8-2022

Early-Exercise Effects on Mice Tendon Remodeling

Miles Valencia

EARLY-EXERCISE EFFECTS ON MICE TENDON REMODELING

A Thesis

Presented to the

Faculty of

California State University,

San Bernardino

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in

Biology

by

Miles Miguel Valencia

August 2022

EARLY-EXERCISE EFFECTS ON MICE TENDON REMODELING

A Thesis

Presented to the

Faculty of

California State University,

San Bernardino

---

by

Miles Miguel Valencia

August 2022

Approved by:

Angela Horner, Committee Chair, Biology

Tomasz Owerkowicz, Committee Member, Biology

Jenna Monroy, Committee Member, Biology, Claremont McKenna College

ABSTRACT

Tendons connect and transmit energy between muscles and bones and play a key role in movement. Tendon remodels by breaking down and absorbing tendon components including collagen fibers and elastin while replacing them with newly formed tendon. These processes can be influenced by short- and long-term factors such as exercise and aging; the magnitude of influence on tendon remodeling remains unclear. I researched the effects of maturation and exercise on tendon remodeling using a mice colony artificially selected for high voluntary wheel running called high-runner mice. Control and high-runner linetype mice were separated into 2 age cohorts that started training at 3- and 9-weeks of age, which correspond to before and after skeletal maturity. Each cohort was divided into 3 training groups that vary in exercise intensity: wheel running (high-frequency, low-impact), jumping (low-frequency, high-impact), and a sedentary group. After 9 weeks of training, gastrocnemii tendons were harvested. I measured gross tendon morphology and applied materials testing for measuring mechanical properties. The results show minimal significant differences between tendon mechanics suggesting that training intensity does not have a significant impact during maturation; however, linetype had a significant effect on tendon length, which is suggestive of cursorial evolution. Cursorial animals have evolved longer, thinner tendons capable of storing more elastic strain energy for more efficient locomotor behaviors, but it remains questionable if this is the case for high-runner mice. Future studies should

investigate whether early-life exercise impacts lifelong mechanical properties measurable in advanced-aged tendon. These studies should be paired with histological methods to quantify concurrent changes in tendon composition.

ACKNOWLEDGEMENTS

I would first like to thank my thesis advisor Dr. Angela Horner. I started this program feeling insecure in my abilities as a junior scientist, but Dr. Horner steadily built my self-confidence throughout my program. She helped me set challenging goals and deadlines that I thought were daunting at first but are now attainable.

I would also like to thank Dr. Jenna Monroy of W.M. Keck Science Department at Claremont McKenna College for lending her servomotor, which was absolutely vital for completing my research.

I would like to acknowledge the Office of Student Research of CSU San Bernardino for providing the travel and research supplies grants. These grants helped finance research equipment and traveling expenses for completing and presenting my research.

DEDICATION

I must express my deep gratitude for my parents, siblings, and wife Margo for your endless encouragement throughout my academic career that helped me move forward even when times got tough. Also, I will never forget the financial support of my Auntie Julie and Uncle Jerry Kline. Minimizing my financial burden allowed me to really immerse myself in my studies and helped me excel in my research. My family has been an incredible source of support, and this accomplishment would not have been possible without them.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

Vertebrates have evolved to occupy aquatic, terrestrial, and aerial environments, which has been possible because of the evolution of diverse musculoskeletal designs. Locomotor abilities have evolved to accommodate these diverse habitats, often with dramatic shifts in morphology. The organization of tendon tissue is a relatively conserved trait, but tendon can adapt to loads with an organism's lifespan by remodeling; i.e., thickening through hypertrophy and molecular changes affect mechanical properties. Tendons are dense, fibrous connective tissue mainly composed of collagen surrounded by an extracellular matrix. They connect and transmit forces between muscles and bones, and play a major role in locomotion. Tendons are capable of resisting high tensile loads, aiding muscle power amplification, energy conservation, and energy dissipation. The morphology and composition of tendon differs among muscle groups, between sexes, across age groups, and can adapt to loading in widely varying mechanical properties. Previous studies have suggested that mammalian tendon may be able to remodel early in ontogeny (i.e. prior to skeletal maturity), but is limited in adapting to loads later in life (Svensson et al., 2016). In order to better understand the interactions of ontogeny and loading on tendon mechanics, I tested the efficacy of different loading regimes for remodeling tendon in juvenile and adult mice.

Tendon Structure

Tendon is a viscoelastic tissue comprised of water, proteoglycans, collagen, and elastin that connects muscle to bone. Collagen has a variety of forms with different roles in fibrillogenesis and movement and has been categorized into 28 different types as a function of these relationships. The main types found in the plantarflexor tendon (i.e., calcaneal, Achilles) are types I, III, and V. Type I collagen is the main structural component (60-90%) contributing to tensile resistance while types III and V (~3%) are characterized by their tendency to act as a precursor for type I formation. Type III has been characterized to co-assemble with other collagen fibers to help growth and repair; Type V collagen is proposed as a foundational unit for type I to begin growth (Birk & Mayne, 1997; Svensson et al., 2016). The ratio of collagen types progressively shifts toward the tendon enthesis, the region that inserts into bone. The proximal, muscular end is composed of greater proportions of type I and III collagen whereas the distal enthesis has greater proportions of type I, II, and X (Tempfer & Traweger, 2015). As collagen fibers are forming, they are bundled into a hierarchical organization with progressively larger groupings of tropocollagen (protein macromolecule), microfibril, subfibril, fibril, fascicle, and tendon (Sharma & Maffulli, 2006) (refer to Fig. 1). Tendon fascicles organize into wavy, or ‘crimped,’ parallel arrangements that also contribute to tendon elasticity.

Collagen fibers are mostly assembled in parallel along a single axis resulting in the formation of cross-links that increase tensile strength. Collagen is

developed as an immature form where collagen fibers increase in length and diameter by forming immature and mature cross-links between collagen segments, respectively (Avery & Bailey, 2005). Both immature and mature collagen are ‘enzymatic cross-links’ that are formed by the cross-linking enzyme lysyl oxidase (LOX), and the number of cross-links is proportional to the mechanical strength of the collagen (Fig. 2). Non-enzymatic cross-links are formed via aging when reactive oxygen species (ROS; - a by-product of cellular respiration) chain-react with glucose molecules and collagen amino groups. The resulting cross-links are called advanced glycation end-products (AGEs), and although they have similar influences on mechanical properties, AGEs are more resistant to degradation. AGEs are accumulated because tendon degradation decreases with aging (Verzijl et al., 2000), thus, allowing ROS to create non-enzymatic cross-links (Wood & Brooks, 2016). Accumulation of AGEs from aging can result in decreased maximum strain, thus, increased brittleness (Gautieri et al., 2017; Nielsen et al., 1998); however, stimulation of AGE production during maturation has increased mechanical strength without decreasing maximum strain (Svensson et al., 2018).

The extracellular matrix (ECM) surrounding collagen contains negatively charged hydrophilic macromolecules, or proteoglycans (PGs; >3%), that aid in fibrillogenesis and affect the viscoelasticity of the tendon. Fibrillogenesis is assisted by PGs because they are paired with glycosaminoglycan side chains (GAGs) that bind to growth factors. Growth factors binding to their respective

receptors are accelerated by PGs, which is essential for collagen formation (Kjaer, 2004). In addition to fibrillogenesis, the compressive resistance of the ECM is increased by the large molecular weight of PGs, which also influences its adhesive effects. These bonding forces include ionic and hydrogen bonds. When tendons are stretched, these passive bonds are progressively broken throughout tensile load bearing, and this time-dependent change in length (deformation) contributes to its viscoelastic properties (Elliott et al., 2003). Both the viscoelastic properties and physical connection between muscles and bones contribute to the role of tendon in locomotion.

Tendon Function

Mechanical work can be stored in tendons and released for amplifying power (Sawicki et al., 2015), conserving energy (Biewener et al., 1988), or dissipating energy (Roberts & Konow, 2013). For example, frog jumping performance is largely powered by tendon recoil: mechanical work is stored in tendons as elastic energy prior to jumping and then released at a faster rate to amplify power (Astley & Roberts, 2012). This enables frog limb power to far exceed the maximal possible from muscle power alone.

Tendons can also conserve energy by storing mechanical energy, thereby reducing muscle work. For example, kangaroos hopping bipedally can effectively conserve elastic energy above 7 km/hour with 35-54% mechanical strain energy (Biewener et al., 1981; Dawson & Taylor, 1973; Gutmann et al., 2013). Muscles contract isometrically to stretch and store elastic energy in tendons. When

tendons recoil, mechanical work is primarily done by tendon, thus, powering movements like hopping and jumping. Similarly, other hopping bipeds such as wallabies show similar energy conservation albeit the energy storage potential decreases with smaller size (Rankin et al., 2018). Cursorial animals further reduce energy expenditure with long tendons and short-fibered muscles. Long and slender tendons are more easily stretched, resulting in an increased potential to store elastic energy (Roberts, 2016) while proximally located muscles reduce energy demand when cycling limbs (Ellerby & Marsh, 2006). Contrarily, the hypothesis that tendons reduce muscle work was recently challenged with research showing no significant differences in cost of force production between isometrically contracting muscles and muscles contracting concentrically and eccentrically (Holt et al., 2014).

Muscle damage can be avoided during rapid and forceful movements when tendons act as attenuators by absorbing mechanical work and moderating its transition into muscle strain (Roberts & Konow, 2013). Energy stored by tendon is released at a slower rate than muscle length change, allowing muscles more time to function within optimal operating lengths. Researchers demonstrated this phenomena with wild turkeys showing that tendons absorbed 2/3 of the total impact (Konow et al., 2012), and fascicle lengthening was near consistent when dropped from a series of heights (Konow & Roberts, 2014).These studies are constant in showing tendon slowing the rate of muscle

strain, thus, allowing longer isometric contractions to counter and reduce peak forces experienced.

In order to prevent rupture, tendon must maintain a mechanical strength greater than any anticipated load over its lifetime. This safety factor is typically three to five times maximal load in animal tendons and apodemes (Alexander, 1981), though some species vary considerably (Biewener & Blickhan, 1988). For example, during kangaroo rat escape responses, the tendons may experience forces as great as 36 MPa compared to the forces during steady-state hopping (5-10 MPa); thus, a relatively high tendon safety factor of ten is necessary (Biewener et al., 1988). Cursorial animals like dogs and kangaroos tend to have lower safety factors because greater elasticity allows more elastic energy return through tendon recoil (Alexander, 1981). Vertebrate tendon is likely an evolutionarily conserved trait; when materials properties were compared among vertebrates ranging from 0.5 to 545 kilograms, there were no significant difference in elastic modulus or energy lost, even between flexors and extensors (Pollock & Shadwick, 1994); however, in a previous study, the physiological functions of flexor and extensor tendons were found relevant for influencing tendon elasticity (Shadwick, 1990).

Tendon Mechanical Properties

Tendons are viscoelastic tissues. When tensile forces are applied to purely elastic materials, they deform a proportional amount. However, tendons are composed of both elastic (i.e. collagen and elastin) and viscous materials

(i.e. water and proteoglycans) that make its deformation rate time-dependent. Interactions between collagen-collagen and collagen-ECM are influenced by the viscous properties of the ECM that slow the rate of stretching and cause time-dependent characteristics (Hulmes et al., 1973). Viscosity of the ECM can be altered intrinsically by negatively charged compounds such as proteoglycans and glycosaminoglycans (GAG).

Ramp-to-failure mechanical tests show that tendons can withstand high tensile loads while exhibiting time-dependent length changes. Tendon fascicles at rest are in crimped formation, but when the tendon is stretched, the crimp angles are straightened before the tendon must actively resist tensile loads. When plotting stress against strain, the straightening of crimp angle corresponds to the exponential curve at the beginning of the graph, called the 'toe' region (Doral et al., 2010). This is followed by the elastic, linear region where reversible length changes occur as more stress is applied, and the increase (slope; Δstress/Δstrain) becomes relatively constant (II; Fig. 3). When the tendon sustains irreversible deformation, it enters the plastic region, and the transition value where the slope of the elastic region begins to decrease is called the yield stress (III; Fig. 3). When the tendon ruptures, this value is the failure stress or mechanical strength and is identified by the dashed line when the stress-strain curve ends (Fig. 3).

Unlike muscle, tendon performance is relatively insensitive to fluctuations in temperature. Studies have suggested that tendon mechanical properties are

not significantly altered at temperatures ranging from 0 to 41° C (C.-Y. Huang et al., 2009; Rigby et al., 1959; Wang et al., 1991). When tendons exceeded average body temperatures, it exhibited reduced energy conservation and deformation before failure. Furthermore, cyclic loading beyond 30 minutes suggested deleterious temperature effects on stress-relaxation behaviors (C.-Y. Huang et al., 2009; Rigby et al., 1959). In addition to tendon, temperature effects have been studied on other collagenous tissues including chameleon tongues and ligaments. Chameleon prey capture utilizes ballistic tongue projection powered by elastic recoil whereas tongue retraction is powered by muscle. A decrease of 10°C resulted in tongue retraction speeds slowed by 42% while elastic recoil decreased only by 10-19% (Anderson & Deban, 2010). A study on canine ligaments suggested an inverse relationship between mechanical properties and temperature (Woo et al., 1987) whereas other studies on sheep and canine ligaments suggested no relationship (Dorlot et al., 1980; Hasberry & Pearcy, 1986). Overall, more studies have suggested negligible temperature effects on tendon and collagenous tissues at room and body temperatures.

Tendon Remodeling

Tendon remodeling is described by the growth, repair, and general compositional change in tendon tissue (Sharma & Maffulli, 2006). During tendon growth and maturation, collagen and ECM-proteins are upregulated to increase the quantity and quality of tendon tissue. Tendon cells called fibroblasts produce collagen fibrils throughout tendon growth and maturation, and the collagen

progressively increases in diameter and density (Ansorge et al., 2011; Goh et al., 2008). Type I and III collagen fibers are initially distributed throughout the tendon, but type III is gradually replaced by type I (Birk & Mayne, 1997). Lysyl oxidase creates cross-links between the forming collagen fibers and results in altered tendon mechanical properties (Avery & Bailey, 2005). Mechanical testing have demonstrated that maturation increases tendon strength (Cribb & Scott, 1995; Goh et al., 2008) as well as stiffness and modulus (Ansorge et al., 2011; Viidik et al., 1996; Wood et al., 2011). At the onset of aging, studies have demonstrated that aged tendon accumulates non-enzymatic cross-links (AGEs) and decreases ECM components such as proteoglycans and water content (Avery & Bailey, 2005; Kannus et al., 2005). The increased amount of cross-links is associated with an increase in stiffness and modulus, which is well agreed upon in the literature (Nielsen et al., 1998; Viidik et al., 1996; Wood et al., 2011; Wood & Brooks, 2016). With advanced age, however, failure stress has been found to increase (Nielsen et al., 1998; Viidik et al., 1996) or decrease (Dressler et al., 2002; Goh et al., 2008). Despite differences in results, it is generally accepted that both tendon mechanical strength and stiffness increase during maturation whereas only stiffness increases during aging due to the accumulation of AGEs. Mechanical strength of tendon have generally increased with exercise, though (Heinemeier et al., 2012). Training has varying effects on aged tendon with some studies reporting failure stress and stiffness to increase (Heinemeier et al., 2012;

Viidik et al., 1996), decrease (Wood & Brooks, 2016), or to have no significant effect (Nielsen et al., 1998).

