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THE EFFECT OF CHRONIC MUSCLE DISUSE ON ACHILLES TENDON MATERIAL PROPERTIES IN AN AVIAN BIPEDAL MODEL

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ABSTRACT

Research has been devoted to the effect exercise and disuse has on the material properties of tendons, specifically on stiffness. This research has mostly concerned the period following the growth period, with little research devoted to examining stiffness during the growth period. Previous research has shown that botulinum-toxin-A can be used to create disuse of tendons. When injected during the growth period in rats, no significant difference in tendon stiffness has been viewed when compared to the control group (Eliasson et. al., 2007; Khayyeri et. al., 2017). Because of the little research examining stiffness during the growth period and lack of botulinum-toxin-A used in a bipedal model, this study was performed. Overall, no significant differences were found in tendon stiffness between the control and experimental groups, but a larger pool of animals would have been needed to make any definitive conclusions. The results did not align with my original hypothesis. Despite this, the feasibility of this experiment and human implications with botulinum-toxin-A in the treatment of cerebral palsy allows for multitudes of future research.

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Chapter 1

Introduction

Hierarchy of Tendon Morphology and Anatomy

Tendons are composed of fibrous connective tissue, with the purpose of attaching muscle to bone (Thorpe and Screen, 2016). Tendons characteristically are white and shiny in color, and fibrous yet elastic in texture. A tendon is attached to the muscle by a myotendinous junction, and to the bone by a structure known as an osteotendinous junction (Aro, Vidal & Pimentel, 2012). Tendon morphology is characterized by a distinct hierarchy, as shown in Figure 1, of tissues ranging in length scale from macroscopic structures to collagen molecules.

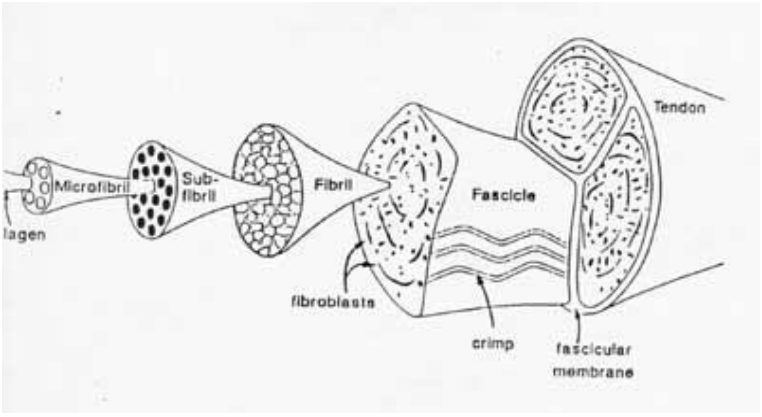

Figure 1. Tendon Hierarchy (Harvey, Thompson, Cochlin, Raju, Cui, Cornell & Brady, 2009)

On the most superficial and macro-scale level, tendons are surrounded by a layer of loose connective tissue known as the paratenon (Thorpe and Screen, 2016). This layer allows the tendon to move against other layers of tissue, and also provides the blood supply for the deeper layers, such as the epitenon, the layer below the paratenon (Aro et.al, 2012). The epitendon serves to link

fascicles, contributing to the structure of the tendon. This layer has continuity with the layer directly deep to the epitenon, the endotenon, which lines fascicles comprised of bundles of collagen fibers. The endotenon also contains blood vessels, with these blood vessels remaining outside of the bundles of collagen fibers (Aro, et. al, 2012).

In terms of the hierarchy at the tendon level, the epitendon surrounds the whole tendon, and is continuous with the intrafasicular matrix (IFM), which surrounds each fascicle at the fascicle level. Individual fascicles, the largest subunits of tendons, are visible to the human eye with diameters ranging between 150 to 500 μm. The fascicles are composed of collagen fibres; following the fibre level is the fibril level, as collagen fibres are formed from the aggregation of collagen fibrils, which serve as a structural subunit in tendons as well as other tissues (Thorpe and Screen, 2016). Collagen fibrils, ranging from 10 to 500 nm, are formed from pentafibrils, groups of crosslinked collagen molecules (Thorpe and Screen, 2016).

Collagen Structure

Tendons are comprised of 55-70% of water by weight. Of the dry weight of the tendon, 60-85% is attributed to collagen, with some non-collagenous proteins as well. Mostly type I collagen fibers, in addition to a few other types of collagen in much smaller amounts, are arranged lengthwise and parallel within the tendon, and individual collagen molecules assemble together increasingly into fibril subunits, fibrils, fibers, and fascicles, forming the tendon as a whole (Screen, Berk, Kadler, Ramirez & Young, 2015). With 90% of the collagen fibers in tendons being type I collagen fibers, type III collagen fibers can make up to 10% of the remaining collagen fibers in the tendon, regulating the size of the type I collagen fibers. Type V collagen fibers are found at

the core of type I collagen fibers, with a suspected function of providing a template for the synthesis of new collagen fibers. Types XII and XIV function to bridge type I collagen fibers to other molecules found the in the extracellular matrix (ECM) and also function in tendon development (Thorpe and Screen, 2016).

The levels of these types of collagen vary between different types of tendons. Type III collagen fibers are much more abundant in energy-storage tendons in comparison to tendons found in muscles that provide positional control, which do not have energy-storing capacity, but function to position the body segment to which they insert (Thorpe and Screen, 2016). The collagen fibers in a tendon are organized into a pattern known as a crimp, characteristic of type I collagen fibers and collagen fibers in general, which function to help the tendon to adapt to mechanical stress or loading the tendon may encounter (Screen, Lee, Bader & Shelton, 2004).

