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Age-dependent effects of treadmill exercise during a period of inactivity

P. Arora¹, A.D. Husom¹, D.A. Ferrington², and L.V. Thompson¹

¹Department of Physical Medicine & Rehabilitation, University of Minnesota, Minneapolis, Minnesota, USA

²Department of Ophthalmology, University of Minnesota, Minneapolis, Minnesota, USA

Abstract

The objective of this study was to investigate the effect of a treadmill exercise protocol to prevent muscle weakness, atrophy and alterations in calcium regulation in adult, old and very old rats. Adult (7–12 months), old (29–30 months) and very old (34–36 months) F344BNF₁ rats were randomly assigned to weight bearing (WB), weight bearing exercise (WBX), non-weight bearing (NWB) and non-weight bearing exercise (NWBX) groups. The WB group was considered the sedentary-control animals. NWB rats were hindlimb unweighted for 14 days. WBX and NWBX groups were exercised on a treadmill for approximately fifteen minutes four times daily. The contractile properties [diameter, peak active force (P_0), specific tension (P_0/CSA)] of single myosin heavy chain type II fibers and Ca regulation [Ca^{+2} dependent ATPase activity] were determined. Fiber diameter reduced by 24% in the very old rats with NWB. P_0 and P_0/CSA declined in the young adult and very old rats with NWB. NWBX attenuated these changes in the young and very old rats. Ca^{+2} dependent ATPase activity increased with treadmill exercise during non-weight bearing in the young animals. In conclusion, the treadmill exercise is beneficial in attenuating the non-weight bearing induced changes in the individual MHC type II muscle fibers of the gastrocnemius muscle.

Keywords

treadmill exercise; single fiber contractile properties; calcium regulation

INTRODUCTION

Exercise training partially attenuates the deleterious effects of inactivity (Alley and Thompson, 1997; Brown and Hassler, 1995; Brown and Taylor, 2005; Herbert et al., 1988; Hurst and Fitts, 2003; Sandmann et al., 1998; Thompson, 2002). Several studies have focused on preventing the inactivity-induced skeletal muscle weakness by examining the benefits of intensive exercise protocols in adult aged rats. These exercise protocols included a daily short-duration high-load exercise training program, resistance training, conditioning and isometric exercises (Brown and Taylor, 2005; Herbert et al., 1988; Hurst and Fitts, 2003; Pierotti et al., 1990). Although the intensive exercise protocols were beneficial to varying degrees to the muscles, these

Corresponding author: LaDora V. Thompson, PhD, PT, University of Minnesota, MMC388, 420 Delaware St SE, Minneapolis, MN 55455, Phone: (612)-626-5271, Fax: (612)-625-4274, thomp067@umn.edu.

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rigorous protocols may not be appropriate for muscles of older rats due to the risk of muscle injury.

Mild exercise, in the form of weight bearing or isometric exercise, is thought to prevent the deconditioning associated with inactivity (Stiller, 2007; Morris, 2007). There is support for the efficacy of weight bearing exercise (Alley and Thompson, 1997; Brown and Hassler, 1995; Brown and Taylor, 2005; Sandmann et al., 1998). Weight bearing exercise (standing) of 15 minutes duration, four times per day during a period of inactivity has been shown to attenuate inactivity-induced decrements in whole muscle and single fiber function (Alley and Thompson, 1997; Sandmann et al., 1998). The attenuation is muscle and fiber-type specific, such that the myosin heavy chain isoform (MHC) type I fibers (slow-twitch fibers) in the soleus and gastrocnemius muscles improve in contractile function.

Weight bearing exercise appears to preferentially stimulate or recruit the MHC type I fibers (Sandmann et al., 1998). In contrast, the MHC type II fibers (fast-twitch fibers) continue to exhibit the detrimental effects of inactivity and therefore require a different exercise protocol or intervention (Alley and Thompson, 1997; Sandmann et al., 1998).