Tendon remodeling encompasses the simultaneous growth and degradation of collagen to ensure that tendons are composed of healthy mature collagen. Integrins within the cellular membrane detect loading and stimulate cellular pathways for the maintenance of the ECM by producing new or degrading old collagen (Kjaer, 2004). Loading via exercise induces variable responses in tendon mechanical properties and morphology depending on training parameters and muscle-tendon units. Researchers have utilized different protocols such as treadmill and wheel running (Buchanan & Marsh, 2001; Heinemeier et al., 2012; Legerlotz et al., 2007; Wood & Brooks, 2016), forced water treading (Simonsen et al., 1995), and strength training (Klitgaard, 1988; R. C. Marqueti et al., 2018; Simonsen et al., 1995). Training has been found to result in an increase in stiffness and modulus (Arampatzis et al., 2010; Buchanan & Marsh, 2001; Couppé et al., 2008; Heinemeier et al., 2012; Kubo et al., 2006) or to have no effect (T.-F. Huang et al., 2004; Karamanidis & Arampatzis, 2006; Rosager et al., 2002). Mechanical strength is also variably impacted suggesting increases (Nakagaki et al., 2007; Woo et al., 1981) or no exercise effects (T.-F. Huang et al., 2004; Inhofe et al., 1995). In addition, training has alluded to increase tendon cross-sectional area (CSA) (Couppé et al., 2008; Kaux et al., 2013; Sommer, 1987) while others have shown no effect (Buchanan & Marsh, 2001; T.-F. Huang et al., 2004). As a result of aging, tendon remodeling rates

decline, and the accumulation of AGEs tends to increase tendon stiffness and modulus (Avery & Bailey, 2005); however, this makes tendon more brittle (Gautieri et al., 2017; Nielsen et al., 1998). With exercise, the mechanical properties of aged tendon – weight-bearing or not – may revert to a state similar to a healthy adult tendon (Lacroix et al., 2013; Wood & Brooks, 2016). Stiffness and modulus tend to decrease with training, and tendon remodeling increases (Viidik et al., 1996; Wood & Brooks, 2016).

Although tendon is capable of remodeling to accommodate for injuries and loading patterns, tendon remodeling is limited to the peripheral region (Fig. 4). Researchers have studied the life-long tissue renewal of tendon and other collagenous tissues by using the carbon-14 (14C) bomb-pulse method. This method utilizes the nuclear testing done during the 1950s and 1960s that resulted in an increase of atmospheric 14C levels. 14C from atmospheric CO2 is absorbed into all living organisms via their diet, and it becomes incorporated into their tissues from remodeling. Tissues with slow turnover contain these trace elements for longer periods of time before being replaced. Researchers have used the 14C bomb-pulse method to analyze tendon renewal and found that tissue turnover within the core was extremely limited after reaching skeletal maturity (Heinemeier et al., 2013, 2018). Other collagenous tissues, like cartilages and menisci, share these low remodeling rates (Heinemeier et al., 2016; Våben et al., 2020); however, studies have reported that the peripheral region of tendon has relatively higher rates of collagen turnover (Gumucio et al.,

2014; Langberg et al., 1999, 2001, 2007). The heterogeneity of tendon remodeling is possibly due to vascularization. Blood is supplied through the myotendinous junctions and periphery of the paratenon, which is the loose connective tissue surrounding the tendon (Doral et al., 2010; O'Brien, 2005). Tendons are penetrated with blood vessels, but the tendon core has reduced vascular density compared to the paratenon. Vascular density has been shown to increase with exercise, however (R. C. Marqueti et al., 2018).

Although studies have researched numerous combinations of loading frequencies and magnitudes to investigate the effects of exercise intensity on tendon remodeling, results have been contradictory. Varying levels of mechanical load on tendons have resulted in differences in tendon mechanical properties (Arampatzis et al., 2010; Legerlotz et al., 2007). Differences in exercise intensity have shown conflicting results about tendon mechanical properties (Buchanan & Marsh, 2001; Klitgaard, 1988; Wood & Brooks, 2016). Exercise regimes with varying patterns of loading and frequency result in different tendon adaptations to optimally respond to mechanical loads. Both resistance training and uphill running have been effective in increasing mechanical strength in rats albeit to different extents (Heinemeier et al., 2012; Kaux et al., 2013). Resistance training for humans has also resulted in stronger tendons, but endurance training showed no significant effect compared to untrained individuals (Karamanidis & Arampatzis, 2006). Tendon research has established a body of literature focusing on different exercise effects at different life stages, but researchers

rarely address the interaction effects within the same study (R. C. Marqueti et al., 2018). As a result, exercise and aging studies are typically consolidated into review articles attempting to identify physiological, morphological, and mechanical patterns (Heinemeier & Kjaer, 2011; Svensson et al., 2016; Thampatty & Wang, 2018).

In order to investigate the effects of exercise type and magnitude on tendon mechanics and remodeling, I used a mouse line selectively bred for high activity – the high runner mouse model. Dr. Ted Garland and colleagues have artificially selected for high voluntary wheel activity behavior in lab mice – now called high-runner (HR) mice – since the 1990s, and the artificially selected line exhibit a suite of behavioral, morphological, and physiological traits correlated to higher running behavior (Swallow, Carter, et al., 1998; Swallow, Garland, et al., 1998). At generation 10, HR lines ran 75% more than control lines, and at generation 49, total revolutions per day progressed to a 3-fold difference between line types (Meek et al., 2009; Swallow, Carter, et al., 1998). This difference in total revolutions is possibly linked to HR behavioral, anatomical, and physiological traits including: intermittent wheel running (Girard et al., 2001), hindlimb symmetry (T. Garland & Freeman, 2005), and maximum aerobic performance (Rezende, Garland, et al., 2006; Rezende, Gomes, et al., 2006). Because numerous studies have explored the morphological and physiological traits of these high activity mice line types, the mice colonies from the Garland

lab make a great candidate for studying the influence of genetics on exercise-mediated tendon remodeling.

HR linetypes have distinct morphological traits in juxtaposition to control mice, and two HR lines have developed a mini-muscle phenotype. On average, HR mice have reduced overall body mass(Koteja et al., 1999), increased relative kidney mass, and reduced relative triceps surae muscle mass (Jr. Garland Theodore et al., 2002; Koteja et al., 1999). Two of these HR lines developed triceps surae muscles that are 44% smaller than controls lines along with relatively larger kidneys, livers, and ventricles (Jr. Garland Theodore et al., 2002). In addition, mini-muscle mice exhibited longer and slenderer pelvic limb bones (Kelly et al., 2006). These HR lines were excluded from this study because their distinct morphological differences and increased muscle fatigue resistance (Syme et al., 2005), which may convolute isolating exercise effects on tendon remodeling.

To investigate the effects of maturation on tendon, I introduced training regimens to mice both before and after reaching skeletal maturity. Laboratory mice reach puberty between five and six weeks of age (Falconer, 1984), and their skeletal growth begins to approach an asymptote at nine weeks of age, reaching complete skeletal maturity at ~ 12 weeks (Beamer et al., 1996). In order to compare the ability of the tendon to remodel before and after puberty, mice were separated into young (3-weeks) and adult (9-weeks) cohorts before

implementing training protocols designed to span these two important time points.

In the present study, I investigated the impacts of maturation (young vs. adult) and exercise intensity (jump vs. wheel vs. sedentary) on tendon remodeling measured via morphology and materials properties. I used two lines of mice (Mus domesticus), a control line representing ‘normal’ activity levels, and a HR linetype that is at least three times more active than typical lab mice, and three different levels of exercise: wheel running, forced jumping, and sedentary. My aims were to determine 1) if pre-adolescent exercise induced tendon remodeling, 2) if exercise type correlated to extent of tendon remodeling, and 3) if the HR mice selection phenotype is evident in tendon morphology and mechanics. Previous studies have demonstrated that exercise can increase the mechanical properties of maturing tendon and revert aged tendon to a healthy adult tendon state; however, how tendons respond to different types of loading, or at different stages of life, is highly variable and difficult to interpret.

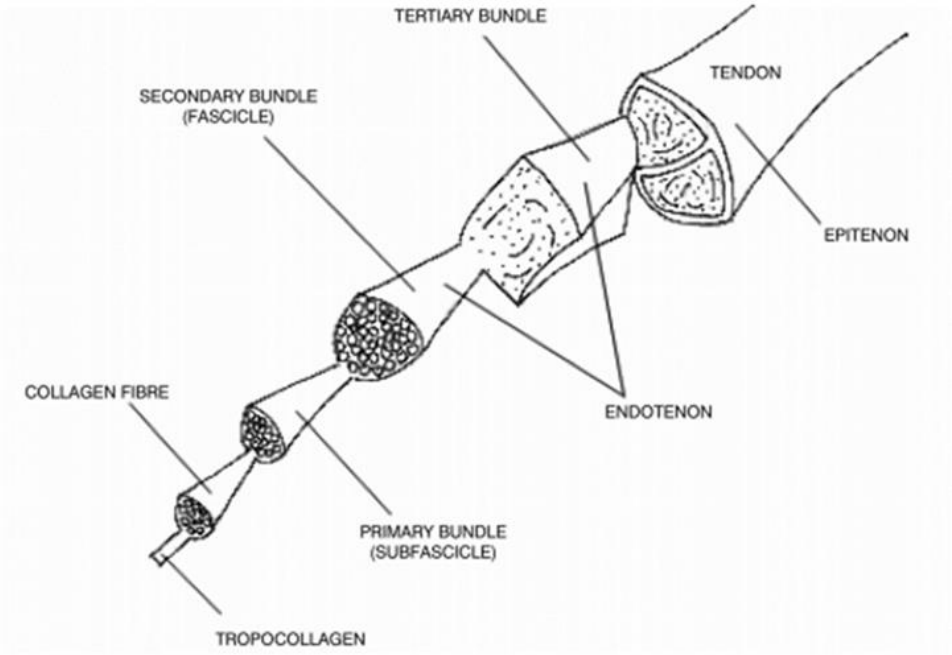

Figure 1. Tendon Structure. Hierarchical organization of tendon starting from proteins chains (tropocollagen) to whole tissues (tendon) (Sharma & Maffulli, 2006).

Figure 2. Cross-Links and Aging. Graph illustrates the general trend in cross-link concentration in regards to maturation & aging and type of cross-linkages. Enzymatic cross-links are formed via lysl oxidase while advanced glycation end-products (AGEs) are formed via reactive oxidative species. (Svensson et al., 2016).

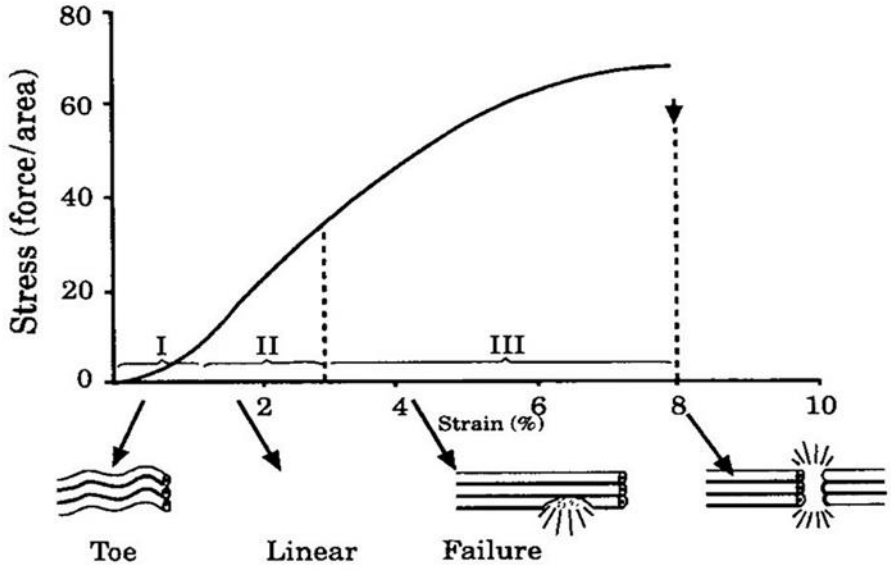

Figure 3. Tendon Mechanical Properties. The schematic diagram shows the generalized pattern of the stress-strain curve when a ramp-to-failure test is applied to tendon (Doral et al., 2010).

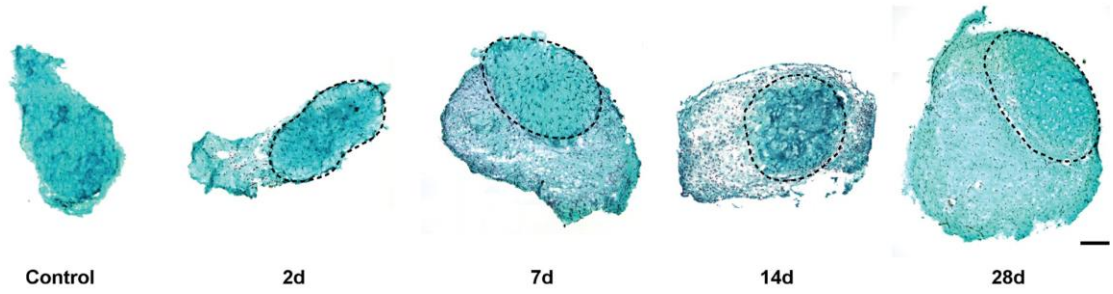

Figure 4**.** Regional Tendon Remodeling. Cross sections of plantaris tendon stained with fast green and hematoxylin. The outlined region shows the tendon core. Chronological sections compare remodeling within peripheral and core regions of the tendon, which show more tendon remodeling in the peripheral region. Scale bar for all panels = 100 μm (Gumucio et al., 2014).

CHAPTER TWO

MATERIALS AND METHODS

Animals

Mice were from colonies artificially selected for high voluntary wheel-running behavior. The complete design of the artificial selection experiment for high voluntary wheel-running behavior in mice has been described in detail (T. Garland, 2003; Swallow, Carter, et al., 1998), but briefly: a population of Hsd:ICR mice (Mus domesticus) were used as progenitors for eight closed lines. Four lines of control mice are selected at random for breeding while four HR lines are selected for high voluntary wheel activity levels (Fig. 5). These selected lines were given access to Wahman-type activity wheels (1.12 m circumference; Layfayette Instruments) for 6-8 weeks before the week of selection. Male and female mice with the highest total wheel revolutions on day 5 and 6 were selected to reproduce. For both control and selected lines, breeding was kept within their respective colonies, and siblings were not allowed to mate.

120 female mice from generations 93-96 were weaned at 21 days of age and then housed in cages specific to exercise treatment. Sixty mice were housed immediately in exercise cages while the other sixty were aged in standard housing for six weeks beforehand. Mice from line 7 were used as the HR group and line 1 as the control group. Following IACUC protocol (19-020) at CSUSB and veterinarian approval, all mice were given food and water ad libitum; room

temperature was kept at (21.5°C) and photoperiod at 12h:12h, with lights on at 07:00h.

Experimental Design

Mice (n = 120) were categorized by linetype, age, and exercise treatment. Mice from the HR linetype were classified as the high-activity group while the control linetype served as the low- to normal-activity group. Each linetype was separated into two cohorts corresponding to before (young, 3-weeks) and after (adult, 9-weeks) skeletal maturity. Exercise treatments were implemented with varying intensities including voluntary wheel running (low mechanical load, high-frequency), involuntary jumping (high mechanical load, low frequency), and sedentary groups. Both exercise treatments have been shown to be effective in stimulating morphological and mechanical changes to the plantarflexor tendons, but rarely within the same study (Legerlotz et al., 2007; R. C. Marqueti et al., 2011). During a pilot study, I elicited a response in tendon properties during a pilot study training mice for four weeks. I subjected mice to training for eight weeks to induce changes in material properties (unpublished data). Activity data was collected in 30-min intervals for wheel mice using activity wheels (Columbus Instruments; 9.2 cm diameter). Jump cages were not equipped with activity-tracking platforms; however, a pilot study suggested the cage setup prompted a significant change in tendon properties (unpublished data).

