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Effects of tendon compliance on the economy of cyclic contractions

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by

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Abstract

The use of compliant tendons to store elastic strain energy and release it during the subsequent cycle lowers the mechanical work output and has been suggested to lower the metabolic energy consumed. In this thesis, I examined and compared the activation conditions, work output and force generation of muscle lifting a load an equal distance in the presence of a compliant and non-compliant tendon, and then replicated those movements and activation within an oxygen chamber to test for the differences in energy consumption. It was found that for a single contraction cycle, a cyclically contracting muscle in series with a compliant tendon did less than half of the shortening and lengthening work, nearly twice the net work, resulted in a reduced impulse, required different activation conditions and consumed 17% less energy than a muscle oscillating a load with a non-compliant tendon, suggesting it is responsible for more economical movement.

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Chapter One: Introduction

1.1 The Mechanics and Energetics of Animal Locomotion

Locomotion is the ability to move from one place to another. It is an ability that is employed in a variety of methods across the animal kingdom, and for many animals it is a necessity for survival. However, locomotion is not without cost. Given that even a stationary animal consumes energy, the act of locomotion can be very energetically demanding. Certain types of locomotion are more economical than others with respect to energy consumption, an aspect of locomotion that among other factors (eg. environmental, physiological, etc.) is influenced by anatomical characteristics. One anatomical characteristic that has been widely suggested to reduce the costs associated with cyclic locomotion is the presence of series compliance, notably tendons connecting the muscle to the skeleton. Studies that examined the locomotion of kangaroos (Dawson and Taylor, 1973; Alexander and Vernon, 1975), wallabies (Biewener et al., 1998) and horses (Alexander, 1984; Biewener, 1998) have all suggested that tendons play a major role in recovering mechanical work and likely influence the metabolic energy savings of the muscles involved in powering movement.

The structure of skeletal muscle allows it to generate the force necessary for movement. Muscle is composed of a collection of bundles called fascicles, which themselves are bundles of individual fibres. Within an individual fibre, there are many cylindrical myofibrils, which are composed of repeating units called sarcomeres. The sarcomeres constitute the contractile component of muscle, and generate force during a contraction when thick filaments (myosin) attach and slide along the length of thin (actin) filaments (Huxley, 1957).

The type of contraction a muscle performs determines the amount of force it will generate. Muscle generates force but does not always transmit it to the skeleton directly; the series elastic component and parallel elastic component, elements that cannot produce force on their own but are able to transmit force generated by the contractile component to the skeleton, also contribute to movement. When stretched, the effects of these elastic elements on muscle are additive to either the muscle length (series component) or muscle force (parallel component). A muscle is able to generate the force required for movement through contraction, which occurs when individual muscle fibres are stimulated by the nervous system. The contraction of the muscle can occur without a change in muscle length as during an isometric contraction, or it can change length, where muscle lengthening occurs during an eccentric contraction and muscle shortening occurs during a concentric contraction. As Hill originally proposed (1938), the force generated by a muscle is dependent on the shortening velocity of muscle. Accordingly, an isometric contraction, which does not change length and therefore has a shortening velocity of zero, generates a greater amount of force than a concentric contraction. An eccentric contraction results in a greater generation of force than an isometric contraction, but requires an external input to stretch the active muscle.

The type of contraction a muscle performs impacts the amount of energy used to do so, and the series compliance can thus potentially play an important role in influencing muscle contraction and energy use. While a concentric contraction will generate some amount of force for a given shortening velocity resulting in work being done, during an isometric contraction muscle length does not change and therefore no work is done. The absence of work being done does not mean there is no energy required to perform that contraction, however, relative to a high work-producing or high power-producing contraction, an isometric contraction has been

proposed to use less energy (Fenn, 1924), suggesting it would be advantageous for a muscle to perform contractions without shortening to reduce the costs of locomotion. But, if a muscle is not shortening then no work is being done, and therefore something else must be responsible for doing the work necessary to achieve locomotion.

Tendons have been suggested to aid in cyclical movement through the storage and release of mechanical energy during running, trotting and hopping (Biewener and Baudinette, 1995; Cavagna et al., 1977; Heglund et al., 1982; Roberts, 1997), and thus could be responsible for limb movement without muscle shortening. This idea is illustrated by an analogy of a bouncing ball (Roberts, 2002); there is a transfer from kinetic and potential energy to strain energy that occurs when the ball hits the ground and is deformed, however, the elastic properties of the ball cause it to recoil back to its original shape, while the strain energy is converted back to kinetic and potential energy, powering its movement upwards. Following this analogy, the muscle does not have to expend as much mechanical energy to power subsequent contractions because of the tendon.

Serving as a link between muscle and bone, tendons are composed mostly of collagen and elastin (Alexander, 1984), which gives them compliant and elastic properties. The compliance (ability to stretch) of a tendon is influenced by its length and cross-sectional area with the most compliant tendons usually occurring in series with highly pennate, distal limb muscles that generate high forces but are not capable of large amounts of shortening (Ker et al., 1988; Pollock and Shadwick, 1994). The elastic properties of tendon are exhibited when a tendon recoils to its original length once the external force responsible for the stretch is removed. These tendon properties are responsible for a tendon's ability to store and release strain energy during the repeated cyclical contractions of locomotion.

The ability to passively store and release energy during locomotion is influenced by the characteristics of the tendon involved. Research examining the tendons of diminutive kangaroo rats showed elastic energy storage was low in their small, thick tendons and thus is likely less important for smaller terrestrial animals (Biewener et al., 1981). It has been suggested (Biewener and Blickhan, 1988) that the shorter and thicker tendons of the kangaroo rat are used in stabilization during acceleration to withstand greater forces associated with rapid acceleration, potentially to avoid predation. Therefore, their capacity for energetic savings is reduced in favour of other characteristics that likely aid in their survival. A larger animal such as the tammar wallaby does not face the same predatory pressures and so it uses its long, thin tendons for mechanical energy storage to reduce costs of locomotion at the expense of rapid acceleration (Biewener and Baudinette, 1995; Biewener et al., 1998).

The capacity to store and release energy has implications for muscle energetics as the importance of tendon elasticity is exhibited by the influence it has on muscle length changes during movement (Cavagna et al. 1977; Roberts, 2002). Analysis of the leg muscles and tendons of hopping wallabies showed that muscular force production and elastic energy savings increased at greater speeds (Biewener and Baudinette, 1995), suggesting cyclical contractions producing high forces and little work could be used to power movements with a compliant tendon. Since high force contractions have been shown to occur at lower shortening velocities (Hill, 1938), this situation may be reminiscent of an individual hopping on a pogo stick. Similar to the bouncing ball analogy, hopping on a pogo stick stores and releases elastic strain energy within the pogo spring, and therefore can result in movement even with minimal shortening of the leg muscles (e.g. perhaps only to account for hysteresis (loss of energy) that might occur within the spring). Since isometric contractions have been suggested to consume less energy than shortening

contractions (Fenn, 1924), the idea that locomotion could occur with minimal shortening of the muscles involved is advantageous as it implies metabolic energy savings.

1.1.1 Energy consumption of a cyclically contracting muscle

Energy as a usable currency can be provided through aerobic and anaerobic respiration. Both processes consume substrates to produce adenosine triphosphate (ATP). The final and most energetically-lucrative phase of respiration, oxidative phosphorylation, is responsible for the relatively large production of ATP within a cell's mitochondrion. Per molecule of glucose, anaerobic respiration produces less ATP and is therefore used for short bursts of high activity (e.g. sprinting) while during sustained movements (e.g. locomotion over longer distances) greater quantities of ATP are produced by aerobic respiration, cyclical movements could not be sustained by anaerobic means and require aerobic respiration to produce the energy necessary for animal locomotion, making oxygen consumption a good measure of energy used by muscle for sustained activity.

The consumption of oxygen as a measure of energy use has been employed in many instances for terrestrial locomotion of large mammals (Dawson and Taylor, 1973; Baudinette, 1992), small mammals (Taylor et al., 1970; Thompson et al., 1980), running birds (Fedak et al., 1974; Roberts et al., 1998) and frogs and turtles (Baudinette et al., 2000; Zani and Kram, 2008). In an earlier study by Taylor and his colleagues (1970), an analysis of running speed and oxygen consumption proposed a linearly increasing relationship between the two variables for mammals such as dogs, mice, squirrels and kangaroo rats. However, in a further study measuring the oxygen consumption of hopping red kangaroos at increasing speeds, oxygen consumption actually decreased (Dawson and Taylor, 1973). Similarly, another species of macropodidae, the

tammar wallaby, displayed slightly reduced oxygen consumption while hopping at greater speeds on a treadmill (Baudinette et al., 1992; Biewener and Baudinette, 1995). Both groups of researchers suggested that the variance from the increase with speed was a result of elastic energy storage and release occurring in the long hindlimb tendons present in both marsupials.

In addition to influencing oxygen use, the influence of tendons on the work done by their associated muscles has been observed in other forms of bipedal locomotion such as running in turkeys (Roberts, 1997) and hopping in wallabies (Baudinette et al., 1992; Biewener and Baudinette, 1995). Roberts (1997) showed the lateral gastrocnemius tendon of a turkey can be responsible for storing and subsequently recovering greater than 60% of the work produced by the muscle and tendon in series. The ability of the tendon to conserve energy for a subsequent leg movement reduces the amount of work the contracting muscle must perform and thus is proposed to reduce metabolic energy use. As suggested, if an organism were able to reduce the amount of work done by a locomotory muscle while still generating the necessary force required to counter-act gravity, it could reduce the number of activated muscle fibres and thus limit the muscle's energy consumption (Roberts, 1997). This suggests that near isometric contractions combined with sufficient elastic energy savings of an appropriate tendon could reduce both the amount of work done by the muscle and energy consumed by the muscle during locomotion. However, such a mechanism is difficult to test empirically, as it is not readily feasible to add, remove, or otherwise alter the series compliance in the limbs of animals to test its effects. Consequently, arguments about the role of tendons as energy saving mechanisms are highly speculative.