Non-Collagen Structure

Among the non-collagenous fibers present in the tendon, proteoglycans are the most prominent (Aro, et. al, 2012). These proteoglycans are a type of glycoprotein that have complex sugar sidechains, which function to draw water into the tendon. Decorin is the most abundant proteoglycans present in tendons, a small leucine rich proteoglycan (SLRP) making up 80% of the total (Aro et. al, 2012). SLRPs as a whole play an important role in the development of collagen fibrils, with some types playing a role during growth, while others, such as decorin, have been discovered to have a role later in the maturation process. SLRPs consist of a core protein and sidechains, which attach to specific binding sites on collagen fibrils, forming interfibrillar bridges.

SLRPs vary in the way they are distributed throughout the tendon with the highest concentration found in tensional regions (Screen et. al, 2015).

The glycoprotein cartilage oligomeric matrix protein (COMP) also plays a role in the tendon having five subunits which each attach to type I collagen fibers, with no known function (Aro, et. al, 2012). Tenascin-C, found in relatively small quantities in the tendon, like COMP does not have a known function, but may play a role in elasticity. Additionally, concerning elasticity, elastic fibers are also present in the tendon, making up less than 10% of the tendon's dry weight, functioning in the transfer of energy and resistance to fatigue (Thorpe and Screen, 2016).

Characteristic Mechanical Properties of Tendons

The mechanical testing of tendons demonstrates the many characteristic mechanical properties of tendons such as stiffness, Young's modulus, strength and toughness, among many others. Many of these properties can be examined through the use of a stress-strain curve. A study was performed to determine the regions that create the stress-strain curve and their relation to the characteristic properties of tendons (Gosline, 2018). The stress-strain curve has a characteristic "J-shape" beginning with a toe region, followed by a linear region, yield region 1 and yield region, 2 respectively, as shown in Figure 2 (Gosline, 2018).

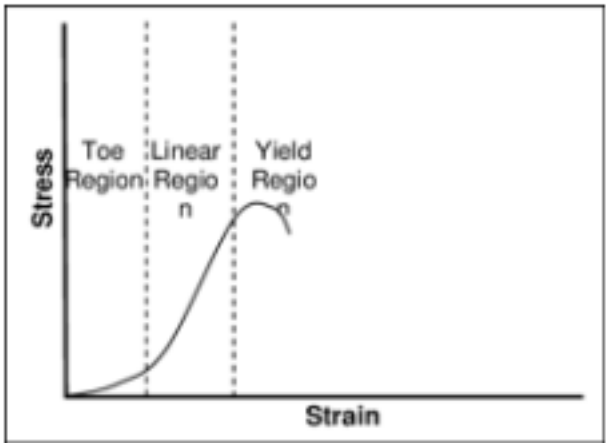

Figure 2. Typical Stress-Strain curve (Freeman & Kwansa, 2008)

The toe region demonstrates a region with low stiffness and slight increases in elastic modulus. Also known as Young's modulus named after Thomas Young, a founder of material testing, elastic modulus is the slope of the stress-strain curve, also known as the ratio between tensile stress and strain. The elastic modulus constant serves as the measure of the normalized stiffness of a material (Wainwright, 1982). Stiffness can also be defined, in regards to tendons, as the necessary force entailed in stretching a tendon per a designated unit of distance (*Mechanical Properties*, 1980).

The next region, known as the linear region, is also known as the region of fully reversible elasticity, as materials in this region can stretch and following stretching, return to their original state (Gosline, 2018). This region demonstrates the characteristic property of energy storage. As a tendon deforms and reforms energy is absorbed during loading and released to drive movement by the tendon. Energy storage in a tendon can be measured using the area under the stress-strain curve (Wainwright, 1982). Another mechanical property focused on the loading of tendons and changes in energy in the tendon is hysteresis, also referred to as energy dissipation. This property arises because the stress-strain curves will differ for loading of the tendon versus unloading of the tendon. This difference is caused by the differing amounts of energy lost and gained during loading

and unloading. This property of tendon can be attributed to the fact that tendons are viscoelastic (Robi, Jakob, Matevz & Matiaz, 2013).

Viscoelasticity is time-dependent, in that stress-strain relationship is dependent on the time of the loading or displacement, rather than being constant. In addition to hysteresis, viscoelastic materials also demonstrate the properties of creep and stress relaxation. Creep is characterized by the way in which a tendon continues to deform when a constant load is applied (Robi, et.al, 2013). Creep is a critical aspect of viscoelastic properties in the way in which they differ from elastic materials, which show no changes in length despite the application of a constant load over a period of time. The final of the three properties of viscoelastic materials such as tendons is stress relaxation, which is defined as the reduction of stress as the result of the constant deformation of a tendon (Robi, et. al, 2013).

Following this region, the slope of the stress-strain curve experiences a slight drop and enters yield region 1 as seen in Figure 2, in which the strain experienced by the material can cause permanent changes to the physical and mechanical properties of the tendon. Following yield region 1 is yield region 2, in which the tendon or material being tested is stretched to a point of failure. This is demonstrated by the common principle that as load is increased on any type of material it will eventually break or fail (Gosline, 2018). As stress is added to a tendon, the tendon can resist the applied stress to a maximum limit, also known as the strength of the tendon (*Mechanical Properties*, 1980).

In order to avoid failure, tendons have a safety factor, which under normal loading conditions, causes the tendon to resist reaching maximum stress or strain, and therefore avoid failure (Wainwright, 1982). The safety factor can also be defined as the ratio of tensile stress to the maximum in vivo stress experienced by the tendon during normal activity. Tendons high in

strength and stiffness, such as those involved in locomotion, function at very high strains, and due to this, have low safety factors, allowing the potential for tendon failure (Gosline, 2018).

