Differential effects of mild therapeutic exercise during a period of inactivity on power generation in soleus type I single fibers with age

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Kim JH, Thompson LV. Differential effects of mild therapeutic exercise during a period of inactivity on power generation in soleus type I single fibers with age. J Appl Physiol 112: 1752–1761, 2012. First published March 15, 2012; doi:10.1152/japplphysiol.01077.2011.—The purpose of this study was to investigate the effects of mild therapeutic exercise (treadmill) in preventing the inactivity-induced alterations in contractile properties (e.g., power, force, and velocity) of type I soleus single fibers in three different age groups. Young adult (5- to 12-mo-old), middle-aged (24- to 31-mo-old), and old (32- to 40-mo-old) F344BNF1 rats were randomly assigned to three experimental groups: weight-bearing control (CON), non-weight bearing (NWB), and NWB with exercise (NWBX). NWB rats were hindlimb suspended for 2 wk, representing inactivity. The NWBX rats were hindlimb suspended for 2 wk and received therapeutic exercise on a treadmill four times a day for 15 min each. Peak power and isometric maximal force were reduced following hindlimb suspension (HS) in all three age groups. HS decreased fiber diameter in young adult and old rats (~21 and ~12%, respectively). Specific tension (isometric maximal force/cross-sectional area) was significantly reduced in both the middle-aged (~36%) and old (~23%) rats. The effects of the mild therapeutic exercise program on fiber diameter and contractile properties were age specific. Mild treadmill therapeutic exercise attenuated the HS-induced reduction in fiber diameter (+17%, 93% level of CON group) and peak power (μN·fiber length·s⁻¹) (+46%, 63% level of CON group) in young adult rats. In the middle-aged animals, this exercise protocol improved peak power (+60%, 100% level of CON group) and normalized power (kN·m⁻²·fiber length·s⁻¹) (+45%, 108% level of CON group). Interestingly, treadmill exercise resulted in a further reduction in shortening velocity (~42%, 67% level of CON group) and specific tension (~29%, 55% level of CON group) in the old animals. These results suggest that mild treadmill exercise is beneficial in attenuating and preventing inactivity-induced decline in peak power of type I soleus single fibers in young adult and middle-aged animals, respectively. However, this exercise program does not prevent the HS-induced decline in muscle function in the old animals.

permeabilized single fibers; force-velocity relationship; skeletal muscle

PERIODS OF INACTIVITY [i.e., bed rest, immobilization, space flight, and hindlimb suspension (HS)] result in significant muscle atrophy and contractile dysfunction (8, 14). Particularly, muscles in the absence of weight-bearing activity show a heightening susceptibility to damage (43). Many of the physiological responses associated with inactivity or non-weight bearing in skeletal muscle are also observed during the aging process. For example, sarcopenia, a degenerative loss of muscle mass and strength with age, is a common feature in the elderly. The deleterious changes associated with inactivity are further exacerbated in the elderly due to the presence of sarcopenia (10). Therefore, the combination of inactivity and age may result in poor rehabilitation potential, limiting the return to independent living.

Muscle power is a key contractile parameter measuring the work performed per unit time, and the deterioration of this parameter correlates with a loss of strength and/or contraction speed (20). A loss of muscle power occurs with aging and is associated with greater fall injury, decreased gait speed, morbidity, mortality, and poor quality of life (5, 9, 20, 30). Despite the multitude of investigations focused on inactivity, few studies, however, demonstrate the effect of inactivity on the contractile parameter “power” in individual skeletal muscle fibers, and even fewer studies investigate age-associated effects. Because the inactivity-induced detrimental changes in muscle contractility are further exacerbated by the aging process, a comprehensive evaluation of power generation will provide information requisite in the development of rehabilitation exercise protocols.

Exercise training is suggested as one of the powerful interventions to attenuate detrimental effects of inactivity on muscle function. These exercise protocols include a daily, short-duration, high-load exercise (16), intermittent weight-bearing exercise (1, 2, 26), resistance exercise (33), and isometric exercise program (17). The degree of effectiveness of exercise during a period of inactivity on muscle functions, including power generation, varies depending on frequency, intensity, time, and type of exercise (exercise prescription). Although the exercise prescription is very important, the effectiveness of the exercise program is dependent on the age of the individual, because there is a difference in the muscle’s adaptive potential (19, 32, 40). For instance, a high-intensity exercise (resistive or isometric) appears to be beneficial in preventing inactivity-induced functional impairments, including power generation in single-muscle fibers in young adults (17, 47). However, the attenuation of fiber size, force, and power generation with high-intensity resistance in the young is blunted in the old population (27). Likewise, endurance exercise training does not attenuate the decrease in contractile function in the aged (34). In a recent study that focused on old rats, a mild intermittent treadmill exercise (isometric exercise with weight bearing: walking) during a period of inactivity was used (2). This exercise protocol stimulated or recruited the type II fibers and improved type II fiber function of the gastrocnemius muscle in the old animals (2). However, it is unknown whether this therapeutic treadmill exercise during a period of inactivity improves contractile functions, including power generation in type I single-muscle fibers from different aged rats. Recent studies show that many of the age-related alterations are of similar magnitude in the largely slow-twitch soleus muscle as in the largely fast-twitch gastrocnemius muscle (4, 29). Thus, although the majority of studies in the field of aging muscle to date have focused on age-related changes in type II fibers, the...
field now recognizes the importance of type I fibers. Understanding the impact of aging and inactivity on type I fibers is essential to develop the most effective therapies for sarcopenia.