1.2 Research Objectives and Hypotheses

As mentioned previously, there is a sizable amount of research surrounding animal locomotion that suggests tendon plays a major role in reducing metabolic energy consumption through the storage and subsequent release of elastic strain energy. Based on measurements of mechanical work recovery, terrestrial locomotors such as horses (Biewener, 1998), kangaroos (Dawson and Taylor, 1973), tammar wallabies (Biewener and Baudinette, 1995), turkeys (Roberts, 1997) and even humans (Ker et al., 1987) have all been suggested to benefit from the elastic properties of their tendons, specifically in the hindlegs. The suggestion is that these species utilize the elastic properties of their tendons by storing strain energy within the tendon as it is stretched during landing, and subsequently release that energy upon recoil to power the subsequent jump, resulting in a reduction in the mechanical work done by the muscle. This observation has led to the idea that the metabolic energy consumption of a muscle might also be reduced through the action of a compliant tendon. While it is generally believed to be the case, research investigating the effects of tendon on muscle energy consumption is somewhat limited. Lichtwark and Barclay (2010) measured the energetic efficiency of a cyclically contracting mouse soleus and found that increasing the compliance of a tendon allowed a muscle to shorten at speeds that increase energy efficiency. However, further research needs to be undertaken to better understand how tendon affects the economy of movement. To test this, ideally an *in vivo* study would be undertaken that involved an animal with a removable tendon whose metabolic energy measurements could be taken as it moved along a path, and then be repeated as it moved along the exact same path once the tendon was removed. For obvious ethical reasons this is not an option, but the idea behind the removable tendon played a role in the conception of this investigation.

1.2.1 Questions

The first question I attempted to answer with this research was whether the force and work produced by a cyclically contracting muscle differed if it moved a load an equal distance in the presence of a compliant and non-compliant tendon.

This approach involved a cyclically contracting muscle *in vitro* moving a load in series with a compliant material serving as a tendon, or a non-compliant material as in the absence of a tendon (Chapter 2). In continuing with the analogy of a hopping animal, the distance of each jump, or in this case the amplitude of load movement, would be equal in the presence of a compliant/non-compliant tendon to facilitate a comparison for the effort required to move. Quantifying or defining the effort of the contracting muscle was achieved by measuring the impulse and work done by the muscle that constituted movement of the load. The work done by the muscle was expressed for a single contraction cycle and as a rate (power), and included measurements during the shortening and lengthening of the muscle in addition to the net result. Likewise, the impulse was expressed for a single cycle and as a rate. Activation conditions of cycle frequency and stimulus duty cycle that resulted in equal load movements were also quantified and compared between tendons.

The second question posed in this investigation was whether the energy consumption of a cyclically contracting muscle while moving a load the same distance differed in the presence of a compliant and non-compliant tendon.

To determine the energy consumption, the muscle movements and activation conditions from the jumping trials were replicated in an oxygen chamber, where measurement of the oxygen consumed by a working muscle could later be converted to a value for the energy

consumption (Chapter 3). The reason for replicating the movements and activations in the oxygen chamber, rather than measuring energy consumption from the muscle while it actually moved a load via a compliant or non-compliant tendon, was that it is not practical to make both measures at the same time. Thus, separating the experiment into two components (the jumping measurements and the oxygen measurements) allowed for measurements during the jumping trials that were not encumbered by simultaneous measures of oxygen use, and precise measures of oxygen consumption that would not be possible if a tendon and load were incorporated into the measurement apparatus.

1.2.2 Hypothesis

The suggestion that a compliant tendon influences metabolic energy consumption during cyclic locomotion is based on observations of mechanical work output and oxygen consumption. Reductions observed in the work done during cyclic locomotion of some terrestrial species (Alexander, 1984; Roberts, 1997) have suggested a tendon may allow locomotory muscle to produce force while reducing muscle shortening, which is notable since slower contractions consume less metabolic energy (Fenn, 1924). Likewise, the tendons of hopping macropodids (Dawson and Taylor, 1973; Baudinette et al., 1992; Biewener and Baudinette, 1995) were suggested to have played a role in decreased levels of oxygen consumption observed during hopping at increasing speeds, an outcome in opposition to previously suggested correlations for speed of movement and oxygen consumption in quadripedal mammals (Taylor et al., 1970). The reductions in mechanical work and oxygen consumption suggest a compliant tendon may influence the metabolic energy consumption of a cyclically contracting muscle and thus are central to my hypothesis.

My hypothesis is that a compliant tendon will allow a cyclically contracting muscle to do less work and consume less energy than a cyclically contracting muscle in series with a non-compliant tendon when load displacement is equal.

1.2.3 Significance of Research

This research will add to the current understanding of the effects of tendon compliance on the economy of movement by supporting or refuting the idea that metabolic energetic savings occur during locomotion, a notion so far only suggested through indirect measurement. Furthermore, this research will offer quantified values of the work done, force produced, and the expected energetic savings per cycle and per unit time that a muscle incurs through the use of a compliant tendon during cyclical contractions. Again, information that has thus far has not been available other than through speculation.

Chapter Two: Cyclical Contractions In The Presence Of A Compliant And Non-Compliant Tendon

2.1 Introduction

Studies examining the mechanics of animal locomotion have suggested a compliant tendon may influence mechanical energy production during movement. By storing strain energy in the stretch of tendon and releasing that energy during recoil, a muscle may not have to shorten as much as it otherwise would, and therefore would do less work to power moving (Roberts et al., 1997). Since a muscle shortening a shorter distance uses less metabolic energy (Fenn, 1924), using a tendon to reduce the muscle shortening without reducing the overall distance of movement appears to be a more economical form of locomotion.

The central focus of this thesis was to measure and understand the influence of tendon compliance on the economy of movement. To better understand the influence of the tendon, the aim of the experiments covered in this chapter was to quantify and compare the amount of energy used by a muscle cyclically contracting with (compliant) or without (non-compliant) a tendon, when the displacement of the load is the same under the two conditions. In this way, potential savings to the cost of locomotion incorporating a compliant tendon can be assessed.

To attain these measurements, the movements of a muscle activated to repeatedly lift a load while in series with either a compliant or non-compliant tendon. Measurements of the work, impulse and activation conditions that resulted in oscillation of the load are examined in this chapter, with a hypothesis that the presence of a compliant tendon would result in less work being done by a cyclically contracting muscle to move a load any given distance compared with a muscle moving the same load the same distance without a tendon. The measurements made in

this chapter were also essential for eventual measurement of the energy consumed replicating these movements within an oxygen chamber.

2.2 Materials and Methods

2.2.1 Experimental species and muscle

The leopard frog, *Rana pipiens*, was the animal of choice for this experiment and has been used extensively in studies of muscle physiology. The leopard frog is normally a ballistic jumper that uses hopping as its method of locomotion in terrestrial environments.

The muscle used in this set of experiments was the sartorius muscle. The sartorius originates on the anterior ilium and inserts onto the medial portion of the tibia allowing for flexion of the knee and rotation of the hips. The sartorius has only a small amount of relatively non-compliant connective tissue at either end of the muscle, so that the majority of length changes seen in the preparation will be due to only changes in muscle length and not the tendon, allowing the use of an additional articial tendon to account for the majority of the series compliance. The sartorius is favourable for use in oxygen consumption experiments because oxygen is able to diffuse uniformly throughout the long, thin muscle, resulting in predominantly aerobic respiration under appropriate stimulation parameters. The sartorius is robust, and being a superficial muscle, the ease with which it is dissected makes it ideal for this experiment.

2.2.2 Muscle dissection and jumping trials apparatus

All procedures were approved following animal care guidelines of the Canadian Council on Animal Care and the University of Calgary. Adult frogs of either sex were killed by decapitation and pithing. The skin from the lower extremities was removed and the lower limbs

were placed in a petri dish containing physiological saline (in $mmol^{-1}$: 115 NaCl, 3 KCl, 2 $CaCl_2$, 20 $NaHCO_3$, 2 NaH_2PO_4, 5 glucose, pH 7.8 at 20°C). The dissection then occurred underneath a microscope on a stage chilled to 5°C. The sartorius was removed keeping small portions of bone at the proximal and distal ends for attachment. A stainless steel wound clip was clamped to the ischial bone fragment, while a knot was tied on the tendinous area between the muscle and tibial bone fragment using 5-0 surgical silk suture.

The jumping trials were performed using a custom-built apparatus that allowed the muscle-tendon-load to move vertically with little resistance and limited movement to only the vertical axis (Figure 2.1). The dissected muscle was hung directly from a force transducer (ELG-V-500G, Entran Sensors and Electronics, Fairfield, NJ, USA) and was connected in series via stainless-steel cylinders with the tendon and load. The muscle was attached to the transducer via a small gold loop soldered to the end of the wound clip (clamped onto the ischial bone fragment). The other end of the muscle was tied to a small stainless steel hook to allow for attachment to the cylinder that connected the muscle to the tendon (Figure 2.1). To prevent dessication of the muscle, two muscle-sized sections of KimWipe scientific cleaning wipes (Kimberly-Clark, Irving, TX, USA) were placed on either side of the muscle and doused in physiological saline. Saline was then continuously pumped over the muscle for the duration of the experiment using a peristaltic pump. To stop saline from dripping down the cylinders and potentially increasing resistance to muscle movement, a 16-gauge needle attached to a vacuum line was positioned directly below the muscle to collect saline after it ran down the muscle.

