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Effects of Life-Long Wheel Running Behavior on Plantar Flexor Contractile Properties

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EFFECTS OF LIFE-LONG WHEEL RUNNING BEHAVIOR ON PLANTAR FLEXOR CONTRACTILE PROPERTIES

A Thesis

Presented to the

Faculty of

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In Partial Fulfillment

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Master of Science

in

Biology

by

Alexander Nicholas Beechko

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ABSTRACT

Aging in skeletal muscle is characterized by a loss in muscular performance. This is in part related to the direct loss of muscle mass due to senescence, known as sarcopenia. With age, skeletal muscles lose force production, contractile speed, and power production. The force velocity relationship of muscle is a product of force production and contraction speed, both of which decline with age; however, the mechanisms and trajectory of this decline are not well understood. Exercise has positive effects on muscle, and thus may assist in maintaining performance in old age. However, few long-term studies have been performed to examine the effects of life-long exercise on muscle contractile performance. In order to test the potential for life-long exercise to reduce the effects of again on muscle contractile performance, muscle performance was determined in control mice and mice selected for high voluntary wheel running at baseline, adult, and old ages. Peak isometric force declined with age in control (C) mice without exercise ($P<0.05$), but high runner mice (HR) mice without wheels did not differ significantly with age. With age pooled, HR mice had significantly greater muscle quality (N/g) than C mice, regardless of wheel access ($P<0.05$). Mass specific peak power production (W/g) was significantly greater in old mice that had access to wheels compared to those that did not, regardless of selection ($P<0.05$). The findings of this study support the hypothesis that voluntary lifelong exercise behavior attenuates losses in

important performance metrics, such as mass-specific power production and muscle quality.

ACKNOWLEDGEMENTS

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Lastly, but certainly not least, I would like to dedicate this thesis to my wife, Jessica Beechko, for without her unconditional love and support, none of this would have been possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

Aging of Muscle Tissue

Biological tissues degrade via senescence. Aged skeletal muscle tissue experiences sarcopenia, the loss of muscle mass during senescence (Narici et al., 2003). Significant reductions in muscle mass begin in humans near the fifth decade of life (Janssen et al., 2000), coinciding with a significant reduction in force (Narici et al., 1991). By the seventh decade, quadriceps cross-sectional area (CSA) is 25-33% smaller than that of individuals in their second to third decade of life (Lexell et al., 1988; Young et al., 1984). While skeletal muscle loses its capacity to generate force with aging (Doherty, 2003; Porter et al., 1995), the magnitude of this deficit is directly correlated with the activity of the muscle during its lifetime. Unused fibers degrade due to disuse atrophy or sarcopenia , while regularly recruited muscle fibers preserve force production via hypertrophy of existing fibers (E.P. Widmaier et al., 2004; Hill et al., 2004; Lieber, 1992). However, studies report that even well into the eighth decade of life, short term exercise in the form of sprint training results in isometric force production to be comparable with that of individuals in their fifth decade of life (Korhonen et al., 2006). Studies like these highlight the plastic nature of skeletal muscle and their ability to remodel themselves through active loading, even in the end stages of life.

The load and total activity muscles experience determine the nature and extent of homeostatic response due to exercise (i.e. muscle adaptation). The number of fibers recruited during exercise is also an important factor in determining the extent of adaptation (e.g. sprint training compared to endurance running) (Lieber, 1992). For example after endurance training, skeletal muscles can experience as much as two-fold increases in mitochondrial enzyme activity, indicative of increased aerobic capacity (Baldwin et al., 1972). Post strength training, muscles may increase their cross-sectional area via hypertrophy of existing fibers, thereby also enhancing force production (Bell et al., 2000).

Muscles connect to tendon to impose movement on the skeleton, and therefore changes in tendon mechanics with aging may also alter muscle-tendon unit (MTU) mechanics. Increases in MTU stiffness associated with aging show adverse effects on normal locomotor function (Kovanen et al. 1987; Kragstrup et al. 2011; Shadwick 1990). Alterations in either muscle or tendon can affect the functionality of the entire MTU and impact locomotor performance (Brainerd and Azizi 2005, Azizi 2008, Randhawa 2013, Azizi and Roberts 2014, Carrier 1998). Thus, understanding how aging affects the muscle tendon unit is important in considering the mechanisms of declining locomotor performance with age.

Force-Velocity Relationship, Aging, and Endurance Training

The force-velocity relationship of muscles is dependent on many factors, including fiber length, muscle mass, and fiber type composition.

With age, there is typically a reduction in both shortening velocity and maximal force production, caused by loss of mass and a general shift towards a predominant type I fiber composition, which have greater oxidative capacity, small in cross-sectional area, and relatively slow contractile speed (Close, 1972; Laughlin et al., 1990). Interestingly, eccentric (active lengthening) strength is preserved rather than concentric (active shortening) strength (Porter et al. 1995), suggesting non-contractile elements like extracellular matrix (ECM), tendon, and collagen cross-linkages may have significant role in preserving force (Pousson et al., 2001).

Reduction in motor unit activation capacity and decreases in single fiber specific tension contribute to reduced muscle function with late stage aging (Narici et al., 2003). Though there are many compounding effects that contribute to late life muscle decline, alterations in maximal shortening velocity (V_{max}), peak isometric force production (P_o), peak power (P_{max}), and MTU stiffness are likely to impact mobility the most. With aged muscle, V_{max} reductions can be attributed to reduction in sarcomeres in series (Narici et al. 2003; Raj et al. 2010). Several studies consistently report a range of 20-40% reduction in V_{max} with age (Morse et al., 2005; Raj et al., 2010; Thom et al., 2005; Valour et al., 2003) compared to younger, healthy adults. This reduction in shortening velocity impacts other aspects of muscle functionality, such as a muscle's architectural gear ratio (Azizi et al., 2008; Holt et al., 2016). Longitudinal human studies using isokinetic dynamometers to test contractile velocities between 30^o/s and 300^o/s show

strength declines with age, with patients experiencing 10-22% loss in vastus lateralis strength by the sixth decade of life (Aniansson et al., 1986; Frontera et al., 2000; Gajdosik et al., 1999; Grimby, 1995; Winegard et al., 1996). Reductions in peak power production (along with contractile velocity) are indicative of declining muscle performance with age and are reported to be at minimum 30% less than those of healthy young adults and can be as great as 80% less (Narici et al. 2005; Thom et al. 2005; Toji and Kaneko 2007; Valour et al. 2003). Non-contractile elements play a significant role in declining muscle function with age (Gajdosik et al., 1999). Alterations in series elastic elements (tendons and aponeuroses) from aging result in stiffer muscle-tendon units due to increases in tendon collagen content and changes in microstructure of collagen fibers (Kovanen et al. 1987; Kragstrup et al. 2011; Lieber and Ward 2013; Mays et al. 1988)