The Effect of Load History on Tendon Properties

Buchanan and Marsh (2001) studied the Achilles tendon of adult guinea fowl placed either in a group in which the birds performed either level or downhill running. Following the training protocol, the tendons of each bird were tested using an in-situ procedure. The researchers found that both groups experienced a significant increase in tendon stiffness, with a greater than 40% increase in stiffness for the exercise group. Buchanan and March concluded that this increase in stiffness may be a biological mechanism to resist potential tendon damage and fatigue. Additionally, it was found that there was no increase in the cross-sectional area (CSA) of the tendons nor any signs of hypertrophy. These findings were particularly important as this determined that a change in material properties occurred as opposed to accrual of tissue, and the change was not due to tendon damage (Buchanan and Marsh, 2001).

Maganaris and Paul (2002) performed an in-vivo procedure on five healthy adult men using electrical stimulation to test the mechanical properties of the human tibialis anterior tendon. Through their experimentation, they discovered tendon force followed an increasing curvilinear trend as a function of displacement, while stress followed the same trend as a function of strain. Tendon stiffness and Young's modulus were found to increase from rest to the maximal isometric load applied (Maganaris & Paul, 2002).

Also spearheaded by Maganaris and Paul (2000) a similar in-vivo study was performed on six adult men testing the tensile properties of the gastrocnemius tendon. The stiffness was found

to increase as a result of the tendon force, following a curvilinear trend, with Young's modulus mirroring the trend as a function of stress. When comparing these results to those of the human tibialis tendon previously discussed, the values calculated for Young's modulus as well as hysteresis were similar, despite the different forces and loads experienced. This similarity in data prompted the researchers to believe that mechanical properties of tendons are independent of loads and functions of the various types of tendons (Maganaris & Paul, 2000).

An additional study combined both in-vivo and ex-vivo components in experimentation on the Achilles and patellar tendons (Westh, et. al., 2008). A group of researchers tested 10 male runners, 10 female runners, and 10 female non-runners, all of which were adults. The researchers used both imaging as well as electrical stimulation in an effort to determine how the structural and mechanical properties of the tendons were affected by habitual exercise. The runners had an Achilles tendon with a larger CSA than non-runners, with male runners having the largest CSA, and similar patellar tendon CSAs for both female groups, but a larger CSA for the male runners. Similarly, the male runners had the highest patellar tendon stiffness, followed by female runners, and the non-runner group (Westh et. al., 2008).

A longitudinal study in elite teenage volleyball athletes examined changes in the patellar tendon through the use of MRI and ultrasound, following a protocol including cycling, squats, and isometric knee contractions (Helland et. al., 2013). In addition to increases in tendon force and increased morphological changes in the tendon compared to the muscle, it was found that an increase in the CSA of the tendon corresponded to an increase in stiffness of the tendon. This finding was particularly applicable to athletes involved in constant jumping movements, as increased stiffness can improve performance during vertical jumps (Helland et.al., 2013).

Also focused on the patellar tendon, a group of researchers examined habitual loading in adult badminton and fencing athletes, as these specific sports involve lunging movements requiring the load to be greater in the tendon patellar tendon of one knee when compared to the other (Couppe et. al., 2008). The researchers examined the differences between recreational and elite athletes in these sports, as well as the differences in the lead-extremity versus non-lead extremity. Between groups, the recreational athletes did not demonstrate great differences in tendon CSA between the extremities, while the difference in tendon CSA for the elite group of athletes was significant, indicating tendon hypertrophy. Additionally, tendon force and stiffness, which were determined at maximal force, were both higher in the lead extremity, while the maximal stress was higher in the non-lead extremity. Significant differences between the extremities was not found for Young's modulus or strain (Couppe et. al., 2008).

A similar study concerning jumping examined the relationship between Achilles tendon stiffness and ground contact time (GCT) during drop jumps in healthy adult males (Abdelsattar, Konrad & Tilp, 2018). After the participants performed drop jumps on a force plate and tendons were examined using ultrasound. Researchers found an inverse relationship between tendon stiffness and GCT. This study further concluded that athletes with a stiffer Achilles tendon participating in sports with short GCTs have an athletic advantage compared to those with more a more compliant Achilles tendon as well as a lower chance of injury to the Achilles tendon and surrounding muscles (Abdelsattar et. al., 2018).

The Effect of Botox on Tendon Stiffness

Tendon testing using botulinum toxin has been performed on animals such as rats. A group of researchers injected botulinum toxin into the calf muscle of female rats during the growth period to cause unloading of the Achilles tendon (Eliasson, Fahlgren, Pasternak & Aspenberg, 2007). The rats were randomly assigned to groups including unloaded, unloaded with the botulinum toxin or control. The results of the study revealed that stiffness increased across all groups (unloading itself did not alter stiffness) leading to the conclusion that if the botulinum toxin is administered during the growth period, tendons will continue to grow (Eliasson et. al., 2007). A similar study also examined the effect of botulinum toxin in the Achilles tendon of rats. The findings were in contrast with those of Eliasson and colleagues, as stiffness increased in the tendons unloaded due to the botulinum toxin (Khayyeri et. al., 2017).