Therefore, the primary purpose of this study was to examine the effect of a mild therapeutic exercise during a period of inactivity on contractile properties, specifically power generation of soleus type I single fibers from young adult, middle-aged, and old rats. We hypothesized that 1) inactivity-induced decline in muscle functions, including power generation, would be different with age; and 2) therapeutic treadmill exercise would prevent the detrimental changes associated with inactivity, and the benefits of the therapeutic exercise on skeletal muscle would depend on the age of the rat.

METHODS

Animals

Young adult (5–12 mo), middle-aged (24–31 mo), and old (32–40 mo) male Fischer-344 Brown Norway F1 Hybrid (F344BNF1) rats were purchased from National Institute on Aging colony (Harlan, IN). Young adult, middle-aged, and old groups were determined by average survival rates derived from large populations of the F344BNF1 species, representing survival rates of 95%, 80–50%, and <50%, respectively (42). Each animal was housed individually (40 in² × 7 in cage) under 12:12-h light-dark cycles at 20°C and provided a diet of Purina Rodent Chow and water ad libitum. After 1 wk of acclimatization, rats were randomly assigned to one of the following three experimental groups: 1) weight-bearing control (CON; total n = 19), 2) non-weight-bearing (NWB; total n = 18), and 3) non-weight-bearing and exercise (NWBX; total n = 16) group. The experiments were approved by the University of Minnesota Institutional Animal Care and Use Committee.

CON. The rats in this group were allowed to move freely in the cage with weight bearing on all limbs. NWB with HS. To examine the effect of inactivity on fiber size and contractile properties in young adult, middle-aged, and old rats, we used the HS model. HS is the preferred model to simulate bed rest immobility and the clinical condition of inactivity (1). The rats in this group were defined as NWB because their hindlimbs could not touch the cage floor. The procedure of HS has been previously described (1, 48). Briefly, a harness with orthopedic traction tape was tied to the proximal two-thirds of the tail, and the hindlimbs were elevated to a spinal orientation of 40–45° above horizontal. The height of the suspension was adjusted to lift the hindlimbs so that only forelimbs maintained contact with the cage floor, thus allowing rats to move and obtain food and water. The animals were suspended for 2 wk.

Therapeutic exercise (treadmill). To determine the effects of mild therapeutic treadmill exercise during a period of inactivity on contractile properties in young adult, middle-aged, and old rats, the animals exercised on a motor-driven treadmill four times a day, ~15 min each, during 2 wk of HS. Exercise training started every morning from around 8–10 AM during a light cycle, and each training protocol was repeated every 2 h over a period of 8 h daily. Once animals were taken down from suspension, the animals were placed on the treadmill, and exercise was initiated (with a goal of 15 min). The treadmill exercise speed of the rats was 342 cm/min at a 0° incline. This treadmill exercise speed was tolerated by all three different age groups. Sometimes the animals were encouraged to exercise with physical methods (i.e., air pump, soft stick), but these methods were used minimally to avoid unnecessary stress. Animals were removed from the treadmill when there were signs of resistance to walking. The walking exercise time for each animal per day was recorded. The average training time was calculated for each rat per day and subsequently averaged for each experimental group.

Tissue Preparation and Permeabilized Single-fiber Preparations

After 2 wk, rats were anesthetized with pentobarbital sodium (35 mg/kg body mass; intraperitoneal injection). The soleus muscles were rapidly removed, trimmed free of excess fat and connective tissue, weighed, and prepared for single-muscle-fiber contractile measures (49). Briefly, immediately after dissection, the muscle was placed in ice-cold relaxing solution, as described below. The muscle was separated into small bundles composed of ~50 fibers by using fine scalpels and scissors under microscope. Each end of the bundles was tied with surgical suture to glass capillary tubes to maintain optimal length of the muscle fibers and stored in glycercinated skinning solution containing 20 mM imidazole (pH = 7.0), 125 mM potassium-proprionate, 2 mM EGTA, 4 mM ATP, 1 mM MgCl₂, and 50% glycerol (vol/vol) at ~20°C.

Relaxing and Activating Solutions

Relaxing solution (pCa = 9.0) contained 20 mM imidazole (pH = 7.0), 7.0 mM ethylene glycol-bis-(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), 5.4 mM MgCl₂, 14.5 mM creatine phosphate, 4.7 mM ATP, and CaCl₂. Activating solution (pCa = 4.5) contained 20 mM imidazole, 7.0 mM EGTA, 5.4 mM MgCl₂, 14.5 mM creatine phosphate, 4.7 mM ATP, and CaCl₂. The pH of both solutions was finally adjusted to 7.0 with KOH.