Effects of Endurance Training on Age and Musculoskeletal Performance

Endurance training has been shown to reduce the decline of musculoskeletal performance with age as well as recoup lost muscle mass in late stage aging (Aagaard et al., 2010; Chamari, K. et al., 1995; Gollnick et al., 1973; Gosselin et al., 1998; Kirkendall and Garrett, 1998; Kovanen et al., 1984; Lieber, 1992). Force production can be preserved based on a training regimen that utilizes endurance based cardiovascular training (Aagaard et al., 2010; Gollnick et al., 1973), while strength training elicits hypertrophy of existing muscle fibers along with increased muscle fiber recruitment (Kraemer et al., 1995). Traditional

resistance training, power training, and eccentric loading training are deemed the most effective at preserving strength and contractile speed (Ferri et al. 2003; Suetta et al. 2008; Fronterra et al. 1988; Reeves et al. 2005; Labarque et al. 2002; Morse et al. 2007; Petrella et al. 2007). Endurance training is an effective combination of each of these effects for preserving not only overall muscular health (Chamari et al. 1995; Kirkendall and Garrett 1998), but cardiovascular health, cellular health, and collagen fiber content in mice and rats (Allen et al. 2001; Hambrecht et al. 1997; Kovanen et al 1984; Kovanen et al. 1987). Endurance training staves off declines in aerobic power production, showing a significantly smaller reduction compared to anaerobic power production (Chamari et al., 1995). Therefore, endurance training may be used as an effective way to maintain muscle force and power production throughout the process of aging.

Parsing Disuse Atrophy and Aging

Muscles that are not actively used undergo atrophy, wherein energetically costly muscle fibers are metabolized to maintain a relatively low basal metabolic rate (BMR) (Lieber 1992; Lieber and Ward 2013). Muscle atrophy results in reductions in physiological cross-sectional area (PCSA), muscle strength, shortening velocity, and fiber number (Aagaard et al., 2010; D'Antona et al., 2007; Lexell, 1995; Lexell et al., 1988). While disuse atrophy symptoms are often reversible with regular exercise and training, loss of muscle mass via sarcopenia

is non-reversible (Raj et al. 2010). Therefore, a central concern in musculoskeletal aging studies is how to distinguish the effects of disuse atrophy from sarcopenia.

Studies utilizing human models tend to be dominated by either short-term longitudinal (Narici et al. 2005; Thom et al. 2005,2007; Toji and Kaneko 2007; Valour et al. 2003; Labarque et al. 2002) or cross-sectional studies (Narici et al. 2005; Gadjosik et al. 1999; Hortobagyi et al. 1995; Pousson et al. 2001). Although humans are the ideal model organism for biomedical studies, there are shortcomings with using humans as a model organism to investigate the aging process. First, cross-sectional studies do not track the same individual through their entire lifetime, and as such miss crucial behavioral patterns that are not ideal for modeling aging such as compounding injuries that can conflate disuse atrophy with sarcopenia, or CSA changes due to longstanding disuse atrophy symptoms (Narici et al. 2005; Gajdosik et al. 1999; Hortobagyi et al. 1995; Pousson et al. 2001). Due to human longevity, longitudinal studies usually rely on patients self-reporting exercise activity, which is unlikely to be accurate or precise. Additionally, elderly individuals are often sedentary in nature and suffer from a compounding effect of disuse atrophy and sarcopenia (Thom et al. 2007; Toji and Kaneko 2007; Valour et al. 2003; Labarque et al. 2002). Animal models are limited with respect to translational utility, and animals housed in cages with little enrichment for activity also suffer from disuse atrophy (Allen et al. 2001; Brooks and Faulkner 1988; Gosselin et al. 1998; Holt et al. 2016). However,

rodent models are well characterized with regard to muscle anatomy and physiology (Charles et al., 2016; Holt et al., 2016; Horner et al., 2011; Morse et al., 2005), and experience senescence within a feasible observation period of 24-36 months.

Hypotheses

In this study I use a novel model organism, mice selectively bred for high levels of voluntary wheel running behavior, to test the effects of aging on muscle contractile performance. Because the mice used in this study were derived from a breeding experiment wherein mice are selectively bred to exhibit heightened voluntary wheel-running activity the effects of disuse atrophy may be effectively eliminated. These High Runner (HR) mice differ from control (C) counterparts in several aspects including: wheel-running activity, decreased body fat content, increased maximum aerobic capacity, and muscle phenotype showing greater percentages of Type 1 fibers (Houle-Leroy et al., 2003; Meek et al., 2009; Rezende et al., 2006; Swallow et al., 1998). Although previous studies have investigated the combined effects of aging with short periods of training (Narici et al. 2005; Gajdosik et al. 1999; Hortobagyi et al. 1995; Pousson et al. 2001), the effects of maintaining levels of high voluntary aerobic activity throughout ontogeny are not well studied.

This experiment uses voluntary wheel exposure for HR and control mice to test the hypothesis that extreme lifelong voluntary aerobic activity can mitigate

the effects of aging on muscle performance, specifically in maintaining total isometric force production, muscle-tendon unit stiffness, shortening velocity, and power production. If high levels of voluntary activity are sufficient to minimize the impact of aging on muscle performance, then HR mice with lifelong wheel access should demonstrate a less severe reduction in power production and muscle quality than both control groups (with wheel access and without), as well as HR counterparts without wheel access, at all stages of ontogeny.

CHAPTER TWO

MATERIALS AND METHODS

Mouse History and Care

Female mice were obtained from an artificial selection experiment for high voluntary wheel-running activity conducted at the University of California, Riverside. The complete design of the selection protocol is described in detail elsewhere (Swallow et al. 1998; Garland 2003). Mice from an initial population of laboratory mice from the Hsd:ICR strain were used to establish eight separate lines. For each subsequent generation, 6-8 week old mice were solitarily housed with access to Wahman-type activity wheels (1.12m circumference) for 6 days. Daily revolutions were recorded in one-minute intervals. Selection protocol was based on the number of total revolutions on days 5-6. In the four selected (HR) lines, the males and females from each family that had completed the greatest number of revolutions were chosen as breeders. For the four control lines breeders were chosen without regard to the number of revolutions run. Sibling pairing was not allowed. Mice obtained for this study represent generations 72, 74, 79, and 81 of the selection experiment. All experiments were carried out at CSU San Bernardino and were performed in accordance with Institutional Animal Care and Use Committee protocol 14-003 approved prior to the beginning of the experiment. Mice were housed individually for wheel exposure (Columbus Instruments, 30.5cm circumference) and in groups of three to four for non-wheel exposure. Animals were monitored every other day and were kept under

controlled lighting and environmental conditions with a 12h:12h photoperiod. Total daily wheel revolutions were recorded every other day, and water and food were given *ad libitum*.

Experimental Design

The mice for this experiment were separated into treatment groups by selection (HR vs C), age (5 mo., 9 mo., 13 mo.+), and exercise (wheel access vs no wheel access). There was a total of 11 young mice aged 5 months (6 control, 5 HR), 16 adult mice aged 9 months (8 control, 8 HR), and 15 old mice aged 13-35 months (8 C, 7 HR).