Human Implications of Botox

This study does provide implications for clinical use. In addition to the human use of botulinum-toxin-A for cosmetic use, botulinum-toxin-A has also been tested in patients with cerebral palsy, especially in children with the disease. One of the primary symptoms of this disease is muscle spasticity, which often leads to a variety of secondary issues associated with the disease (Molenaers, Campenhout, Fagard, De Cat & Desloovere, 2010). Botulinum-toxin-A has become an effective, safe, and widely accepted form of treatment to manage the symptoms of the disease, as it causes a reduction in muscle tone, decreasing the muscle stiffness caused by the disease. In a study focused on the spasticity of the lower limb in children with cerebral palsy,

botulinum-toxin-A injections improved the condition of the children with cerebral palsy as a whole when the treatment was administered at a young age (Molenaers et. al., 2010). A review of literature on the use of botulinum-toxin-A in children with cerebral palsy found agreement that this treatment is extremely effective in reducing spasticity of muscles, but not all muscles need to be injected and must be regulated to prevent adverse symptoms (Strobl, Theologis, Brunner, Kocer, Viehweger, Pascual-Pascual & Placzek, 2015).

Statement of the Problem

As reflected in the literature referenced above, most studies concerning stiffness in tendons are performed following the growth period, with few examining the effect of tendon loading history on stiffness and material properties during the growth period. The purpose of this study is to examine changes in tendon stiffness during growth in response to different levels of loading. As animals grow, they receive varying load stimuli, depending on their exercise. This study aims to discover if the tendon develops differently if there is not adequate load stimulus. Tendon stiffness following growth has had great implications in the potential for failure or injury. It is unknown how disuse during growth affects the musculoskeletal system following the growth period.

Statement of the Hypothesis

It is hypothesized that the tendons receiving botulinum-toxin-A as treatment will be more compliant (less stiff) than the control group, as the botulinum-toxin-A would cause unloading of the tendon. Because stiffness is calculated using force and displacement, it is also hypothesized that force and displacement data between the two groups will show significant differences.

Chapter 2

Methods

Animals and Experimental Treatment

Six guinea fowl (*Numida meleagris*) were acquired from a local breeder (GuineaFarm, Ohio). For four weeks, the guinea fowl were not separated, allowing a brooding period in which the guinea fowl matured under careful temperature control. For the next twenty-two weeks, the guinea fowl were randomly separated into control and botulinum toxin groups. The control group animals examined in this study consisted of two birds, and the botulinum toxin group consisted of four birds (groups consisted of a total of 16 birds but not all animals were included for analysis in this pilot study). Both groups were cage-reared, provided food and water *ad libitum*, and were raised in a 12h:12h light:dark cycle (Salzano et. al., 2018). Achilles tendon disuse was achieved by chronic muscle paralysis to the gastrocnemius muscles using botulinum-toxin-A.

Starting at 6-7 weeks, botulinum toxin-A was injected into the lateral and medial gastrocnemius of the disuse group guinea fowl and repeated every five weeks over the course of 20 weeks (Salzano et. al., 2018). After treatment, the guinea fowl were then euthanized at 27-28 weeks of age. The guinea fowl tendons were dissected, removing the surrounding muscles and tendons, while ensuring the tendon was attached to tarsometatarsus bone distal of the ankle joint.

Material Testing Rig

Figure 3 displays the MTS Bionix 858 Mini Bionix II used to test the tendons. The proximal portion was attached to the rig via the aponeurosis in the proximal clamp. The distal portion of the tendon remained attached to the tarsometatarsus bone, with the bond held in place using another clamp.

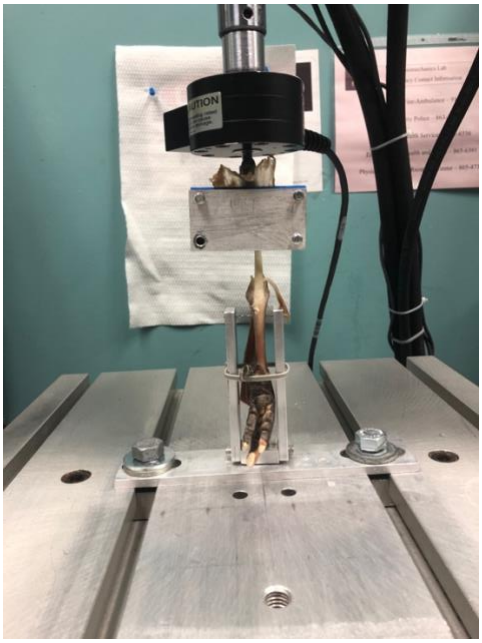

Figure 3. Material testing rig with clamped specimen

Each tendon was kept frozen prior to testing and thawed one day before testing. Each tendon was rehydrated for the 30 minutes prior to experimentation and throughout testing with water as well.

Tendon Conditioning

Before material testing each tendon, the tendon underwent a warm-up conditioning period. The tendon was first measured from the lowest point of the upper clamp to the beginning of the tendon. The tendon was stretched from 0.1% to 0.4% of its original length with a frequency of 0.1 Hz for 10 cycles. The conditioning allowed for the collagen fibers to orient correctly and function as it would in-vivo, as freezing can cause the fibers and proteins to kink. Allows tendon to function normally as it would in-vivo.

Tendon Force-Length Testing

The tendon length was then remeasured. The tendon then underwent 20 cycles of stretching from 0.1% to 5% of the remeasured length with a frequency of 0.1 Hz. Data including the force, in Newtons, the displacement, in millimeters, and the capture frequency was collected during testing.

Data Analysis

The force and displacement data from the last five cycles of tendon loading was used to calculate the stiffness of each tendon. The force was plotted against the displacement of the tendon,

with the linear portion of the slope used to calculate stiffness in N/mm. The stiffness value determined was the average stiffness across the last five cycles of tendon loading. The standard deviations were calculated for the average stiffness value of each tendon. The average stiffness values for the control and botulinum-toxin-A groups were then averaged to find the average among each experimental group and the standard deviation for each group was calculated as well. A t-test was performed for the overall group averages to determine if there was statistical significance between the average stiffness values for each group.