Isolation of Single-muscle Fibers and Determination of Fiber Length and Fiber Diameter

Single-muscle fibers (2- to 3-mm segment) were cautiously isolated from a fiber bundle with micro-forceps under a dissecting microscope and transferred to an experimental bath filled with relaxing solution immediately. The individual fiber was securely mounted between a force transducer (Cambridge model 400A, sensitivity 2 mN/mg) and a DC motor lever controller (Cambridge model 300H) using micro-tweezers and cuffs. The experimental bath was mounted to the stage of an inverted microscope. A micrometer at ×600 magnification was used to determine fiber length (FL) (optimal length) and diameter after setting the sarcomere length at 2.5 μm (48). A total of 363 single fibers were analyzed, corresponding to approximately 7 fibers per animal. The fiber diameter was determined as the average size of three places along the length of the fiber and the fiber cross-sectional area (CSA) was calculated by assuming a circular cross section (2).

Determination of Isometric Maximal Force and Specific Tension

To determine isometric maximal force (P₀), “baseline force” of the permeabilized single fiber was monitored in relaxing solution (pCa = 9.0). The fiber was then transferred to activating solution (pCa = 4.5) to induce muscle contraction. P₀ (mg) was determined when the force output reached a plateau. This procedure was repeated five to six times, and the trial that gave the greatest force was selected to calculate the P₀. Specific tension (P₀/CSA; kN/m²) was calculated as P₀ normalized by fiber CSA.

Determination of Power

To determine single-muscle fiber power generation, the isotonic load-clamping test was utilized (45). Briefly, when each fiber was fully activated in activating solution, the fiber was subjected to three successive submaximal isotonic load steps. The time interval of each load step was 100 ms, and shortening velocity and force were measured over the last 30 ms in each step. This procedure was repeated 5–6 times at different loads so that each single fiber was submitted to a total of 15–18 isotonic contractions. The data points obtained from the isotonic contractions were fit using the Hill equation: (P = a(V + b) = (P₀ + a)b, where P is the force during load clamping, V is velocity, P₀ is developed before the submaximal load clamps, and a and b are constants of force and velocity. Only
individual experiments in which \( r^2 \) was >0.98 were accepted. Power was determined in terms of the fitted force-velocity parameters \([P_o, \text{ maximum shortening velocity } (V_{\text{max}}), a/P_o]\). Absolute peak power (\( \mu \text{N·FL·s}^{-1} \)) was defined as the product of force (\( \mu \text{N} \)) and shortening velocity (FL/s). Normalized power (\( \text{kN·m}^{-2} \cdot \text{FL·s}^{-1} \)) was defined as the product of normalized force (force per CSA; kN/m²) and shortening velocity (FL/s).

**Determination of Myosin Heavy Chain Isoforms**

To determine myosin heavy chain (MHC) isoforms of each single fiber (total = 363), sodium dodecyl sulfate (SDS)-PAGE gel electrophoresis and silver staining methods were used (49). After single-fiber muscle physiology measurement, each fiber was solubilized in 50 \( \mu \text{l} \) of sample buffer containing 60 mM Tris (pH 6.8), 24 mM EDTA, 1% SDS, 5% β-mercaptoethanol, and 15% glycerol, 2 mg/ml bromophenol blue and then stored at \(-80^\circ \text{C}\). On the experiment day, the samples were heated at 95°C for 4 min and centrifuged at 8,000 rpm for 2 min. Ten microliters of the sample were loaded on the SDS-PAGE electrophoresis system composed of a 4% of stacking gel and 8% of separating gel. MHC isoform expression of each fiber was determined by comparing the migration of sample proteins with standards were made from homogenates of rat tibialis anterior muscle. Standards were made from homogenates of rat tibialis anterior muscle.

**Statistical Analysis**

A two-way ANOVA was used to determine the effects of different ages (young adult, middle aged, old) and conditions (CON, NWB, NWBX) on each of the following variables: body mass, muscle mass, diameter, \( P_o, \text{ Po}/\text{CSA}, V_{\text{max}}, \text{ peak power, and normalized power} \). Tukey’s post hoc test was used when significant interaction between ages and conditions was noted. All statistical analyses were performed using the SPSS software program (version 18.0), and significance was set at \(<0.05\). Only the data from MHC type I fibers are reported.

**RESULTS**

Table 1 summarizes the animal characteristics (body mass, soleus muscle wet mass, soleus muscle mass-to-body mass ratio), exercise time, and sample sizes for analysis in control and experimental groups.

**Age**

To determine the effect of aging on the body mass and muscle mass, the CON rats in different age groups were evaluated. Body mass did not change with age. The soleus muscle mass was not different between young adult and middle-aged rats, but was significantly reduced in the old compared with young adult (\( P = 0.04 \)) and middle-aged rats (\( P = 0.008 \)). Similarly, relative muscle mass (muscle weight/body weight) was not different between young adult and middle-aged rats, but significantly lower in the old than in the young adult rats (\( P = 0.014 \)), confirming that sarcopenia is evident in these rats.