The improvements associated with mild treadmill exercise (isotonic exercise with weight bearing; walking) to prevent inactivity-induced muscle dysfunction have not been investigated. However, the gastrocnemius muscle is an ankle plantar flexor and is recruited during walking exercise (stance phase of the gait cycle) (Meinders et al., 1998). Thus it is possible that mild treadmill exercise stimulates both the MHC type I and the MHC type II fibers present in the gastrocnemius. The primary objective of the present study is to evaluate the effect of treadmill exercise during a period of non-weight bearing on MHC type II fibers, which have not been previously investigated.

The deleterious changes associated with inactivity are greatly compounded in the older animal due to the presence of age-induced sarcopenia. Studies have shown that when adverse changes of inactivity are superimposed on age-induced sarcopenia there is further physiological deconditioning (Brown et al., 2004; Morris, 2007). To date, there is little information or evidence regarding the effectiveness of treadmill exercise as an intervention during periods of non-weight bearing to reduce skeletal muscle atrophy and preserve contractile function, especially in the older animal. Consequently a second objective of this study is to test the hypothesis that a treadmill exercise intervention consisting of four daily bouts for fifteen minutes each would prevent muscle weakness, atrophy and alterations in calcium regulation in the type II (fast-twitch) fibers from gastrocnemius muscle of adult, old and very old rats.

MATERIAL AND METHODS

Animals

Care, Housing and Ages—The animal protocol for this study was approved by the Institutional Animal Care and Use Committee at the University of Minnesota and follows the guidelines of the National Institutes of Health. Male Fischer 344 Brown Norway F₁ Hybrid rats (purchased from the National Institutes on Aging Aging Rodent Colonies) were housed in individual cages (40 in² × 7 in) under a 12-hour light/dark cycle at 20°C, maintained on a diet of Purina Rodent Chow and water ad libitum. The rats were acclimated for 7 days prior to initiating the experimental protocol.

This study used rats from 3 age groups that cover the lifespan as determined by survival rates derived from large populations of the Fischer 344 Brown Norway F₁ Hybrid species

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www.nia.nih.gov/ResearchInformation/ScientificResources/AgedRodentColoniesHandbook/StrainSurvivalInforma

). The young adult age group (7 to 12 months) represent 100% survival rate. The old adults (29 to 30 months) represent 68% survival rate and the very old group (34 to 36 months) represents 44% survival rate. Thus, the very old rats are considered in senescence.

Activity Levels—To determine the effects of mild treadmill exercise on muscle contractility properties and calcium regulation the rats for each age group were divided into four categories based on activity levels. These activity levels consisted of sedentary – control weight bearing rats (WB), hindlimb unweighted rats (NWB) that were non-weight bearing, sedentary - control weight bearing rats that were exercised on the treadmill (WBX), and hindlimb unweighted rats that were exercised on the treadmill (NWBX). Each of these categories is explained further and the exact protocols of hindlimb unweighting and treadmill exercise are described below. Animal characteristics and number of rats are summarized in Table 1.

Sedentary – control weight bearing rats (WB)—The sedentary-control weight bearing groups were allowed free movement within the cage bearing weight on all hindlimbs and forelimbs.

Hindlimb Unweighted Non -Weight Bearing (NWB)—Hindlimb unweighting is a model of inactivity (Alley and Thompson, 1997; Sandmann et al., 1998). The hindlimb unweighted rats were defined as non-weight bearing when the rats did not bear weight on their hindlimbs. This protocol has been previously described (Alley and Thompson, 1997; Zhong et al., 2006; Zhong and Thompson, 2007). Briefly the animals were partially unweighted with a tail harness for two weeks. The harness was tied to an overhead rod, and the height of the suspension was adjusted so that the hindlimbs were not touching the cage floor. The forelimbs maintained contact with the cage floor, thus allowing rats to obtain food and water. Each cage housed a single rat.