Mice from the 5 mo. group were not exposed to wheels and served as baseline data for muscle performance. Adult mice were exposed to wheels for four to five months starting at 4 months of age, and old mice were exposed to wheels for 13 - 35 months starting at 4 months of age. Computer software recorded the home cage wheel activity (Columbus Instruments) every 10 minutes, and these data were compiled to calculate total distance run, average nightly activity, and change in running activity with age. Mice not exposed to wheels were housed in group cages of 3-4 mice for the duration of their lifetime. Two of the HR lines are observed to have a significant reduction in muscle mass compared to “normal” muscled individuals. This “mini-muscle” phenotype was originally present in the base population and has reached fixation in one HR line and an ~50% frequency in another HR line. Particular care was taken to avoid

the mini-muscle phenotype, which has been shown to result in 45-54% decreased muscle mass throughout ontogeny (Syme et al., 2005).

Surgical Setup and Procedure

Experiments were performed on the plantar flexor muscle group (medial and lateral gastrocnemii, soleus, and plantaris) of baseline, adult, and old female mice. Muscle contractile performance and passive properties were measured *in situ.* Mice were anesthetized prior to each *in situ* surgical procedure for three to five minutes and maintained at 2.5 – 3.5% isoflurane using closed system anesthesia (Parkland Scientific, Coral Springs, FL, USA). Mice were placed supine on a small animal heating pad. The sciatic nerve was exposed via a small caudal incision on the thigh. A stimulating nerve cuff was placed around the nerve, and the nerve severed proximally. The area of the nerve was sutured and a pocket was left for administration of mineral oil *ad libitum*. The calcaneal tendon was then isolated and severed from the foot with the insertion at the calcaneus intact, and the plantar flexor muscle group freed from the shank. Kevlar thread was tied around the calcaneal tendon, and a small incision then made on the lateral aspect of the thigh to expose the femur for placement of a bone clamp. The mouse was then moved to a small stage, where the femur clamp was connected to a stationary arm to minimize off-axis rotation, and the calcaneal tendon attached to the lever arm of a servomotor (305 C-LR, Aurora Scientific Inc., ON, Canada). The muscle group was kept wet with saline and the core temperature of the animal was maintained at 37^0C.

Force-Velocity Acquisition and Data Analysis

Force velocity measurements were recorded and calculated in real time using a custom-made procedure in Igor Pro Ver.6.0. Muscle contractions were administered via a supramaximal square wave pulse (duration 20 ms, frequency 80-100Hz) to the sciatic nerve (Holt and Azizi, 2014). Twitch contractions were administered to determine optimal voltage, and optimal muscle length. All subsequent contractions were performed at the optimal voltage and length. An isometric tetanic contraction was performed to determine maximal force (F_0), with subsequent isotonic tetanic contractions performed at a range of forces between 0.1-0.9 F_0 (Fig 1&2). V_{max} was calculated by using the Hill equation (Hill, A.V., 1938).

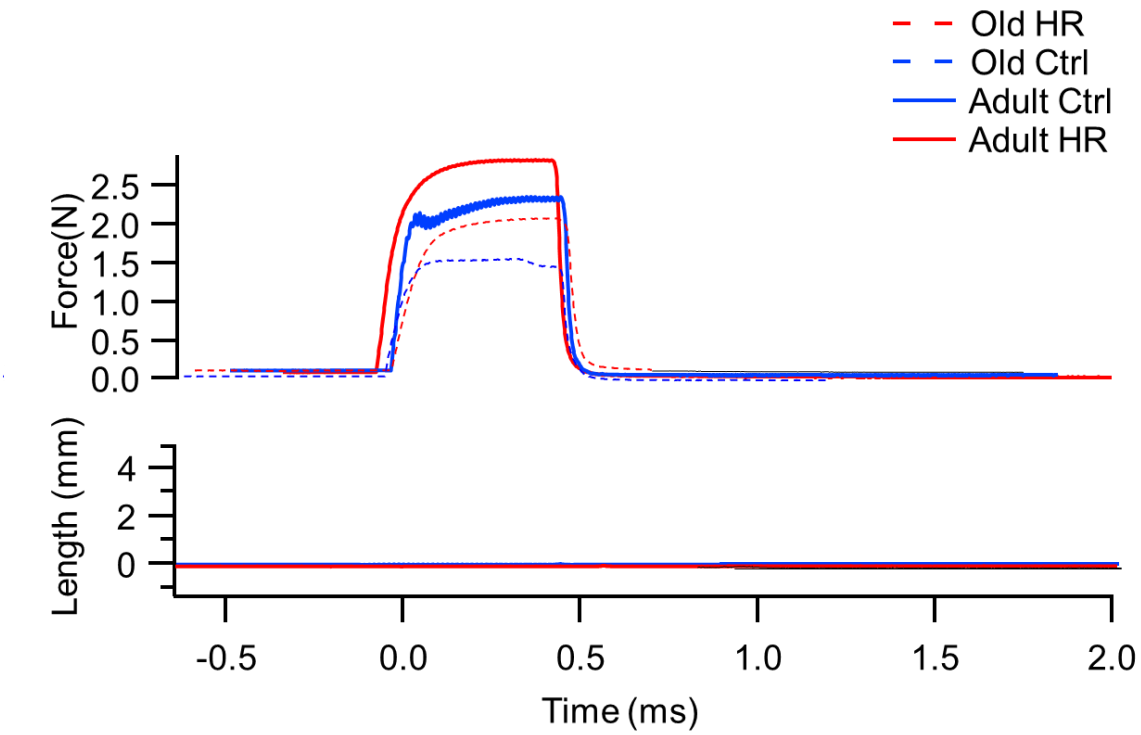

Figure 1. Sample isometric contractions of the plantar flexor muscles for representative untrained HR and C mice, both adult and old at resting muscle length (Lo).

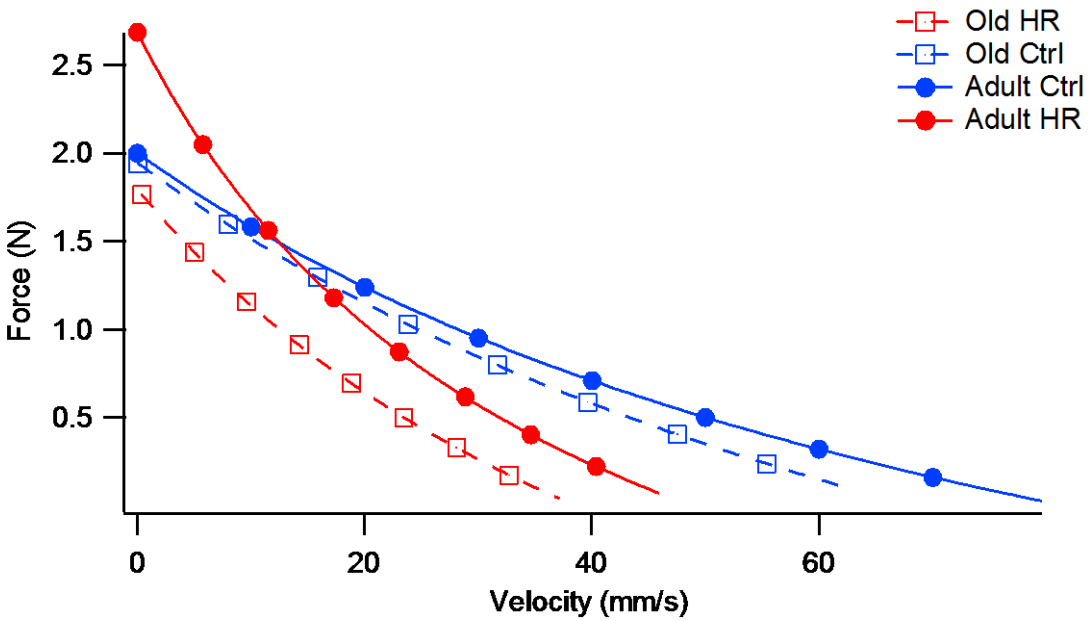

Figure 2. Experimentally derived force-velocity curve of the plantar flexor muscles for representative individuals of untrained HR and C mice, both adult and old.