**Inactivity**

To determine the effect of inactivity on the body mass and muscle mass, the CON and NWB rats in different age groups were evaluated. Body mass did not change with 2 wk of HS. The soleus muscle was significantly decreased with HS in young adult and middle-aged rats by 37 and 26%, respectively, but not in old rats.

**Therapeutic Exercise**

To determine the effect of therapeutic exercise on the body mass and muscle mass, control and experimental rats in different age groups were evaluated. Body mass did not change

| Table 1. Animal and soleus muscle characteristics from young adult (5–12 mo), middle aged (24–31 mo), and old (32–40 mo) rats |
|----------------------------------|-----------------|-----------------|
|                                  | CON             | NWB             |
| Young adult (5–12 mo)            |                 |                 |
| BW, g                           | 423.00 ± 33.49  | 378.5 ± 30.57   | 431.00 ± 33.49 |
| MW, g                           | 0.19 ± 0.01     | 0.12 ± 0.01#    | 0.18 ± 0.01†   |
| MW/BW, mg/g                     | 0.44 ± 0.03     | 0.32 ± 0.02#    | 0.41 ± 0.02    |
| Exercise time, min/day           |                 |                 | 45.7 ± 1.84    |
| Animals, N                      | 5               | 6               | 5              |
| MHC type I fibers, N             | 28              | 39              | 25             |
| Middle aged (24–31 mo)          |                 |                 |
| BW, g                           | 494.86 ± 28.30  | 433.75 ± 37.44  | 448.6 ± 33.49  |
| MW, g                           | 0.19 ± 0.01     | 0.14 ± 0.01#    | 0.14 ± 0.01*# |
| MW/BW, mg/g                     | 0.37 ± 0.02     | 0.31 ± 0.03     | 0.30 ± 0.02*   |
| Exercise time, min/day           |                 |                 | 44.8 ± 1.91    |
| Animals, N                      | 7               | 4               | 5              |
| MHC type I fibers, N             | 28              | 23              | 31             |
| Old (32–40 mo)                   |                 |                 |
| BW, g                           | 444.57 ± 28.30  | 411.13 ± 26.48  | 396.17 ± 30.57 |
| MW, g                           | 0.14 ± 0.01*#   | 0.12 ± 0.01     | 0.13 ± 0.01*   |
| MW/BW, mg/g                     | 0.32 ± 0.02*    | 0.29 ± 0.02     | 0.34 ± 0.02    |
| Exercise time, min/day           |                 |                 | 42.52 ± 1.64   |
| Animals, N                      | 7               | 8               | 6              |
| MHC type I fibers, N             | 29              | 40              | 46             |

Values are means ± SE; N, sample size. CON, weight-bearing control group; NWB, 2-wk hindlimb suspended group; NWBX, group of rats that exercised on treadmill for ~15 min, four times daily, with each therapeutic exercise bout occurring every 2 h over a period of 8 h. The body mass (BW) and soleus muscle wet mass (MW) are reported in grams. The soleus muscle mass-to-body mass ratio (MW/BW) is calculated in mg/g. The treadmill exercise time is defined as the average training time per day over the entire 2 wk of exercise (min/day). MHC, myosin heavy chain. Significantly different from #age-matched CON group, †age-matched NWB group, * condition-matched young adult group, and ‡condition-matched middle aged group; significance was set at \( P < 0.05 \).
with exercise. Therapeutic treadmill exercise attenuated the HS-induced decrease in relative muscle mass in young adult rats. In contrast, in middle-aged and old rats, treadmill exercise did not reduce the relative muscle mass loss in soleus muscle. These results indicate that treadmill exercise during a period of HS is effective in maintaining muscle weight in young adult rats, but not older rats.

**Absolute Peak Power (μN·FL·s⁻¹)**

To determine single-muscle fiber performance, we used absolute power as a key contractile parameter because it is a physiological and functional measurement of maximum level of work performed per unit time. There was a significant interaction between age and condition for absolute power \( (F = 8.33, P < 0.001) \) (Fig. 1). In the young adult group, HS resulted in a significant decline in absolute power \( (−57\%, P < 0.001) \). Mild treadmill exercise during a period of HS significantly attenuated the HS-induced decline in absolute peak power \( (+46\%, P = 0.017) \), but absolute peak power was still significantly lower than that in the CON group \( (P < 0.001) \). In the middle-aged group, the absolute power was significantly decreased with HS \( (−38\%, P = 0.002) \), and the exercise completely reversed this decline in absolute power generation \( (+60\%, P = 0.002) \). In the old group, absolute peak power was significantly lower in the NWB group compared with the CON group \( (−43\%, P < 0.001) \). Interestingly, treadmill exercise did not change the HS-induced reduction in absolute peak power.