Weight bearing with treadmill exercise (WBX)—The weight bearing rats with mild treadmill exercise bore weight on both their forelimbs and hindlimbs and received the exercise training to account for muscle changes due to the treadmill exercise. The rats walked on a treadmill for approximately fifteen minutes four times daily with each training bout occurring every two hours over a period of eight hours. The average training times were thus calculated for each rat and then for each experimental group (Table 1). The average treadmill exercise speed of the rats was 342 cm/minute at an incline of zero degrees. This treadmill exercise speed was tolerated by the three age groups.

Hindlimb Unweighted Non – Weight bearing with treadmill exercise (NWBX)—The goal of including NWBX group in the study was to determine the effects of mild treadmill exercise on the contractile properties of the skeletal muscle fibers and calcium regulation during a period of inactivity. This group of rats performed daily treadmill exercise during the two weeks of non-weight bearing. The hindlimb unweighting and treadmill exercise protocol are described above. The actual times walked by the rats are shown in Table 1.

Single Fiber Physiologic Experiments

Tissue collection/muscle preparation—After two weeks of treatment, rats were weighed and anesthetized with an intraperitoneal injection of sodium pentobarbital (35mg/kg body weight). The deep lateral portion of the gastronemius muscles was isolated, rinsed with cold Ringer's solution, trimmed free of excess fat and connective tissue and weighed. The remaining hindlimb muscles composed of both type I and type II MHC fibers were used to purify the sarcoplasmic reticulum calcium ATPase (described below).

Single fiber preparation—The skinned single fiber has the sarcolemma chemically removed, which allows for accurate determination of the force-generating capacity (Alley and Thompson, 1997). The preparation was done as described by Sandmann and Thompson (1998). Briefly, the gastronemius was placed in a cold (4°C) relaxing solution containing: 20mM imidazole (pH 7.0), 7.0 mM ethylene glycol-bis(B-amino-ethyl ether)- N,N,N',N' – tetraacetic acid (EGTA), 5.4mM MgCl₂, 14.5mM creatine phosphate, 4.7 mM ATP and CaCl₂ to achieve pCa ($-\log[\text{Ca}^{2+}]$) 9.0. This composition contained enough KCl to achieve an ionic strength of 180 mM. The muscle was divided into small bundles comprised of approximately 50 fibers and placed in the relaxing solution. Each bundle was tied to a glass capillary tube with surgical suture at its approximate anatomic length. Thus prepared, the bundles were stored in glycerinating solution containing 20mM imidazole (pH 7.0), 125 mM K-propionate, 2mM EGTA, 4mM ATP, 1mM MgCl₂, and 50% glycerol (vol/vol) at –20°C. The glycerinated fibers can be stored up to four weeks.

Isolation of single fibers—The single skinned fiber muscle preparation and experiments have been described in detail in the previous studies (Alley and Thompson, 1997; Sandmann et al., 1998). Briefly, at the time of the experiment a single skinned fiber was isolated from a fiber bundle under a dissecting microscope and transferred to the experimental chamber containing relaxing solution (4°C). A 2 to 3 mm segment of muscle fiber was mounted between a force transducer (Cambridge Model 400A; sensitivity 2mV/mg) and a DC motor lever (Cambridge Model 300H).

Fiber diameter and optimum length—The fiber was observed through an inverted microscope (X600) with a calibrated eyepiece micrometer. Sarcomere length (SL) was adjusted to 2.5µm (Zhong and Thompson, 2007). Three separate measurements were used to calculate the mean fiber diameter. The individual fiber cross-sectional area was calculated from the diameter assuming a circular cross-section.