The muscle was stimulated through magnet wires soldered onto the wound clip and metallic hook tied at the distal end of the muscle. These wires were connected to a stimulator (Isostim A320, World Precision Instruments, Sarasota FL, USA) through which the muscle could

be activated with 1ms pulses at a frequency of 100 Hz. Using a program custom-written in LabView 6.1 (National Instruments, Austin, TX) and a PCI-MIO-16-E4 data acquisition and control card (National Instruments, Austin, TX, USA), the muscle was activated to achieve cyclic oscillation of the load near an observed harmonic frequency of the tendon/load. The number of cycles the muscle was activated (usually 5-7 in each series, enough to establish that a steady-state or near steady-state had been achieved), the cycle frequency as approximated from the resonant frequency of the tendon/load, and the duration of the stimulus within each cycle of contraction were all controlled via the LabView program. Force was recorded at 5 kHz.

The two hollow, 7cm long, steel cylinders acted as markers for the high-speed camera to track the movements of the muscle and load, as well as stabilizing the muscle-tendon-load system so that it moved only in the vertical direction. For tracking purposes, each cylinder was painted matte white to contrast the single black ring painted at the ends adjacent to the tendon. Each cylinder moved through close-fitting holes drilled in teflon bushings, and were oriented such that the black rings on the cylinders remained visible to the high-speed camera at all times. The bushings allowed the cylinders to move in only the vertical direction. Each bushing was anchored to a support column and could be adjusted up or down the column to account for greater stretch of a compliant tendon. The support column was always adjusted such that the muscle and load were stationed directly in line with the holes of the bushings to minimize any lateral movement of the cylinders or load.

The load was a teflon tube with a cap that could be removed to add varying masses of lead weights and sand. The load was attached to the overhanging cylinder via a small gold loop. The tube and cylinder mass were accounted for when setting the load mass for maximal-power.

2.2.3 Determination of tendon and load mass

To simulate the presence or absence of a tendon in series with a muscle during the jumping experiments, synthetic materials were used to mimic compliant and non-compliant tendons. The compliant tendon was made of lycra knitting elastic (H.A. Kidd and Company, Toronto, ON, Canada) and had an approximate cross-sectional area of 5.4×10^{-8} m^2. The stress-strain properties of this material were measured by stretching the tendon various lengths while suspended from the force transducer (Figure 2.2). The non-compliant tendon was made of spectra fishing line (50 lb. test; PowerPro, Irvine, CA, USA), a fibre with a low degree of compliance, but for the purposes of this investigation, was considered non-compliant and thus representative of the absence of a tendon. At both ends of the compliant and non-compliant tendons, a gold hook was attached and used to connect the tendon in series with the muscle and load via the two steel cylinders.

The resonant frequency of the tendon and load was an important variable in providing an estimate of the cycle frequency needed to activate a muscle in a manner that might oscillate a load harmonically. To measure this, a load and tendon were suspended in series, released from a short height (just below the site anchoring the tendon), and allowed to oscillate until coming to rest. This process was repeated for tendons of various length connected to loads with various masses. The oscillations were recorded using a high-speed camera (500 Hz sampling frequency; Hi-Spec 2G Mono, Fastec Imaging, San Diego, CA, USA) and later analyzed using Lolitrack software (Loligo Systems, Tjele, Denmark) to determine the average time between peaks of oscillation and thus establishing relationships between tendon length, load mass and resonant frequency (Figure 2.3). Among the compliant tendons, since there was no difference in the material or cross-sectional area, only a difference in length would result in a change in the

compliance. The length of the compliant tendon that was expected to function ideally would be stiff enough to lift a load but still capable of stretching. The tendon chosen was 12.85 mm in length and had a parabolic relationship between the resonant period and load mass. The non-compliant tendon, with structural properties that are independent of length, measured 25.0mm in length, deliberately made slightly longer than the expected length of a stretched compliant tendon so that minimal adjustment of the bushings would be required when interchanging tendons.

The force-velocity relationship for the sartorius muscle (n=4) was determined by measuring the force generated at various shortening velocities (in muscle lengths per second, ML s^{-1}) to determine what load mass should be used for a given muscle (Figure 2.4). To ensure consistency throughout experiments and across different frog sartorii, the load mass that resulted in maximal power output was chosen as the standard mass to use for the duration of the jumping trials. To determine this value, a Hill equation was fitted to the force-velocity curve to generate an equation describing the relationship between force and velocity. The force was multiplied by the velocity, which generated an equation for the power-velocity relationship. Then, taking the first derivative of this power-velocity curve and setting the equation equal to zero allowed for calculation of a velocity that elicited a maximal power response from the muscle. Referring back to the force-velocity equation, the force generated at this velocity could be determined, which represents the fraction of isometric force at which the sartorius muscle generated maximal power (34.6% isometric force; Figure 2.4). Thus, once an isometric contraction was performed for a given muscle, the load mass that resulted in the maximal power output could be determined. Determination of the load mass also allowed for a calculation of the resonant frequency for the compliant tendon, as described previously (Figure 2.3).

2.2.4 Jumping trials

The aim of the jumping trials was to activate a muscle in such a way that it resulted in a near-harmonic and stable oscillation of the load in the presence of a compliant or non-compliant tendon. Matching the amplitude of load oscillation when using a compliant and non-compliant tendon would allow the work done by the muscle under comparable circumstances of lift height to be quantified and compared between tendons. The jumping trials would also allow a comparison of the activation conditions required to achieve the desired movements, while the muscle length changes in the presence of either tendon could be measured for replication during the oxygen consumption trials.

Once the muscle was set up to perform the jumping trials, the stimulus current and muscle length were adjusted to elicit maximal isometric force. The stimulus current was increased until further increases did not result in greater force; this value was then increased by 50% to ensure maximal activation of the muscle. The muscle length was increased until isometric force attained a plateau. As such, during contractions the muscle tended to shorten over the plateau of the length-tension relationship. The muscle was then stimulated tetanically for 400ms using the non-compliant tendon and a load mass of about 100g, resulting in an isometric, tetanic contraction. The load mass that resulted in maximal power was determined from this isometric force (i.e. 34.6% of this force), which subsequently yielded the resonant frequency of the compliant tendon at that mass, as described above. It was found that this frequency proved to be slightly too fast to allow for full excursion of the load (i.e. an excursion that matched that obtained with the non-compliant tendon). However, contractions at approximately 80% of the calculated resonant frequency led to steady oscillations of the load with an amplitude that

matched those with a non-compliant tendon. This lower frequency of contractions was thus used during experiments.

The cycle frequency was adjusted for each preparation such that the amplitude of oscillation of the load became symmetrical between cycles and as large as possible. If the cycle frequency was too slow, the shortening of the muscle occurred after the recoil of the tendon had begun to move the load upwards again. If the cycle frequency was too fast, the muscle would shorten without allowing the load to achieve its full displacement and thus limiting the impact of the tendon recoil. In an animal, an analogous situation would involve contraction of muscles at intervals either shorter or longer than normal, resulting in movements of limbs disruptive to overall locomotion. Therefore, depending on the individual muscle, trials using a compliant tendon were performed at frequencies between 75-85% of the measured resonant frequency of the tendon/load to achieve harmonic and coordinated movements.

Trials with a non-compliant tendon required slower cycle frequencies to achieve a similar displacement of the load as occurred with a compliant tendon. A cycle frequency between 40-50% of the resonant frequency with a compliant tendon typically resulted in a similar displacement of the load. The non-compliant tendon trials appeared to be more detrimental to the force production of the muscle (e.g. resulted in a faster decline in the isometric force) and therefore only five contractions per cycle were performed, as opposed to the seven per trial performed with a compliant tendon.

After selecting an appropriate cycle frequency for the compliant tendon, the stimulus duration was varied to achieve the greatest oscillation in load amplitude while maintaining near-harmonic movement. The stimulus duration of trials involving a non-compliant tendon was then varied to match the load amplitude of the trials with a compliant tendon for each preparation. As

a result, a range of stimulus durations was used, with compliant values typically ranging from 25-45% of the period of the resonant frequency, while non-compliant values ranged from 45-60%.

2.2.5 Movement and work analysis

Once the experiment was completed, the muscle was excised from the portions of bone and connective tissue at either end and placed on a piece of filter paper to absorb excess moisture. The mass of the muscle was measured using a Sartorius CP124S analytical balance.

The muscle, tendon and load movements were analyzed using Lolitrack software, yielding pixel positions and the associated times of the two markers on the cylinders in each frame. The pixel positions were converted to millimetres using a calibration factor, then, by subtracting the initial position at time zero, the movement of the two cylinders could be resolved relative to the zero point. The calibration factor was the relationship between pixel position and shortening distance, calculated by including a ruler in the same plane as the muscle during recordings. From the movement of the markers on the two cylinders, changes in muscle length, load displacement, and tendon length could be calculated. In the presence of the compliant tendon the muscle-tendon-load system reached steady-state oscillation by the third cycle, with little deviation in amplitude of the load or muscle during subsequent cycles. Thus, the third cycle was typically used when calculating load movement, tendon length change and muscle length change. Trials with the non-compliant tendon did not result in an amplitude of load movement that was as consistent as oscillations with the compliant tendon, however, the third cycle was typically indicative of the series average (for cycles 2-4) and thus was used for analysis.