**Normalized Power (kN·m⁻²·FL·s⁻¹)**

In single-fiber physiology experimentation, absolute peak power is normalized to the CSA of the fiber. This normalization is important in identifying whether the functional change associated with inactivity and/or age is due to muscle atrophy or intrinsic changes in contractile proteins \( (41) \). There was a significant interaction effect between age and conditions for normalized power \( (F = 4.33, P = 0.002) \) (Fig. 2). In the young adult group, normalized power was significantly lower in the NWB group than in the CON group \( (−32\%, P < 0.001) \). Treadmill exercise did not change the HS-induced decline in power, and it was still lower than that in the CON group \( (−25\%, P = 0.016) \). In the middle-aged group, the normalized power was significantly decreased in the NWB group compared with the CON group \( (−30\%, P = 0.02) \), and treadmill exercise during the HS period significantly reversed the HS-induced decline in power generation \( (+45\%, P = 0.013) \). In the old group, normalized power was significantly lower in the NWB group compared with the CON group \( (−27\%, P = 0.031) \); however, mild treadmill exercise during HS period did not change normalized power. Overall, mild treadmill exercise...
attenuated HS-induced decline in power generation of type I soleus single fibers in middle-aged animals.

\(P_o\) (Peak Force)

\(P_o\) is one of the contractile properties to evaluate the force-generating function in single-muscle fibers. Our two-way ANOVA analysis indicated that there was a significant interaction between age and condition in \(P_o\) (\(F = 4.52, P = 0.001\)) (Fig. 3). In the young adult group, HS resulted in a significant decline in \(P_o\) (−41%, \(P < 0.001\)). Treadmill exercise did not change the HS-induced decline in \(P_o\). In the middle-aged group, \(P_o\) in the NWB group was significantly lower than that in the CON group (−41%, \(P < 0.001\)), but treadmill exercise did not reverse the HS-induced reduction in \(P_o\). In the old group, a significant decline in specific tension was noted with 2 wk of HS (−41%, \(P < 0.001\)). Treadmill exercise resulted in a 29% reduction in specific tension compared with that in the NWB group (\(P = 0.002\)).

\(V_{max}\)

\(V_{max}\) is another functional parameter of single-muscle fiber contraction. \(V_{max}\) is determined in terms of extrapolating hyperbolic nonlinear force-velocity data and represents the cycling rate of myosin-actin interaction (3). Our two-way ANOVA analysis indicated that there was a significant interaction between age and condition (\(F = 7.305, P < 0.001\)) (Fig. 5). In the young adult group, \(V_{max}\) in the NWB group was not different from that in the CON and NWBX groups. However, the values in the NWBX group were higher compared with those in the CON group (40%, \(P = 0.006\)). In the old group, there was no difference in \(V_{max}\) between CON and NWB.
groups. In contrast to the young adult and middle-aged groups, $V_{\text{max}}$ in the NWBX group of the old rats was significantly lower compared with that of the age-matched CON ($-33\%, P = 0.039$) and NWB groups ($-42\%, P < 0.001$). These results indicate that treadmill exercise during inactivity period deteriorates fiber contraction speed in the old age group.

**\(a/P_o\)**

The $a/P_o$ is a unitless parameter describing the curvature of the force-velocity relationship. Our two-way ANOVA analysis indicated that there was a significant interaction between age and condition ($F = 6.574, P < 0.001$) (Fig. 6). In the young adult group, the $a/P_o$ in the NWB group was significantly lower than that in the CON group ($P = 0.001$). The $a/P_o$ in the NWBX group was not different compared with that in the NWB group. In the middle-aged group, the $a/P_o$ was not different among the three different conditions. In the old group, the $a/P_o$ was significantly lower in the NWB compared with the CON group ($P = 0.005$). The $a/P_o$ was significantly increased (therefore less curvature) as a result of treadmill exercise ($P < 0.001$).

**Fiber Diameter**

There is a significant interaction between age and condition for fiber diameter (fibers with MHC type I isoform only) ($F = 3.97, P = 0.004$) (Fig. 7). In the young adult group, single-muscle fiber diameter of the NWB group was significantly smaller than that of the CON group, resulting in $21\%$ atrophy ($P < 0.001$). Therapeutic mild treadmill exercise during the HS period resulted in a significant increase in fiber diameter compared with the NWB group ($+17\%, P < 0.001$). Therefore, therapeutic treadmill exercise during a period of HS in adult age has a positive effect in preventing HS-induced muscle atrophy. In the middle-aged group, no significant muscle atrophy or hypertrophy was observed in the experimental groups. In the old group, there was a significant reduction in fiber diameter with HS ($-12\%, P = 0.007$), but treadmill exercise did not reverse the HS-induced reduction in fiber diameter. Thus, from a therapeutic perspective, treadmill exercise during a period of HS showed a positive effect, preventing single-muscle fiber atrophy more in the young adult age than in the middle-aged or old group.