Training

Exercise treatments were implemented at two life stages. Each line type (n = 60) was separated into two age cohorts (n = 30) that were placed into exercise cages at three weeks (young cohort) and nine weeks (adult cohort) of age (Fig. 6). These timepoints correspond with before and after skeletal maturity (Beamer et al., 1996). Each age cohort was divided into three training groups (n = 10) with varying degrees of mechanical loading: jump, wheel, and sedentary groups. The jump treatment replicated an exercise of high mechanical load with low frequency while the wheel treatment exemplified a low mechanical load with high frequency; the sedentary group served as the control. Jump cages were equipped with an elevated platform (14 cm; mouse hip height is ~ 4 cm) with access to water while food was accessed from the bottom platform; this ensured mice must jump between top and bottom platforms to eat and drink. Wheel cages had activity wheels (30.47 cm circumference; Columbus Instruments) for voluntary wheel running that measured daily wheel activity in 30-minute intervals. After eight weeks of exercise, mice were euthanized using carbon dioxide asphyxiation and then stored at -20° C for dissection and mechanical testing at a later date. Studies have shown no difference in mechanical properties after comparing tendon stored at -20° C and freshly dissected tendon (Goh et al., 2010), nor tendons stored at -20° C and tested up to 360 days later (Boon-Ho Ng & Siaw-Meng Chou, 2003). To further ensure mechanical properties were not altered, tendons were kept frozen until mechanically tested (Chen et al., 2011).

Morphometrics

Mice plantarflexor tendons were extracted for lateral and ventral digital images to calculate tendon cross-sectional area and length. The plantarflexor tendons are connected to the plantarflexor muscles including the medial and lateral heads of the gastrocnemius, soleus, and plantaris that contribute to the extension of the ankle joint; an essential movement for the wheel and jump treatments. The soleus and plantaris tendons were removed from the plantarflexor tendon group for this study (Fig. 7). The plantarflexor tendon of the right hindlimb was separated from the surrounding connective tissue, but kept intact for in situ or in vitro pictures; the tendon was kept moist throughout imaging and testing using phosphate-buffered saline solution. Either the tendon was positioned in front of a mirror angled at 45° so that a camera (Canon EOS Rebel T3) captured images of the tendon from dorsal and lateral views; or the tendon was pictured within the testing rig described in the following section (Fig. 8). These pictures were analyzed with an image processing software (ImageJ, NIH) to obtain two diameter measurements (dorsal and lateral) and the tendon length. I assumed the tendon cross-section was elliptical and calculated the CSA using the area of an ellipse formula:

$$(1)\ CSA = \frac{1}{2}d_1 * \frac{1}{2}d_2 * \pi$$

After pictures were taken, the right hindlimb tendon was extracted for mechanical testing, and the left plantarflexor muscles were separated from the surrounding tissue. The mechanical testing tendon was dissected to include the

portion of the calcaneus where the plantarflexor tendon inserts and the muscle-tendon junction where the tendon becomes an aponeurosis and spreads into the muscle. The hindlimbs of HR mice have symmetrical anatomy, so this was assumed for all individuals (T. Garland & Freeman, 2005). To analyze whether changes in tendon morphometrics corresponded to muscle hypertrophy, gastrocnemii muscles of the left leg were isolated and weighed.

Mechanical Testing

For clamping the mouse tendon, I used and modified an experimental setup as described by Probst and colleagues (2000). Briefly, I cut out a wooden block with a conical slot that holds the calcaneus, thus increasing the total measurable distance of the distal region of the tendon. This wooden block was secured in a fixed position. The proximal region just distal to the muscle-tendon junction was bound with a 2-0 silk surgical knot that was reinforced with VetBond to prevent slippage (Komatsu et al., 2006). Then the thread was attached to a dual-mode servomotor (305C-LR, 10.0 N, Aurora Scientific Inc., Ontario, Canada) for in vitro materials testing (Fig. 8). The servomotor was fixed to a three-axis manipulator that adjusted the initial length of the tendon. The time and force measurements from the servomotor were recorded at 1000 Hz using a 16-bit A/D converter (National Instruments, TX, USA) and LabChart 8 software program (LabChart 8 v. 8; AD Instruments). The deformation of compliant structures attached to the testing rig can be falsely recorded as tendon deformation by the servomotor (Arruda et al., 2006; Cui et al., 2009), so I isolated

tendon deformation using visual tracking. To visually track deformation, I used a 10/0 detail paint brush to apply two dots of India ink: one distally near the calcaneal insertion and one proximally near the knot (Fig. 8). A high-speed camera (XC-2, XCitex) equipped with a macro lens (Sigma 105 mm F2.8 EX DG OS HSM) recorded the dorsal view at 300 fps using ProCapture software program (ProCapture, XCitex). All data were synchronized using a common external trigger. The India Ink dots were tracked using ProAnalyst software program (ProAnalyst, XCitex) and then exported as x-y coordinates for data analysis.

Tendon mechanical properties were measured by applying materials tests including cyclic loads and ramp-to-failure tests. Before loading, the tendon was extended to approximate in vivo resting tension. Three cyclic loads for 2.5 seconds were applied in 5.5 g increments starting at 5.5 g in order to reorient collagen fibers along the long axis of the tendon (Schatzmann et al., 1998). Before each cyclic load, the resting length was recorded to confirm lengthening and reorientation of collagen. Lastly, the ramp-to-failure test applied 549.45 g over 2.5 seconds for the tendon to rupture (Fig. 9). Mechanical tests were conducted at room temperature (23° C) because previous studies have shown negligible temperature effects on the mechanical properties of tendons (C.-Y. Huang et al., 2009; Wang et al., 1991).

Data Analysis

Tendon mechanical properties were calculated using morphometric values and materials testing results. Length change (deformation) was measured by calculating the distance between the proximal and distal India ink dots using custom code in Igor Pro Software (Wavemetrics Inc., OR, USA.). The length while under resting tension for the second or third cyclic load was measured as the resting length (L0). A smoothing spline was applied to the cyclic loading tests prior to measuring L0 and deformation. Stiffness was calculated by graphing a force-length curve and measuring the slope of the linear region (2). Morphometric measurements including cross-sectional area (CSA) and resting length (L0) were used to convert stiffness to elastic modulus (3). Stress is force divided by CSA, and strain is deformation divided by L0. Peak forces experienced during ramp-to-failure tests were recorded as the failure force, which was then converted to stress (4).

$$(2)\ Stiffness = force\ (g)/\Delta length$$

$$(3)\ Elastic\ Modulus = stress\ (g * cm^{-2})/strain$$

$$(4)\ Failure\ Stress = Peak\ Force\ (g)/CSA\ (cm^2)$$

Running was measured on activity wheels (Columbus Instruments; 9.2 cm diameter) by recording wheel revolutions in 30-minute intervals. The number of wheel revolutions recorded in each interval was added up to calculate total wheel revolutions in a day, week, and for the full training duration. These values were

correlated with the mechanical properties of the corresponding individuals in linear regressions.

Statistical Analyses

Statistical analyses were performed using the statistical program JMP (SAS Institute Inc., Carey, NC), and figures were produced using the package ggplot2 (Wickham, 2016) within the program R (R Core Team, 2020). I compared morphometric and mechanical properties using linear regressions and 3-way Analysis of Variances (ANOVA) utilizing the least squares means method. Linetype (HR/control), age (young/adult), and training (wheel/jump/sedentary) were treated as main effects. Statistical significance was determined at a 95% confidence ($P<0.05$). Before analyzing the data, eleven mice were excluded due to complications and errors in materials testing such as VetBond covering the tendon and the proximal knot unraveling before tendon rupture. Outliers were identified using the outlier screening tool in JMP, and outliers beyond the interquartile range scaled at 1.5 were excluded prior to statistical tests.

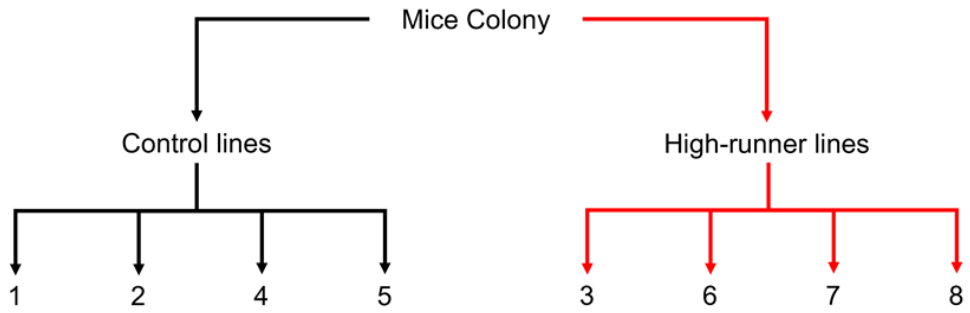

Figure 5. Selected Mice Colonies. Mice colonies were artificially selected for high voluntary wheel running as described in the materials & methods. Lines were separated into control (1, 2, 4 & 5) and high-runner (3, 6, 7 & 8) linetypes.

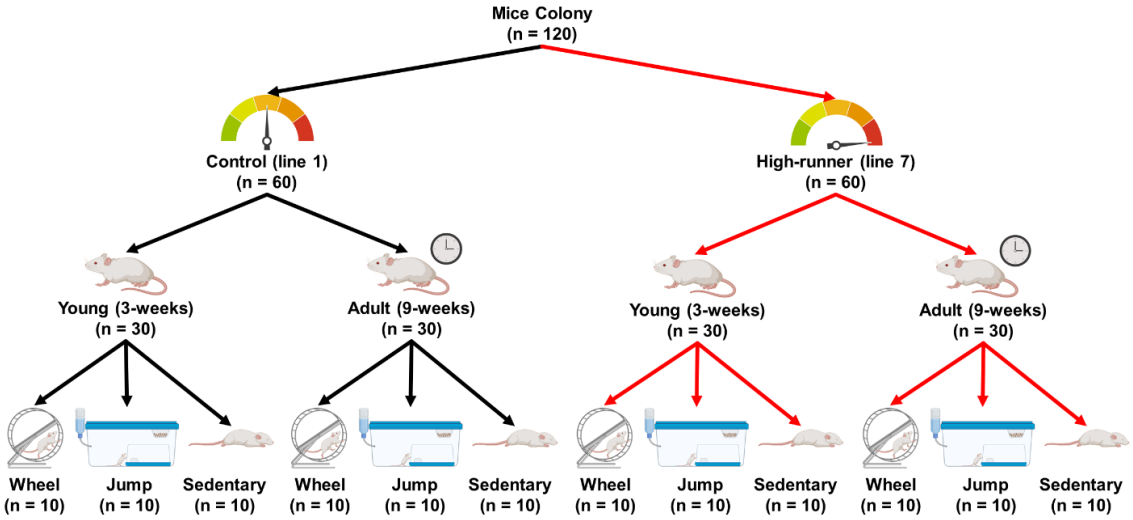

Figure 6. Experimental Design. Mice from control and high-runner lines (normal-/high-activity) were separated into two age cohorts (young/adult) corresponding to before and after skeletal maturity. Each cohort within each linetype was separated into three training groups: voluntary wheel exercise, involuntary jump exercise, and sedentary.

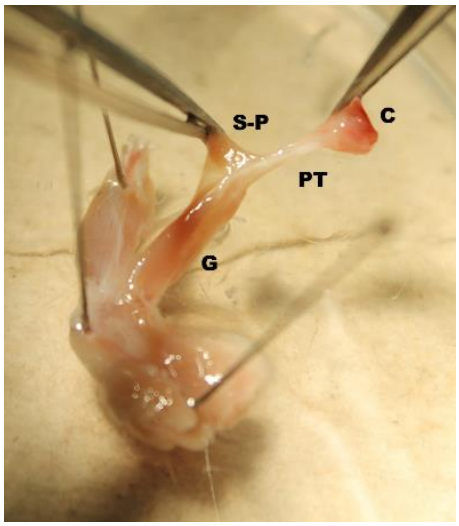

Figure 7. Mouse Plantarflexor-Tendon Unit. The plantarflexor tendon (PT) is distally attached to the calcaneus (C) and proximally attached to the plantarflexor muscles. The soleus-plantaris complex (S-P) is separated from the medial and lateral gastrocnemii muscles (G), which the S-P muscles are later separated.

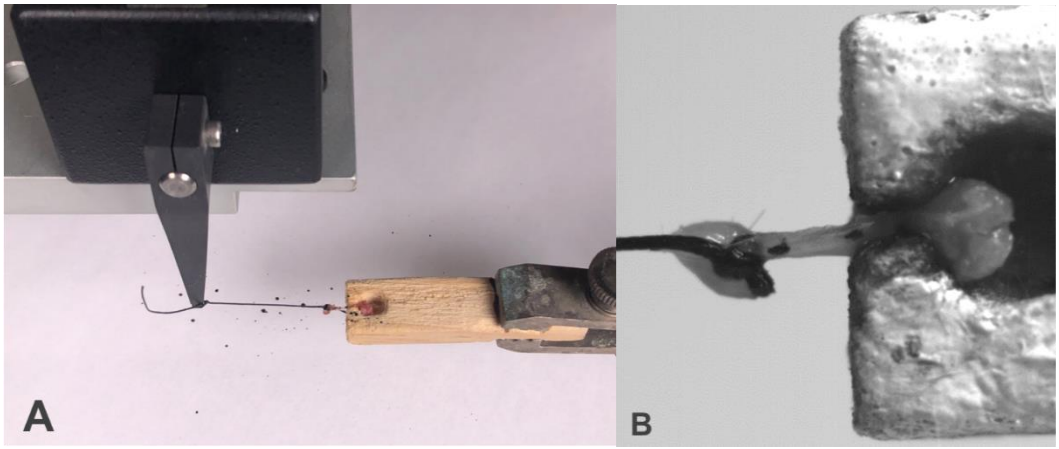

Figure 8. Materials Testing Setup. (A) The distal tendon is attached to the calcaneus that is wedged within the conical slot of the wooden rig. The proximal tendon is bound by a silk thread that is attached to the servomotor. The tendon is placed in the lateral position for taking pictures, which are then used for morphometric measurements. (B) The tendon is in the dorsal position for mechanical tests. Two India ink dots are visible that are used as visual markers for the distal and medial positions. The knot was tied distal to the muscle-tendon junction, so it was used as the proximal region of the tendon.

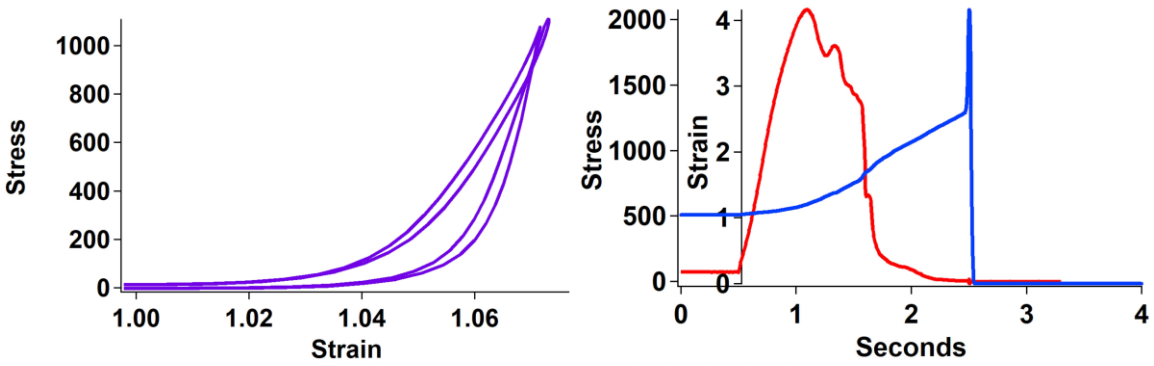

Figure 9. Representative Materials Testing Outputs. Representative trials showing (A) hysteresis loops from cyclic loading and (B) tendon rupture from ramp-to-failure tests. Force measurements recorded via the servomotor and deformation measured via high-speed videos were normalized using morphometric measurements including tendon cross-sectional area (CSA) and length to visualize stress (force/CSA) and strain (Δlength/resting length) curves. (B) Stress is shown in red and strain in blue.