Once the muscle movements were measured, the shortening work, lengthening work, net work and impulse of the muscle was determined for each trial. Work was determined by integrating the force with respect to length, assuming all force was parallel to the axis of movement. Net work is the sum of the work done while shortening (positive value) and the work done while the muscle is being stretched (negative value). The impulse was measured by integrating the force with respect to time. Cycle impulse was defined as the impulse measured over one complete cycle of oscillation of the load. The rate of impulse was defined as the impulse divided by the period of oscillation, which resulted in the impulse per second and is equal to the average force generated during the oscillation. All work and impulse values were standardized to the mass of the individual muscle used during those trials.

The work done by muscles oscillating a load at an equal amplitude in series with either a compliant or non-compliant tendon was then quantified for a single cycle and compared. Furthermore, the activation parameters required to attain an equal amplitude of load movement could be compared between the non/compliant tendons. In comparing these parameters, the stimulus duration was expressed as the duty cycle, which is the stimulus duration as a percentage of the cycle period.

2.2.6 Determination of parameters for oxygen consumption

The jumping trials were essential for the completion of the oxygen consumption trials, where the stimulus parameters and the muscle length changes while oscillating a load in the presence of a compliant or non-compliant tendon would be recorded and later replicated within a sealed chamber while measuring oxygen consumed by the muscle. However, the conditions that

resulted in matching load amplitudes were not always equal across preparations. Therefore a methodology to determine which movements to replicate within the chamber was required.

As a first approach, relationships between various parameters of amplitude of load oscillation, stimulus duty cycle, etc. were explored to determine if a clear relationship existed that might direct the selection of conditions to replicate while measuring oxygen consumption (see Results). A clear relationship between load amplitude and any of the other variables was not readily identifiable. Thus, clusters of points from the plot of the relationship between stimulus duty cycle versus load amplitude were identified and used to differentiate between trials with low and high amplitude oscillations of the load, and these were then further separated into three clusters of low, medium or high duty cycles. This clustering was performed on data from both compliant and non-compliant tendons. Thus, for both the compliant and non-compliant trials (Figure 2.5), there were six different sets of activation conditions to compare, comprising low and high amplitude oscillations of the load and the associated low, medium and high duty cycles of activation.

To replicate the movement of the muscle from the jumping trials in the oxygen chamber, the muscle strain (trajectory and amplitude), phase of activation (the specific point within the strain trajectory that activation commenced), cycle frequency and stimulus duration (duty cycle) were required. The stimulus duration and cycle frequency were calculated by averaging the data within each of the clusters, as assigned above. Muscle strain amplitude was calculated as the maximal change in length of the muscle relative to the average length during the steady-state contractions. The percent strain for each muscle was determined, and then averaged amongst all trials within a cluster.

The strain trajectories from each trial of interest were associated with a unique cycle frequency and trajectory, similar to others in the cluster but not identical. Thus, before averaging the strain trajectories within clusters for use in the oxygen measurements, each record was processed using a program custom-written in LabView 6.1 (by D.Syme and A.Hume) to standardize the amplitude and the time base to match that of the cycle frequency to be used for that cluster. This program was designed to adjust the records of strain trajectory so that they contained the proper number of data points for use in another program that would control muscle length during measures of oxygen consumption. It also scaled the values of muscle length such that the maximum and minimum muscle length had values of 1 and -1, respectively. These standardized records of strain trajectory could then be used by another program to impose a desired strain amplitude at a desired frequency onto other muscles during measures of oxygen consumption. The original length traces recorded by the camera contained points recorded at an interval of 2 ms and the cycle frequency of each trial was slightly different (based on the load and resonant characteristics of the tendon used). However, the apparatus that would control the muscle length during measures of oxygen consumption required points at intervals of 0.2ms and the cycle frequency (and thus period of the strain cycle) was equal to the average within each cluster of points defined above, and not necessarily that obtained in any given jumping trial. Thus, the program instructed to select a single cycle and interpolated data points such that the output file would contain an appropriate number of data points to result in the proper cycle frequency when output at a rate of one point per 0.2ms. All such files within a cluster were then averaged, resulting in a single, scaled, strain trajectory from each cluster to be used during measures of oxygen consumption.

In addition to manipulating the length traces, the program also allowed for calculation of the stimulus phase (i.e. the time elapsed from initiation of a cycle until the stimulus commences). Calculation of the phase occurred simultaneously to rescaling the length traces as detailed above, reading in the original trace of the muscle length changes and the accompanying stimulus trace. For each trial, a cursor was placed at the initial instance of stimulus, and upon calculating the midpoint of the muscle strain oscillation, the number of data points until the stimulus occurred was displayed. Dividing this value by the number of points in a single cycle of oscillation yielded the phase of stimulus (Figure 2.6).

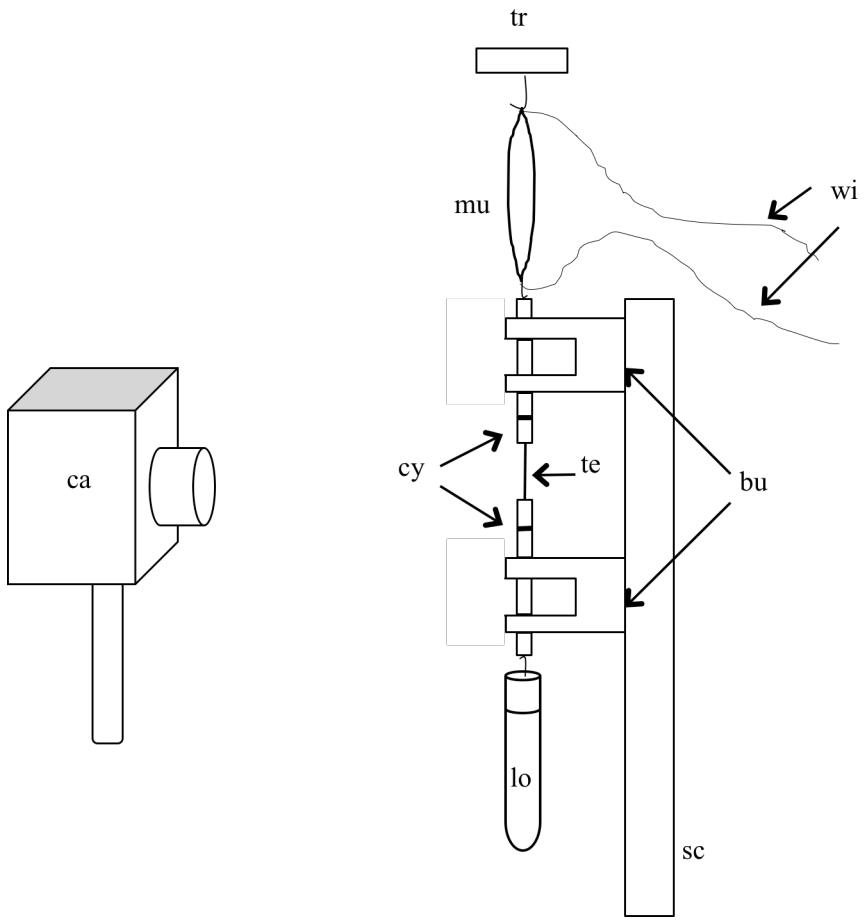


Figure 2.1. Experimental apparatus used to conduct jumping trials. Muscle (mu) was suspended from the force transducer (tr) and attached in series to a tendon (te) and load (lo) via two hollow cylinders (cy). To limit the horizontal movement of the muscle-tendon-load, the cylinders moved vertically through two bushings (bu) mounted to a support column (sc). The cylinders had markers (black line) on the ends near the tendon attachment, which allowed movement of the muscle and load to be tracked by a high-speed camera (ca). The muscle was stimulated via magnet wires (wi) attached to hooks at both ends of the muscle. The load had a detachable lid which allowed the mass to be adjusted.

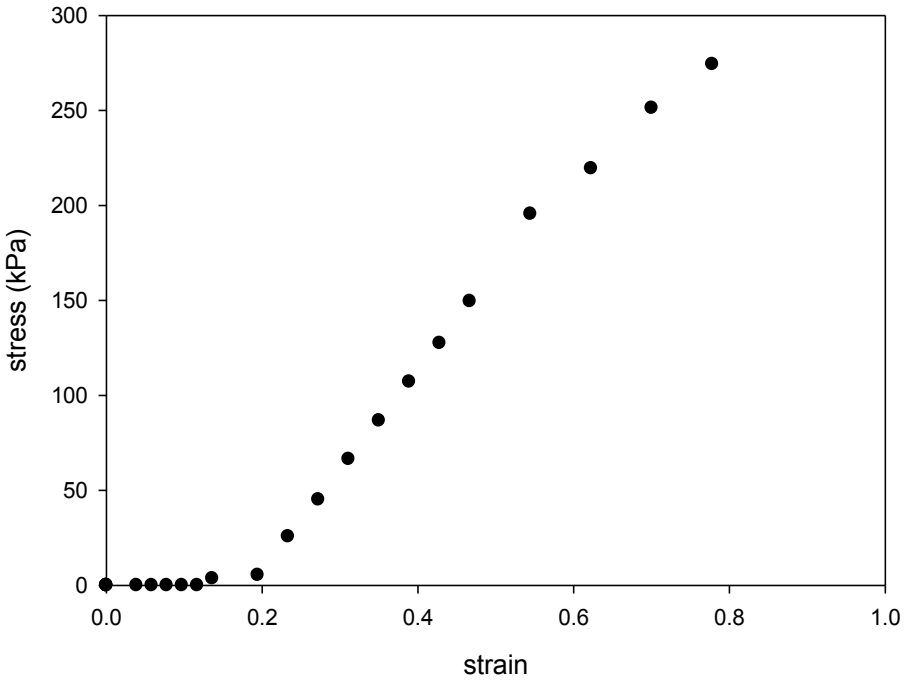


Figure 2.2. Stress-strain relationship for the compliant tendon used during jumping trials (12.85mm length; 5.4×10^{-8} m^2 cross-sectional area). The force exerted and length of the tendon while suspended from the force transducer was measured for various load masses.