**DISCUSSION**

The purpose of this study was to investigate the effects of a mild therapeutic exercise, in the form of treadmill walking, during a period of inactivity on single-fiber contractile properties (e.g., power, force, and velocity) from the soleus of three different age groups (young adult, middle age, old). We hypothesized that 1) inactivity-induced decline in muscle func-

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**Fig. 5.** Maximal shortening velocity ($V_{\text{max}}$), determined from the Hill plot of the force-velocity curve, generated by single muscle type I fibers from young adult (5–12 mo), middle-aged (24–31 mo), and old (32–40 mo) rats. $V_{\text{max}}$ is expressed in FL/s. Values are means ± SE. Significantly different from #age-matched CON group, †age-matched NWB group, "condition-matched young adult group, and β condition-matched middle-aged group: significance was set at $P < 0.05$.

**Fig. 6.** $a/P_o$ in single muscle type I fibers from young adult (5–12 mo), middle-aged (24–31 mo), and old (32–40 mo) rats. The $a/P_o$ is unitless. Values are means ± SE. Significantly different from #age-matched CON group, †age-matched NWB group, "condition-matched young adult group, and β condition-matched middle-aged group: significance was set at $P < 0.05$. 

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**Fig. 7.** Maximal shortening velocity ($V_{\text{max}}$), determined from the Hill plot of the force-velocity curve, generated by single muscle type I fibers from young adult (5–12 mo), middle-aged (24–31 mo), and old (32–40 mo) rats. $V_{\text{max}}$ is expressed in FL/s. Values are means ± SE. Significantly different from #age-matched CON group, †age-matched NWB group, "condition-matched young adult group, and β condition-matched middle-aged group: significance was set at $P < 0.05$. 

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**Fig. 8.** $a/P_o$ in single muscle type I fibers from young adult (5–12 mo), middle-aged (24–31 mo), and old (32–40 mo) rats. The $a/P_o$ is unitless. Values are means ± SE. Significantly different from #age-matched CON group, †age-matched NWB group, "condition-matched young adult group, and β condition-matched middle-aged group: significance was set at $P < 0.05$. 

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**Fig. 9.** Maximal shortening velocity ($V_{\text{max}}$), determined from the Hill plot of the force-velocity curve, generated by single muscle type I fibers from young adult (5–12 mo), middle-aged (24–31 mo), and old (32–40 mo) rats. $V_{\text{max}}$ is expressed in FL/s. Values are means ± SE. Significantly different from #age-matched CON group, †age-matched NWB group, "condition-matched young adult group, and β condition-matched middle-aged group: significance was set at $P < 0.05$. 

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**Fig. 10.** $a/P_o$ in single muscle type I fibers from young adult (5–12 mo), middle-aged (24–31 mo), and old (32–40 mo) rats. The $a/P_o$ is unitless. Values are means ± SE. Significantly different from #age-matched CON group, †age-matched NWB group, "condition-matched young adult group, and β condition-matched middle-aged group: significance was set at $P < 0.05$. 

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**Fig. 11.** Maximal shortening velocity ($V_{\text{max}}$), determined from the Hill plot of the force-velocity curve, generated by single muscle type I fibers from young adult (5–12 mo), middle-aged (24–31 mo), and old (32–40 mo) rats. $V_{\text{max}}$ is expressed in FL/s. Values are means ± SE. Significantly different from #age-matched CON group, †age-matched NWB group, "condition-matched young adult group, and β condition-matched middle-aged group: significance was set at $P < 0.05$. 

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**Fig. 12.** $a/P_o$ in single muscle type I fibers from young adult (5–12 mo), middle-aged (24–31 mo), and old (32–40 mo) rats. The $a/P_o$ is unitless. Values are means ± SE. Significantly different from #age-matched CON group, †age-matched NWB group, "condition-matched young adult group, and β condition-matched middle-aged group: significance was set at $P < 0.05$. 

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**Fig. 13.** Maximal shortening velocity ($V_{\text{max}}$), determined from the Hill plot of the force-velocity curve, generated by single muscle type I fibers from young adult (5–12 mo), middle-aged (24–31 mo), and old (32–40 mo) rats. $V_{\text{max}}$ is expressed in FL/s. Values are means ± SE. Significantly different from #age-matched CON group, †age-matched NWB group, "condition-matched young adult group, and β condition-matched middle-aged group: significance was set at $P < 0.05$.
Fig. 7. Diameter of single muscle type I fibers from young adult (5–12 mo), middle-aged (24–31 mo), and old (32–40 mo) rats. The unit for fiber diameter is μm. Values are means ± SE. Significantly different from #age-matched CON group, †age-matched NWB group, *condition-matched young adult group, and β condition-matched middle-aged group: significance was set at \( P < 0.05 \).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Young Adult</th>
<th>Middle Aged</th>
<th>Old</th>
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<tr>
<td>Diameter (μm)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CON</td>
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<td>60 ± 2</td>
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<td>80 ± 4</td>
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**Absolute Peak Power**

Previously, the inactivity-induced decline in peak power, at the whole muscle level (soleus) (17, 45) and at the single-fiber level (type I) (3, 21), was investigated in rats with specific body masses (i.e., 250–275 or 300–350 g in Sprague-Dawley) or rats with specific ages (i.e., adult). Although our finding is consistent with previous data showing that 2 wk of inactivity reduced absolute peak power of type I fibers in the adult rats (3, 21), our comprehensive evaluation shows a period of inactivity is detrimental to power generation, regardless of the age of the animal, young or old. The dramatic loss in power generation has potential to hinder or limit an individual’s return to independence, since reduced power generation contributes to slower walking, increased injuries following falls, and increased comorbidities (5, 9, 20, 30).