Chapter 3

Results

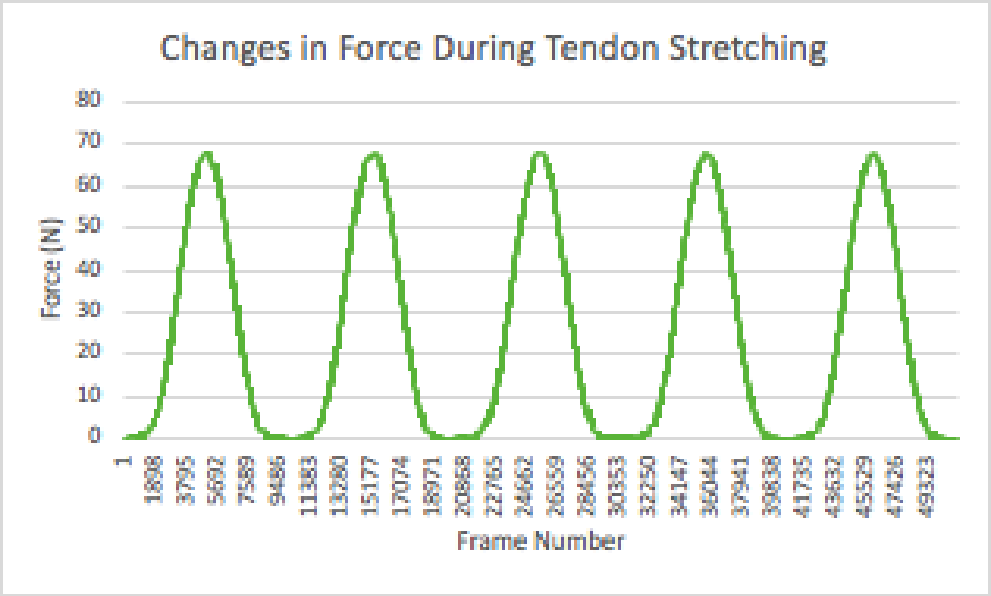

Figure 4. Example of the changes in force (N) over time during material testing

Figure 4 shows the cycling of stretching of each tendon and the changes in force throughout testing. The five waves represent the five cycles in which force data was collected. As shown by the equal peaks of each cycle, the force changed consistent over the course of the five cycles.

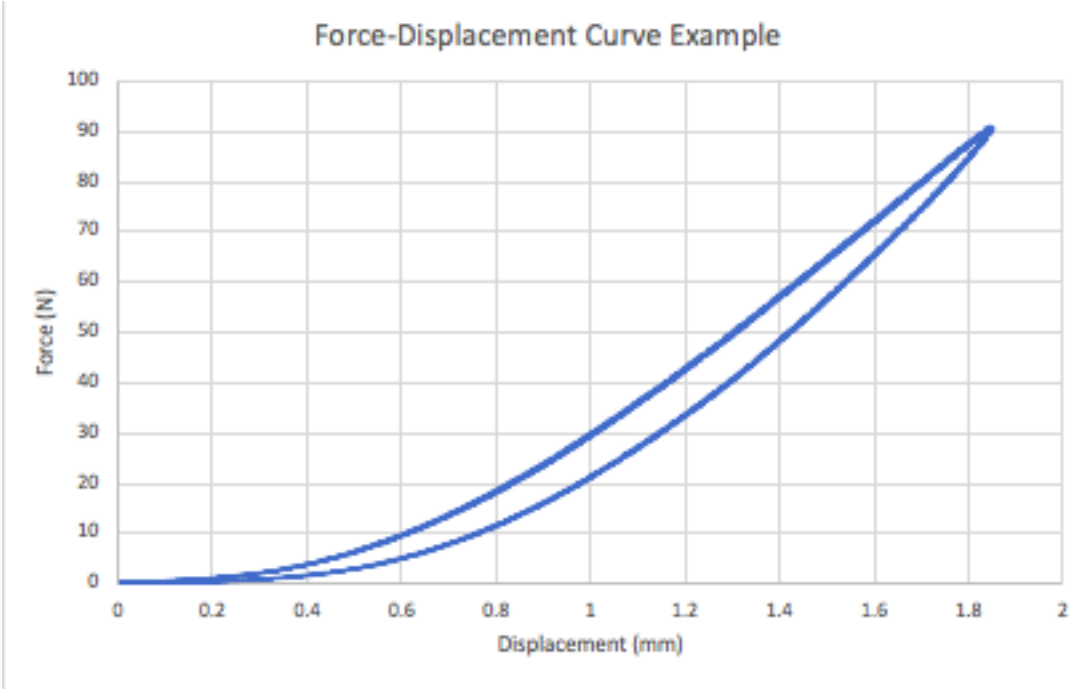

Figure 5. Example of the force-displacement relationship during tendon loading and unloading

The relationship between force and displacement through material tendon testing is displayed in Figure 5. This figure demonstrates the curve produced by the relationship between force, measured in Newtons, and displacement, measured in millimeters. The curve is the compilation of the five cycles used during testing. The five individual curves are superimposed in Figure 5.