CHAPTER THREE

RESULTS

Wheel Training

For both age cohorts, high-runner mice showed a three-fold difference in activity levels compared to the control linetype. Young, control mice ran on average 1.70 km/day compared to 6.32 km/day for HR mice. For the adult cohort, control mice ran on average 2.31 km/day compared to 7.32 km/day for HR mice (Fig. 10). At the end of training, the young, control linetype averaged 65.7 km total in contrast to 237 km for the HR linetype. For the adult cohort, the control mice averaged 380 km total versus 1226 km for HR mice. Linetype had a significant effect on average kilometers ran per day ($F_{1, 1} = 75.6044$; $p < 0.0001$) and total kilometers ($F_{1, 1} = 37.1287$; $p < 0.0001$). Age and the interaction between linetype and age showed no significant effect on average kilometers ran per day ($F_{1, 1} = 1.1015$, $p = 0.3018$; $F_{1, 1}$ 0.0233, $p = 0.8797$, respectively). However, total kilometers was significantly affected by age ($F_{1, 1} = 53.5347$; $p < 0.0001$) and showed a significant interaction effect between linetype and age ($F_{1, 1} = 14.6217$; $p = 0.0006$); thus, verifying the difference in activity levels between linetypes.

Morphology

Morphometric analyses demonstrated that age, linetype, and exercise treatments significantly affected tendon morphology (Fig. 11, Table 3). Tendon

length was significantly affected by age ($p = 0.0318$) showing adult mice had 7.24% longer tendons (Table 1&3). Linetype had no significant effect on tendon length ($p = 0.2625$) where HR mice had 3.59% longer tendons (Table 1&3). Exercise showed no significant effect on tendon length ($p = 0.1925$); however, significant interaction effects were present between linetype and exercise ($p = 0.0318$) and linetype and age ($p = 0.0388$; Table 3). HR, sedentary mice had 16.50% longer tendons than the control linetype, the jump exercise exhibited a larger increase in the adult cohort compared to the other exercise treatments (Fig. 11; Table 1).

Tendon CSA was significantly affected by age ($p = 0.0499$) showing that young mice had 11.07% thinner tendons than adult mice (Table 1&3). Linetype significantly affected tendon CSA ($p = 0.0210$) where HR mice had 13.69% thinner tendons than control mice (Table 1&3). Exercise showed no significant effect on tendon CSA ($p = 0.8332$) in addition to all interaction effects ($p > 0.05$). Furthermore, tendon morphology showed no significant correlation with average kilometers ran per day for both linetypes (all $p > 0.05$; Fig. 15).

I found evidence that body mass was significantly affected by age, linetype, and exercise treatments, and only found a significant interaction effect between linetype and exercise ($p = 0.0002$). Significantly smaller body masses were found in the young cohort ($p < 0.0001$), HR linetype ($p < 0.0001$), and exercise groups ($p = 0.004$; Table 3). Furthermore, linetype and exercise treatments showed interactive effects on final mass where HR, jump mice had

15.10% lower body masses compared to the control, jump mice (Fig. 11); all other comparisons for final body mass were not significant. No statistical tests were conducted on gastrocnemii mass because insufficient number of samples were collected from each group (Table 3).

Materials Properties

Results from materials testing demonstrated minimal significant effects on raw measurements (Fig. 12/13/14, Table 4). Tendon yield forces increased in the adult cohort by 6.35% compared to the young cohort ($p = 0.1964$), and HR mice by 5.85% compared to the control linetype ($p = 0.2327$; Fig. 12; Table 2&4). The jump treatment resulted in the greatest difference compared to the sedentary group showing a 10.82% lower yield force albeit not significant ($p = 0.1171$; Table 4). When accounting for tendon CSA, linetype had a significant effect on yield stress ($p = 0.0113$) showing a 21.46% higher value in HR mice compared to control (Table 2&4). Tendon yield strain was significantly affected by age ($p = 0.0273$) but not linetype ($p = 0.3296$) or exercise ($p = 0.2931$; Table 4). The interaction effect between linetype and age was significant for both yield force and strain ($p = 0.0270$ & $p = 0.2670$, respectively; Table 4). However, no other combination of interactions between treatments showed significant effects on tendon yield force, strain, or stress ($p > 0.05$; Fig. 16).

Results showed no significant differences in failure forces between age ($p = 0.9740$) or linetype ($p = 0.3025$) albeit shown between exercise treatments ($p = 0.0290$; Fig. 13; Table 4). When accounting for tendon CSA, failure stress was

significantly affected by linetype ($p = 0.0144$) but not age ($p = 0.2539$) nor exercise ($p = 0.3224$; Table 4). Young mice had significantly longer failure strains compared to adult mice ($p = 0.0122$), showing a 10.55% longer failure strain (Table 2&4). Failure strain was not significantly affected by linetype ($p = 0.5907$) or exercise ($p = 0.1850$; Table 4). Stiffness was not significantly affected by linetype ($p = 0.8064$), age ($p = 0.6152$), nor exercise treatments ($p = 0.1967$; Fig. 14; Table 4); however, when accounting for tendon CSA, modulus was significantly affected by linetype ($p = 0.0485$; Table 4). The interaction effect between linetype and age approached significance for stiffness ($p = 0.0525$) while the interaction effect between linetype and exercise was significant for modulus ($p = 0.0454$; Table 4). All other combination of interactions between treatments showed no significant effects on failure force, strain, or stress, stiffness, or modulus. In addition, tendon mechanical properties showed no significant correlation with average kilometers ran per day for both linetypes (all $p > 0.05$; Fig. 15&16).

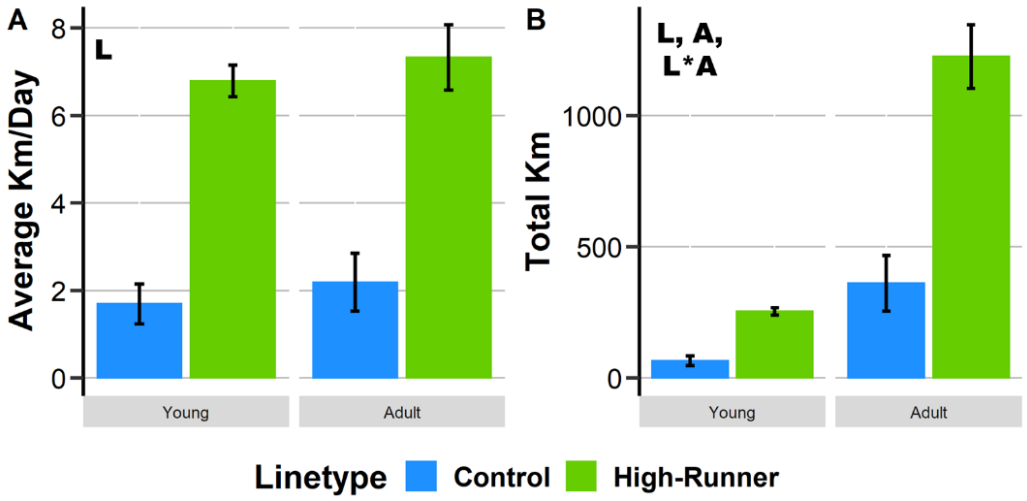

Figure 10. Wheel Running Between Linetypes. Wheel revolution results showing (A) average kilometers run per day and (B) total kilometers with error bars. High-runner mice consistently ran 3-fold the amount compared to the control line, and an age and interaction effect between linetype and age was found when comparing total kilometers ran. L = significant effect by linetype; A = significant effect by age; L*A = significant interaction effect between linetype and age.

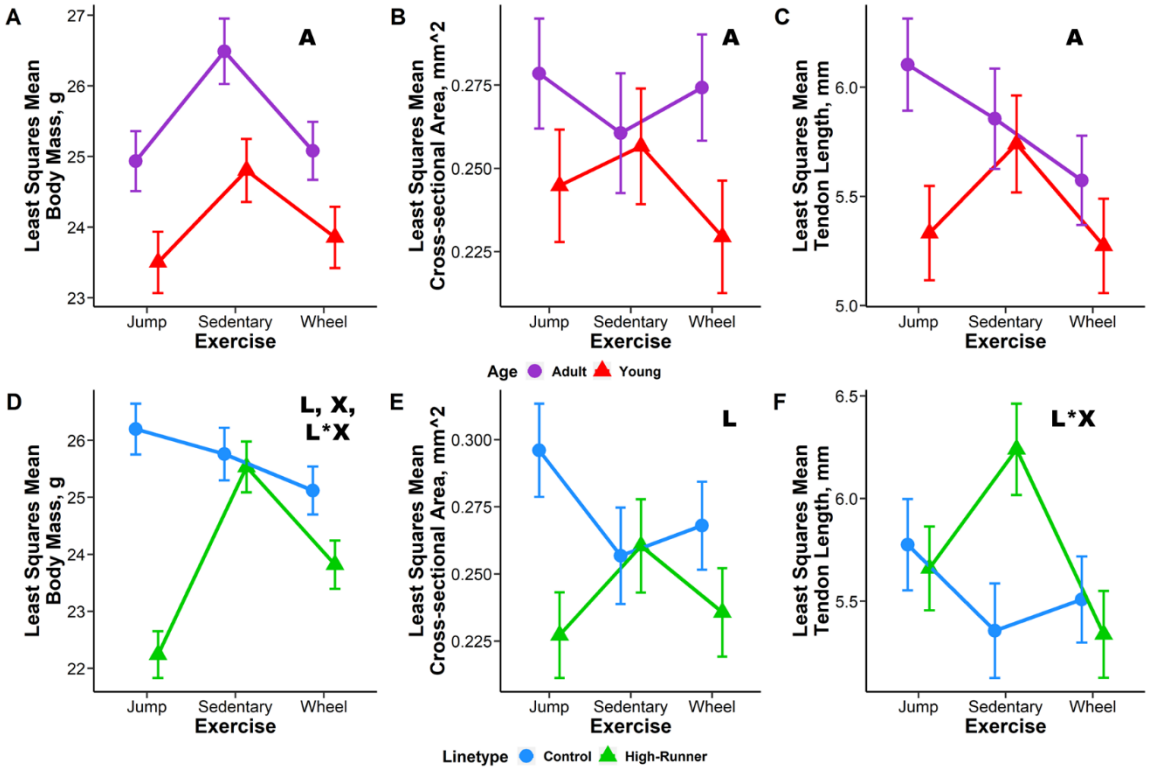

Figure 11. Tendon Morphology. Morphometric results showing the least squares means for (A, D) body mass, (B, E) tendon cross-sectional area, and (C, F) tendon length. Interaction plots visualize age and exercise group comparisons (A-C) as well as linetype and exercise group comparisons (D-F). Age and linetype consistently affected morphology where linetype changes manifested more as interaction effects with exercise. L = significant effect by linetype; A = significant effect by age; X = Significant effect by exercise; L*X = significant interaction effect by linetype and exercise.

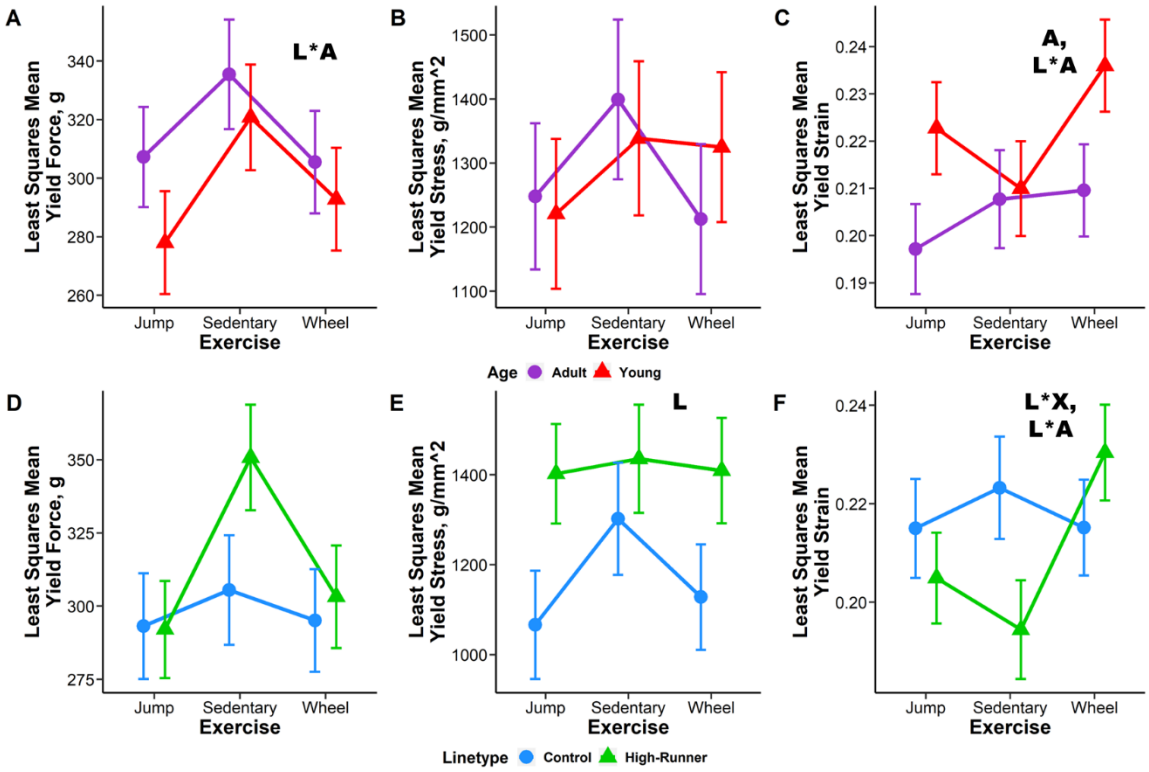

Figure 12. Tendon Mechanical Properties – Yield Force/Stress/Strain. Mechanical properties results showing the least squares means for (A, D) yield force, (B, E) yield stress, and (C, F) yield strain. Linetype had no effect on yield force, but normalizing for tendon morphology showed a significant effect on yield stress. Age had a significant effect on yield strain showing that young mice had more compliant tendons compared to adult mice. L = significant effect by linetype; A = significant effect by age; L*A = significant interaction effect between linetype and age; L*X = significant interaction effect by linetype and exercise.

