve adult (6-mo-old) and aged (old; 36-mo-old) male Fischer 344 rats were obtained from the National Institute on Aging (NIA) and randomly assigned to either control (n = 6) or functional overload (OV; n = 6) treatments. Rats were barrier housed individually in an American Association of Accreditation of Laboratory Animal Care-approved vivarium. Housing conditions consisted of a 12:12-h dark-light cycle, and temperature was maintained at 22 ± 2°C. Animals were provided food and water ad libitum and were allowed to recover from shipment for at least 2 wk before experimentation began. During this time, rats were weighed weekly and were carefully observed for signs of failure to thrive, such as precipitous weight loss, disinterest in the environment, or unexpected gait alterations.

Because muscle atrophy and dysfunction in humans accelerates during the eighth decade of life, probability of survival curves generated by the NIA were employed to ensure that old rats used corresponded roughly to humans in their eighth decade of life. The probability of survival for old rats was ~20%.

Surgical procedures. As described previously (13), rats were anesthetized with a ketamine-xylazine cocktail (50 mg/kg ip) and supplemented as necessary for reflexive response. In a sterile aseptic environment, the dorsal surface of the hindlimb was shaved and cleaned, and the superficial musculature was exposed by means of a proximal-to-distal incision through the skin and blunt separation of the skin and fasciae. The medial gastrocnemius and the proximal two-thirds of the lateral head of the gastrocnemius were carefully isolated by blunt manipulation of the tissues and were removed bilaterally. Care was taken to leave the nerve and vasculature supply to the remaining musculature undisturbed. Incomplete removal of the synergists was done to ensure that the nerve and vascular supply remained intact. Control animals did not undergo sham procedures, because previous research has demonstrated that sham operations had no effect on muscle mass in control animals (16). After recovery from anesthesia, animals were returned to their cages and were maintained in barrier housing for 8 wk. Thus, by the time of death and data collection, the rats were 8.5 (adult) and 38.5 (old) mo of age, respectively.

The plantaris muscle was chosen because previous studies suggest that muscle atrophy during aging is greatest in type II fibers (9). The plantaris, composed predominantly of type II fibers, offers a better opportunity to observe the maximal physiological and anatomic effects on fast-twitch fibers. In addition, the plantaris muscle of young animals undergoes a large amount of hypertrophy in response to OV imposed by the surgical ablation of synergists (10, 11, 16, 17, 25, 26).

Measurement of contractile properties. Measurements of isometric muscle function were made in vitro at 24°C in oxygenated (95% O2-5% CO2) Krebs-Ringer bicarbonate (pH 7.4) supplemented with 25 μM tubocurarine chloride (11, 18). The distal tendon of the plantaris muscle was sutured (2.0 silk) to a piezoelectric force transducer (model 200B, Piezoelectronics, New York, NY) with the proximal end (origin) attached to a rigid post. The proximal end of the plantaris muscle was secured by cutting the femur proximal to the knee and placing a ligature (2.0 silk) around the femoral condyle. To further secure the femur, an additional drape clamp was subsequently attached by means of a C clamp to the top surface of the testing chamber. Optimal muscle length (L0), defined as the length at which twitch tension was peak (P0), was determined within 2 min of immersion of the muscle in the chamber. P0 was measured at L0, with 0.2-ms duration, supramaximal voltage, and stimulation frequencies of 40, 60, 80, 100, and 150 Hz (model 548 stimulator, Grass Industries, Cambridge, MA). The stimulus train duration was 500 ms. The time between stimulation trains was 2 min. Data acquisition was accomplished by interfacing the piezoelectric force transducer with a Nicolet two-channel digital oscilloscope (model 720B, Oxford, UK) along with a CompuAdd 386 personal computer.

Morphometric analysis. After measurement of contractile properties on the right limb, muscles were blotted dry, trimmed of visible fat and tendon projections, and immediately weighed. Muscles were then fixed at L0 and coated with embedding compound (OCT, Fisher Pharmaceutical, Cincinnati, OH) and immediately frozen in isopentane cooled by liquid nitrogen. Muscles were stored below −80°C pending subsequent analysis. Whole muscle CSA was estimated by using the following algorithm (11)

\[
\text{CSA (mm}^2\text{)} = [\text{muscle mass (mg)} \times \cos \text{angle of pinnation}] / [L_f (\text{mm}) \times \text{muscle density (mg/mm}^3\text{)}]
\]

The angle of pinnation was assumed to be 15° and 17°, for control and experimental rats, respectively, (11), and fiber length (Lf) was estimated as follows

\[
L_f \text{(mm)} = (L_m \times L_0 / L_f)
\]