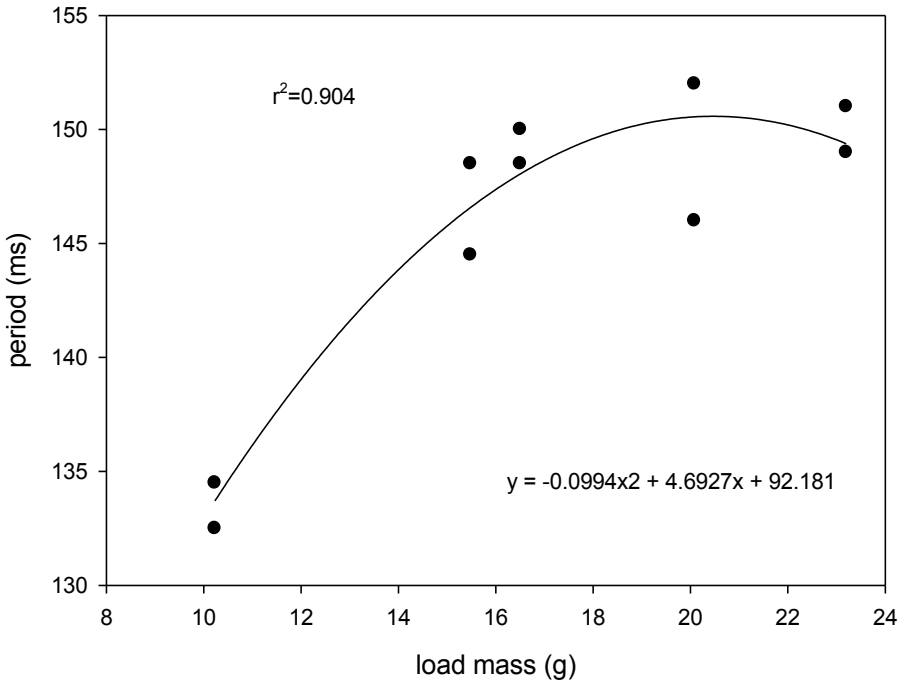


Figure 2.3. Resonant period of the compliant tendon used during jumping trials (12.85 mm in length) as it oscillated various load masses. Period was measured by allowing a load attached to the tendon to oscillate and measuring the time between peaks. The fitted curve is quadratic.

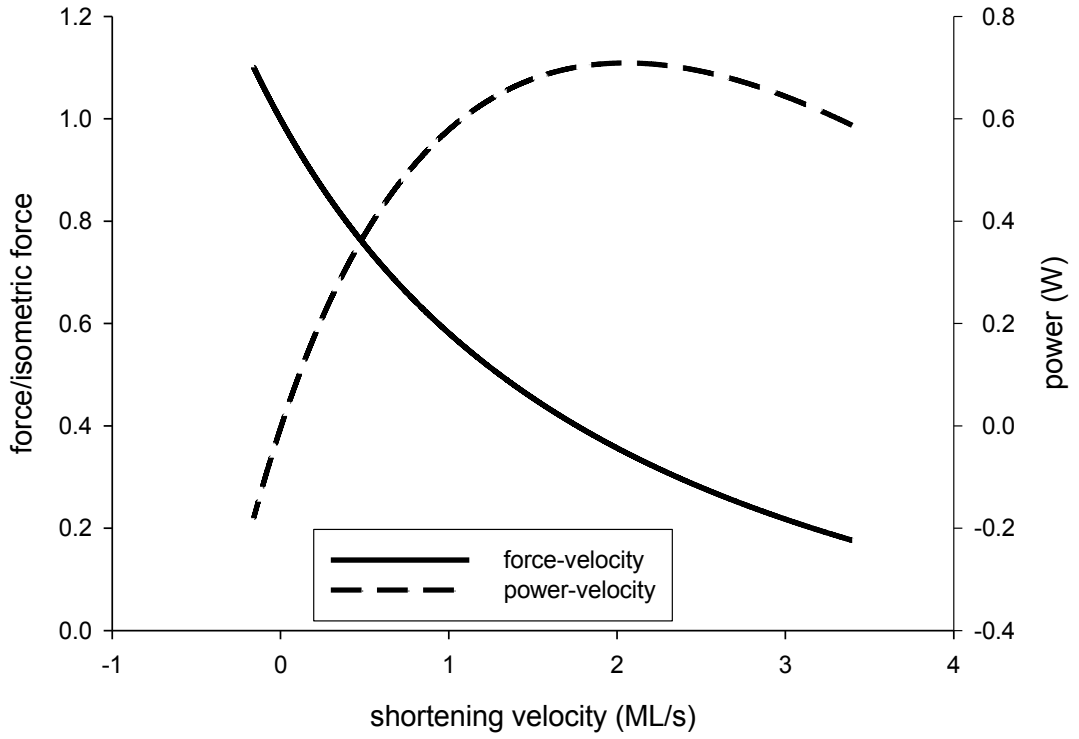


Figure 2.4. The force-velocity and power-velocity curves used to determine the load mass for use during jumping trials (n=4). The solid line represents the force calculated at various velocities while the dashed line represents the power calculated for various velocities.

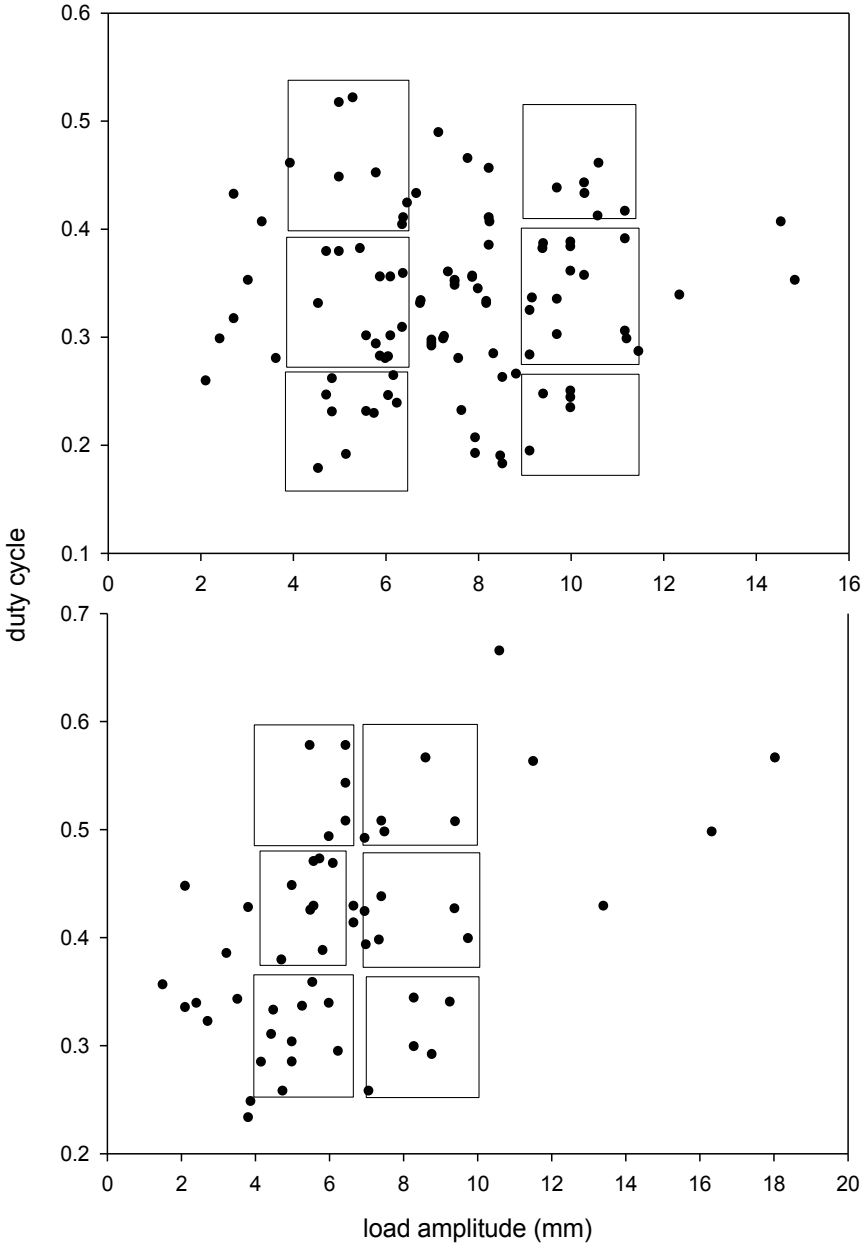


Figure 2.5. The stimulus duty cycle at various amplitudes of load movement during compliant (upper) and non-compliant (lower) jumping trials. The boxes demarcate high and low amplitude, and high, medium and low duty cycles. The left column of boxes contained trials with low amplitude while the right column contained trials of high amplitude. The uppermost row of boxes contained high duty cycle trials, while the middle contained medium duty cycle trials and the bottom row contained low duty cycle trials.

