SHORT COMMUNICATION

Changes in force by repeated stretches of skeletal muscle in young and old female Sprague Dawley rats

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ABSTRACT. We examined whether the decline in isometric force and peak stretch force during repeated stretches of activated plantar flexor muscles was larger in old (24 months, N=5) compared with young (4 months, N=5) female Sprague Dawley rats. Thirty stretches were imposed on isometric contractions (stimulation time 1.9 s, rest periods 180 s) by ankle rotation from 90° to 40° at $50° \cdot s^{-1}$. Even though muscle weights were similar [2094±54 (young) vs *2033±73 mg (old) (mean±SE)], the isometric force (IF) at 90° before stretch 1 and peak stretch force (PSF) at 40° for stretch 1 were lower in old rats [18.9±0.5* vs *15.5±1.4 N* (p=0.024) *and 25.8±1.3* vs *20.5±0.9 N* (p=0.017)]. *After the stretches, the IF deficit was similar [38.5±3.0% (young)* vs *39.8±3.4% (old)] and did not recover after 1 hour of rest [35.1±4.1% (young)* vs *36.9±4.3% (old)]. The decline in PSF was also similar [36.0±5.2% (young)* vs *26.6±1.8% (old)]. Skeletal muscles of 24-month-old female Sprague Dawley rats were weaker than 4-month-old rats but had similar susceptibility to develop IF deficits after stretches of activated muscles.*
(Aging Clin. Exp. Res. 12: 478-481, 2000)

INTRODUCTION

When activated muscles are exposed to unaccustomed stretches, an isometric force (IF) deficit results (1); the IF deficit is associated with muscle injury, and its magnitude appears to be predominantly a function of the peak stretch force (2).

With old age in humans and mice, the ability to produce IF declines, thus producing weakness, but the force produced during stretches of activated muscles is maintained (3, 4). Maintenance of peak stretch forces in the presence of muscle weakness might lead to an increased susceptibility to muscle injury with increasing age. The IF deficit after single stretches was shown to be dependent on age and length change for EDL muscles *in situ* of male mice, and EDL muscle fibers of male rats (5). In addition, larger IF deficits were observed after 75 stretches of 20% length change in EDL muscles *in situ* of old male mice (6). However, stretch-induced force deficits for soleus muscle of male rats after 20 stretches of 20% length change were not age-dependent (7). Observations on the susceptibility to stretch-induced injury in skeletal muscles *in situ* from old male rodents are equivocal. Information on such susceptibility of skeletal muscles in intact female rodents is absent.

The purpose of this study was to examine the changes in IF and peak stretch force (PSF) in response to stretches of activated plantar flexor muscles in young (4 months) and old (24 months) intact female rats in the same range of joint motion.

MATERIALS AND METHODS

Animal care and preparation

Female Sprague Dawley rats were 2-3 months of age upon arrival at West Virginia University. Water and laboratory chow were provided *ad libitum*. The 24-month survival rate of *ad libitum* fed female Sprague Dawley is about 40% (8). Use and care of rats were approved by, and followed the guidelines of the West Virginia University Animal Care and Use Committee (WVU-ACUC #9809-02). Rats used for this study complied with Animal Welfare Act P.L.

Key words: Aging, isometric force, muscle injury, peak stretch force.
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Received March 14, 2000; accepted in revised form August 3, 2000.

91-579, and DHHS Guidelines governing the care and use of laboratory animals. Rats were kept anesthetized with sodium pentobarbital (75 mg·kg^{-1} i.p.), and euthanized by an intracardial injection of sodium pentobarbital.

Experiments were performed on plantar flexor muscles (i.e., soleus, plantaris and gastrocnemius muscles) in 4-month (N=5) and 24-month (N=5) old rats. Six of eleven rats allowed to age showed obvious tumour growth before reaching 24 months of age, and were excluded from the study. Details on the dissection procedure for nerve cuff placement, animal positioning, force recording under the sole of the foot and dynamometer are described elsewhere (9, 10). Briefly, electrodes of the nerve cuff were connected to a stimulator (Grass SD9 stimulator, Grass Medical Instruments, Quincy MA, USA). The dynamometer involves a DC permanent magnet servomotor (Model 1410C), and a Unidex 1 single axis motion controller (Aerotech Inc, Pittsburgh, PA, USA), and allows controlled ankle rotations with respect to range of motion and angular velocity. The angle between the tibial longitudinal axis and the plantar surface of the foot represents ankle angle, e.g., 90°.