Following the final contraction, passive sine waves were generated at varying lengths and frequencies (amplitude= 1.0,1.5mm, frequency= 2.5Hz) to assess passive stiffness and resilience of the plantar flexor MTU (Fig 3).

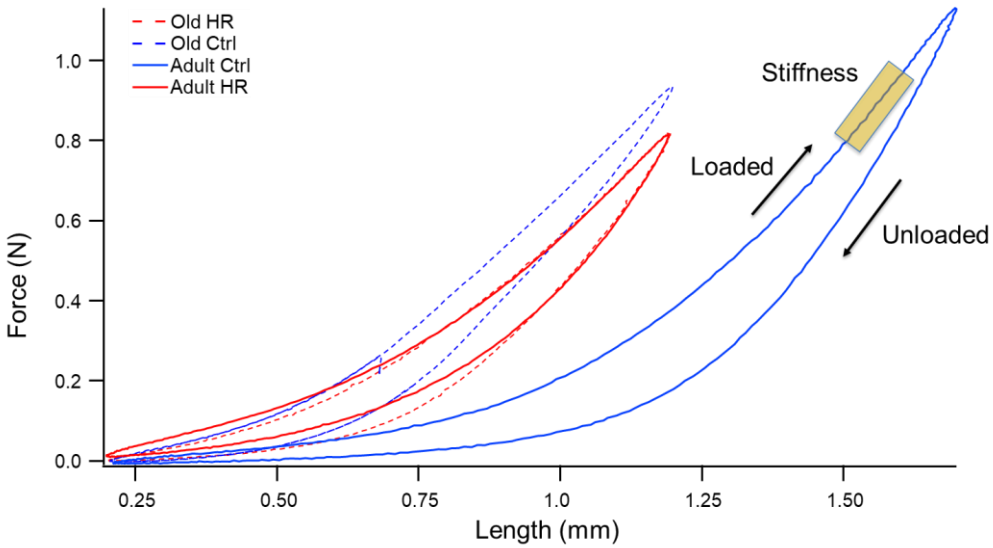

Figure 3. Representative passive force length curve**.** Stiffness was calculated via the slope of the steepest part of the loaded portion of the curve and reported in N/mm in Table 1 and Fig 4.

Mice were euthanized via a coelomic overdose injection of sodium pentobarbital. Plantar flexor length at optimum passive tension was measured. Plantar flexor muscles were dissected out for weighing (±0.001g). ANOVAs were

performed with age (5-32 mo.), training (Wheel access/No wheel), and line (HR/C) as main effects, and within group and between group comparisons were made in JMP Version 7.2.

CHAPTER THREE

RESULTS

A summary of means for contractile and passive properties of the plantar flexor group for selection, wheel access, and age is displayed in Table 1.

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Peak Isometric Force

Peak isometric force production (P_0) was significantly greater within C mice in adult compared to young (P=0.0021) and adult compared to old (P=0.017). There was no significant wheel access effect on P_0 for C mice. When normalized to mass, muscle quality in adult C mice was greater than old mice (P=0.0012) and young mice (P<0.0001). For HR mice, P_0 did not differ significantly for either age or wheel access. Muscle quality in HR mice was significantly greater in adult mice than old mice (P=0.018). For baseline mice, C mice had significantly lower P_0 than HR mice (P=0.0169).

Muscle Quality

Baseline C mice had significantly lower muscle quality than young HR mice (P=0.00017). For adult mice, there were no significant differences for selection or wheel access on peak isometric force. However, adult C mice had significantly lower muscle quality than HR mice (P=0.013). For old mice, there were no significant differences for selection or wheel access for either peak

isometric force or muscle quality. When pooled, young mice P_0 was significantly less than adult P_0 (P=0.0094) and adult P_0 was significantly greater than old P_0 (P=0.0043). There were no significant differences based on wheel access or line type. However, muscle quality was significantly greater in HR mice than C mice (P=0.0006) regardless of age or wheel access.

Shortening Velocity

V_{max} did not differ significantly in C mice for either age or wheel exposure. V_{max} did not differ significantly in HR mice for either age or wheel exposure. In old age without wheel access, C mice had significantly greater V_{max} than HR mice (P=0.026).

Peak Power

For C mice, peak power was significantly greater in adult mice compared to old mice (P=0.038). This was also true when power was normalized to muscle mass (P=0.026). Peak power did not vary significantly between young and adult or young and old. Peak power did not vary significantly due to wheel access. For HR mice, peak power did not vary due to either age or wheel access. Peak power did not differ significantly with age. For young mice, peak power did not differ significantly between C or HR. For adult mice, there were no significant differences in peak power production for either selection or wheel access. There were no significant differences in old mice for power production due to selection or wheel access. When controlled for muscle mass, Old mice that had access to

wheels had significantly greater peak power production than those without, regardless of selection (P=0.034).

MTU Stiffness

For C mice, young mouse MTU stiffness was less than adult mice (P=0.045). HR mouse MTU stiffness did not differ significantly in either age or wheel access. Young C mice had significantly less stiff MTU's than young HR mice (P=0.013). There were no significant differences in adult mice. There were no significant differences in old mouse MTU stiffness.

Body Mass

Body mass in C mice was significantly greater in old mice compared to adult mice (P=0.038). It did not significantly differ with wheel access. Adult HR mice were significantly heavier than young HR mice (P=0.02), but did not differ significantly from old counterparts. Pooled body mass did not differ in young or adult ages for either selection or wheel access. However, old HR mice body mass was significantly less than old C mice (P=0.02).

Muscle Mass

C mice muscle mass did not differ significantly with either age or wheel access. HR muscle mass did not differ significantly with either age or wheel access. C mice had significantly more mass than HR mice at young age (P=0.039). Muscle mass did not differ significantly at adult ages for either line

type or wheel access. Muscle mass did not differ significantly in old age for either line type or wheel access.

Plantar Flexor Length

For C mice, plantar flexor whole muscle group length was significantly longer in adults compared to young ($P=0.0078$). Adult muscle length was also significantly longer than old mice ($P=0.0067$). Wheel exposed mice had significantly longer muscles than those not wheel exposed ($P=0.0044$). For old mice, mice that were exposed to wheels had significantly longer muscles than those without ($P<0.0001$). For HR mice, there were no significant differences in muscle length for either age or wheel exposure. For young mice, there were no significant differences in muscle length. For adult mice, there was a significant selection difference, with HR mice having shorter muscles than C mice ($P=0.019$). For old mice, ones that were exposed to wheels had significantly longer muscle length, regardless of selection ($P=0.049$).