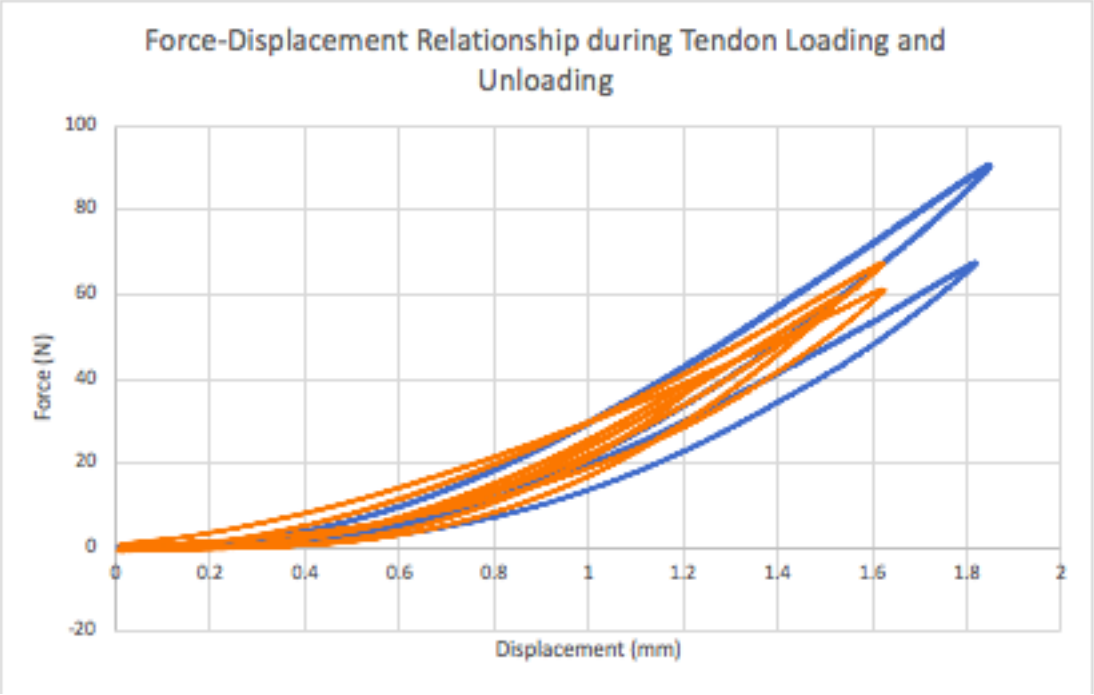

Figure 6. The force-displacement curve for each of the six tendons that underwent testing

Displayed in Figure 6 are the force-displacement curves produced from the final five cycles of testing for each of the six guinea fowl tendons tested. The colors used indicate the treatment the groups received, with blue representing the control group and orange representing the group that received botulinum-toxin-A.

Table 1. Stiffness and Standard Deviation Values for Each Tendon and Averages for Each Experimental Group

Guinea Fowl Number	793	786	758	796	788	757
Group	Control	Control	Botox	Botox	Botox	Botox
Average Stiffness (N/mm)	57.70	72.42	63.38	54.40	59.29	48.87
Standard Deviation	0.23	0.46	0.42	0.59	0.18	0.29
Average Stiffness for Each Group		Control: 65.06				Botox: 58.89
Average Standard Deviation for Each Group		Control: 10.41				Botox: 6.35

Table 1 displays the average stiffness values found over the five cycles of data for each individual tendon, as well as their standard deviations. These values were then used to calculate the average stiffness values and standard deviations for the control group and experimental group, respectively.

The average stiffness values for each experimental group was used to perform a t-test, producing a p-value of 0.13 at the 0.05 level. The p-value indicates that there is no statistically significant difference in stiffness between the tendons of the control and experimental groups.

Chapter 4

Discussion

This study examined the effect of chronic muscle disuse during growth on tendon stiffness. Overall, the experimental design and procedures produced reliable analyses of tendon mechanics. A full understanding of the biological effect of unloading on tendon stiffness in this study will become more clear with additional analyses in the remaining specimens not measured in this thesis.

Figure 4 shows the settling of the force during the last five of the twenty cycles of material testing for each tendon. This stabilization of the force during testing is notable as force decays throughout testing, specifically in this case during the first fifteen cycles of testing. Figure 4 demonstrates how the peak force during each of the cycles is consistent and stabilized across the last five cycles. The regularity of the force during the cycles is important as this is the data used to calculate stiffness, indicating that the stiffness data produced is also accurate and consistent.

As shown in Figure 6, the force-displacement curves for each of the tendons resembled the expected example curve from only one of the tendons tested in Figure 5. Despite the expected outcome that the curves for the tendons of the control and experimental groups would differ, Figure 6 shows that the curves for the birds from both groups are similar in stretch-relaxation shape, with the force-displacement curves from both groups mostly overlapping and hard to distinguish if they were not separated by color.

This presents an interesting point, when compared to the stiffness values in Table 1. The average stiffness values among the two guinea fowl in the control group were farther apart at 57.70 N/mm and 72.42 N/mm, respectively, with the average stiffness of one of the tendons, 57.70 N/mm, falling within the average stiffness values obtained from the guinea fowl tendons of the experimental group. The experimental group also showed variation in the average stiffness values calculated from testing, ranging from 48.88 N/mm to 63.38 N/mm for the individual tendons. The average stiffness values for the control group and experimental group, 65.06 N/mm and 58.90 N/mm respectively, demonstrated some difference in stiffness. As the average stiffness value for the experimental group is lower, this may indicate that the injection of botulinum-toxin-A may have caused the tendons to become more compliant when compared to the control group.

On the other hand, the overall average standard deviations for each group may lead to the conclusion that no significant difference exists in tendon stiffness between the groups. With the stiffness of the control group being 65.06 ± 10.41 and the experimental group being 58.90 ± 6.35, the standard deviations show that there may not be a statistically significant difference between the groups, as the standard deviations are large enough that the averages of the two groups may overlap. This conclusion may be likely, as it is supported by the literature on the injection of botulinum-toxin-A in rats during the growth period. The rats injected with botulinum-toxin-A during the growth period in two studies showed similar stiffness values compared to a group of rats that did not receive the experimental treatment (Eliasson et. al., 2007; Khayerri et. al., 2017).