Experimental procedure

Thirty stretches were initiated 600 ms after the onset of tibial nerve stimulation [200 μs pulse duration, 80 Hz, 6.0±0.5 V (mean±SE)] from an ankle position of 90° by dorsiflexion to 40° at a velocity of 50°·s^{-1}. The passive return of the foot was performed after the end of each stimulation. The first movement with muscle activation was preceded by a movement of the same magnitude without activation to measure passive force throughout the range of ankle motion. Stimulation time was 1.9 s for contractions with rest periods of 180 s between contractions. Using such stimulation protocols, muscle fatigue is negligible because: a) isometric contractions of 1.9 s with rest periods of 180 s resulted in a force decline of ~ 0.5% per contraction (1); and b) during isometric contractions of 1.5 s, decreases in force at the end of the plateau-phase were small [4.4±0.4% (4 months) and 8.9±0.9% (24 months) (mean±SE)].

One hour after the stretches, recovery was tested by performing isometric contractions three times each at an ankle position of 90°. Stimulation time of isometric contractions was 600 ms with rest periods of two minutes.

Data collection and analysis

Force values for stretches were calculated by subtracting the force during dorsiflexion without muscle activation from the total force during dorsiflexion with muscle activation. Data analysis provided 1) IF at 90° before each stretch and one hour after the series of thirty stretches, and 2) PSF at 40° during each stretch.

Statistics

Student's *t*-test and two-way ANOVA were used for statistical analysis with significance accepted at $p<0.05$. *Post-hoc* testing was done with a Bonferroni test to determine where specific differences had occurred. Values are presented as mean±SE for N=5 per group.

RESULTS

Body weight of 24-month-old rats was 36% larger than 4-month-old rats, but weights of plantar flexor muscles were similar (Table 1). In 24-month-old rats, both IF at an ankle position of 90° and PSF at 40° were 18% and 21% lower, respectively, than 4-month-old rats.

Repeated stretches of activated plantar flexor muscles

Force traces for contraction number one and thirty during the stretch protocol are illustrated in Figure 1 (arrows indicate the IF and the PSF during the active stretch). The decline in IF, normalized for each group to the IF before the first stretch, was not different (two-way ANOVA) (Fig. 2), and total decline in IF was similar (Fig. 2) [38.5±3.0% (4 months) *vs* 39.8±3.4% (24 months)]. For both groups, following one hour of rest, IF remained similar [35.1±4.1% (4 months) *vs* 36.9±4.3% (24 months)].

Throughout the stretch protocol, the decline in relative PSF was similar for both 4-month-old rats and

Table 1 - *Body weight, muscle weights and force data of plantar flexor muscles of 4-month-old and 24-month-old female Sprague Dawley rats.*

	4 months	24 months	%Δ
Body weight (g)	262±11	357±20*	+36
Weight SO (mg)	125±6	132±11	
Weight PLAN (mg)	340±16	343±16	
Weight GAST (mg)	1629±41	1557±54	
Weight plantar flexors (mg)	2094±54	2033±73	
IF90° (N)	18.9±0.5	15.5±1.4*	-18
PSF40° (N)	25.8±1.3	20.5±0.9*	-21

SO: soleus muscle; PLAN: plantaris muscle; GAST: gastrocnemius muscle; IF90°: isometric force at an ankle position of 90°; PSF40°: peak stretch force at an ankle position of 40°.
Values are means±SE. *significantly different from 4-month-old female rats. Student's *t*-test with a significance level of $p<0.05$.

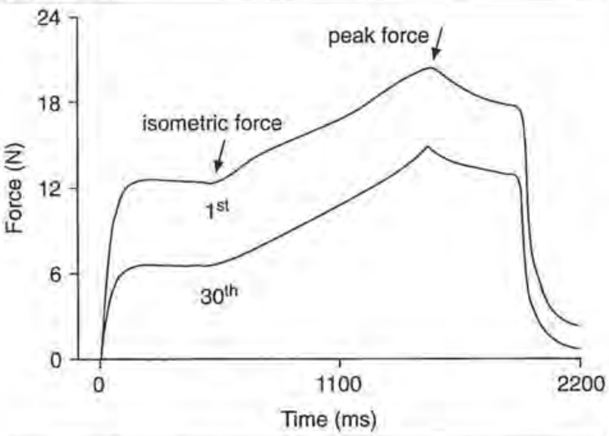

Figure 1 - *For plantar flexor muscles in intact female rats, typical example of force-time traces during the 1st and 30th contraction consisting of an isometric contraction followed by a stretch of active muscles (i.e., ankle dorsiflexion). Arrows indicate the isometric force (IF) before the stretch and peak stretch force (PSF) during a stretch of active muscles.*