Table 1. Animal, muscle and connective tissue properties that were normalized to triceps surae mass in young, adult, and old mice**.** Mean values (Avg) with Standard Error of Measurement (S.E.M.). (*) indicates P< 0.05 (pair-wise comparison).

	Young				Adult				Old			
No Wheel	Control (n=6)		HR (n=5)		Control (n=4)		HR (n=4)		Control (n=3)		HR (n=3)	
	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
Animal Mass (g)	29.957	1.317	26.956	0.932	30.203	2.001	32.357	1.687	36.597	2.953	33.975	1.160
Muscle Mass (g)	0.160	0.108	0.130	0.011	0.146	0.013	0.137	0.014	0.117	0.014	0.122	0.010
Triceps Surae Length (mm)	15.537	0.108	15.348	0.338	16.518	0.135	14.212	0.668	14.160	0.204	14.283	0.177
Peak Isometric Force (N)	1.400	0.094	1.982	0.162	2.260	0.153	2.489	0.315	1.146	0.108	1.391	0.417
Vmax(mm/s)	68.403	6.051	57.519	6.148	64.218	3.530	68.377	7.847	54.825	6.868	58.783	12.884
Muscle Quality (N/gmuscle)	8.820	0.662	15.539	1.287	15.604	0.757	17.154	1.447	10.109	1.316	10.476	2.618
Peak Power (W/gmuscle)	0.136	0.016	0.160	0.017	0.192	0.023	0.135	0.057	0.092	0.031	0.111	0.039
MTU Stiffness	0.388	0.020	0.532	0.040	0.590	0.044	0.530	0.086	0.498	0.072	0.561	0.014

	Adult				Old			
Wheel	Control (n=4)		HR (n=4)		Control (n=4)		HR (n=3)	
	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
Animal Mass (g)	32.975	0.665	29.708	1.334	36.633	2.225	26.827	0.585
Muscle Mass (g)	0.157	0.003	0.122	0.013	0.160	0.010	0.126	0.012
Triceps Surae Length (mm)	15.345	0.238	14.530	0.566	16.083	0.355	14.893	0.869
Peak Isometric Force (N)	2.205	0.092	2.301	0.322	1.933	0.072	1.845	0.066
Vmax(mm/s)	66.216	2.178	55.538	5.716	72.542	3.226	69.819	8.305
Muscle Quality (N/gmuscle)	14.015	0.382	18.650	0.754	12.207	0.732	15.141	1.713
Peak Power (W/gmuscle)	0.195	0.011	0.155	0.026	0.175	0.020	0.202	0.026
MTU Stiffness	0.548	0.042	0.733	0.034	0.404	0.026	0.425	0.015

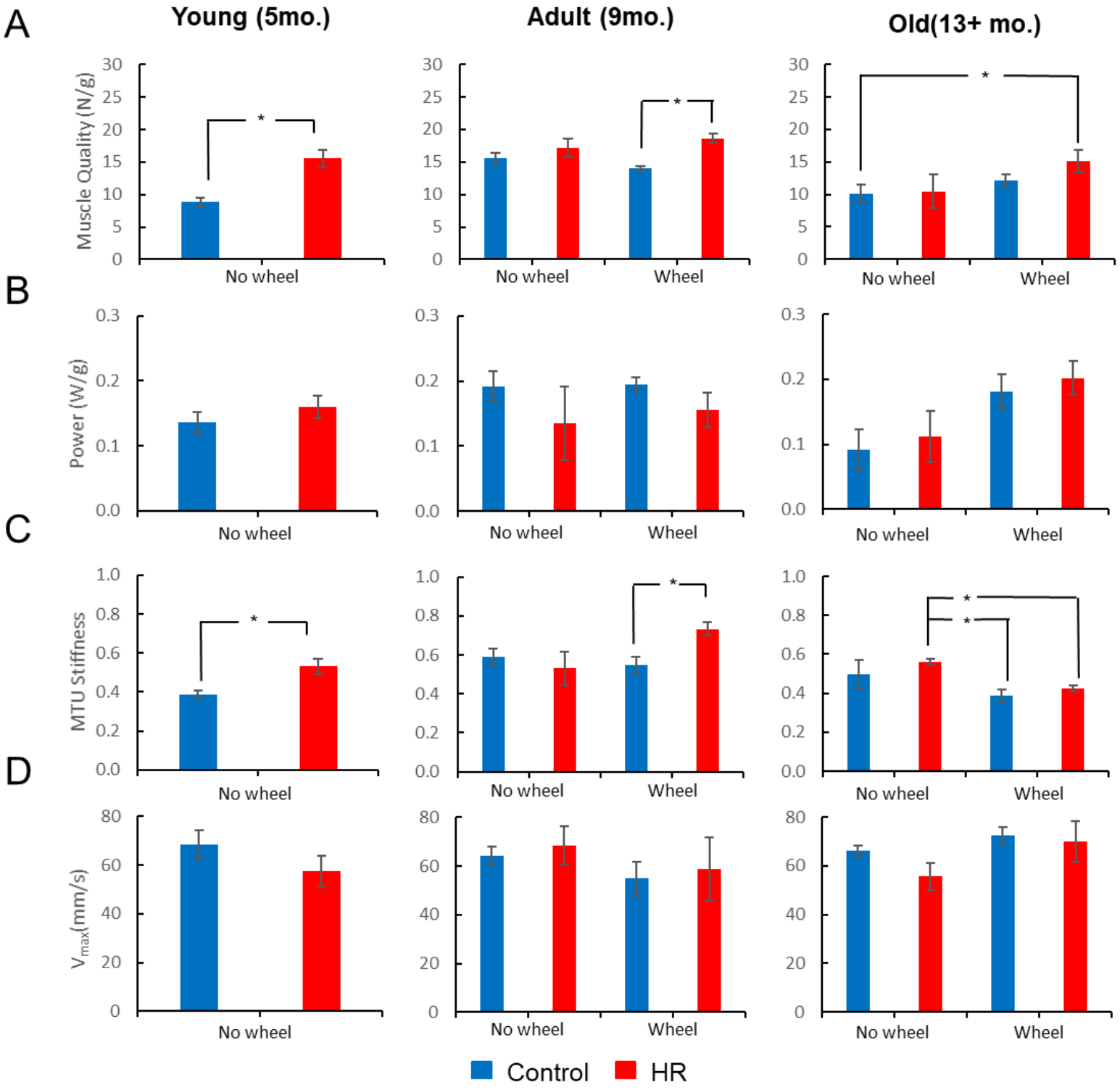

Figure 4. Measurements of muscle quality, shortening velocity, peak power, and MTU stiffness as a function of age and training. Triceps surae muscle quality (N/g)(A), Peak Power (W/g)(B), Muscle Tendon Unit Stiffness (MTU)(C), and Maximal shortening velocity (mm/s)(D) are shown for young, adult, and old C and HR mice, with and without wheel access . Bar graphs show average values with error bars reflecting S.E.M. Significances of $P<0.05$ are shown with a (*).