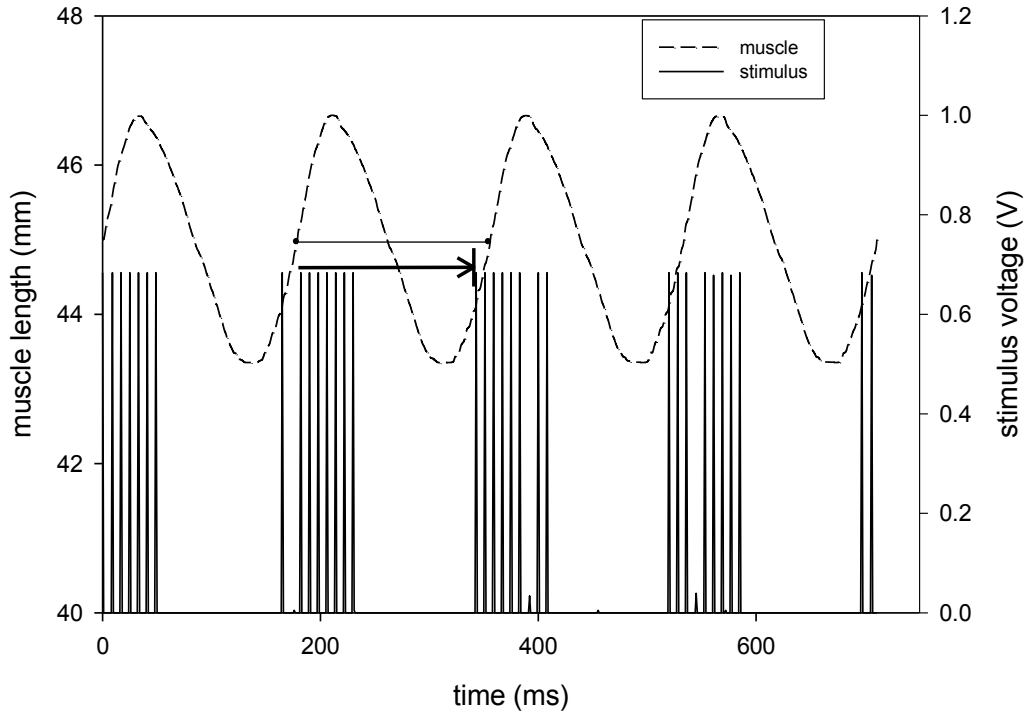


Figure 2.6. An example of stimulus and length for a muscle contracting cyclically in the presence of a compliant tendon. Muscle length is represented by a dashed line,the stimulus voltage is represented by the solid lines, the duration of a single cycle is represented by the thin line connecting two endpoints, while the period of time between the start of a cycle until the stimulus commences is represented by the thicker line with an arrow. The stimulus phase is the time from the onset of a cycle until the stimulus begins, and is represented as a percentage of the cycle period. A cycle's origin was defined as the midpoint of the muscle length amplitude during lengthening. In this example, the phase was 93%.

2.3 Results

Twelve muscles from 12 frogs were used during the jumping trials. The mean mass of the muscles was 97.2 ± 28.1 mg and the mean muscle length was 43.9 ± 2.9 mm. All work and power values are normalized for muscle mass (J kg^{-1} and W kg^{-1} respectively) so they could be compared with values from other muscle preparations. All cycle impulse and rate of impulse values are normalized for muscle cross-sectional area (kNs m^{-2} and kN m^{-2} respectively).

During a series of contractions with a compliant tendon connecting the muscle to the load, initially the muscle would contract, stretch the tendon and lift the load, and the tendon would begin to recoil as the load approached its apogee (Figure 2.7). As the muscle relaxed the load would fall, and as the muscle was stimulated to commence a subsequent cycle the tendon would stretch against the falling load until the load again began to rise. Consequently, over a series of cycles, the load would be pulled upwards by both recoil of the tendon and shortening of the muscle as it contracted, fall as the muscle relaxed, then stretch the tendon as the muscle was activated again, and upon reaching a point of maximal excursion, be pulled upwards by the contraction of the muscle. In this way, the extent of muscle shortening was less than the extent of movement of the load, with stretch and recoil of the tendon being responsible for much of the movement of the load.

In the presence of a non-compliant tendon the movement of the load was approximately equal to the movement of the muscle (Figure 2.7), with any differences being due to measurement error or a small amount of slack in the connections. When the muscle was stimulated it shortened and lifted the load an identical distance. Once the stimulus ended, the muscle would relax and lengthen as the load fell, returning toward its original length. Upon approaching its original length the falling load would pull on the muscle, slightly stretching the

muscle and resulting in a small bounce of the load. This is reflected as a pair of peaks in the length traces (Figure 2.7) before the load was again lifted in the subsequent cycle. The cycle frequency was adjusted such that the muscle and load had sufficient time to rise and fall before a subsequent contraction occurred and the amplitude of oscillation of the load was the same as that with a compliant tendon.

2.3.1 Work Production

The amplitude of load movement was used as the independent variable in comparing the work done or the impulse of the muscle in the presence of either tendon. However, a range of amplitudes of load oscillation resulted from using different cycle frequencies and stimulus duty cycles, and it was not always possible to exactly match amplitudes with compliant and non-compliant tendons in any given experiment. Thus, to facilitate comparisons, work and impulse values were grouped by amplitude of load oscillation at one millimetre intervals and presented as a mean value for each range (e.g. all work values from contractions with a load amplitude between 8.00mm and 8.99mm were grouped, etc.). A 2-way ANOVA was used to test for differences between the compliant and non-compliant values for the work and impulse measures while a 1-way ANOVA was used to test for differences within each tendon type (e.g. compliant or non-compliant). In further discussion, work values resulting from a muscle acting in series with a compliant tendon will be referred to as compliant values, while values resulting from a muscle acting in series with a non-compliant tendon will be referred to as non-compliant values. All values of work and impulse were taken from a single contraction cycle at a point where a steady state oscillation of the load had been achieved.

Comparing the effect of each tendon on work, the non-compliant shortening work was significantly greater than the compliant shortening work (2-way ANOVA, $P<0.001$; Figure 2.8), with non-compliant values increasing from approximately twice those of the compliant tendon at lower amplitudes (21.1 J kg^{-1} vs. 10.2 J kg^{-1}, 4.5mm load amplitude), to almost three times the compliant shortening work at higher amplitudes (41.9 J kg^{-1} vs. 14.8 J kg^{-1}, 8.5mm load amplitude). This increase was seen even though the shortening work for both compliant and non-compliant increased significantly (1-way ANOVA, $P<0.001$) as the load amplitude was increased.

The non-compliant lengthening work was significantly greater than the compliant lengthening work (2-way ANOVA, $P<0.001$; Figure 2.9), with non-compliant values increasing from over twice those of the compliant tendon at lower amplitudes (-17.5 J kg^{-1} vs. -7.4 J kg^{-1}, 4.5mm load amplitude), to nearly four times the compliant lengthening work at higher amplitudes (-37.7 J kg^{-1} vs. -10.9 J kg^{-1}, 8.5mm load amplitude). Lengthening work is expressed as a negative value because it refers to work being done on the muscle. Within tendon, both compliant and non-compliant lengthening work also increased significantly (1-way ANOVA, $P<0.001$) with an increased load amplitude.

Unlike the relationship seen for shortening and lengthening work, the net work done by the muscle was greater in the presence of a compliant tendon than with a non-compliant tendon (2-way ANOVA, $P<0.001$; Figure 2.10). Since net work is equal to the difference between the shortening and lengthening work, it appears that while a greater magnitude of shortening and lengthening work is done with a non-compliant tendon, the greater similarity seen between these measures for a non-compliant tendon resulted in a comparatively lower net work. Net work values for both tendons were small at low load amplitudes, but only the compliant net work

significantly increased as the amplitude of load movement was increased (1-way ANOVA, P=0.027). There was not a significant change seen with respect to load amplitude in the net work of the non-compliant tendon (1-way ANOVA, P=0.059). Two values representing only single observations for the non-compliant tendon (at 16 and 18mm load amplitude) were omitted from Figure 4 as uncharacteristically large and thus suspect outliers.

The rate at which a muscle does work (power, measured in Watts) is another useful measure that, unlike the work, takes into account the time required to complete a single contraction cycle. The power of the muscle was expressed while shortening, lengthening and as a net value. Factoring in the cycle frequency reduced the difference between non-compliant and compliant shortening work values, however, the difference in shortening power (Figure 2.11) between compliant and non-compliant was still significant (2-way ANOVA, P<0.001). Shortening power for both compliant and non-compliant increased significantly as the amplitude of load movement increased (1-way ANOVA, P<0.001).

Calculation of the lengthening power (Figure 2.12) yielded a similar result, as the non-compliant was significantly greater than the compliant (2-way ANOVA, P<0.001) and both values increased significantly with increasing amplitude of load movement (1-way ANOVA, P<0.001).

As was seen with the work, the compliant net power was significantly greater than the non-compliant (2-way ANOVA, P<0.001; Figure 2.13), yet neither the compliant (1-way ANOVA, P=0.097) nor non-compliant (1-way ANOVA, P=0.074) power values showed a statistically significant increase at increasing amplitudes of load movement.