Muscle length (Lm) was measured at L0, and Lf/Lm was assumed to be 0.42 (11). Muscle density was assumed to be 1.06 mg/mm3 (11, 18). Sectioning for all histological staining was performed in a cryostat (American Optical) chilled to −20°C. Sections of the midbelly of the right plantaris were cut at a thickness of 10 μm, collected on glass slides, and stored in airtight containers at −20°C until use. Fiber-type distribution was determined by using standard myosin ATPase staining as described elsewhere (pH 9.4, 4.6, 4.3) (8). A preliminary study showed that slides preincubated at a pH of 4.6 offered a clear separation of three fiber types. ATPase-stained sections were used for the determination of fiber areas. The area of at least 100 intact fiber of each type, if present, was measured for the determination of fiber areas.

Muscle fiber cross sections were projected at an objective magnification of ×25 to a computer (CompuAdd 316s) equipped with software to measure fiber CSA (J. ava) and Video Analysis Software, Corte Madera, CA). The captured image of a fiber was traced on a video monitor by using a handheld mouse. The software package was calibrated to the area in micrometers squared. Fiber CSA was determined for type I, IIA, and IIX/IIB fiber types. Average fiber CSA was determined for each muscle from the mean of all fibers traced for that muscle. To reduce experimental bias in the selection of fibers for measurement, all of the fibers on randomly selected screens were quantified. All area measurements were performed with the researcher blinded to the treatment of each respective section. Tracing of fibers was practiced until a coefficient of variation of ~5% was repeatedly achieved. A 15- to 20-mg section of the midbelly of the muscle was used for analysis of myosin heavy chains (MHCs) with sodium dodecyl sulfate-polyacrylamide gel electrophoresis as previously described (13, 22).

Statistical analysis. Data are presented as means ± SE. A two-way ANOVA was used to assess the effects of age and mechanical load on the dependent variables, and Scheffé’s post hoc test was used where appropriate. The level of significance accepted a priori was P < 0.05.
RESULTS

Indexes of aging. During the course of the 8 wk of OV treatment, one old rat was lost in both the control and OV treatments. To balance the groups, one adult animal was randomly removed from both the control and OV treatments. Plantaris muscle mass and CSA (Table 1), as well as the CSA of type I, IIA, and IIX/IIB fibers (Fig. 1), were lower in old rats, compared with adult animals. The MHC composition of the plantaris muscle indicated a loss of faster MHC isoforms with concomitant increases in slower MHC (Fig. 2). Compared with adult rats, plantaris muscle wet weight, muscle CSA, and average plantaris muscle fiber CSA were found to be decreased by 44, 42, and 39%, respectively, in old animals (P < 0.05). Aging decreased type I, IIA, and IIX/IIB fiber CSA by 49, 32, and 39%, respectively (P < 0.05). Aging decreased type IIB MHC from 28.4 ± 2.7% in adult rats to 18.4 ± 0.6% in old animals (P < 0.05). Aging increased type I and IIA MHC from 4.6 ± 0.7 and 22.3 ± 2.4%, respectively, in adult rats to 7.1 ± 0.5 and 30.3 ± 2.0%, respectively, in old animals (P < 0.05). Compared with muscles in adult rats, Po decreased 84% (P < 0.05). In addition, specific tension decreased 73% for plantaris muscles in old compared with adult rats (P < 0.05).

Effects of mechanical load. Compared with muscles in age-matched controls, OV increased plantaris wet weight, whole muscle CSA (Table 1), and total fiber CSA (Fig. 1) by 53, 63, and 92%, respectively, in adult-OV rats (P < 0.05). Compared with old rats, plantaris muscle CSA increased by 20% in old-OV animals, but this difference was not significant (P = 0.08). The CSAs of type I, IIA, and IIX/IIB fibers were 91, 76, and 103% greater, respectively, for muscles in adult-OV rats compared with fibers in muscles of adult control rats (P < 0.05). In contrast, in old rats, OV of plantaris muscles resulted in 31, 35, and 39% decreases in type I, IIA, and IIX/IIB fiber CSAs, respectively, compared with fibers in age-matched control animals (P < 0.05). Compared with muscles in age-matched controls, OV increased Po by 39% in adult rats (P < 0.05), and specific tetanic tension decreased 15%, but this latter difference was not significant. In muscles from old rats, OV had no effect on either Po or specific tension.