A

	Age (mo.)	Body mass(g)	Muscle Mass(g)	Muscle Length (mm)	P_0(N)	V_{max} (mm/s)	Muscle Quality (N/g)	MTU Stiffness (N)	Peak Power (W/g)
Age (mo.)	-	0.35	0.17	**0.008***	0.71	0.07	0.25	**0.001***	**0.006***
Body mass(g)		-	0.96	**0.024***	0.59	0.78	0.55	0.26	0.37
Muscle Mass			-	0.23	0.35	0.77	0.29	0.82	0.61
Muscle Length (mm)				-	**0.009***	0.78	0.077	0.34	0.073
P_0(N)					-	0.91	**0.0007***	0.07	**0.049***
V_{max} (mm/s)						-	0.98	0.67	**0.016***
Muscle Quality (N/g)							-	0.11	0.058
MTU Stiffness (N)								-	0.36
Peak Power (W/g)									-

B

	Age (mo.)	Body mass(g)	Muscle Mass(g)	Muscle Length (mm)	P_0(N)	V_{max} (mm/s)	Muscle Quality (N/g)	MTU Stiffness (N)	Peak Power (W/g)
Age (mo.)	-	0.07	0.10	0.29	0.69	0.07	0.81	0.63	0.89
Body mass(g)		-	0.86	**0.047***	0.19	0.99	**0.039***	0.98	0.48
Muscle Mass			-	0.43	**0.0003***	0.34	**0.0002***	0.41	0.66
Muscle Length (mm)				-	0.78	0.12	0.43	0.49	0.21
P_0(N)					-	0.62	0.11	0.83	0.14
V_{max} (mm/s)						-	0.18	0.94	**0.004***
Muscle Quality (N/g)							-	0.90	**0.007***
MTU Stiffness (N)								-	0.22
Peak Power (W/g)									-

C

	Age (mo.)	Body mass(g)	Muscle Mass(g)	Muscle Length (mm)	P_0(N)	V_{max} (mm/s)	Muscle Quality (N/g)	MTU Stiffness (N)	Peak Power (W/g)
Age (mo.)	-	0.19	0.86	0.77	0.35	0.27	0.14	**0.0014***	0.091
Body mass(g)		-	0.089	0.83	0.24	0.60	0.84	0.27	0.18
Muscle Mass			-	0.18	**0.0028***	0.97	**0.0028***	0.15	0.06
Muscle Length (mm)				-	0.56	0.41	0.85	0.82	0.60
P_0(N)					-	0.99	0.46	0.56	0.72
V_{max} (mm/s)						-	0.18	0.54	**0.0044***
Muscle Quality (N/g)							-	0.20	0.87
MTU Stiffness (N)								-	0.38
Peak Power (W/g)									-

D

	Age (mo.)	Body mass(g)	Muscle Mass(g)	Muscle Length (mm)	P_0(N)	V_{max} (mm/s)	Muscle Quality (N/g)	MTU Stiffness (N)	Peak Power (W/g)
Age (mo.)	-	0.69	0.34	0.59	0.41	0.28	0.40	0.94	0.70
Body mass(g)		-	0.20	0.24	0.19	0.79	0.12	0.96	0.62
Muscle Mass			-	0.43	**0.0003***	0.34	**0.0002***	0.41	0.66
Muscle Length (mm)				-	0.78	0.13	0.43	0.49	0.20
P_0(N)					-	0.62	0.11	0.83	0.14
V_{max} (mm/s)						-	0.18	0.91	**0.0064***
Muscle Quality (N/g)							-	0.9316	**0.0049***
MTU Stiffness (N)								-	0.22
Peak Power (W/g)									-

E

	Age (mo.)	Body mass(g)	Muscle Mass(g)	Muscle Length (mm)	P_0(N)	V_{max} (mm/s)	Muscle Quality (N/g)	MTU Stiffness (N)	Peak Power (W/g)
Age (mo.)	-	**0.039***	**0.0046***	0.58	**0.028***	0.12	**0.027***	0.28	0.14
Body mass(g)		-	0.96	0.063	0.85	0.88	0.53	0.10	0.76
Muscle Mass			-	0.086	**<0.0001***	0.21	**<0.0001***	0.48	0.29
Muscle Length (mm)				-	0.13	**0.025***	**0.025***	0.86	**0.014***
P_0(N)					-	0.37	**0.0019***	0.41	0.66
V_{max} (mm/s)						-	**0.0031***	0.64	**<0.0001***
Muscle Quality (N/g)							-	0.11	**0.0002***
MTU Stiffness (N)								-	0.08
Peak Power (W/g)									-

F

	Age (mo.)	Body mass(g)	Muscle Mass(g)	Muscle Length (mm)	P_0(N)	V_{max} (mm/s)	Muscle Quality (N/g)	MTU Stiffness (N)	Peak Power (W/g)
Age (mo.)	-	0.68	0.64	0.79	0.51	0.60	0.47	0.1	0.68
Body mass(g)		-	0.97	0.38	0.89	0.19	0.82	0.73	0.38
Muscle Mass			-	0.27	**<0.0001***	0.4	**<0.0001***	0.78	0.087
Muscle Length (mm)				-	0.18	0.42	0.12	0.76	0.61
P_0(N)					-	0.20	0.31	0.11	0.87
V_{max} (mm/s)						-	0.14	0.46	**0.0012***
Muscle Quality (N/g)							-	**0.0027***	0.45
MTU Stiffness (N)								-	0.43
Peak Power (W/g)									-

Figure 5. Data tables reflecting P values for ANOVA analyses for within group and between group comparisons. A = C mice without wheels. B = C mice with wheels. C = HR mice without wheels. D = HR mice with wheels. E = C vs HR mice without wheels. F = C vs HR mice with wheels.

CHAPTER FOUR

DISCUSSION AND CONCLUSIONS

This study sought to elucidate the effects of life-long aerobic training on contractile properties in muscle to test the hypothesis that extreme lifelong voluntary aerobic activity can mitigate the effects of aging on muscle performance, specifically in maintaining total isometric force production, muscle-tendon unit stiffness, muscle quality, and power production.

In the present study, all HR mice with access to wheels had higher muscle quality than controls without wheel access throughout ontogeny. HR mice with wheel exposure did not differ significantly from their control counterparts in either adult or old groups for peak isometric force, but old age, wheel-exposed mice had greater overall force production than non-wheel exposed lines. However, for muscle power, old HR mice that had been exposed to wheels did not differ significantly from adults who had the same treatment, where control mice from the same cohort experienced a large decline in peak power reduction with age and this same group had greater muscle power in old age compared to non-wheel lines (Table 1, Fig.4C), suggesting that there is a selection and training effect on peak power production in old age. These data suggest that voluntary wheel running significantly impacts muscle quality in late age, and limits peak power production loss with age.