Peak active force (P₀) and Specific Tension (P₀/CSA)—The baseline force of a single skinned fiber was monitored in the relaxing solution (pCa 9.0). To determine the maximal force the fiber was transferred to the activating solution containing: 20mM imidazole (pH 7.0), 7.0 mM ethylene glycol-bis(B-amino-ethyl ether)-N,N,N',N' –tetraacetic acid (EGTA), 5.4mM MgCl₂, 14.5mM creatine phosphate, 4.7mM ATP and CaCl₂ to achieve pCa ($-\log[\text{Ca}^{2+}]$) 4.5 and enough KCl to achieve an ionic strength of 180 mM. The individual fiber was activated five times (5 trials) and the trial that gave the greatest force was selected to calculate the peak active force (P₀). P₀ was calculated in each fiber by subtraction of the baseline force from the maximal force (Alley and Thompson, 1997). P₀ was normalized by dividing the fiber's cross-sectional area (P₀/CSA) to determine the specific tension.

Myosin heavy chain isoform analysis—After contractile measurements, the individual fiber was solubilized in 50 µl of sample buffer [60 mM Tris (pH 6.8), 24 mM EDTA, 1% sodium dodecyl sulphate (SDS), 5% b-mercaptoethanol, 15% glycerol, 2 mg/ml bromophenol blue] and stored at –80°C. MHC isoform expression of each fiber was determined by separating proteins using gel electrophoresis and comparing the migration of sample proteins with standard proteins of MHC type I, IIa, IIb on silver-stained gels (Zhong and Thompson, 2007). The SDS polyacrylamide gel was composed of a 4% (wt/vol) stacking gel and 8% separating gel.

Sarcoplasmic Reticulum (SR) Experiments

Isolation of SR—The SR vesicles were isolated using differential centrifugation, with minor modifications as described previously (Ferrington et al., 1996). The isolated vesicles were suspended in a medium consisting of 20mM MOPS (pH 7.0) and 0.3 M sucrose and stored at

–70°C. The protein concentration of the SR preparations was determined using the BCA assay (BioRad) following the addition of 0.1% SDS. Bovine serum albumin was used as the standard.

Sarcoplasmic Reticulum Ca-ATPase activity (Vmax)—SR Ca-ATPase activity was measured at 25°C using an enzyme linked, continuous spectrophotometric assay (Ferrington et al., 1996). The standard sample mix contained 2–4µg of SR vesicles in 175µL of buffer containing 40mM Imidazole (pH 7.0), 0.6M sucrose, 2mM NaN₃, 0.5mM CaCl₂, 1mg/ml A23187. EGTA was used for a blank control. A substrate mix of 0.1M Imidazole (pH 7.0), 0.25M potassium chloride(KCl), 0.1M MgCl₂, 10mM EGTA, 0.4mM NADH, 1.0mM phosphoenolpyruvate, 5.0mM ATP, 100 IU pyruvate kinase, 100 IU lactate dehydrogenase (10 IU for standard curve) was added to a 96-well plate. Activity was determined by varying the calcium concentration from pCa 5.0 to 7.5. Addition of the SR sample mix to the substrate mix started the assay. The absorbance of NADH was monitored at 340 nm to determine the rate of ATP hydrolysis. Ca-ATPase activity (Vmax) was determined from the fit of experimental data to the Hill Equation using the analytical software in Origin (OriginLab, Corp.).

Statistical analysis

Contractile parameters from the individual fiber experiments containing MHC type II isoform (diameter, peak active force and specific tension) were compiled for each rat by calculating a mean for each parameter. Thus, the sample size was based on the number of rats investigated per experimental group (5–7 rats) rather than the number of individual fibers investigated (505 fibers).

To determine the normality of the outcome variables, descriptive statistics were performed on the data values of fiber diameter, peak active force, specific tension and the Ca-dependent ATPase activity (Vmax). The data for the outcome variable Vmax was transformed to fit a normal distribution. The statistical analysis was then performed on the fiber diameter, peak active force and specific tension and on transformed data for Ca ATPase activity.

Means and standard error of the means (SEM) of different activity levels in each age group were compared using a one – way analysis of variance (ANOVA) with treatment as the main effect. The significance level was set at a $p \leq 0.05$. Tukey- Kramer multiple comparison analysis was performed to determine the differences between groups.