DISCUSSION

The intent of this study was to determine whether the plantaris muscle of adult and very aged Fischer 344×Brown Norway rats undergoes similar alterations in muscle mass, muscle fiber CSA, and muscle physiology in response to OV induced by surgical ablation of synergistic muscles. Similar to previous research in elderly humans, 38-mo-old male rats were found to undergo large age-associated decreases in muscle mass, fiber CSA, and contractile function (6, 7, 12, 24, 30, 31). Because similar findings have not been
shown in other aging rats (9, 28), these data suggest that aging Fischer 344 × Brown Norway rats may better model the age-associated alterations in muscle morphology and function seen in elderly humans. Furthermore, results of the present study demonstrate that the plantaris muscle in very aged rats is unable to hypertrophy in response to 8 wk of OV and in fact underwent accelerated atrophy (Fig. 1). Our data suggest that atrophic skeletal muscle in very aged rats loses its capacity to enlarge in response to an increase in mechanical load.

Aging effects. Loss of muscle strength with advancing age is associated with decreased mobility and an increased risk of falls in the elderly (29). Loss of strength in elderly humans (12) and aging rats (3) has been associated with decreased muscle mass and CSA. Aging was found to significantly decrease plantaris muscle mass (Table 1) and CSA of all fiber types (Fig. 1), as well as to decrease contractile function. In the present study, the magnitude of age-associated decreases in plantaris muscle mass and contractile function appears greater than previously reported in aged Fischer 344 × Brown Norway rats (3). Differences in the age of the animals at death may help explain this discrepancy. Animals used in the present study were killed at 38 mo of age, whereas previous investigations have examined rats up to 36 mo of age. Plantaris muscle mass is not different between animals aged 6 and 28 mo old, but by 36 mo of age 34% of plantaris mass is lost (3). Longitudinal data in humans have indicated that muscle mass and strength decline more rapidly after 60 yr of age, with a further acceleration of this decline during the eighth decade of life (19). A similar age-related acceleration in skeletal muscle wasting and dysfunction may also be present in aging male Fischer 344 × Brown Norway rats. Although loss of whole muscle and fiber CSA was dramatic in the present study, loss of maximal isometric strength (Table 1) exceeded loss of muscle CSA (Fig. 1).

In the present study, plantaris muscle CSA (Table 1) and average fiber CSA (Fig. 1) decreased by ~40% in old rats, and \( P_0 \) decreased by ~80% (Table 1). Consequently, specific tension decreased ~70%. Previous research examining the effects of aging on specific tension have yielded conflicting results (1, 3). In humans, maximum specific strength has been suggested to decrease with age for men but not for women (30, 31). In rodents, no age-related decreases in specific tension have been reported for the plantaris muscle of 36-mo-old male Fischer 344 × Brown Norway rats (3); however, loss of maximal contractile force exceeds the loss of muscle mass for the soleus and extensor digitorum longus muscles in aged mice (1) and rats (3). As mentioned previously, discrepancies between this study and previous investigations may be due to the fact that the rats used in present study were older than those used previously. In addition, Carmelli and Reznick (4) indicate that there is a large increase in connective tissue in aging muscle. Therefore, with advancing age, a greater percentage of muscle mass is composed of noncontractile tissue, which decreases measurement of specific tension.

Muscle plasticity with aging. Muscle hypertrophy and increased muscle strength are characteristic results of the surgical ablation of synergists (10, 11, 13, 16, 17). As previously reported, OV increased plantaris mass (Table 1) and average fiber CSA (Fig. 1) by 53 and 92%, respectively, in adult rats. Ianuzzo and Chen (10) demonstrated an ~40% increase in the average fiber CSA after enlargement produced by surgical ablation of synergists, whereas Timson and co-workers (26) reported that the CSA of both type I and II fibers increased by ~50% after ablation of synergists. Furthermore, OV increased \( P_0 \), ~40% in adult rats, but unlike in previous reports (11) specific tension was not affected by OV. Specific tension has been shown to increase in response to resistance training (12), decrease after compensatory hypertrophy (11, 17), or remain unchanged after compensatory hypertrophy (16). Discrepancies between measurements of specific tension between the present study and others employing the surgical ablation procedure may be due to differences
between animal strains, gender, age of the animals used, or duration of the OV procedure. However, unique to the present investigation was the lack of an increase in either plantaris muscle mass or contractile function in very aged male Fischer 344 × Brown Norway rats.