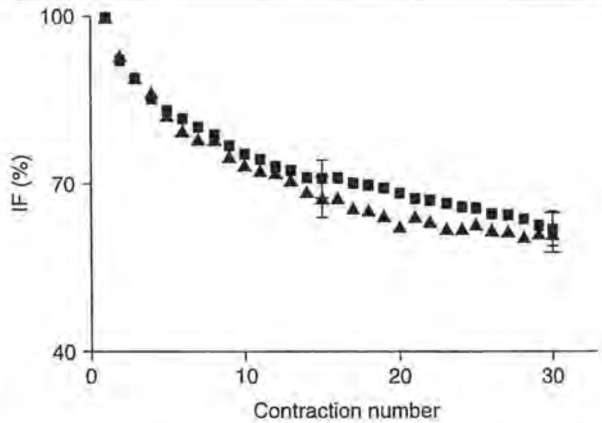

Figure 2 - *The isometric prestretch force (IF) of female rat plantar flexor muscles vs contraction number expressed as a percentage of the IF before the first stretch for young (4 months) rats (■) and old (24 months) rats (▲). Data are presented as mean±SE for five animals. For clarity, only the SE's of contraction numbers 15 and 30 are plotted.*

24-month-old rats (Fig. 3), terminating with 36.0±5.2% for young and 26.6±1.8% for old rats for the last stretch.

DISCUSSION

This study examined changes in IF, PSF, and stretch-induced force deficits of activated plantar flexor muscles in young (4 months) and old (24 months) female Sprague Dawley rats. IF and PSF were lower in the old rats, albeit produced with similar muscle weights. Similar weights of plantar flexor muscles have been reported for female Fischer 344 rats between 12 and 24 months (11), and also for EDL muscles of old male mice (26-27 months *vs* 2-3 months) with a decrease in maximum IF (10%) (6). The decrease in PSF in our study could result from the slow angular velocity of ankle rotations (i.e., $50°\cdot s^{-1}$) during the stretches. For isolated soleus muscle of old mice (28-31 months), stretch force was decreased at low velocities of stretching, and maintained during high velocities of stretching (4). It is concluded that plantar flexor muscles of 24-month-old female Sprague Dawley rats were weaker than 4-month-old rats; the weakness manifests itself by decreased IF production.

Decline in force by stretches of activated skeletal muscles

As far as we know, the susceptibility of skeletal muscles to stretch-induced force deficits with aging has been examined only in male rodents. In the latter, the decline in IF by single stretches of active skeletal muscle was dependent on age and muscle length change. For example, for *in situ* EDL muscles of male mice, the decline in IF was larger in old (25-28 months) compared to adult mice (9-12 months) after single stretches with length changes of 40 and 50%, but not after single stretches with length changes of 10, 20 and 30% (5). In the same study, single permeabilized fibers from EDL mus-

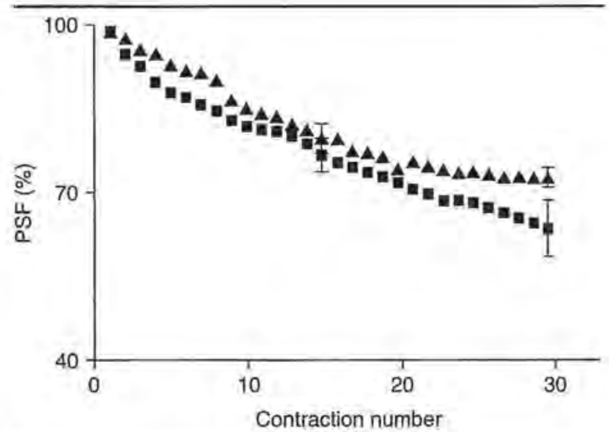

Figure 3 - *The peak stretch force (PSF) of female rat plantar flexor muscles vs contraction number expressed as a percentage of the PSF for the first stretch for young (4 months) rats (■) and old (24 months) rats (▲). Data are presented as mean±SE for five animals. For clarity, only the SE's of contraction numbers 15 and 30 are plotted.*

cles of old male F344 or F344 X Brown Norway rats (27-34 months) had larger declines in IF than young male rats (5-6 months) after single stretches with length changes of 10 and 20%, but not after single stretches with a length change of 5%. Interestingly, soleus muscle from 3-month-old and 23-month-old male Fischer 344 rats had similar IF deficits following repeated stretches (7). In the present study, plantar flexor muscles of 24-month-old Sprague Dawley female rats which were weaker did not develop larger IF deficits after stretches in the physiological range of motion compared with 4-month-old rats. It is thus possible that: 1) the length changes during stretches of activated plantar flexor muscles are small in intact rats; 2) plantar flexor muscles of old rats are less susceptible to stretch-induced muscle injury than dorsiflexor muscles of old rats; and 3) age and gender may interact differently in different strains of rats regarding the susceptibility of rat muscles to stretch-induced muscle injury.

ACKNOWLEDGEMENTS

This work was supported by the National Institute of Occupational Safety and Health, Centers for Disease Control (R01-OH02918).

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