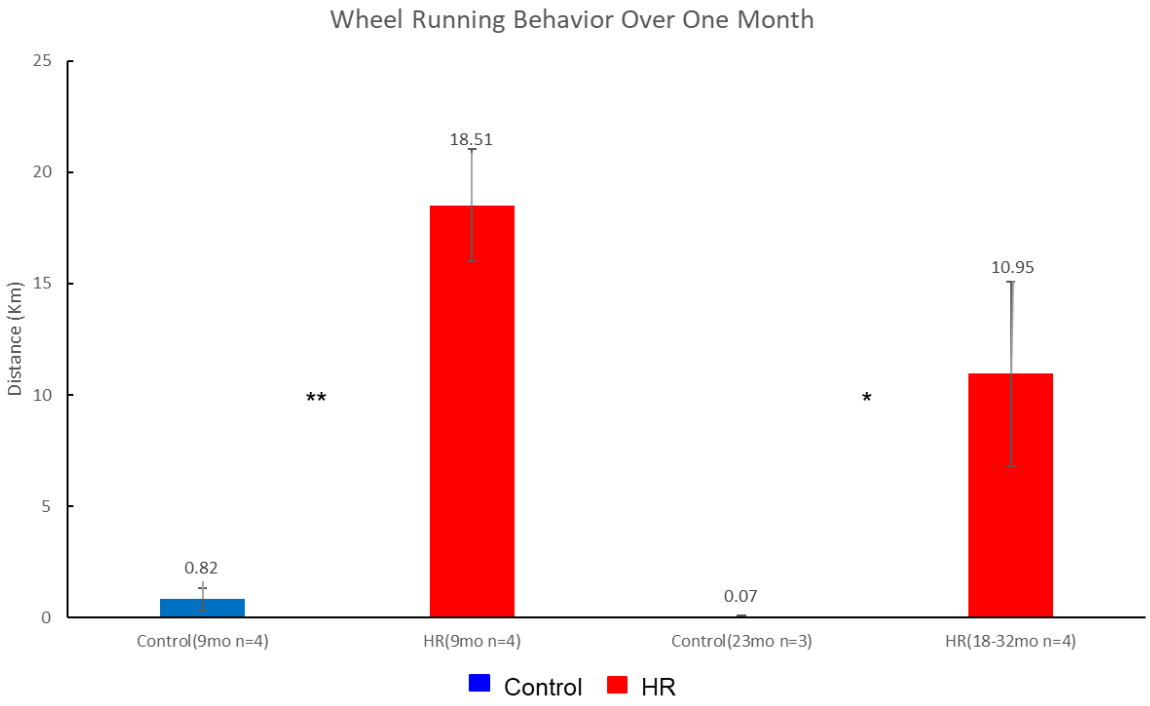

Figure 6. Average total running distance in the last month of life before *in situ* procedure for wheel exposed control and selected mice. Distance run is total kilometers in the last month of life.
*P < 0.05, **P <0.01. Error bars reflect S.E.M.

The HR lines of mice typically run about three times as much as C mice at young adult ages (Rezende et al., 2009). HR mice accomplish this by running longer, farther, and faster than their C mice counterparts in more recent generations (Hiramatsu and Garland, 2018; Singleton and Garland, 2019). Traditional endurance studies expose animals to anywhere from 45-60 minutes a day for 8 to 15 weeks (Davies et al., 1981; Gomez-Cabrera et al., 2008; Gosselin et al., 1998; Green et al., 1983; Guasch et al., 2013; Mazzeo et al., 1984), with one study undergoing life-long forced endurance training for up to two years (Kovanen et al., 1987). The utilization of a lifelong voluntary exercise model as a

metric of evaluating sarcopenia is lacking in the literature. The data in figure 5 represent the average total distance run in the last month of life for a subset of C and HR mice at both adult and old age ranges. Given the choice, C mice have significantly lower wheel running behavior at as early as 9 months, compared to HR mice which maintain significantly higher levels of running behavior ($P<0.0001$) (Fig. 5). HR mice differ in that the duration of intermittent bouts of locomotion is much longer compared to C mice. As evidenced in Fig. 6, on a monthly basis, HR mice are out running C mice by more than two orders of magnitude in late age.

The results from this study corroborate previous findings that peak isometric force, maximal shortening velocity, and power production peak at maturation and decline with late age (Gajdosik et al., 1999; Hortobágyi et al., 1995; Narici et al., 2005; Thom et al., 2005) (Fig.4, Table1&2). The mice in this study show a downward and leftward shift in the force-velocity relationship, indicating a loss in force production at slower contractile velocities, but with without any noticeable declines in force production at greater contractile velocities.(Table 1&2) (Raj et al., 2010). Previous studies show that with age, type II fibers decrease in CSA, resulting in dramatic declines in force production (Lexell, 1995). The higher peak isometric force generated at young and adult ages within the HR mice could mean HR mice have larger type II fibers (which have larger CSA compared to type I fibers) than their control counterparts, who appear to follow the traditional etiology of muscle force production with age

(Frontera et al., 2000). In a prior study (Guderley et al., 2008), HR mice expressed increased percent type IIA fibers and increased surface area of type I fibers than control counterparts. These changes would be expected to result in greater muscle force production at lower contractile speeds, with some likely tradeoff in force production at greater contractile speeds. It has also been noted that in advanced age, skeletal muscles tend to co-express myosin heavy chain (MHC) isoforms in various percentages (Andersen et al., 1999). While the clinical significance of this is uncertain, it could be an underlying factor in old muscle performance, especially in the HR lines.

Maximal shortening velocity typically decreases with advanced age and increases with exercise (Kraemer et al., 1995; Thom et al., 2005). Maximal shortening velocity has no single anatomical basis, although it is related to the number of fibers arranged in series and fiber type. Rate of ATP consumption is faster in fast-glycolytic fibers (type IIb) than that of slow-oxidative or fast-oxidative-glycolytic (type I and type IIa, respectively), and as such type IIb have faster fibers than type IIa or type I. Therefore, fiber composition and fiber length both influence the force-velocity relationship. The findings of this study coincide with expressed V_{max} values of soleus muscles in young, adult, and old mice (Brooks and Faulkner, 1988). Previous studies routinely report that muscle fascicle length decreases with age, thus producing force over a smaller muscle length range, and therefore result in reduced maximum shortening speeds (Blazevich and Sharp, 2005; Morse et al., 2005; Narici et al., 2003). However, in

this study V_{max} did not differ significantly with age. While young control mice had higher V_{max} than young HR mice, this effect was reversed in adults (Fig.4D, Table 1). Though these factors were not significant, they follow expected trends with age from previous studies (Narici et al., 2005; Thom et al., 2005). V_{max} is expected to decline with age, as sarcomeres are lost in series (Lexell et al., 1988; Raj et al., 2010) and overall fiber length decreases (Korhonen et al., 2006; Larsson et al., 1997; Ochala et al., 2007). Throughout ontogeny, V_{max} did not differ significantly between groups. This phenomenon could be explained due to muscle fiber length in the plantar flexors showing minimal change in late age (Gajdosik, 2001; Morse et al., 2005). While fiber type changes in a single muscle fiber typically shift to a significantly lower V_{max} with age, entire muscle groups can respond differently. The plantar flexors in particular are of common interest. Several studies have looked at the entire muscle group in humans, and found that V_{max} declines in old age, but is comparable to young individuals being at 84-86% capacity of adults (Narici et al., 2005; Thom et al., 2005).