RESULTS

Table 1 summarizes the characteristics (body weight, gastrocnemius weight, gastrocnemius to body weight ratio) of the rats by experimental group. Two weeks of hindlimb unweighting does not significantly alter body weight in the three age groups. Although the gastrocnemius muscle mass decreases by 30% and 35%, respectively in the young and old rats, calculation of the muscle to body weight ratio confirms the preferential loss in gastrocnemius muscle mass; ratios for young and old rats were reduced 19% and 29% respectively. Gastrocnemius muscle mass and muscle to body weight ratio in the very old group did not change with hindlimb unweighting. Treadmill exercise partially attenuates the hindlimb unweighting-induced decline in muscle mass, as evidenced by the partial recovery of muscle mass to body weight ratio.

To determine the effect of the treadmill exercise as an intervention during a period of non-weight bearing (hindlimb unweighted rats), individual MHC type II fiber diameter, peak active force, specific tension and Ca⁺² ATPase activity were measured.

Fiber Diameter

In the young adult group, no significant atrophy or hypertrophy was observed across the four activity levels (Fig 1A). Treadmill exercise had no effect on the fiber diameter in the young rats.

In the old group, a significant difference across the activity levels was found ($p < 0.05$, $F = 7.21$, critical value = 3.997, Fig 1A). The post-hoc test showed that the fiber diameter in the non-weight bearing exercise group (67 μm) was significantly less than the weight bearing controls (81 μm). In NWB rats, there was about 17% reduction in diameter. The extent of atrophy in rats performing treadmill exercise during non-weight bearing was essentially equivalent (64 μm). Thus, no exercise effect was observed with treadmill exercise.

In the very old group, there was a significant difference between the various activity levels ($p < 0.05$, $F = 5.10$ and critical value = 3.997). Following the post-hoc test, the non-weight bearing group showed a significant 23% atrophy of fibers. Atrophy was attenuated in the NWBX group with the intervention of treadmill exercise. Thus, the very old rats showed that the treadmill exercise during a period of hindlimb unweighting was beneficial in reversing the non-weight bearing-induced atrophy.

Peak Active Force

In young adult rats, non-weight bearing results in a significant decline in the force generating capacity of individual fibers (Fig 1B, $p < 0.05$, $F = 5.53$ and critical value = 4.02). The force generating capacity of individual fibers from the gastrocnemius of the non-weight bearing group decreased by 29% and treadmill exercise during non-weight bearing recovered about 13% of the force generating capacity.

In the old rats, the force generating capacity in individual fibers decreased 18% with non-weight bearing ($p < 0.05$, $F = 3.13$, critical value = 3.958) (Fig 1B). Treadmill exercise does not reverse this change.

In the very old rats, significant differences in NWB compared with WB controls were noted ($p < 0.05$, $F = 5.60$, critical value = 3.997; Fig 1B). The most dramatic change in force generating capacity was observed for NWB and there was attenuation due to treadmill exercise. Inactivity caused a 61% loss in force and ambulation recovered most of the force.

Specific Tension

In the young adult rats, non-weight bearing results in a significant 18% decline in specific tension (Fig 1C, $p < 0.05$, $F = 3.99$, critical value = 3.997). Treadmill exercise recovers about 12% of the decline in specific tension of single fibers. In the old rats, the specific tension was not different across the various activity levels.

In the very old rats, there were significant differences between WB controls and WBX as well as NWB (Fig 1C, $p = 0.05$, $F = 14.55$, critical value = 4.046). Non-weight bearing resulted in a 34% decline in specific tension. Moreover treadmill exercise in NWBX rats increased the specific tension of individual fibers in the gastrocnemius approximately 21% over NWB. In contrast to the other age groups, the very old rats had a significant exercise-induced improvement in the WBX group (33% improvement).

Sarcoplasmic Reticulum Ca-ATPase activity (Vmax)

In the young rats hindlimb unweighting does not affect the Ca-dependent ATPase activity (Vmax). However, exercise stimulated ATPase activity; there was a 31% and 53% increase in

activity in WBX and NWBX, respectively, (Fig 2). In both old and very old rats physical activity had no effect on the Ca- dependent ATPase activity (Fig 2).