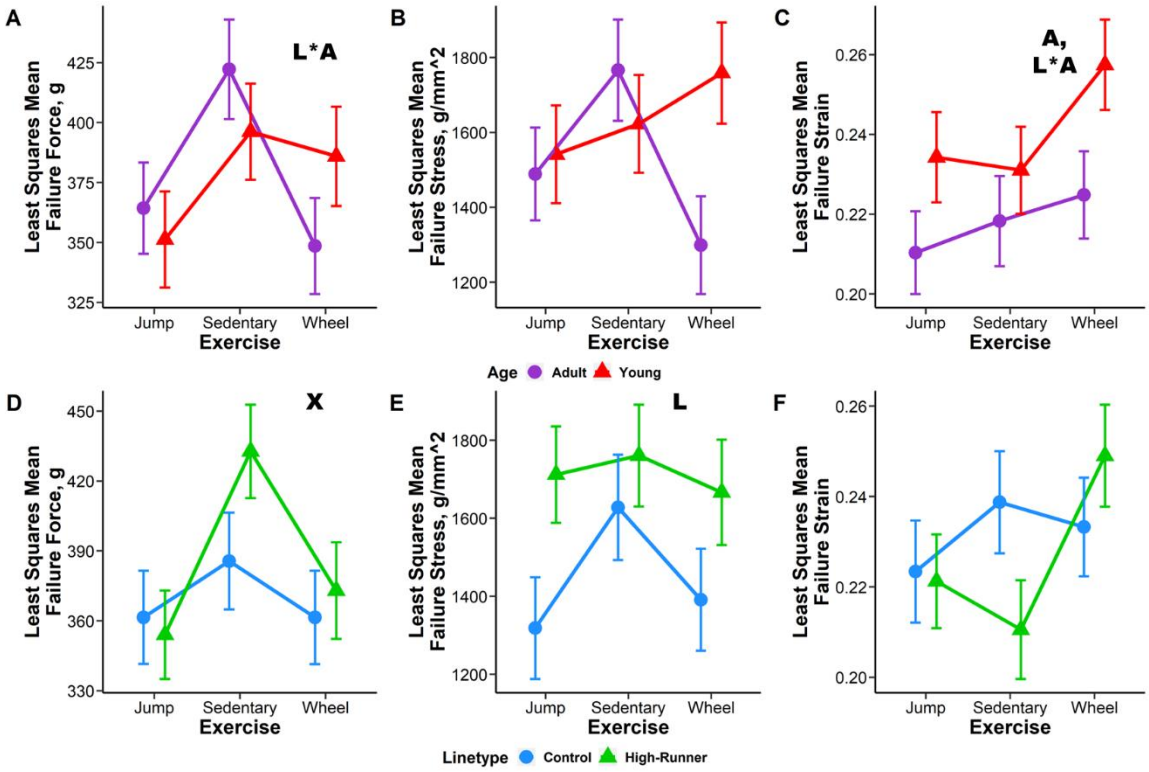

Figure 13. Tendon Mechanical Properties – Failure Force/Stress/Strain. Mechanical properties results showing the least squares means for (A, D) failure force, (B, E) failure stress, and (C, F) failure strain. Exercise significantly affected failure force, and normalizing for tendon morphology revealed linetype to significantly affect failure stress. Age had a significant effect on failure strains showing young mice had significantly longer failure strains compared to adult mice. L = significant effect by linetype; A = significant effect by age; X = Significant effect by exercise; L*A = significant interaction effect between linetype and age.

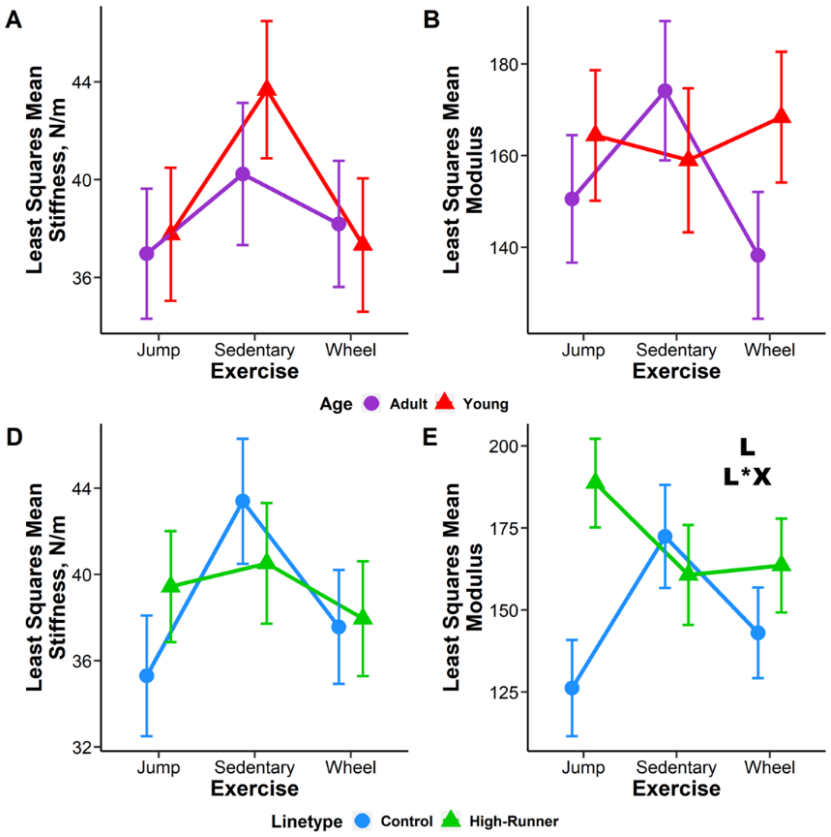

Figure 14. Tendon Mechanical Properties – Stiffness and Modulus. Mechanical properties results showing the least squares means for (A, C) stiffness, (B, D) and modulus. Stiffness was not significantly affected by linetype, age, or exercise, but normalizing for tendon morphology revealed a significant effect by linetype and an interaction effect by linetype and exercise. L = significant effect by linetype; L*X = significant interaction effect by linetype and exercise.

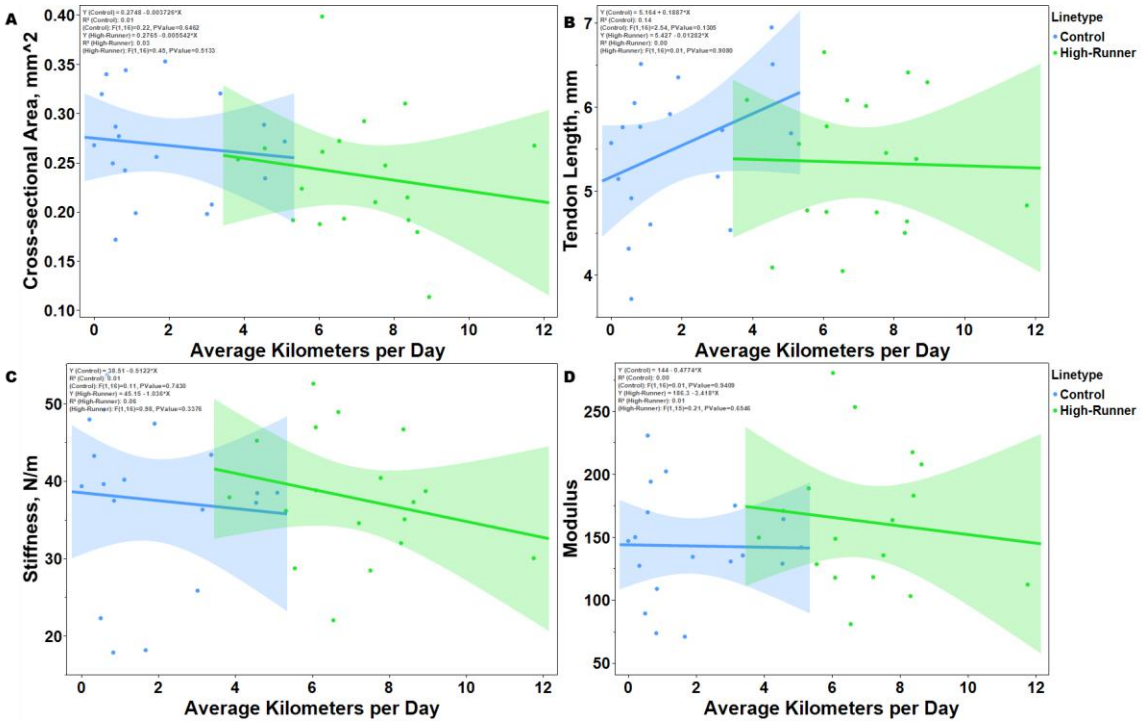

Figure 15. Linear Regression Plots Correlating Wheel Running with Tendon Morphology and Elasticity. Linear regressions comparing average kilometers ran per day to (A, B) tendon morphology and (C, D) mechanical properties. HR mice (green) clearly had higher activity levels compared to the control linetype (blue) with only a few overlapping individuals. Neither morphology nor mechanical properties showed significant relationships with average kilometers ran per day for both linetypes (all $p > 0.05$).

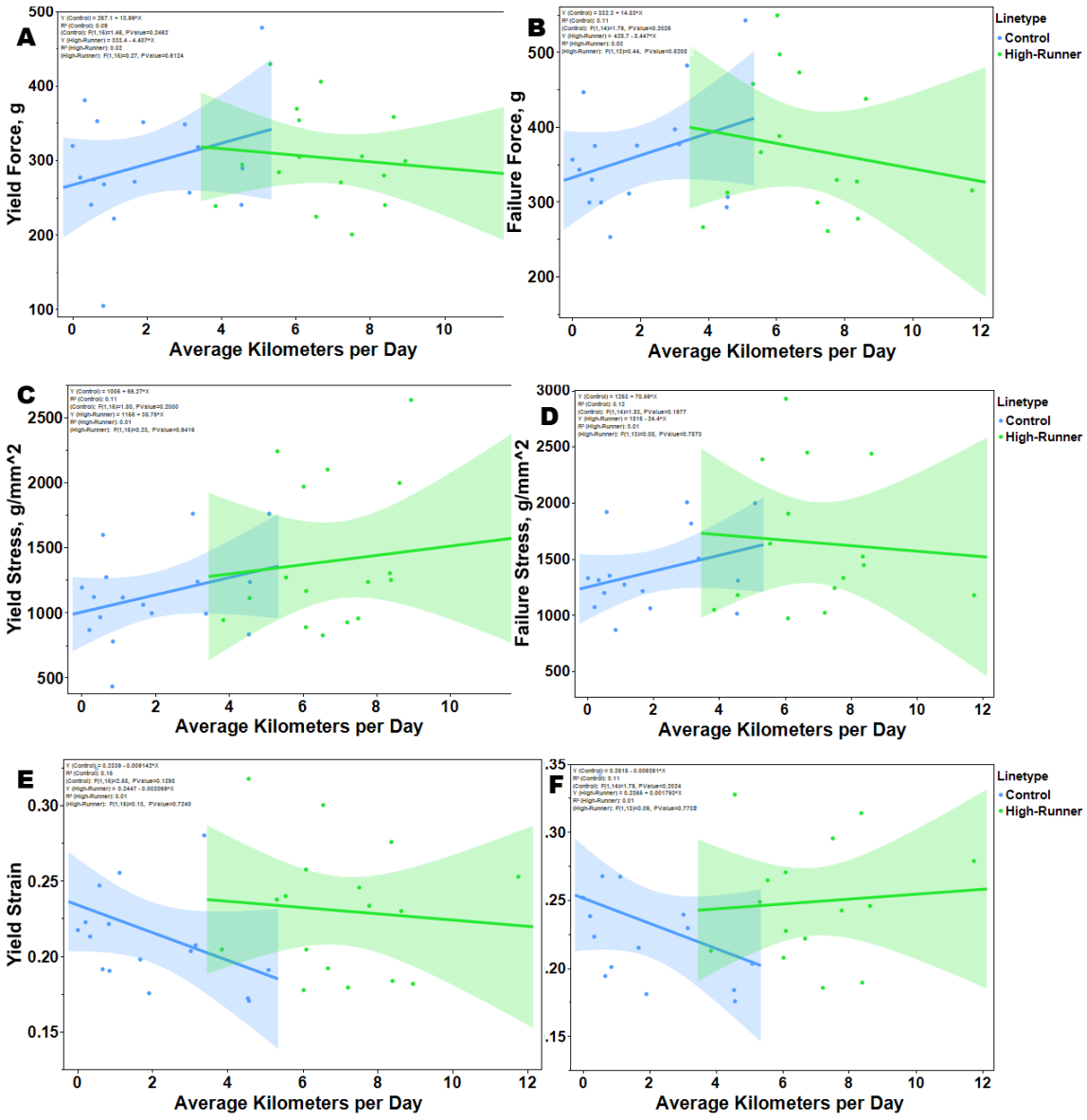

Figure 16. Linear Regression Plots Correlating Wheel Running with Tendon Mechanical Properties. Linear regressions comparing average kilometers ran per day to (A, C, E) yield force, stress, and strain, and (B, D, F) failure force, stress, and strain. HR mice (green) clearly had higher activity levels compared to the control linetype (blue) with only a few overlapping individuals. Average kilometers ran per day showed no significant relationships with any mechanical properties for both linetypes (all $p > 0.05$).

Table 1. Summary of Tendon Morphological Properties. Sample sizes and least squares means of linetype, age, and exercise groupings for tendon morphology.

Group	N	Body Mass, g	N_1	Gastrocnemii Mass, g	N_2	Cross-sectional Area, mm^2	N_3	Tendon Length, mm
Control	49	25.693 ± 0.256	22	0 ± 0	49	0.274 ± 0.01	49	5.547 ± 0.128
High-Runner	53	23.865 ± 0.247	21	0.147 ± 0.004	53	0.241 ± 0.01	53	5.746 ± 0.123
Adult	52	25.503 ± 0.25	18	0 ± 0	52	0.271 ± 0.01	52	5.844 ± 0.124
Young	50	24.055 ± 0.253	25	0.148 ± 0.004	50	0.244 ± 0.01	50	5.449 ± 0.126
Jump	35	24.22 ± 0.303	18	0.152 ± 0.004	35	0.262 ± 0.012	35	5.718 ± 0.151
Sedentary	31	25.647 ± 0.321	16	0.153 ± 0.005	31	0.259 ± 0.012	31	5.798 ± 0.16
Wheel	36	24.47 ± 0.299	9	0 ± 0	36	0.252 ± 0.012	36	5.424 ± 0.149

Note:
Each grouping lists the sample sizes (N) associated with the corresponding morphological traits.
Each variable is listed with The least squares means followed by the standard error.
Insufficient number of samples were collected of gastrocnemii muscles for each group, resulting in the inability to calculate least square means for all groups.

Table 2. Summary of Tendon Mechanical Properties. Sample sizes and least squares of means of linetype, age, and exercise groupings for tendon mechanical properties.

Group	N	Failure Force, g	N_1	Failure Strain	N_2	Failure Stress, g/mm^2	N_3	Modulus	N_4	Stiffness	N_5	Yield Force, g	N_6	Yield Strain	N_7	Yield Stress, g/mm^2
Control	47	369.59 ± 11.702	46	0.232 ± 0.006	47	1446.102 ± 76.257	48	147.253 ± 8.518	49	38.757 ± 1.607	48	297.947 ± 10.452	48	0.218 ± 0.006	48	1165.72 ± 69.724
High-Runner	49	386.619 ± 11.509	49	0.227 ± 0.006	49	1713.372 ± 75.006	51	171.019 ± 8.274	53	39.306 ± 1.547	52	315.371 ± 10.051	52	0.21 ± 0.006	52	1415.894 ± 67.049
Adult	49	378.373 ± 11.509	49	0.218 ± 0.006	49	1518.303 ± 75.006	51	154.324 ± 8.274	52	38.469 ± 1.567	50	316.097 ± 10.277	50	0.205 ± 0.006	50	1286.737 ± 68.558
Young	47	377.836 ± 11.702	46	0.241 ± 0.006	47	1641.172 ± 76.257	48	163.947 ± 8.518	50	39.594 ± 1.587	50	297.221 ± 10.23	50	0.223 ± 0.006	50	1294.876 ± 68.24
Jump	34	357.818 ± 13.805	33	0.222 ± 0.008	34	1515.51 ± 89.966	35	157.494 ± 9.966	35	37.374 ± 1.902	35	292.64 ± 12.261	35	0.21 ± 0.007	35	1234.528 ± 81.79
Sedentary	31	409.239 ± 14.414	31	0.225 ± 0.008	31	1694.62 ± 93.937	29	166.574 ± 10.919	31	41.957 ± 2.016	31	328.155 ± 12.993	31	0.209 ± 0.007	31	1369.07 ± 86.676
Wheel	31	367.258 ± 14.414	31	0.241 ± 0.008	31	1529.082 ± 93.937	35	153.34 ± 9.936	36	37.763 ± 1.874	34	299.183 ± 12.408	34	0.223 ± 0.007	34	1268.822 ± 82.769

Note:
Each grouping lists the sample sizes (N) associated with the corresponding mechanical properties.
Each variable is listed with The least squares means followed by the standard error.