The results of the t-test also supported the previous literature, as the p-value of 0.13 supports no statistically significant difference in average stiffness between the two groups. The

current study is comprised of only six animals and therefore clear evidence of the effect of unloading is hard to establish. More data points may potentially drive the p-value below 0.05 and therefore indicate a difference in stiffness caused by the botulinum-toxin-A.

Although the data presented above may lead to conflicting conclusions, no definitive conclusions can be drawn due to the data collected on such a small pool of animals. The number of tendons used to collect data was only six, which does not give enough data to determine if there is a difference in the stiffness of tendons between the groups. Especially as only two animals made up the data for the control group, which had dissimilar average stiffness values, as shown in Table 1. Since only these two data points were used to create an average for the control group as a whole, if either of these numbers was an outlier, the data would potentially be skewed. In order to make more definitive conclusions concerning the stiffness of these tendons, this experimental procedure will need to be performed on the tendons of many additional guinea fowl.

Despite the inability to draw definitive conclusions from this data, this study demonstrated that the procedures are feasible. Using the small sample of tendons, data collection was successful as stiffness was able to be calculated from the accurate force and displacement data gathered during the experimental procedure. Additionally, botulinum-toxin-A was successful in creating an experimental procedure that produced restricted movement. This smaller-scale study has shown that this procedure can be successful in determining stiffness values and can be used on a larger scale. As this data collection was successful in this particular animal model, this procedure also may be applicable to tendon material testing in other animals and bipedal models.

Through this study, it is unclear as to whether tendon stiffness may have been affected by tendon damage. The possibility exists that no significant differences in tendon stiffness were observed between the experimental groups due to tendon damage. With no way of knowing if tendon damage occurred in either the control group or as a result of the botulinum-toxin-A injection, some measure of cross-sectional area may be beneficial in future research.

It is also possible that changes in the material properties of the tendon has occurred, but that changes in the cross-sectional area of then tendon has negated these effects. Previous studies have examined both tendon stiffness and the modulus of elasticity through the examination of the force-length behavior together with cross-sectional area measurements. For example, Buchanan and Marsh (2001) found that changes in tendon stiffness were the sole result of the changes of material properties in the tendons (Buchanan and Marsh, 2001).

The results of this research have various implications in humans. For example, this study can be viewed in terms of athletic performance. Studies have shown that increased tendon stiffness can help improve performance and prevent injuries in athletes performing vertical jumping movements such as in the sport of volleyball (Helland et.al., 2013). Another study confirmed this in healthy adult males concerning ground contact time, as those with stiffer tendons had a shorter ground contact time. The stiffer tendon and short ground contact time gave these participants an athletic advantage, as well as a lesser chance of injury to the Achilles tendon and surrounding muscles (Abdelsattar et. al., 2018). These findings are notable in terms of this research, as no statistically significant difference in stiffness was found between the tendons affected by botulinum-toxin-A and control groups. Performing this study in the future, but with the addition of an experimental group that participates in additional exercise including vertical jumping movements may help to determine the effect stiffness has on sport performance

and injury prevention. The findings of this study that conflict with the literature in humans recognize the need for more research concerning tendon stiffness in bipedal models.

The application of the use of botulinum-toxin A in the tendons of guinea fowl can also provide some insight into the role in the use of botulinum-toxin-A in humans. The research performed in this study has shown that botulinum-toxin-A injections may not have an effect on tendon stiffness. This may be important in children with cerebral palsy, as botulinum-toxin-A can be used to decease muscle spasticity without changing tendon stiffness. This is especially relevant as the botulinum-toxin-A was administered during the growth period, just as it is in many cerebral palsy patients.

In terms of the future research that could emerge from this project, larger-scale experiments using the same procedure could easily be reproduced and aid in the development of making definitive conclusions about the effect of botulinum-toxin-A on tendon stiffness. Additionally, due to the feasibility shown in this experiment, this experiment shows the potential for duplication in other animal models with small procedural changes. This future research may also be beneficial in ensuring the maintenance of tendon stiffness during the treatment of muscle spasticity in children with cerebral palsy.

BIBLIOGRAPHY

Abdelsattar, M., Konrad, A., & Tilp, M. (2018). Relationship between Achilles Tendon Stiffness and Ground Contact Time during Drop Jumps. *Journal of sports science & medicine*, *17*(2), 223.

Aro, A. A., Vidal, B. D., & Pimentel, E. R. (2012). Biochemical and anisotropical properties of tendons. *Micron,43*(2-3), 205-214.

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.

Couppe, 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*.

Eliasson, P., Fahlgren, A., Pasternak, B., & Aspenberg, P. (2007). Unloaded rat Achilles tendons continue to grow, but lose viscoelasticity. *Journal of applied physiology*.

Freeman, J. W., & Kwansa, A. L. (2008). Recent advancements in ligament tissue engineering: The use of various techniques and materials for ACL repair. *Recent Patents on biomedical engineering*, *1*(1), 18-23.

Gosline, J. M. (2018). *Mechanical Design of Structural Materials in Animals*. Princeton: Princeton University Press.

Harvey, A. K., Thompson, M. S., Cochlin, L. E., Raju, P. A., Cui, Z., Cornell, H., ... & Brady, J. M. (2009). Functional imaging of tendon. *Ann. Brit. Machine Vision Assoc*, *2009*, 1-11.

Helland, C., Bojsen-Møller, J., Raastad, T., Seynnes, O. R., Moltubakk, M. M., Jakobsen, V., ... & Bahr, R. (2013). Mechanical properties of the patellar tendon in elite volleyball players with and without patellar tendinopathy. *Br J Sports Med*, *47*(13), 862-868.