DISCUSSION

The purpose of the study was to examine the effects of mild treadmill exercise during a period of inactivity in young, old and very old rats. We hypothesized that the treadmill exercise would reverse the detrimental changes in muscle size, contractile properties and Ca-dependent ATPase activity associated with non-weight bearing. We found effects of weight bearing and exercise were influenced by the age of the rat. In young rats there was no atrophy associated with non-weight bearing. However, we observed a significant decrease in force generating capacity that was not totally reversed by mild treadmill exercise. Additionally, while non-weight bearing had no effect on Ca-dependent ATPase, exercise stimulated ATPase activity. Thus, in young rats the mechanism behind the decline in force associated with non-weight bearing cannot be explained by a loss in muscle mass. In contrast, atrophy accounts for all or at least a major part of the non-weight bearing decline in force for old and very old rats, respectively. Additionally non-weight bearing and exercise had no effect on Ca-ATPase activity. Notably treadmill exercise had a significant positive impact on force generation in very old muscles suggesting exercise as a potentially valuable exercise intervention for the aged animal.

Non-weight bearing Induced Changes

In adult rats, the detrimental effects of non-weight bearing on the force generating capacity has been reported at both the whole muscle level as well as in single fibers. At the whole muscle level, a 20 to 23% decline in the tetanic tension of the gastrocnemius after hindlimb unweighting has been shown (Templeton et al., 1985). In single fibers, force declined 20% (Herbert et al., 1998; Gardetto et al., 1989; Simard and Lacaille, 1988). Thus, the 29% decline in the force generating capacity of individual fibers from adult rats in the current study is consistent with previous published work (Gardetto et al., 1989; Simard and Lacaille, 1988).

Previous studies examining old rats show a statistically significant decline in muscle strength with inactivity (Sandmann et al., 1998; Simard and Lacaille, 1988). In the current study mean values for single fibers exhibited 18% decline in force. While the ANOVA indicates a statistical significance ($p < 0.048$) the considerable variability in this measure prevented the identification of group differences following the post-hoc analysis. However, in the NWB rats, four out of six rats investigated demonstrated a 30 to 40% drop in the force generating capacity, which is in accordance with previous studies (Sandmann et al., 1998; Simard and Lacaille, 1988). In contrast to the well documented adaptations to inactivity in the adult and old animals, there is a dearth of literature for the very old rats. The novel finding from this study was the 61% decrease in force generating capacity with non-weight bearing or inactivity.

In the current study, the Ca-dependent ATPase activity of muscle composed of mixed MHC fiber types showed no change with non-weight bearing in the three age groups. These results are in contrast to the previous report of increased Ca-dependent ATPase activity following inactivity in the soleus muscle, which is predominantly composed of the MHC type I and SERCA 2a slow isoforms of myosin and the SR Ca-ATPase, respectively (Peters et al., 1999; Schulte et al., 1993). The increased ATPase activity was attributed to the non-weight bearing-induced increase in the SERCA1 (fast) isoform expression in the soleus (Peters et al., 1999; Schulte et al., 1993). After 15 days of hindlimb unweighting Stevens et al., (1990) also showed increased calcium uptake and release by the SR in the soleus muscle that functionally were comparable to fast muscle. Calcium release from the SR is regulated by the voltage-sensitive dihydropyridine receptor, which triggers the release of SR calcium by the ryanodine receptors. Kandarian and colleagues showed non-weight bearing caused increased expression

of these SR calcium release proteins in the soleus (Kandarian et al., 1992). In addition to the SR proteins, the inactivity-induced transition from slow to fast isoforms in the soleus has also been documented for myosin (Diffie et al., 1991). Thus, inactivity stimulates transformation of slow-twitch muscle fibers to a fast-twitch phenotype.