In humans, cross-sectional data indicate that men in their seventh decade of life who participated in resistance training for up to 17 yr had an attenuated age-associated decline in leg extensor CSA, strength, and specific tension (12). These data strongly support the lifelong benefits of resistance training to attenuate age-associated muscle dysfunction. Furthermore, men and women who were nearly 90 yr of age reportedly increased thigh muscle CSA, improved their one-repetition maximum, and increased their walking speed after just 8 wk of progressive resistance training (6). However, in a larger study with very elderly individuals (mean age 87.1 yr) 10 wk of progressive resistance training improved muscle function but did not increase thigh CSA (7). Therefore, the extent to which very aged skeletal muscle can adapt to an increase in mechanical load remains unclear.

Previous research examining the capacity for muscle hypertrophy in aged rats has indicated that aged muscle retains much of its ability to undergo hypertrophy in response to either OV (15, 28) or isotonic exercise training (2). In contrast, findings of the present study indicate that OV was not able to increase plantaris muscle mass or improve contractile function in male Fischer 344 × Brown Norway rats between 36 and 38 mo of age. The reasons for differences between past and present investigations are unknown; however, in previous investigations, aged rodents did not exhibit age-associated muscle atrophy (9, 15, 23).

Animals used in the present study represent the oldest animals used in gerontological research of muscle plasticity to date. Earlier studies using aging rodents did not control for pathogen-free housing (3, 12, 15) and employed either Wistar (12) or Sprague-Dawley rats (2, 15), which have been questioned as appropriate for gerontological research by the NIA (21). Finally, although there is no universally accepted standard for correct use of the term “old,” rats used in the present study were 36.5 mo old at the time of surgery and 38.5 mo of age when final data were collected. The age of the oldest rats examined previously ranged from 19 to 30 mo of age. These animals used previously exhibited modest signs of muscle weakness but no evidence of significant age-associated muscle wasting or sarcopenia. Because of the differences in the design of past and present studies, direct comparisons are difficult; however, data of the present study indicate that the ability for very aged rodent skeletal muscle to adapt to an increased contractile demand is diminished subsequent to manifestation of sarcopenia. Further research is needed to determine the underlying mechanisms associated with this inability for very aged rodent muscle to undergo hypertrophy.

In the present study, OV increased plantaris wet weight and CSA by 16 and 20%, respectively, in old rats, although these differences were not significant (Table 1). The mechanism for this increased mass is unclear; however, the increase in mass was not reflected by an increase in the CSA of individual fiber types (Fig. 1), nor was contractile function increased. This increased mass may have been due to edema associated with hypertrophy (11) and increased connective tissue associated with aging (4). In contrast to muscle wet weight and CSA, OV decreased the CSA of all fiber types in old rats ~30%. This latter observation suggests that the capacity for muscle hypertrophy is compromised in skeletal muscle from very aged rodents, and in fact the OV stimulus accelerated muscle atrophy.

Although well established as a successful model for increasing muscle mass (5, 10, 11, 13, 16, 17, 25, 26), some have argued that, because the stimulus provided by surgical ablation of synergists is chronic, this model may represent a pathophysiological rather than a physiological model of skeletal muscle hypertrophy (23, 26).

Because of the fact that the stimulus is chronic, and because the model may induce certain pathophysiological stresses, it is possible that very aged atrophic muscle studied presently may no longer retain the means to adapt in a positive fashion to this type of chronic stress. However, it should be noted that chronic treadmill training, which results in repeated eccentric contractions, resulted in significant muscle atrophy in 27-mo-old rats (20). Other have observed that muscles of old rodents are more susceptible to contraction-induced damage than are their younger counterparts (32), and the regeneration process after damage has been shown to be impaired (1). Therefore, independent of concerns about the pathophysiological stress of functional overload, data from past and present investigations indicate that skeletal muscle fibers from very aged rodents lose their ability for positive adaptation. Further investigations are needed to determine whether muscle fibers in very aged humans are similarly compromised in their capacity for positive adaptation to resistance exercise.

Summary. Aging was found to have a profound effect on the mass, average fiber CSA, and contractile characteristics of the plantaris muscle from 38-mo-old male Fischer 344 × Brown Norway rats. Furthermore, aging was found to prevent the adaptive response of the plantaris muscle to 8 wk of OV induced by surgical ablation of synergists. Because the age of these animals corresponded roughly to humans in their eighth decade of life, these data support the utility of the Fischer 344 × Brown Norway rat as a tool to investigate the large age-associated decrements in muscle structure, function, and plasticity seen in the frail elderly. Finally, because aging Fischer 344 × Brown Norway rats undergo a large amount of age-associated atrophy and fail to exhibit substantial muscle growth after a strong hypertrophic stimulus, this model offers the unique opportunity for the investigation of muscle adaptation prior to and subsequent to naturally occurring age-associated atrophic processes.
REFERENCES