2.3.2 Impulse Measurements

Impulse is another measure used to quantify the effort exerted by a muscle to move a load, calculated as the product of force and time. In this study, impulse was calculated for a single cycle (Figure 2.14) and as a rate (Figure 2.15) for both tendons at various load amplitudes.

The cycle impulse (Figure 2.14) was significantly greater in the presence of a non-compliant tendon than a compliant tendon (2-way ANOVA, $P<0.001$). This disparity was greatest at low amplitudes and appeared to diminish at increasing load amplitudes. The compliant cycle impulse increased significantly at higher load amplitudes (1-way ANOVA, $P=0.011$) while there was no significant difference seen within the non-compliant values at different amplitudes (1-way ANOVA, $P=0.216$).

Cycle impulse measures the force exerted during one cycle of contraction and is thus a useful comparison of effort for a single jump. However, measuring cycle impulse for a single cycle does not account for potential differences in the cycle period between contractions with compliant and non-compliant tendons and so would not be representative of the effort required to sustain movements over a given period of time. Therefore, the impulse per second, or rate of impulse as it will be referred to, was also measured (e.g. the cycle impulse multiplied by the frequency) (Figure 2.15). In contrast to the cycle impulse, the rate of impulse for the compliant tendons was significantly greater than for the non-compliant tendons (2-way ANOVA, $P=0.003$). Although the rate of impulse appeared nearly equal for both tendon types at low amplitudes (2-3mm), the difference between tendon types was most pronounced at higher load amplitudes (8-9mm) where compliant rate of impulse was over 30% greater than the non-compliant rate of impulse. This divergence was a result of a significant increase in the compliant rate of impulse at

increasing load amplitudes (1-way ANOVA, P=0.034) while the non-compliant did not change significantly (1-way ANOVA, P=0.739).

2.3.3 Activation conditions

An analysis of the activation parameters used to attain the various load amplitudes with compliant versus non-compliant tendons was performed on the cycle frequency and stimulus duty cycle. As with work and force, the cycle frequency and stimulus duty cycle values were binned based on load amplitude, and then expressed as a mean value for each bin. Of the two tendons, the cycle frequencies used to achieve cyclic motion with a compliant tendon were significantly greater as they never decreased below 5.20Hz, while the non-compliant frequencies did not surpass 4.20Hz at any load amplitude (2-way ANOVA, $P<0.001$; Figure 2.16). Within tendons, there was a significant decrease in the compliant cycle frequency as load amplitude increased (1-way ANOVA, P=0.042), but no significant change was seen for non-compliant frequencies as the load amplitude increased (1-way ANOVA, P=0.056).

The stimulus duty cycle represents the fraction of the cycle period during which the muscle was stimulated (Figure 2.17). By taking into account the cycle period, a relative measure of the time a muscle is active is given, as opposed to the absolute time which can be misleading when there are notable differences in cycle frequency, as seen between tendons types. The non-compliant stimulus duty cycle was significantly greater than the compliant (2-way ANOVA, $P<0.001$). In other words, to lift a load an equal distance, a muscle contracting in the presence of a non-compliant tendon requires stimulation for a greater percentage of its cycle than a muscle contracting with a compliant tendon. Also, there was no significant change in the duty cycle with

increasing load amplitude for either the compliant tendon (1-way ANOVA, P=0.355) or non-compliant tendon (1-way ANOVA, P=0.171).

2.3.4 Work loops

Work loops are not only an effective manner of depicting the force exerted by a muscle as it lengthens and shortens over a cycle, but are also useful to interpret and compare how muscles with compliant and non-compliant tendons are used to power movement. Initially introduced by Josephson (1985) for use with synchronous muscle, they allow a graphical depiction of the work done by a muscle. Example work loops at low and high load amplitudes are shown for a muscle contracting in the presence of a compliant (Figure 2.18) and non-compliant tendon (Figure 2.19). Activation conditions and muscle optimal lengths are shown in the figure captions.

For the high amplitude, non-compliant work loop, the bow-shape at the greatest length is a result of the small rebound that occurred after activation ended and the muscle was stretched slightly by the falling load pulling on the non-compliant tendon (also see Figure 2.1).

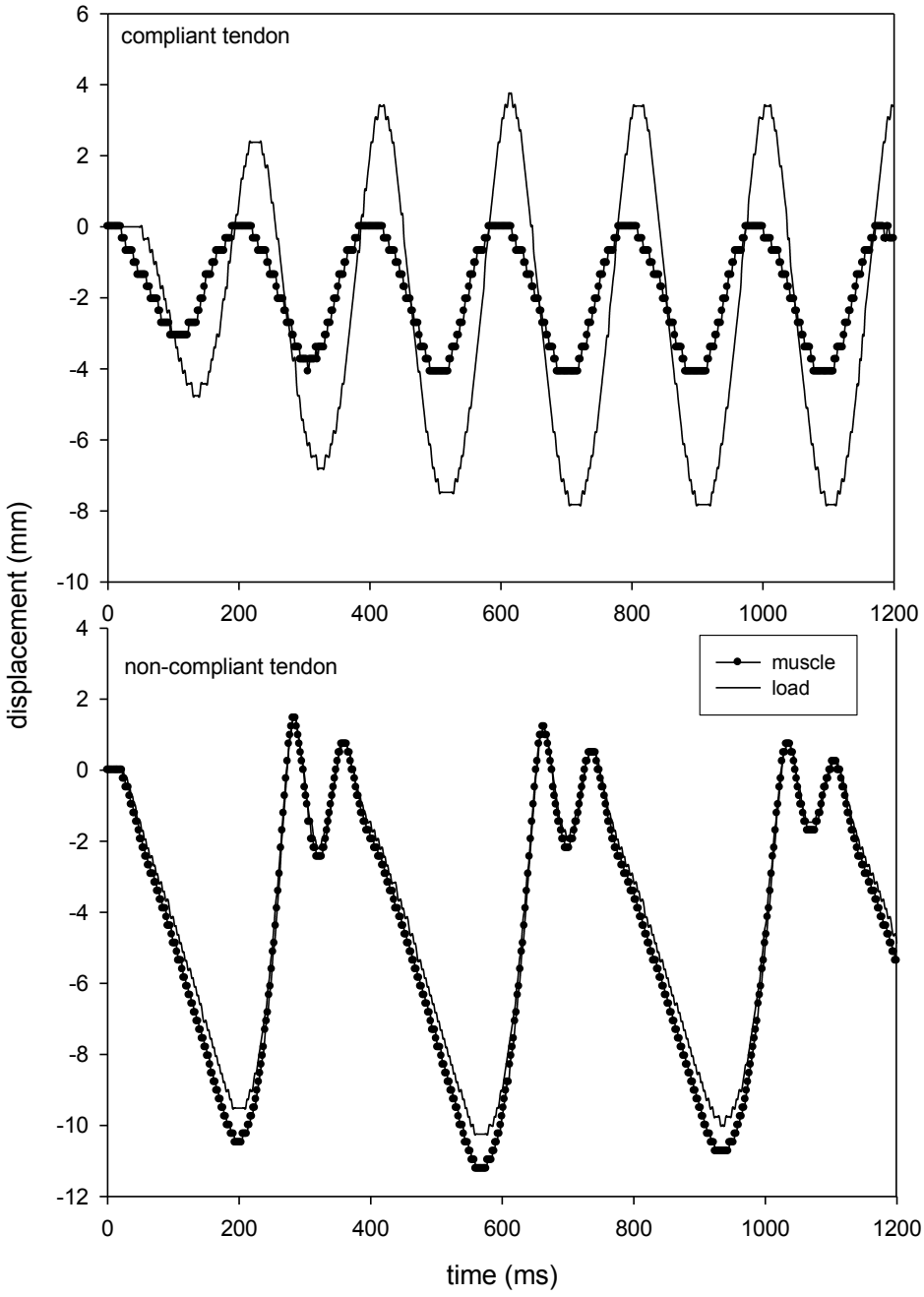

compliant tendon
non-compliant tendon
muscle
load
displacement (mm)
time (ms)
6
4
2
0
-2
-4
-6
-8
-10
-12
0
200
400
600
800
1000
1200

Figure 2.7. Muscle and load movements relative to their initial position for an exemplary compliant and non-compliant trial. The load was oscillated a distance of 11.21mm on average in the presence of both tendons. In the presence of a compliant tendon the cycle frequency was 5.42Hz and the stimulus duty cycle was 0.30, while with the non-compliant tendon the cycle frequency was 2.75Hz and duty cycle was 0.40. Negative values indicate shortening of the muscle and lifting of the load.