In comparison with slow-twitch muscle, fast-twitch muscle is much less responsive to non-weight bearing. In the EDL, composed mainly of fast SERCA and myosin isoforms, minimal changes were observed in muscle protein content, MHC isoform composition, and calcium loading of the SR (Patterson and Dettbarn, 1985; Oishi et al., 1998). The fiber-type difference in response to non-weight bearing could potentially explain results from the current study showing no change in Ca-dependent ATPase activity. The high percentage of fast-twitch fibers in the mixture of hindlimb muscles (Armstrong and Phelps, 1984) used for isolating the SR could have masked non-weight bearing-induced changes in the slow-twitch muscle fibers. Therefore, in order to get a better understanding of the effects of treadmill exercise, calcium regulation and the underlying molecular mechanisms future investigations will involve using preparations from distinct muscles containing mainly fast- or slow-twitch fibers and identifying changes in SERCA isoforms.

Treadmill Exercise induced changes

Several studies have attempted to test the effectiveness of various modes of exercise to attenuate the inactivity-induced muscle weakness. Although exercise benefits have been noted, the exercises are either too aggressive or not suited to the very old rat that might be susceptible to muscle damage (Herbert et al., 1988; Pierotti et al., 1990; Linderman et al., 1994). One study that investigated isometric exercise in rats, an exercise that does not cause muscle damage, did not prevent the inactivity induced decline in gastrocnemius muscle function (Hurst and Fitts, 2003). The results of the current study support the use of mild treadmill exercise for the very old group, which showed the most dramatic exercise effect in both restoration of specific tension and attenuation of inactivity-induced atrophy. It is also important to note the significant improvement in contractile function of the very old weight bearing exercise group. In contrast, the old rats did not respond to the mild treadmill exercise program, suggesting that this type exercise does not provide an adequate stimulus to prevent the detrimental inactivity-induced changes in skeletal muscle function.

Mechanisms—Several mechanisms have been proposed to explain the decline in force generating capacity with unweighting or non-weight bearing. The most logical explanation for this weakness is muscle atrophy. A review of the hindlimb unweighting studies investigating the gastrocnemius muscles from rats of various ages reveals that the extent of atrophy is varied. The extent of atrophy with the hindlimb unweighting model seems to be dependent on the muscle (e.g. plantaris, EDL and gastrocnemius) and duration of inactivity (number of weeks of hindlimb unweighting) (Gardetto et al., 1989; Herbert et al., 1988; Hurst and Fitts, 2003; Sandmann et al., 1998; Simard and Lacaille, 1988). The 5%–23% decrease in fiber diameter in the current study is consistent with previous studies investigating type II fibers from adult, old and very old rats for a similar duration (Gardetto et al., 1989; Simard and Lacaille, 1988).

In order to evaluate other mechanisms (e.g., muscle quality) that contribute to non-weight bearing induced weakness, force generating capacity is normalized to fiber size. This variable is termed specific tension (peak active force/cross-sectional area). If atrophy is the primary underlying mechanism to explain hindlimb unweighted induced decline in force generating capacity, there would be no change in specific tension. In previous studies specific tension is reduced suggesting that atrophy is only a part of the explanation for unweighting induced reduction in force (Alley and Thompson, 1997; Fitts et al., 2001; Sandmann et al., 1998; Stevens et al., 1990). In the current study, there is also a significant decrease in specific tension

with hindlimb unweighting in the young and very old as the diameter and force do not decrease proportionately. Thus, the decrease in diameter in the very old partly explains the decrease in force generating capacity. The other mechanisms suggested for the altered specific tension following unweighting include a decrease in the total number of actomyosin cross bridges, alterations in the structure of myosin and/or a preferential loss of actin (Stevens et al., 1990; Thomason et al., 1989; Zhong et al., 2006). These underlying cellular mechanisms need to be investigated further.