Muscle power peaks in adult age and declines with age (Table 1, Fig. 4B). These trends follow expected results of muscle power changes with age, where muscle power should experience a downward and leftward shift on the power velocity curve, resulting in lower power production at higher contractile velocities (Raj et al., 2010). While there has been some debate on the mechanism for power loss in skeletal muscle with age, a reasonable amount of evidence suggests that reductions in the capacity to produce force are the root cause

(Chamari, K. et al., 1995; Narici et al., 2005; Raj et al., 2010). In control mice that were not exposed to wheels, peak power declined by 52% in old age (Table 1).These results coincide with expected power production in old age, where peak power of plantar flexors decreased between 48-54% of healthy adults (Narici et al., 2005; Thom et al., 2005).

Aging is associated with significant changes in the connective tissue content of muscle and tendon. With age, collagen turnover rates decreases, which may increase stiffness (Gosselin et al., 1994; Kovanen and Suominen, 1988). Also with age, non-contractile material accumulates in muscle, restricting biaxial strain active force production (Kragstrup et al., 2011). Although these phenomena are predicted to cause stiffer MTUs, other studies suggest that tendon becomes more compliant with advanced age (Dressler et al., 2002; Viidik et al., 1996) or not change at all (Nakagawa et al., 1996). The results of this study show stiffness increasing with maturation, and decreasing with advanced age (Fig.4C, Table 1), a trajectory supported by previous studies (Alnaqeeb et al., 1984; Distefano and Goodpaster, 2018). In the baseline cohort, HR mice have significantly stiffer MTUs, but at maturation C and HR mice stiffness is not discernably different. With old age, there is no significant difference in MTU stiffness for selection, and their stiffness values are nearly identical to the baseline mice. MTU stiffness typically increases in age for several reasons. MTU stiffness will increase through adulthood due to MTU CSA increasing and fascicle length increasing minimally. This combined with stiff tendons to facilitate force

transfer between muscle and bone gives rise to increased stiffness in adult age. MTU stiffness can decline in advanced age if training is introduced.

Traditional studies analyzing muscle contractile property response to strength training in humans report increases in peak isometric force at slow and medium contractile speeds, resulting in an upward and leftward shift of the force-velocity curve (Andersen et al., 2005; Häkkinen et al., 1985). However, these studies report that training appears to have negligible effects on unloaded shortening velocity, and that only with subsequent detraining are there gains in unloaded maximal shortening velocity. While shortening velocity increases, force production decreases, and appear to be the result of increased expressions of myosin heavy chain proteins (MHC), MHC II and decreased expressions of MHC I (Andersen et al., 2005). A prior study reports that endurance training in humans helps prevent the loss of muscle power production via maintaining muscle strength, but makes no comparison to a control group (Chamari, K. et al., 1995). Other endurance training studies show that high-intensity endurance training results in no significant increases to muscle force production compared to controls, with significant increases in type IIa/x fiber percentages, along with significant decreases in type I and IIb percentages (Allen et al., 2001; Bell et al., 2000; Harber et al., 2009; Kraemer et al., 1995). These studies also report losses of fiber area of type I and IIx fibers in the endurance trained group. This change has been attributed to aerobic training causing metabolic shifts that require myofibrillar protein degradation in order to facilitate oxygen uptake (Klausen et

al.; Weibel et al., 1981). There are foundational studies that also address the divergent nature of compounded strength and endurance exercise, showing that endurance training often results in depletion of strength gains accrued in strength-focused exercises (Dudley and Djamil, 1985; Hickson, 1980; Hunter et al., 1987).

In this study, voluntary wheel running resulted in significantly greater $P_{0,}$ peak power, and muscle quality than mice without access to wheels (Fig 4 A,C, Table 1). There were no significant training effects on V_{max}. The retention of muscle quality with training in old age by the HR line and not the control line suggests that there is a significant positive training effect on muscle quality in late age, which while it was not analyzed in this study, could be linked to an increase in CSA of type I and type IIa fibers in HR lines. Old HR mice extreme voluntary wheel running through senescence results in comparable muscle power to adult age (Fig.4C, Table 1). However, for relative power (Watts/g), C mice lose power production from adult to old age even when exposed to wheels, while the HR mice gain power production in old age with access to wheels (Table 1). When correlated with behavioral data (Fig. 5), it follows that HR mice will have a greater response to training effects compared to C mice.

Endurance training is associated with increased collagen synthesizing enzymes present in both skeletal and cardiac muscle, without increasing the amount of collagen in the muscle, which suggests that endurance training facilitates high collagen turnover rates within muscle fibers (Kovanen and

Suominen, 1989; Takala et al., 1991). The increased rate of collagen turnover results in decreasing the amount of collagen cross linking that occurs in muscle of older animals, which results in more compliant MTU's (Gosselin et al., 1998). In endurance trained rats, training results in MTU stiffness properties that are equivalent to that of young trained individuals (Gosselin et al., 1998). Here, extreme voluntary endurance training appears to significantly increase MTU stiffness in adult age compared to baseline mice (Fig. 4D), but this effect is lost with old age. In old age, wheel exposure results in significantly more compliant MTU's compared to no wheel access (Fig. 4C, Table 1).

Age related declines in muscle are difficult to assess in isolation of disuse atrophy, which often accompanies the aging process. The use of extreme life-long voluntary wheel running in mice provides insight into the specific effects of the aging process on muscle performance via control of behavior. Peak isometric force and maximal shortening velocity peaked in adult age, then showed decline with advanced age, regardless of selection or training. There were no training effects on P_o and V_{max}, even with extremely active mice (Fig.5) and very old mice (up to 32mo.). P_0 loss occurred in both lines and treatments, but are likely due to different mechanisms. P_0 loss is attributed to loss in muscle CSA, muscle mass, increases in connective tissue, and reduced neural activation (Raj et al., 2010). While most of these metrics were not measured here, muscle mass declined for both C and HR lines with age, regardless of exercise. HR mice had absolutely smaller plantar flexors than C mice for all cohorts and had absolutely greater

muscle quality compared to C mice across all cohorts (Table 1). HR mice possess greater mass-specific force production than controls, which is likely due to HR mice having type II fibers of larger CSA compared to controls. It is possible that control mice experience greater atrophy of existing type II fibers than HR mice (Hunter et al., 2004). . Potentially, HR mice can accrue significant gains in their type II fiber CSA post third trimester of life (Coggan et al., 1992). Lack of significant changes in V_{max} and P_{max} from adult to old age and exercise in this study could be attributed to plantar flexor muscles being resistant to sarcopenia, with old muscles performing comparably to younger ones (Morse et al., 2005). Changes to MTU stiffness is partly due to increased connective tissue content in muscle ECM (Gao et al., 2008; Kjær, 2004; Kragstrup et al., 2011) and fat content (Kent-Braun et al., 2000).

The results of this study show that while life-long voluntary exercise does not significantly preserve peak isometric force production from adulthood to old age, it does attenuate losses in muscle quality and peak power production, making muscle performance in old age comparable to performance in adulthood for female rodents. In this study, life-long voluntary exercise results in positive changes with force production per unit mass in the entire plantar flexor muscle group. Although tissue level mechanisms are difficult to correlate, this likely affects whole animal functionality, such as range of movement, foraging ability, and predator-prey interaction. While this study is limited in tissue-level mechanisms which analyze performance of the plantar flexor complex, future

studies which address individual muscles of animals with life-long voluntary exercise will reveal the degree to which results of this treatment are based on the entire muscle group, or rooted in an individual muscle.

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