Given that reduced power generation is linked to altered muscle performance, it is imperative to design appropriate therapeutic exercise protocols. Few studies tease out the effectiveness of therapeutic exercise protocols during periods of inactivity on power generation (3, 17). We report three major findings. First, we found that 2 wk of inactivity on power generation (3, 17). Some exercise protocols have very positive results, like Hurst and Fitts (17), who used high-intensity isometric exercise, and report its protective effect for peak power at the level of the whole soleus muscle. In contrast, some exercise protocols are ineffective in preventing a loss in power generation, like Bangart et al. (3), who implemented the intermittent weight-bearing standing protocol during 14 days of HS.

There are several lines of evidence indicating that the duration of inactivity and exercise frequency play a role in the effectiveness of the exercise protocol. During a period of 7 days of inactivity, a therapeutic exercise consisting of standing is beneficial, but these simple exercise protocols are less effective when the duration of inactivity is longer (e.g., over 14 days) (16, 37). Short periods of exercise interspersed throughout the day during a period of inactivity are more effective in maintaining muscle size and function (1, 3, 16). Taken together, it is possible that intermittent exercise during 2 wk of HS would be effective in preventing the decline in muscle function.

In the present study, we used an ambulation protocol, namely, the rats walked on a motor-driven treadmill for ~15 min four times daily, with each training bout occurring every 2 h over a period of 8 h. We designed this therapeutic exercise protocol because the intensity was mild and the frequency was intermittent, hence not too vigorous for an older rat. Physiologically, this protocol proved to be beneficial in attenuating inactivity-induced decline in fiber size and force generation for MHC type II fibers in the gastrocnemius muscle from elderly rats (2). However, with inactivity, the soleus muscle is very susceptible to inactivity-induced contractile dysfunction. For instance, a recent study shows that the soleus muscle is not protected from muscle atrophy and functional decline in old rats (4). Therefore, it is important to design exercises that involve the soleus muscle. Similar to the gastrocnemius muscle, the soleus muscle is also an ankle plantar flexor and greatly recruited in the stance phase during walking (24). Because the soleus muscle is active in the stance phase during ambulation and EMG activity of the soleus starts before the EMG activity of the gastrocnemius (7), we hypothesized that the mild treadmill exercise protocol would prevent the detrimental effects.
from HS in the type I fibers from soleus muscle, focusing on single-fiber power generation. The response to therapeutic exercise on power generation in type I single fibers was different among the three age groups. Improvement in peak power was observed in exercising middle-aged and young adult rats, supporting the use of mild treadmill exercise for these two specific age groups. In contrast, this exercise program did not attenuate the inactivity-induced decline in peak power in the old rats, suggesting that this type of exercise does not provide an adequate stimulus to prevent inactivity-induced decline in power generation. While it is not clear as to whether the reduction in power generation would substantially obstruct physical performance, especially in humans, the exercise-induced loss of power generation in the old age group would be expected to limit work capacity and retard the recovery process or the return to a normal lifestyle.

*Peak Force* ($P_o$)

Consistent with other studies, our data confirmed the significant inactivity-induced impairment of single-fiber peak force (type I) in adult (13, 23, 38) and old rats (1, 38, 39). Because our study included three age groups, it is now possible to conclude that inactivity results in a decline in single-fiber force-generating capacity, and the extent of the decline is age independent (about 41% decline in peak force).

Even though our present study shows a significant reduction in peak force with 2 wk of inactivity, which is similar between the three age groups, this conclusion may not be possible with shorter or longer periods of inactivity. For instance, our laboratory has previously reported that type I fibers from the older animal with HS for 1 wk showed greater decline in peak force and specific tension than do single type I fibers from younger animals (38). Therefore, these data suggest that the time course of the detrimental changes in force generation for HS is different with age and would influence peak power generation.

The mild treadmill exercise during inactivity did not attenuate the severe reduction in peak force, suggesting that the therapeutic exercise protocol (duration, intensity, frequency) does not provide an adequate stimulus to increase peak force generation. In fact, the mild exercise during HS appears to further decrease force in the old age group ($-21\%, P = 0.102$). The response to the therapeutic exercise in the old group may be related to poor recovery capacity against inactivity-induced muscle atrophy and damage. Our data indicate that treadmill exercise during a period of HS does not prevent muscle atrophy (i.e., fiber diameter, muscle mass, and relative muscle mass) in the old group. This finding is consistent with the observation of Gallegly et al. (12), who demonstrated an impaired response to an intermittent reloading to attenuate soleus muscle atrophy in old animals. As skeletal muscle atrophies during HS, there are changes in the geometric ultrastructure that predispose muscles to contraction-induced damage (e.g., Z-line streaming, sarcoclemene lesions, inflammatory cell infiltrations). Therefore, it is possible that our therapeutic exercise program in the old rats might work as negative stimulus in type I fibers, which are easily injured and recover more slowly.