Table 3. Statistics Regarding Tendon Morphology. Statistical outputs for 3-way ANOVAs for all analyses on tendon morphology.

Variable	Linetype	Exercise	Linetype*Exercise	Age	Linetype*Age	Exercise*Age	Linetype*Exercise*Age
Body Mass, g	F(1, 90) = 26.4355; p = <.0001	F(2, 90) = 5.8631; p = 0.004	F(2, 90) = 9.6357; p = 0.0002	F(1, 90) = 16.5899; p = <.0001	F(1, 90) = 0.101; p = 0.7513	F(2, 90) = 0.1385; p = 0.8709	F(2, 90) = 1.8389; p = 0.1649
Cross-sectional Area, mm^2	F(1, 90) = 5.5203; p = 0.021	F(2, 90) = 0.1828; p = 0.8332	F(2, 90) = 2.2295; p = 0.1135	F(1, 90) = 3.9493; p = 0.0499	F(1, 90) = 0.0072; p = 0.9325	F(2, 90) = 0.7521; p = 0.4743	F(2, 90) = 0.5884; p = 0.5573
Tendon Length, mm	F(1, 90) = 1.2713; p = 0.2625	F(2, 90) = 1.6785; p = 0.1925	F(2, 90) = 3.5838; p = 0.0318	F(1, 90) = 4.9889; p = 0.028	F(1, 90) = 2.2998; p = 0.1329	F(2, 90) = 1.2078; p = 0.3036	F(2, 90) = 0.4031; p = 0.6694

Note:
F-ratios are followed by 2 numbers denoting degrees of freedom (DF) for the numerator and denominator.
p-values < 0.05 are significantly different.

Table 4. Statistics Regarding Tendon Mechanical Properties. Statistical outputs for 3-way ANOVAs for all analyses on tendon mechanical properties.

Variable	Linetype	Exercise	Linetype*Exercise	Age	Linetype*Age	Exercise*Age	Linetype*Exercise*Age
Failure Force, g	F(1, 84) = 1.0764; p = 0.3025	F(2, 84) = 3.6929; p = 0.029	F(2, 84) = 0.9546; p = 0.3891	F(1, 84) = 0.0011; p = 0.974	F(1, 84) = 5.7976; p = 0.0182	F(2, 84) = 1.3565; p = 0.2631	F(2, 84) = 0.4619; p = 0.6317
Failure Strain	F(1, 84) = 0.2915; p = 0.5907	F(2, 84) = 1.7221; p = 0.185	F(2, 84) = 1.976; p = 0.1451	F(1, 84) = 6.5702; p = 0.0122	F(1, 84) = 4.9325; p = 0.0291	F(2, 84) = 0.4003; p = 0.6714	F(2, 84) = 0.3621; p = 0.6973
Failure Stress, g/mm^2	F(1, 84) = 6.2436; p = 0.0144	F(2, 84) = 1.1474; p = 0.3224	F(2, 84) = 0.5025; p = 0.6068	F(1, 84) = 1.3195; p = 0.2539	F(1, 84) = 2.7289; p = 0.1023	F(2, 84) = 2.6873; p = 0.0739	F(2, 84) = 0.4185; p = 0.6594
Modulus	F(1, 87) = 4.0059; p = 0.0485	F(2, 87) = 0.4128; p = 0.6631	F(2, 87) = 3.2039; p = 0.0454	F(1, 87) = 0.6567; p = 0.4199	F(1, 87) = 2.3907; p = 0.1257	F(2, 87) = 1.1915; p = 0.3087	F(2, 87) = 2.3216; p = 0.1042
Stiffness, N/m	F(1, 87) = 0.0604; p = 0.8064	F(2, 87) = 1.6555; p = 0.1967	F(2, 87) = 0.8058; p = 0.4499	F(1, 87) = 0.2544; p = 0.6152	F(1, 87) = 3.8618; p = 0.0525	F(2, 87) = 0.3081; p = 0.7356	F(2, 87) = 2.6711; p = 0.0747
Yield Force, g	F(1, 88) = 1.4438; p = 0.2327	F(2, 88) = 2.1982; p = 0.1171	F(2, 88) = 0.9286; p = 0.3989	F(1, 88) = 1.6945; p = 0.1964	F(1, 88) = 5.056; p = 0.027	F(2, 88) = 0.134; p = 0.8748	F(2, 88) = 0.0193; p = 0.9809
Yield Strain	F(1, 88) = 0.961; p = 0.3296	F(2, 88) = 1.2445; p = 0.2931	F(2, 88) = 2.4672; p = 0.0907	F(1, 88) = 5.0404; p = 0.0273	F(1, 88) = 5.0768; p = 0.0267	F(2, 88) = 0.9321; p = 0.3976	F(2, 88) = 0.3266; p = 0.7223
Yield Stress, g/mm^2	F(1, 88) = 6.6888; p = 0.0113	F(2, 88) = 0.678; p = 0.5103	F(2, 88) = 0.3818; p = 0.6838	F(1, 88) = 0.0071; p = 0.9331	F(1, 88) = 1.2994; p = 0.2574	F(2, 88) = 0.2997; p = 0.7418	F(2, 88) = 0.2739; p = 0.761

Note:
F-ratios are followed by 2 numbers denoting degrees of freedom (DF) for the numerator and denominator.
p-values < 0.05 are significantly different.

CHAPTER FOUR

DISCUSSION

I aimed to determine if variations in exercise intensity (jump vs. wheel vs. sedentary) and time of implementation (young vs. adult) altered mice (*Mus domesticus*) tendon remodeling as measured via morphological and mechanical adaptations. I hypothesized that both jump and wheel training would lead to stronger tendons compared to mice without exercise, and mice that began training before skeletal maturity would exhibit stronger tendons compared to adult mice with or without exercise. Additionally, I hypothesized that increased activity levels – as seen in HR mice – would exacerbate these changes in tendon materials properties.

The literature has established that high-runner mice have distinct morphological traits separating them from the control linetype. Selected linetypes exhibit reduced overall body mass and triceps surae muscle complex, and larger femoral condyles (Jr. Garland Theodore et al., 2002; T. Garland & Freeman, 2005). HR mice ran three times the distance per day on wheels; although I did not directly quantify jump cage activity for all cages, I observed substantially more activity in HR mice cages than control. Based off of observation, I would estimate that control mice jumped about 200 times per day while HR jumped at least 400 times per day. These stark differences in activity contributed to overall smaller body masses in HR mice, and exercise treatments in both groups significantly reduced overall body mass (Table 1&3). Research has shown HR

linetypes to generally have smaller body masses than control linetypes (Acosta et al., 2015; Jr. Garland Theodore et al., 2002; Girard et al., 2001), indicating that genetics is a major contributor to body mass variation. Additionally, HR mice have generally longer tendons than the control linetype, suggesting that the >90 generations of selection on these mice has resulted in phenotypic changes in limb tendons. These changes – longer tendons – are similar to cursorial animals (Ker et al., 1988). Other lines within the HR colony (not used in this study) have a mini-muscle phenotype that has reached fixation in one line, and remains at 50% frequency in another line (Jr. Garland Theodore et al., 2002). These mini-muscle mice have exhibited longer and thinner femora and tibiafibulae despite no increase in metatarsal-femur ratio, which is a classic indicator of cursoriality (Kelly et al., 2006). These lines achieve longer running distances via increased running speeds and intermittent running (Girard et al., 2001), and increased muscle fatigue resistance (Syme et al., 2005); contrarily, mini-muscle mice have higher cost of transport partly due to increased postural costs (Dlugosz et al., 2009).

Given the differences in body mass and tendon morphology between HR mice and control lines, the fact that raw, non-normalized materials properties did not differ between the groups is remarkable. Previous studies have demonstrated that increased magnitude and strain rate increased materials properties in human Achilles tendon (Arampatzis et al., 2007, 2010). Larger body masses in control lines proportionally increases mechanical strain on tendons, which would

presumably increase tendon mechanical properties. In addition, jump mice are expected to have thinner tendons because a pilot study showed significantly thinner tendons from mice within the same jump cage setup (unpublished data). This is possibly due to tendons being used more as locomotor springs, which has been shown to result in thinner tendons to enable more strain and elastic recoil (Ker et al., 1988; Shadwick, 1990). The similar raw, non-normalized mechanical properties indicates that materials properties responsible for morphological differences in tendon CSA and length should show significant differences.

Previous research has demonstrated that changes in mechanical properties paired with no changes in extrinsic properties can be explained via intrinsic changes. Pollock and Shadwick (1994) have claimed that all mammalian tendons have similar materials properties despite differences in tendon size and morphology. Ker (1988) counters that tendons functioning as locomotor springs develop thinner tendons achieving higher elastic strain. This is supported by research demonstrating hypertrophy in tendons not involved with elastic saving mechanisms and no change in their counterparts (Birch et al., 1999). Thinner tendons capable of resisting increasing mechanical loads suggests intrinsic changes such as increasing collagen fibril diameter and density (Rigozzi et al., 2010; Wood et al., 2011).

Surprisingly, I found minimal significant age-dependent differences in materials properties. No age-dependent differences were found in failure force and stiffness despite research suggesting an increase in failure stress (Goh et

al., 2008) and stiffness (Avery & Bailey, 2005; Buchanan & Marsh, 2001). Yet, when normalizing for size differences, significant differences were revealed between linetypes for failure stress and modulus. The final age gap between cohorts was 6 weeks, so it is possible that the age difference was insufficient to highlight age-dependent changes in raw measurements; however, research on aged tendon have shown that stiffness remained similar between adult and aged tendon (Wood & Brooks, 2016). Although I observed no significant differences between yield and failure forces, young mice showed significantly longer yield and failure strains compared to adult mice suggesting that maturing tendon are more compliant. The literature typically investigates the tensile strength of tendon; however, yield force is arguably more relevant, given that animals perform up to and within the plastic region rather than breaching their tendon safety factor (Alexander, 1981).

Despite not exposing many significant differences in materials properties between age cohorts, I suspect potential long-term effects from training before reaching skeletal maturity. Heinemeier and colleagues (2013) found no changes in the core tendon of 80-year-old humans, which suggests no changes since reaching skeletal maturity. Implementing exercises before skeletal maturity that increase collagen formation (Barone et al., 2009) and cross-linkages (Heinemeier et al., 2007) could influence baseline mechanical properties of the core-tendon. As stated previously, the final age gap between cohorts was six weeks, which might be insufficient to measure the potential for materials properties. This

hypothesized core-tendon could potentially decrease the possibility of tendon ruptures at later life stages by being more resistant to injury from overuse (Magnan et al., 2014). In the future, long-term studies should follow subjects into advanced ages with variations in training periods to help tease apart the intricate relationship between age and exercise.

Several papers have reported similar results that exercise or aging did not augment significant differences in tendon remodeling. Age-dependent differences between adult and old tendon were not significant for tendon length and CSA (Wood et al., 2011), failure load (Nakagawa et al., 1996), and stiffness (Wood & Brooks, 2016). Researchers have also found exercise to have no effect on morphology and mechanical properties (T.-F. Huang et al., 2004; R. de C. Marqueti et al., 2017). When comparing these results with other studies, interpretations are often limited due to incompatible comparisons. Previous research has suggested that mammalian tendons have no significant differences in stiffness and modulus among mammals ranging from 0.5 to 545 kg (Pollock & Shadwick, 1994). In contrast, differences between immature and mature tendons were significant suggesting unequal comparisons between age groups (Shadwick, 1990). Unfortunately, a majority of studies are focused on advanced aged subjects (LaCroix et al., 2013; Nakagawa et al., 1996; Wood & Brooks, 2016) that prove difficult to compare to younger specimens (Kaux et al., 2013; Legerlotz et al., 2007) and especially studies with no listed ages (Hansen et al., 2002; Heinemeier et al., 2012). Studies rarely address training at multiple life

stages (R. C. Marqueti et al., 2018), which results in more puzzle pieces before clearly connecting the current selection. This experiment was designed to add to this subset of studies that addresses the variability of training intensity and maturation effects on tendon remodeling. Having two mice linetypes with different activity levels paired with exercises of varying difficulties establishes many combinations that training can be achieved. In addition, these combinations were applied to two life stages, thus, making reasonable comparisons for identifying maturation and training effects on tendon remodeling.

This study does have methodological limitations despite efforts to reduce them. (1) The wooden testing rig utilized a design by Probst and colleagues (2000) to maximize the testable length of tendon, but the proximal attachment via suture is probably subject to uneven distribution of force during testing. A surgical knot was tied around the proximal region of the tendon and reinforced with VetBond to reduce slippage; however, I noted frequent uneven ruptures that resulted in shearing along the anterior and posterior portions of the tendon. This hints at an uneven distribution of force along the tendon, and therefore, could lead to underestimates of yield and failure forces (Bennett et al., 1986). (2) Morphometric measurements were extracted from photos taken from two different cameras. Correctly calibrating each image should eliminate any discrepancies, but the photos differed with one being in color. Taking morphometric measurements from grayscale images might prove more difficult than colored images such as when discerning tendon from remanent connective

tissue. (3) The lack of quantitative measurements for mice within the jump treatment pose the problem of overstating claims for less than predicted movements. Despite verifying in advance that the jump cage setup elicited a morphological and mechanical response in tendon in half the training time (unpublished data), I cannot quantify the magnitude of jumps that separated exercise and linetype treatments. I can only state with absolutely certainty that the mice accessed this platform because it housed the water supply that is essential to the mice's survival.

In summary, high-runner mice run significantly more than standard lab mice and have smaller body masses, but have relatively strong tendons; this, paired with average longer tendons is suggestive of cursorial evolution. Cursorial animals have evolved longer, thinner tendons capable of storing more elastic strain energy for more efficient locomotor behaviors, but it remains questionable if this is the case for high-runner mice.

REFERENCES

Acosta, W., Meek, T. H., Schutz, H., Dlugosz, E. M., Vu, K. T., & Garland, T. (2015). Effects of early-onset voluntary exercise on adult physical activity and associated phenotypes in mice. *Physiology & Behavior*, 149, 279–286. https://doi.org/10.1016/j.physbeh.2015.06.020

Alexander, R. M. (1981). Factors of safety in the structure of animals. Science Progress, 67(265), 109–130.