CONCLUSION

The mild treadmill exercise comprising of approximately fifteen minutes of exercise four times a day is definitely beneficial to ameliorate the inactivity-induced muscle weakness in type II fibers from the very old and adult animals. This exercise becomes more important for functional capacity as the weight-bearing sedentary-control animals age. Further work needs to be done to identify the mechanisms responsible for attenuating the loss in muscle function.

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Fig. 1. Atrophy, force-generating capacity, and specific tension with inactivity and treadmill exercise in MHC type II fibers

Fiber diameter (mean $\pm$ SEM) (A), Peak active force (mean $\pm$ SEM) (B) Specific Tension (mean $\pm$ SEM) (C). Weight bearing (WB), weight bearing exercise (WBX), non-weight bearing for 14 days (NWB) and non-weight bearing exercise (NWBX) rats in the young (7–12 months), old (29–30 months) and very old (34 to 36 months) groups. * significantly different from weight bearing sedentary-controls ($p \leq 0.05$). † significantly different than non-weight bearing ($p \leq 0.05$).

Fig. 2. Ca^{+2} dependent ATPase activity during inactivity and treadmill exercise in limb skeletal muscle

Activity (mean $\pm$ SEM) with the number of rats in each group indicated in the bar graph. Weight bearing (WB), weight bearing exercise (WBX), non-weight bearing (NWB) and non-weight bearing exercise (NWBX) rats in the young (7–12 months), old (29–30 months) and very old (34 to 36 months). * significantly different from weight bearing controls ($p \leq 0.05$).

Table 1

Animal Characteristics by Age and Activity Level

Means $\pm$ SEM for young adults (7 to 12 months), old (29 to 30 months) and very old (34 to 36 months) rats. Weight bearing (WB) is sedentary-control activity. Weight bearing exercise (WBX) is treadmill exercise for approximately 15 minutes four times daily. Non-weight bearing (NWB) is hindlimb unweighting for 14 days. Non-weight exercise (NWBX) is treadmill exercise for approximately 15 minutes four times daily during hindlimb unweighting. The numbers of animals in each group are provided in parenthesis. Body weight and gastrocnemius muscle are measured in grams. Gas/BW is the gastrocnemius muscle weight normalized to the body weight of the rat. The treadmill exercise times are the average training time per day over the entire 2 weeks of exercise.

	WB	WBX	NWB	NWBX
Young	Body Weight (BW) (g)	442 $\pm$ 18 (7)	384 $\pm$ 22 (5)	379 $\pm$ 21 (5)
	Gastrocnemius (g)	2.23 $\pm$ 0.06	2.06 $\pm$ 0.25	1.55 $\pm$ 0.04*
	Gas/BW (mg/g)	5.07 $\pm$ 0.12	5.30 $\pm$ 0.44	4.11 $\pm$ 0.19*
	Exercise Time (min/day)	48 $\pm$ 1	48 $\pm$ 1	51 $\pm$ 3
Old	Body Weight (g)	534 $\pm$ 28 (7)	480 $\pm$ 37 (5)	481 $\pm$ 13 (6)
	Gastrocnemius (g)	1.74 $\pm$ 0.05	1.66 $\pm$ 0.02	1.12 $\pm$ 0.06*
	Gas/BW (mg/g)	3.31 $\pm$ 0.16	3.50 $\pm$ 0.21	2.35 $\pm$ 0.17*
	Exercise Time (min/day)	46 $\pm$ 1	46 $\pm$ 1	48 $\pm$ 3
Very Old	Body Weight (g)	475 $\pm$ 30 (8)	444 $\pm$ 22 (5)	411 $\pm$ 21 (6)
	Gastrocnemius (g)	1.10 $\pm$ 0.14	1.29 $\pm$ 0.13	1.01 $\pm$ 0.12
	Gas/BW (mg/g)	2.28 $\pm$ 0.18	2.93 $\pm$ 0.31	2.43 $\pm$ 0.19
	Exercise Time (min/day)		39 $\pm$ 1	48 $\pm$ 2

* Significantly different than age-matched WB group.