$V_{max}$

Many former studies report increased contraction speed after a period of inactivity in both animals (3, 38) and humans (6, 44, 46). Most of the studies showing an increase in shortening velocity have used the slack test (maximal unloaded shortening velocity). The slack test is a direct and accurate reflection of the cycling rate of myosin-actin interactions; however, the unloaded muscle contraction is not the physiological form of muscle action during movement such as walking (3, 20). Instead, muscles shorten under tension to produce mechanical work and power required for movement. Experimentally, to determine shortening speed under tension, the load-clamp test is used to characterize the force-velocity relationship. Subsequently, the Hill plot equation calculates the $V_{max}$. In single permeabilized fiber experimentation, the measurement of $V_{max}$ is lower than that of unloaded shortening velocity (21). In review of the literature, few studies report inactivity-induced changes in the force-velocity relationship. For instance, a 31% increase was reported for $V_{max}$ following 2 wk of suspension in soleus from Sprague-Dawley rats (21). In contrast, our study shows no change in $V_{max}$ across the three age groups. Hence, more comprehensive studies would be needed to clarify this discrepancy.

The mild treadmill exercise during a period of inactivity was not enough to increase the $V_{max}$ of type I fibers from young adult and middle-aged rats, but declined the $V_{max}$ in the old rats. These findings imply that age negatively influences the exercise-stimulus response, especially in respect to muscle shortening velocity. Whereas the cellular mechanisms underlying the exercise-induced reduction in $V_{max}$ in old rats remain to be elucidated, it is likely due to muscle damage discussed above that occurs with ambulation.

*Power = Force and Velocity*

Power is the product of force and shortening velocity at which contraction occurs. Peak power is generated at which an optimum force and an optimum velocity are developed. Therefore, any change in force production and/or contraction speed has potential to affect the peak power output of a muscle. In the present study, we investigated force and shortening velocity to identify the underlying causes contributing to changes in peak power output with inactivity and exercise.

The drastic reduction in peak force across the three age groups with 2 wk of inactivity parallels the severe reduction in power generation. Because $V_{max}$ of type I fibers after 2 wk of HS did not show any significant change in all age groups, the dramatic reduction in peak power generation is primarily due to impairment in force generation.

As noted above, therapeutic mild treadmill exercise during a period of HS improves peak power generation in both young adult and middle-aged rats. Because the therapeutic exercise does not statistically improve peak force generation or $V_{max}$ independently, the observed improvement in peak power is likely due to a combination of small increases in both force and $V_{max}$. For instance, in the young adult group, the change in the mean values for force and $V_{max}$ with therapeutic exercise is 15 and 22%, respectively. In combination (force and $V_{max}$), there is a statistical improvement in power generation. The mild treadmill exercise does not prevent the HS-induced decline in peak power in the old rats, because force generation remains impaired, and there is a decline in contractile velocity.
**Normalized Power and Specific Tension**

Normalized power is representative of muscle fiber function because it accounts for force generation, contraction speed, and fiber size, allowing for the evaluation of intrinsic fiber quality (11). Similar with the absolute peak power data, HS results in a reduction of normalized power, which is age-independent, suggesting the reduction in power generation is due to the changes in fiber quality, because specific tension is primarily reduced. The potential cellular mechanisms underlying the change in fiber quality include disproportionate loss of actomyosin cross bridges per fiber CSA (28, 36), alterations in calcium kinetics (i.e., calcium sensitivity, calcium release rate, and calcium transient) (18, 34), and decreased population of myosin heads in the strong-binding structural state during muscle contraction (15, 22, 25, 35). A major finding of this study is the lack of improvement in normalized power and specific tension in old rats with therapeutic exercise. Indeed, exercise appears to further deteriorate normalized power and specific tension in old animals. These results indicate the possibility of alterations in muscle structure and protein damage. Further studies are required to test these underlying possibilities.

**Clinical Perspective**

The mild treadmill exercise program does not appear to be appropriate for improving power generation in the single fibers of the soleus muscle in old rats. However, this exercise program attenuated inactivity-induced impairment of power generation in the young adult and middle-aged rats. The attenuation in peak power generation is likely due to combined changes in shortening velocity and force-generating capacity. Hence, a therapeutic exercise protocol that incorporates both walking and strength may be the optimal therapy for young and middle-aged population.

In conclusion, the benefits of the mild treadmill exercise program during a period of inactivity on contractile properties of type I fibers are age dependent, whereas the detrimental effects of inactivity (i.e., peak power and peak force) are age independent. For young adult rats, the power generation was partially restored, which implied that the stimulus of exercise was not strong enough: for the middle-aged group, the exercise stimulus fits the needs of restoring power level; for the old rats, our protocol might be too vigorous and may cause further fiber injury. Collectively, when designing therapeutic exercise in a rehabilitation setting, the age of the individual requires attention.

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No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