Anderson, C. V., & Deban, S. M. (2010). Ballistic tongue projection in chameleons maintains high performance at low temperature. *Proceedings of the National Academy of Sciences of the United States of America*, 107(12), 5495–5499. https://doi.org/10.1073/pnas.0910778107

Ansorge, H. L., Adams, S., Birk, D. E., & Soslowsky, L. J. (2011). Mechanical, Compositional, and Structural Properties of the Post-natal Mouse Achilles Tendon. *Journal of Biomedical Engineering*, 39(7), 1904–1913. https://doi.org/10.1007/s10439-011-0299-0

Arampatzis, A., Karamanidis, K., & Albracht, K. (2007). Adaptational responses of the human Achilles tendon by modulation of the applied cyclic strain magnitude. *Journal of Experimental Biology*, 210(15), 2743–2753. https://doi.org/10.1242/jeb.003814

Arampatzis, A., Peper, A., Bierbaum, S., & Albracht, K. (2010). Plasticity of human Achilles tendon mechanical and morphological properties in response to cyclic

strain. *Journal of Biomechanics*, 43(16), 3073–3079. https://doi.org/10.1016/j.jbiomech.2010.08.014

Arruda, E. M., Calve, S., Dennis, R. G., Mundy, K., & Baar, K. (2006). Regional variation of tibialis anterior tendon mechanics is lost following denervation. *Journal of Applied Physiology*, 101(4), 1113–1117. https://doi.org/10.1152/japplphysiol.00612.2005

Astley, H. C., & Roberts, T. J. (2012). Evidence for a vertebrate catapult: Elastic energy storage in the plantaris tendon during frog jumping. *Biology Letters*, 8(3), 386–389. https://doi.org/10.1098/rsbl.2011.0982

Avery, N. C., & Bailey, A. J. (2005). Enzymic and non-enzymic cross-linking mechanisms in relation to turnover of collagen: Relevance to aging and exercise. *Scandinavian Journal of Medicine and Science in Sports*, 15(4), 231–240. https://doi.org/10.1111/j.1600-0838.2005.00464.x

Barone, R., Bellafiore, M., Leonardi, V., & Zummo, G. (2009). Structural analysis of rat patellar tendon in response to resistance and endurance training. *Scandinavian Journal of Medicine & Science in Sports*, 19(6), 782–789. https://doi.org/10.1111/j.1600-0838.2008.00863.x

Beamer, W. G., Donahue, L. R., Rosen, C. J., & Baylink, D. J. (1996). Genetic variability in adult bone density among inbred strains of mice. *Bone*, 18(5), 397–403. https://doi.org/10.1016/8756-3282(96)00047-6

Bennett, M. B., Ker, R. F., Dimery, N. J., & Alexander, R. McN. (1986). Mechanical properties of various mammalian tendons. *Journal of Zoology*, 209(4), 537–548. https://doi.org/10.1111/j.1469-7998.1986.tb03609.x

Biewener, A. A., Alexander, R. M. N., & Heglund, N. C. (1981). Elastic energy storage in the hopping of kangaroo rats Dipodomys spectabilis. *Journal of Zoology*, 195(3), 369–383. https://doi.org/10.1111/j.1469-7998.1981.tb03471.x

Biewener, A. A., & Blickhan, R. (1988). Kangaroo rat locomotion: Design for elastic energy storage or acceleration? *The Journal of Experimental Biology*, 140, 243–255.

Biewener, A. A., Blickhan, R., Perry, A. K., Heglund, N. C., & Taylor, C. R. (1988). Muscle forces during locomotion in kangaroo rats: Force platform and tendon buckle measurements compared. *Journal of Experimental Biology*, 137, 191–205.

Birch, H. L., McLaughlin, L., Smith, R. K. W., & Goodship, A. E. (1999). Treadmill exercise-induced tendon hypertrophy: Assessment of tendons with different mechanical functions. *Equine Veterinary Journal*, 31(S30), 222–226. https://doi.org/10.1111/j.2042-3306.1999.tb05222.x

Birk, D. E., & Mayne, R. (1997). Localization of collagen types I, III and V during tendon development. Changes in collagen types I and III are correlated with changes in fibril diameter. *European Journal of Cell Biology*, 72(4), 352–361.

Boon-Ho Ng, B. M., & Siaw-Meng Chou, B. M. (2003). The Effect of Freeze Storage on the Tensile Properties of Tendons. *Journal of Mechanics in Medicine & Biology*, 3(3/4), 299–308. https://doi.org/10.1142/S0219519403000818

Buchanan, C. I., & Marsh, R. L. (2001). Effects of long-term exercise on the biomechanical properties of the Achilles tendon of guinea fowl. *Journal of Applied Physiology*, 90(1), 164–171. https://doi.org/10.1152/jappl.2001.90.1.164

Chen, L., Wu, Y., Yu, J., Jiao, Z., Ao, Y., Yu, C., Wang, J., & Cui, G. (2011). Effect of repeated freezing-thawing on the Achilles tendon of rabbits. *Knee Surgery, Sports Traumatology, Arthroscopy*, 19(6), 1028–1034. https://doi.org/10.1007/s00167-010-1278-y

Couppé, C., Kongsgaard, M., Aagaard, P., Hansen, P., Bojsen-Moller, J., Kjaer, M., & Magnusson, S. P. (2008). Habitual loading results in tendon hypertrophy and increased stiffness of the human patellar tendon. *Journal of Applied Physiology*, 105(3), 805–810. https://doi.org/10.1152/japplphysiol.90361.2008

Cribb, A. M., & Scott, J. E. (1995). Tendon response to tensile stress: An ultrastructural investigation of collagen: Proteoglycan interactions in stressed tendon. *Anatomy*, 187, 423–428.

Cui, L., Maas, H., Perreault, E. J., & Sandercock, T. G. (2009). In situ estimation of tendon material properties: Differences between muscles of the feline hindlimb. *Journal of Biomechanics*, 42(6), 679–685. https://doi.org/10.1016/j.jbiomech.2009.01.022

Dawson, T. J., & Taylor, C. R. (1973). Energetic cost of locomotion in kangaroos. *Nature*, 246(5431), 313–314. https://doi.org/10.1038/246313a0

Dlugosz, E. M., Chappell, M. A., McGillivray, D. G., Syme, D. A., & Garland Jr., T. (2009). Locomotor trade-offs in mice selectively bred for high voluntary wheel running. *Journal of Experimental Biology*, 212(16), 2612–2618. https://doi.org/10.1242/jeb.029058

Doral, M. N., Alam, M., Bozkurt, M., Turhan, E., Atay, O. A., Dönmez, G., & Maffulli, N. (2010). Functional anatomy of the Achilles tendon. *Knee Surgery, Sports Traumatology, Arthroscopy*, 18(5), 638–643. https://doi.org/10.1007/s00167-010-1083-7

Dorlot, J.-M., Ait Ba Sidi, M., Tremblay, G. M., & Drouin, G. (1980). Load Elongation Behavior of the Canine Anterior Cruciate Ligament. *Journal of Biomechanical Engineering*, 102(3), 190–193. https://doi.org/10.1115/1.3149572

Dressler, M. R., Butler, D. L., Wenstrup, R., Awad, H. A., Smith, F., & Boivin, G. P. (2002). A potential mechanism for age-related declines in patellar tendon biomechanics. *Journal of Orthopaedic Research*, 20(6), 1315–1322. https://doi.org/10.1016/S0736-0266(02)00052-9

Ellerby, D. J., & Marsh, R. L. (2006). The energetic costs of trunk and distal-limb loading during walking and running in guinea fowl Numida meleagris II. Muscle energy use as indicated by blood flow. *Journal of Experimental Biology*, 209(11), 2064–2075. https://doi.org/10.1242/jeb.02227

Elliott, D. M., Robinson, P. S., Gimbel, J. A., Sarver, J. J., Abboud, J. A., Iozzo, R. V., & Soslowsky, L. J. (2003). Effect of Altered Matrix Proteins on Quasilinear Viscoelastic Properties in Transgenic Mouse Tail Tendons. *Annals of Biomedical Engineering*, 31(5), 599–605. https://doi.org/10.1114/1.1567282

Falconer, D. S. (1984). Weight and age at puberty in female and male mice of strains selected for large and small body size. *Genetics Research*, 44(1), 47–72. https://doi.org/10.1017/S0016672300026240

Garland, Jr., Theodore, Morgan, M. T., Swallow, J. G., Rhodes, J. S., Girard, I., Belter, J. G., Carter, P. A., & Cheverud, J. (2002). Evolution of a Small-Muscle Polymorphism in Lines of House Mice Selected for High Levels. *Evolution*, 56(6), 1267. https://doi.org/10.1554/0014-3820(2002)056[1267:EOASMP]2.0.CO;2

Garland, T. (2003). Selection experiments: An under-utilized tool in biomechanics and organismal biology. *Vertebrate Biomechanics and Evolution*, 23–57.

Garland, T., & Freeman, P. W. (2005). Selective breeding for high endurance running increases hindlimb symmetry. *Evolution*, 59(8), 1851–1854. https://doi.org/10.1111/j.0014-3820.2005.tb01832.x

Gautieri, A., Passini, F. S., Silván, U., Guizar-Sicairos, M., Carimati, G., Volpi, P., Moretti, M., Schoenhuber, H., Redaelli, A., Berli, M., & Snedeker, J. G. (2017). Advanced glycation end-products: Mechanics of aged collagen from molecule to tissue. *Matrix Biology*, 59, 95–108. https://doi.org/10.1016/j.matbio.2016.09.001

Girard, I., McAleer, M. W., Rhodes, J. S., & Garland, T. (2001). Selection for high voluntary wheel-running increases speed and intermittency in house mice (Mus domesticus). *Journal of Experimental Biology*, 204(24), 4311–4320.

Goh, K. L., Chen, Y., Chou, S. M., Listrat, A., Bechet, D., & Wess, T. J. (2010). Effects of frozen storage temperature on the elasticity of tendons from a small murine model. *Animal*, 4(9), 1613–1617. https://doi.org/10.1017/S1751731110000698

Goh, K. L., Holmes, D. F., Lu, H.-Y., Richardson, S., Kadler, K. E., Purslow, P. P., & Wess, T. J. (2008). Ageing Changes in the Tensile Properties of Tendons: Influence of Collagen Fibril Volume Fraction. *Journal of Biomechanical Engineering*, 130(2), 021011-1-021011–021018. https://doi.org/10.1115/1.2898732

Gumucio, J. P., Phan, A. C., Ruehlmann, D. G., Noah, A. C., & Mendias, C. L. (2014). Synergist ablation induces rapid tendon growth through the synthesis of a neotendon matrix. *Journal of Applied Physiology*, 117(11), 1287–1291. https://doi.org/10.1152/japplphysiol.00720.2014

Gutmann, A. K., Lee, D. V., & McGowan, C. P. (2013). Collision-based mechanics of bipedal hopping. *Biology Letters*, 9(4), 1–5.

Hansen, K. A., Weiss, J. A., & Barton, J. K. (2002). Recruitment of Tendon Crimp With Applied Tensile Strain. *Journal of Biomechanical Engineering*, 124(1), 72–77. https://doi.org/10.1115/1.1427698

Hasberry, S., & Pearcy, M. J. (1986). Temperature dependence of the tensile properties of interspinous ligaments of sheep. *Journal of Biomedical Engineering*, 8(1), 62–66. https://doi.org/10.1016/0141-5425(86)90032-4

Heinemeier, K. M., & Kjaer, M. (2011). In vivo investigation of tendon responses to mechanical loading. *Journal of Musculoskeletal & Neuronal Interactions*, 11(2), 115–123.

Heinemeier, K. M., Olesen, J. L., Haddad, F., Langberg, H., Kjaer, M., Baldwin, K. M., & Schjerling, P. (2007). Expression of collagen and related growth factors in rat tendon and skeletal muscle in response to specific contraction types. *Journal of Physiology*, 582(3), 1303–1316. https://doi.org/10.1113/jphysiol.2007.127639

Heinemeier, K. M., Schjerling, P., Heinemeier, J., Magnusson, S. P., & Kjaer, M. (2013). Lack of tissue renewal in human adult Achilles tendon is revealed by nuclear bomb 14C. *FASEB Journal*, 27(5), 2074–2079. https://doi.org/10.1096/fj.12-225599

Heinemeier, K. M., Schjerling, P., Heinemeier, J., Møller, M. B., Krogsgaard, M. R., Grum-Schwensen, T., Petersen, M. M., & Kjaer, M. (2016). Radiocarbon dating reveals minimal collagen turnover in both healthy and osteoarthritic human cartilage. *Science Translational Medicine*, 8(346), 346ra90-346ra90. https://doi.org/10.1126/scitranslmed.aad8335

Heinemeier, K. M., Schjerling, P., Øhlenschlæger, T. F., Eismark, C., Olsen, J., & Kjær, M. (2018). Carbon-14 bomb pulse dating shows that tendinopathy is preceded by

years of abnormally high collagen turnover. *FASEB Journal*, 32(9), 4763–4775. https://doi.org/10.1096/fj.201701569R

Heinemeier, K. M., Skovgaard, D., Bayer, M. L., Qvortrup, K., Kjaer, A., Kjaer, M., Magnusson, S. P., & Kongsgaard, M. (2012). Uphill running improves rat Achilles tendon tissue mechanical properties and alters gene expression without inducing pathological changes. *Journal of Applied Physiology*, 113(5), 827–836. https://doi.org/10.1152/japplphysiol.00401.2012

Holt, N. C., Roberts, T. J., & Askew, G. N. (2014). The energetic benefits of tendon springs in running: Is the reduction of muscle work important? *Journal of Experimental Biology*, 217(24), 4365–4371. https://doi.org/10.1242/jeb.112813

Huang, C.-Y., Wang, V. M., Flatow, E. L., & Mow, V. C. (2009). Temperature-dependent viscoelastic properties of the human supraspinatus tendon. *Journal of Biomechanics*, 42(4), 546–549. https://doi.org/10.1016/j.jbiomech.2008.11.013

Huang, T.-F., Perry, S. M., & Soslowsky, L. J. (2004). The Effect of Overuse Activity on Achilles Tendon in an Animal Model: A Biomechanical Study. *Annals of Biomedical Engineering*, 32(3), 336–341. https://doi.org/10.1023/B:ABME.0000017537.26426.76

Hulmes, D. J. S., Miller, A., Parry, D. A. D., Piez, K. A., & Woodhead-Galloway, J. (1973). Analysis of the primary structure of collagen for the origins of molecular packing. *Journal of Molecular Biology*, 79(1), 137–148. https://doi.org/10.1016/0022-2836(73)90275-1

Inhofe, P. D., Grana, W. A., Egle, D., Min, K.-W., & Tomasek, J. (1995). The effects of anabolic steroids on rat tendon: An ultrastructural, biomechanical, and biochemical analysis. *American Journal of Sports Medicine*, 23(2), 227–232. https://doi.org/10.1177/036354659502300217

JMP Pro, Version 16.0.0. SAS Institute Inc., Cary, NC, 1989–2021.

Kannus, P., Paavola, M., & Józsa, L. (2005). Aging and Degeneration of Tendons. In Tendon Injuries: Basic science and clinical medicine (pp. 25–31). *Springer, London*. https://doi.org/10.1007/1-84628-050-8_4

Karamanidis, K., & Arampatzis, A. (2006). Mechanical and morphological properties of human quadriceps femoris and triceps surae muscle–tendon unit in relation to aging and running. *Journal of Biomechanics*, 39(3), 406–417. https://doi.org/10.1016/j.jbiomech.2004.12.017

Kaux, J.-F., Drion, P., Libertiaux, V., Colige, A., Hoffmann, A., Nusgens, B., Besançon, B., Forthomme, B., Le Goff, C., Franzen, R., Defraigne, J.-O., Cescotto, S., Rickert, M., Crielaard, J.-M., & Croisier, J.-L. (2013). Eccentric training improves tendon biomechanical properties: A rat model: eccentric training improves tendon properties. *Journal of Orthopaedic Research*, 31(1), 119–124. https://doi.org/10.1002/jor.22202

Kelly, S. A., Czech, P. P., Wight, J. T., Blank, K. M., & Garland, T. (2006). Experimental evolution and phenotypic plasticity of hindlimb bones in high-activity house mice. *Journal of Morphology*, 267(3), 360–374. https://doi.org/10.1002/jmor.10407

Ker, R. F., Alexander, R. Mcn., & Bennett, M. B. (1988). Why are mammalian tendons so thick? *Journal of Zoology*, 216(2), 309–324. https://doi.org/10.1111/j.1469-7998.1988.tb02432.x

Kjaer, M. (2004). Role of Extracellular Matrix in Adaptation of Tendon and Skeletal Muscle to Mechanical Loading. *Physiological Reviews*, 84(2), 649–698. https://doi.org/10.1152/physrev.00031.2003

Klitgaard, H. (1988). A model for quantitative strength training of hindlimb muscles of the rat. *Journal of Applied Physiology*, 64(4), 1740–1745. https://doi.org/10.1152/jappl.1988.64.4.1740

Komatsu, F., Mori, R., & Uchio, Y. (2006). Optimum surgical suture material and methods to obtain high tensile strength at knots: Problems of conventional knots and the reinforcement effect of adhesive agent. *Journal of Orthopaedic Science: Official Journal of the Japanese Orthopaedic Association*, 11(1), 70–74. https://doi.org/10.1007/s00776-005-0973-x

Konow, N., Azizi, E., & Roberts, T. J. (2012). Muscle power attenuation by tendon during energy dissipation. *Proceedings of the Royal Society B: Biological Sciences*, 279(1731), 1108–1113. https://doi.org/10.1098/rspb.2011.1435

Konow, N., & Roberts, T. J. (2014). The series elastic shock absorber: Tendon elasticity modulates energy dissipation by muscle during burst deceleration. *Proceedings of the Royal Society B: Biological Sciences*, 282(i).