Khayyeri, H., Blomgran, P., Hammerman, M., Turunen, M. J., Löwgren, A., Guizar-Sicairos, M., ... & Isaksson, H. (2017). Achilles tendon compositional and structural properties are altered after unloading by botox. *Scientific reports*, *7*(1), 13067.

Maganaris, C. N., & Paul, J. P. (2000). In vivo human tendinous tissue stretch upon maximum muscle force generation. *Journal of biomechanics*, *33*(11), 1453-1459.

Maganaris, C. N., & Paul, J. P. (2002). Tensile properties of the in vivo human gastrocnemius tendon. *Journal of biomechanics*, *35*(12), 1639-1646.

Molenaers, G., Van Campenhout, A., Fagard, K., De Cat, J., & Desloovere, K. (2010). The use of botulinum toxin A in children with cerebral palsy, with a focus on the lower limb. *Journal of children's orthopaedics*, *4*(3), 183-195.

Robi, K., Jakob, N., Matevz, K., & Matjaz, V. (2013). The Physiology of Sports Injuries and Repair Processes. *Current Issues in Sports and Exercise Medicine*.

Salzano, M. Q., Cox, S. M., Piazza, S. J., & Rubenson, J. (2018). High-acceleration training during growth increases optimal muscle fascicle lengths in an avian bipedal model. *Journal of Biomechanics*.

Screen, H. R., Berk, D. E., Kadler, K. E., Ramirez, F., & Young, M. F. (2015). Tendon Functional Extracellular Matrix. *Journal of Orthopaedic Research*,*33*(6), 793-799.

Screen, H. R., Lee, D. A., Bader, D. L., & Shelton, J. C. (2004). An investigation into the effects of the hierarchical structure of tendon fascicles on micromechanical properties. *Proceedings of the*

Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine,218(2), 109-119.

Strobl, W., Theologis, T., Brunner, R., Kocer, S., Viehweger, E., Pascual-Pascual, I., & Placzek, R. (2015). Best clinical practice in botulinum toxin treatment for children with cerebral palsy. *Toxins*, *7*(5), 1629-1648.

Thorpe, C. T., & Screen, H. R. (2016). Tendon Structure and Composition. *Metabolic Influences on Risk for Tendon Disorders Advances in Experimental Medicine and Biology,*3-10.

Wainwright, S. A. (1982). *Mechanical Design in Organisms*. Princeton: Princeton University Press.

The Mechanical Properties of Biological Materials. (1980). Cambridge: Cambridge Univ. Pr.

Westh, E., Kongsgaard, M., Bojsen-Moller, J., Aagaard, P., Hansen, M., Kjær, M., & Magnusson, S. P. (2008). Effect of habitual exercise on the structural and mechanical properties of human tendon, in vivo, in men and women. *Scandinavian journal of medicine & science in sports*, *18*(1), 23-30.

ACADEMIC VITA

MEGAN E. MCPAUL

EDUCATION

The Pennsylvania State University, University Park, PA — Graduation: 5/19
Schreyer Honors College
Bachelor of Science in Kinesiology, Movement Science Option

Honors Thesis: The Effect of Chronic Muscle Disuse on Achilles Tendon Material Properties in an Avian Bipedal Model
Relevant Coursework: Medical Terminology for Health Professionals, The Biology of Molecules and Cells, Introductory Physiology, Fundamentals of Organic Chemistry I & II, Mammalian Anatomy, Elementary Biochemistry

HONORS

Dean's List Recipient: 7 of 7 semesters (3.5 GPA or higher)
The President's Freshman Award (given to freshmen who achieve a 4.0 GPA their first semester)
Health and Human Development Honor Society Member (awarded to students in the college who maintain a 3.3 GPA)

RELEVANT EXPERIENCE

Doylestown Health Cardiology, Meadowbrook, PA — 5/18-8/18
Student Intern

- Aided Medical Assistants in performing EKGs and placing cardiac monitors on patients
- Assisted patients in maneuvering in and out of the office and prepared them for their visits

Abington Jefferson Health, Abington, PA — 5/17-8/17
Hospital Elder Life Program Volunteer

- Facilitated daily activities for patients to promote a quick recovery through an active lifestyle
- Performed mental and physical assessments of patients
- Supported in minor procedures and completed rounds with physicians in the Medical Intensive Care Unit

EXTRACIRRICULAR ACTIVITIES

THON, University Park, PA — 9/15-Present
THON is a student-run philanthropy committed to helping children and families batting childhood cancer through providing emotional and financial support, spreading awareness, and ensuring funding for critical research in pursuit of a cure.

Donor and Alumni Relations: Alumni Engagement, Alumni Group Liaison — *4/18-Present*

- Generates fundraising pages for Penn State Alumni Association Alumni Chapters
- Establishes fundraising success via the management of technical issues and providing tools for successful fundraising
- Communicates with Alumni Chapters about events and incentives available for their participation

Entertainment Committee Member: Operations Leader — *9/17-2/18*

- Designed interactive games and activities to engage spectators at THON events
- Organized and maintained inventory of materials and prizes for activities
- Led and managed committee members during and to and from task-specific shifts

Dancer Relations Committee Member: Theme Hour Chair — *9/15-2/17*

- Ensured the wellness of dancers to stand for 46 hours to raise awareness and funds for pediatric cancer during THON Weekend
- Constructed and led committee members in executing interactive activities for dancers to participate in

Rince Na Leon, Penn State's Irish Dance Club, University Park, PA — 8/15-Present
President — *5/18-Present*

- Leads club practices twice per week and executive board meetings once per week
- Plans and executes performances year-round including an annual showcase
- Choreographs dance routines and instructs club members in learning the routines