Koteja, P., Swallow, J. G., Carter, P. A., & Garland Jr., T. (1999). Energy Cost of Wheel Running in House Mice: Implications for Coadaptation of Locomotion and Energy

Budgets. *Physiological & Biochemical Zoology*, 72(2), 238. https://doi.org/10.1086/316653

Kubo, K., Yata, H., Kanehisa, H., & Fukunaga, T. (2006). Effects of isometric squat training on the tendon stiffness and jump performance. *European Journal of Applied Physiology*, 96(3), 305–314. https://doi.org/10.1007/s00421-005-0087-3

Lacroix, A. S., Duenwald-Kuehl, S. E., Brickson, S., Akins, T. L., Diffee, G., Aiken, J., Vanderby, R., & Lakes, R. S. (2013). Effect of age and exercise on the viscoelastic properties of rat tail Tendon. *Annals of Biomedical Engineering*, 41(6), 1120–1128. https://doi.org/10.1007/s10439-013-0796-4

LaCroix, A. S., Duenwald-Kuehl, S. E., Brickson, S., Akins, T. L., Diffee, G., Aiken, J., Vanderby, R., & Lakes, R. S. (2013). Effect of Age and Exercise on the Viscoelastic Properties of Rat Tail Tendon. *Annals of Biomedical Engineering*, 41(6), 1120–1128. https://doi.org/10.1007/s10439-013-0796-4

Langberg, H., Ellingsgaard, H., Madsen, T., Jansson, J., Magnusson, S. P., Aagaard, P., & Kjær, M. (2007). Eccentric rehabilitation exercise increases peritendinous type I collagen synthesis in humans with Achilles tendinosis. Scandinavian *Journal of Medicine & Science in Sports*, 17(1), 61–66. https://doi.org/10.1111/j.1600-0838.2006.00522.x

Langberg, H., Rosendal, L., & Kjær, M. (2001). Training-induced changes in peritendinous type I collagen turnover determined by microdialysis in humans. *The Journal of Physiology*, 534(1), 297–302. https://doi.org/10.1111/j.1469-7793.2001.00297.x

Langberg, H., Skovgaard, D., Petersen, L. J., Bülow, J., & Kjær, M. (1999). Type I collagen synthesis and degradation in peritendinous tissue after exercise determined by microdialysis in humans. *The Journal of Physiology*, 521(1), 299–306. https://doi.org/10.1111/j.1469-7793.1999.00299.x

Legerlotz, K., Schjerling, P., Langberg, H., Brüggemann, G.-P., & Niehoff, A. (2007). The effect of running, strength, and vibration strength training on the mechanical, morphological, and biochemical properties of the Achilles tendon in rats. *Journal of Applied Physiology*, 102(2), 564–572. https://doi.org/10.1152/japplphysiol.00767.2006

Magnan, B., Bondi, M., Pierantoni, S., & Samaila, E. (2014). The pathogenesis of Achilles tendinopathy: A systematic review. *Foot & Ankle Surgery*, 20(20), 154–159. https://doi.org/10.1016/j.fas.2014.02.010

Marqueti, R. C., Durigan, J. L. Q., Oliveira, A. J. S., Mekaro, M. S., Guzzoni, V., Aro, A. A., Pimentel, E. R., & Selistre-de-Araujo, H. S. (2018). Effects of aging and resistance training in rat tendon remodeling. *FASEB Journal*, 32, 353–368. https://doi.org/10.1096/fj.201700543R

Marqueti, R. C., Prestes, J., Wang, C. C., Ramos, O. H. P., Perez, S. E. A., Nakagaki, W. R., Carvalho, H. F., & Selistre-de-Araujo, H. S. (2011). Biomechanical responses of different rat tendons to nandrolone decanoate and load exercise. *Scandinavian Journal of Medicine & Science in Sports*, 21(6), e91–e99. https://doi.org/10.1111/j.1600-0838.2010.01162.x

Marqueti, R. de C., Almeida, J. A., Nakagaki, W. R., Guzzoni, V., Boghi, F., Renner, A., Silva, P. E., Durigan, J. L. Q., & Selistre-de-Araújo, H. S. (2017). Resistance training minimizes the biomechanical effects of aging in three different rat tendons. *Journal of Biomechanics*, 53, 29–35. https://doi.org/10.1016/j.jbiomech.2016.12.029

Meek, T. H., Lonquich, B. P., Hannon, R. M., & Garland, T. (2009). Endurance capacity of mice selectively bred for high voluntary wheel running. *Journal of Experimental Biology*, 212(18), 2908–2917. https://doi.org/10.1242/jeb.028886

Nakagaki, W. R., Biancalana, A., Benevides, G. P., & Gomes, L. (2007). Biomechanical and Biochemical Properties of Chicken Calcaneal Tendon Under Effect of Age and Nonforced Active Exercise. *Connective Tissue Research*, 48(5), 219–228. https://doi.org/10.1080/03008200701492136

Nakagawa, Y., Hayashi, K., Yamamoto, N., & Nagashima, K. (1996). Age-related changes in biomechanical properties of the Achilles tendon in rabbits. *European Journal of Applied Physiology and Occupational Physiology*, 73(1–2), 7–10. https://doi.org/10.1007/BF00262803

Nielsen, H. M., Skalicky, M., & Viidik, A. (1998). Influence of physical exercise on aging rats. III. Life-long exercise modifies the aging changes of the mechanical properties of limb muscle tendons. *Mechanisms of Ageing and Development*, 100(3), 243–260. https://doi.org/10.1016/S0047-6374(97)00147-4

O'Brien, M. (2005). Anatomy of tendons. *Tendon Injuries: Basic Science and Clinical Medicine*, 3–13. https://doi.org/10.1007/1-84628-050-8_1

Pollock, C. M., & Shadwick, R. E. (1994). Relationship between body mass and biomechanical properties of limb tendons in adult mammals. American *Journal of Physiology - Regulatory Integrative and Comparative Physiology*, 266(3 35-3). https://doi.org/10.1152/ajpregu.1994.266.3.r1016

Probst, A., Palmes, D., Freise, H., Langer, M., Joist, A., & Spiegel, H. U. (2000). A New Clamping Technique for Biomechanical Testing of Tendons in Small Animals. *Journal of Investigative Surgery*, 13, 313–318.

Rankin, J. W., Doney, K. M., & McGowan, C. P. (2018). Functional capacity of kangaroo rat hindlimbs: Adaptations for locomotor performance. *Journal of the Royal Society, Interface*, 15(144), 1–15.

R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rezende, E. L., Garland, T., Chappell, M. A., Malisch, J. L., & Gomes, F. R. (2006). Maximum aerobic performance in lines of Mus selected for high wheel-running activity: Effects of selection, oxygen availability and the mini-muscle phenotype. *Journal of Experimental Biology*, 209(1), 115–127. https://doi.org/10.1242/jeb.01883

Rezende, E. L., Gomes, F. R., Malisch, J. L., Chappell, M. A., & Garland, T. (2006). Maximal oxygen consumption in relation to subordinate traits in lines of house mice selectively bred for high voluntary wheel running. *Journal of Applied Physiology*, 101(2), 477–485. https://doi.org/10.1152/japplphysiol.00042.2006

Rigby, B. J., Hirai, N., Spikes, J. D., & Eyring, H. (1959). The Mechanical Properties of Rat Tail Tendon. *Journal of General Physiology*, 43(2), 265–283. https://doi.org/10.1085/jgp.43.2.265

Rigozzi, S., Müller, R., & Snedeker, J. G. (2010). Collagen fibril morphology and mechanical properties of the Achilles tendon in two inbred mouse strains. *Journal of Anatomy*, 216(6), 724–731. https://doi.org/10.1111/j.1469-7580.2010.01225.x

Roberts, T. J. (2016). Contribution of elastic tissues to the mechanics and energetics of muscle function during movement. *Journal of Experimental Biology*, 219(2), 266–275. https://doi.org/10.1242/jeb.124446

Roberts, T. J., & Konow, N. (2013). How Tendons Buffer Energy Dissipation by Muscle. *Exercise and Sport Sciences Reviews*, 41(4), 1–8.

Rosager, S., Aagaard, P., Dyhre-Poulsen, P., Neergaard, K., Kjaer, M., & Magnusson, S. P. (2002). Load-displacement properties of the human triceps surae aponeurosis and tendon in runners and non-runners. *Scandinavian Journal of Medicine & Science in Sports*, 12(2), 90–98. https://doi.org/10.1034/j.1600-0838.2002.120205.x

Sawicki, G. S., Sheppard, P., & Roberts, T. J. (2015). Power amplification in an isolated muscle-tendon unit is load dependent. *Journal of Experimental Biology*, 218(22), 3700–3709. https://doi.org/10.1242/jeb.126235

Schatzmann, L., Brunner, P., & Stäubli, H. U. (1998). Effect of cyclic preconditioning on the tensile properties of human quadriceps tendons and patellar ligaments.

Knee Surgery, Sports Traumatology, Arthroscopy, 6(1), S56–S61. https://doi.org/10.1007/s001670050224

Shadwick, R. E. (1990). Elastic energy storage in tendons: Mechanical differences related to function and age. *Journal of Applied Physiology*, 68(3), 1033–1040. https://doi.org/10.1152/jappl.1990.68.3.1033

Sharma, P., & Maffulli, N. (2006). Biology of tendon injury: Healing, modeling and remodeling. *Journal of Musculoskeletal Neuronal Interactions*, 6(2), 181–190.

Simonsen, E. B., Klitgaard, H., & Bojsen-M⊘ller, F. (1995). The influence of strength training, swim training and ageing on the Achilles tendon and m. Soleus of the rat. *Journal of Sports Sciences*, 13(4), 291–295. https://doi.org/10.1080/02640419508732242

Sommer, H.-M. (1987). The biomechanical and metabolic effects of a running regime on the Achilles tendon in the rat. *International Orthopaedics*, 11(1), 71–75. https://doi.org/10.1007/BF00266061

Svensson, R. B., Heinemeier, K. M., Couppé, C., Kjaer, M., & Magnusson, S. P. (2016). Effect of aging and exercise on the tendon. *Journal of Applied Physiology*, 121(6), 1353–1362. https://doi.org/10.1152/japplphysiol.00328.2016

Svensson, R. B., Smith, S. T., Moyer, P. J., & Magnusson, S. P. (2018). Effects of maturation and advanced glycation on tensile mechanics of collagen fibrils from rat tail and Achilles tendons. *Acta Biomaterialia*, 70, 270–280. https://doi.org/10.1016/j.actbio.2018.02.005

Swallow, J. G., Carter, P. A., & Garland, T. (1998). Artificial selection for increased wheel-running behavior in house mice. *Behavior Genetics*, 28(3), 227–237. https://doi.org/10.1023/A:1021479331779

Swallow, J. G., Garland, T., Carter, P. A., Zhan, W.-Z., & Sieck, G. C. (1998). Effects of voluntary activity and genetic selection on aerobic capacity in house mice (Mus domesticus). *Journal of Applied Physiology*, 84(1), 69–76.

Syme, D. A., Evashuk, K., Grintuch, B., Rezende, E. L., & Garland, T. (2005). Contractile abilities of normal and "mini" triceps surae muscles from mice (Mus domesticus) selectively bred for high voluntary wheel running. *Journal of Applied Physiology*, 99(4), 1308–1316. https://doi.org/10.1152/japplphysiol.00369.2005

Tempfer, H., & Traweger, A. (2015). Tendon vasculature in health and disease. *Frontiers in Physiology*, 6, 1–7. https://doi.org/10.3389/fphys.2015.00330

Thampatty, B. P., & Wang, J. H. (2018). Mechanobiology of young and aging tendons: In vivo studies with treadmill running. *Journal of Orthopaedic Research*, 36(2), 557–565. https://doi.org/10.1002/jor.23761

Våben, C., Heinemeier, K. M., Schjerling, P., Olsen, J., Petersen, M. M., Kjaer, M., & Krogsgaard, M. R. (2020). No detectable remodelling in adult human menisci: An analysis based on the C14 bomb pulse. *British Journal of Sports Medicine*, 54(23), 1433–1437. https://doi.org/10.1136/bjsports-2019-101360

Verzijl, N., DeGroot, J., Thorpe, S. R., Bank, R. A., Shaw, J. N., Lyons, T. J., Bijlsma, J. W. J., Lafeber, F. P. J. G., Baynes, J. W., & TeKoppele, J. M. (2000). Effect of Collagen Turnover on the Accumulation of Advanced Glycation End Products *.

Journal of Biological Chemistry, 275(50), 39027–39031. https://doi.org/10.1074/jbc.M006700200

Viidik, A., Nielsen, H. M., & Skalicky, M. (1996). Influence of physical exercise on aging rats: II. Life-long exercise delays aging of tail tendon collagen. *Mechanisms of Ageing and Development*, 88(3), 139–148. https://doi.org/10.1016/0047-6374(96)01729-0

Wang, X. T., de Ruister, M. R., Mcn Alexander, R., & Ker, R. F. (1991). The effect of temperature on the tensile stiffness of mammalian tail tendons. *Journal of Zoology*, 223(3), 491–497. https://doi.org/10.1111/j.1469-7998.1991.tb04779.x

WaveMetrics Inc. (1996). Igor Pro User's Guide (Vers.3). Wavemetrics Inc.

Wickham H (2016). ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org.

Woo, S. L.-Y., Gomez, M. A., Amiel, D., Ritter, M. A., Gelberman, R. H., & Akeson, W. H. (1981). The Effects of Exercise on the Biomechanical and Biochemical Properties of Swine Digital Flexor Tendons. *Journal of Biomechanical Engineering*, 103(1), 51–56. https://doi.org/10.1115/1.3138246

Woo, S. L.-Y., Lee, T. Q., Gomez, M. A., Sato, S., & Field, F. P. (1987). Temperature Dependent Behavior of the Canine Medial Collateral Ligament. *Journal of Biomechanical Engineering*, 109(1), 68–71. https://doi.org/10.1115/1.3138645

Wood, L. K., Arruda, E. M., & Brooks, S. V. (2011). Regional stiffening with aging in tibialis anterior tendons of mice occurs independent of changes in collagen fibril

morphology. *Journal of Applied Physiology*, 111(4), 999–1006. https://doi.org/10.1152/japplphysiol.00460.2011

Wood, L. K., & Brooks, S. V. (2016). Ten weeks of treadmill running decreases stiffness and increases collagen turnover in tendons of old mice. *Journal of Orthopaedic Research*, 34(2), 346–353. https://doi.org/10.1002/jor.22824