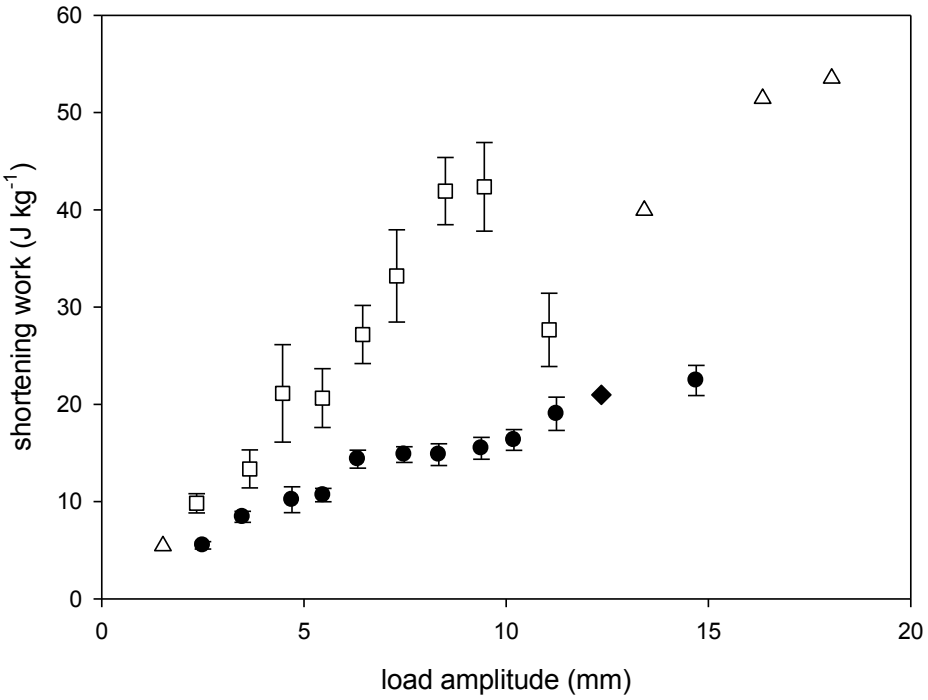


Figure 2.8. Shortening work done by muscle lifting a load at different amplitudes with a non-compliant tendon (open symbols) and compliant tendon (black symbols). Open triangles (non-compliant) and black diamonds (compliant) show data points where only a single preparation is represented. Values are shown as mean +/- standard error. Compliant and non-compliant values are significantly different (2-way ANOVA, $P<0.001$) and both increase significantly as the load amplitude increases (1-way ANOVA, $P<0.001$).

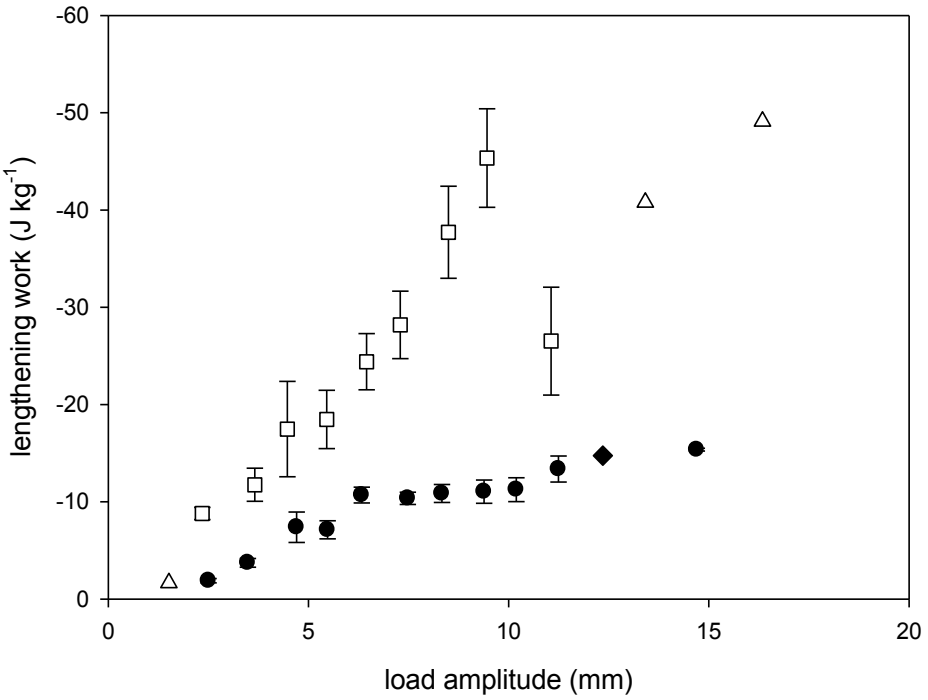


Figure 2.9. Lengthening work done by muscle lifting a load at different amplitudes with a non-compliant tendon (open symbols) and compliant tendon (black symbols). Open triangles (non-compliant) and black diamonds (compliant) show data points where only a single preparation is represented. Values are shown as mean +/- standard error. Compliant and non-compliant values are significantly different (2-way ANOVA, $P<0.001$) and both increase significantly as the load amplitude increases (1-way ANOVA, $P<0.001$).

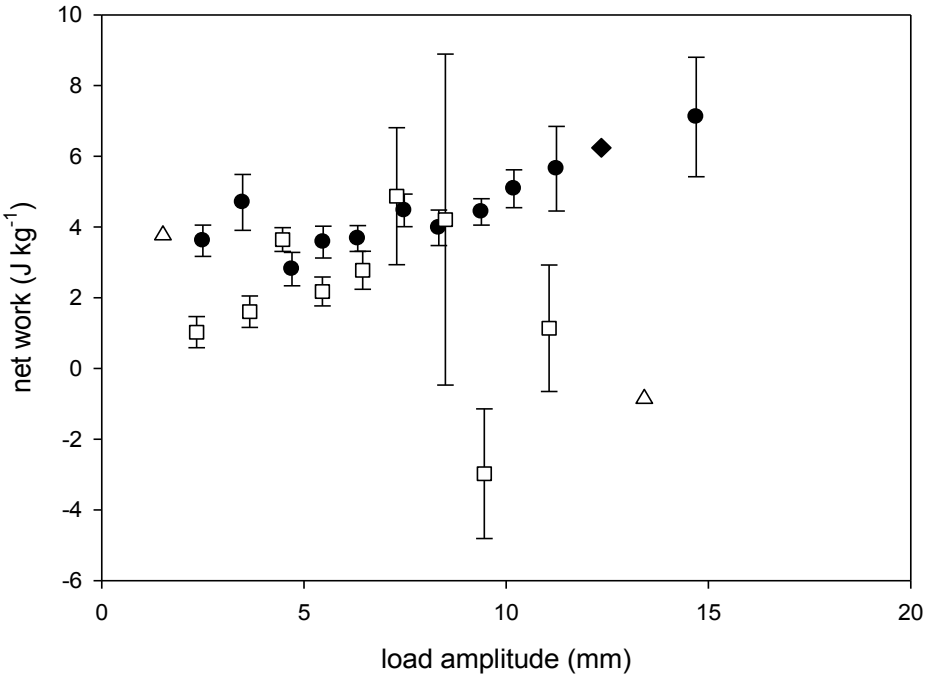


Figure 2.10. Net work done by muscle lifting a load at different amplitudes with a non-compliant tendon (open symbols) and compliant tendon (black symbols). Open triangles (non-compliant) and black diamonds (compliant) show data points where only a single preparation is represented. Values are shown as mean +/- standard error. Compliant and non-compliant values are significantly different (2-way ANOVA, P=0.027) with a significant increase seen within the compliant values (1-way ANOVA, P=0.027) but not the non-compliant (1-way ANOVA, P=0.059). NOTE: two non-compliant values (16 and 18mm amplitude) were omitted from the plot; see text for further details.

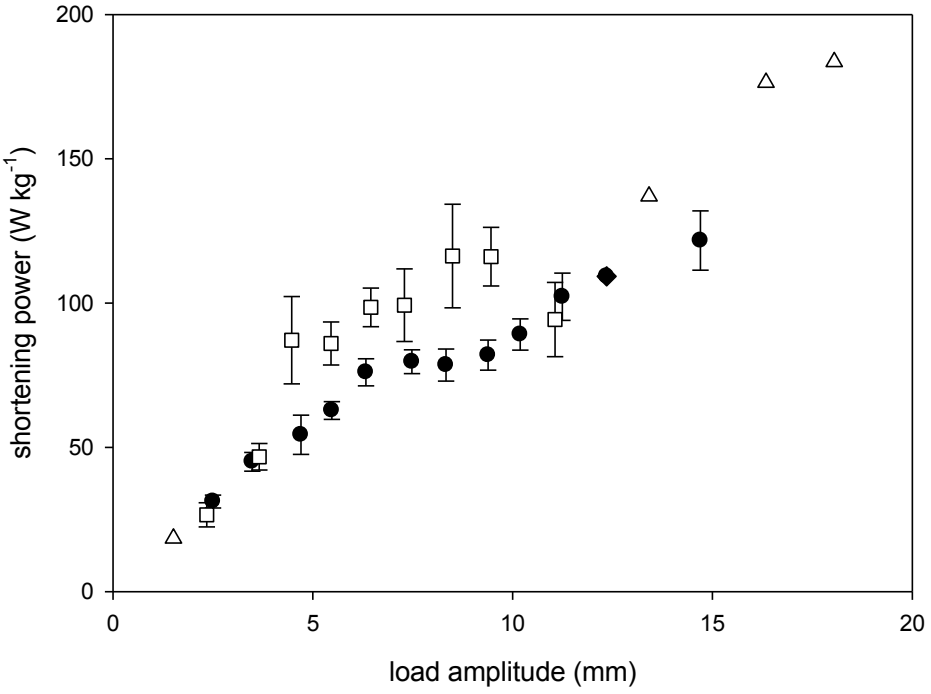


Figure 2.11. Shortening power while lifting a load at different amplitudes with a non-compliant tendon (open symbols) and compliant tendon (black symbols). Open triangles (non-compliant) and black diamonds (compliant) show data points where only a single preparation is represented. Values are shown as mean +/- standard error. There was a significant difference between compliant and non-compliant (2-way ANOVA, $P<0.001$), with a significant increase seen for both tendons (1-way ANOVA, $P<0.001$) with increasing load amplitude.

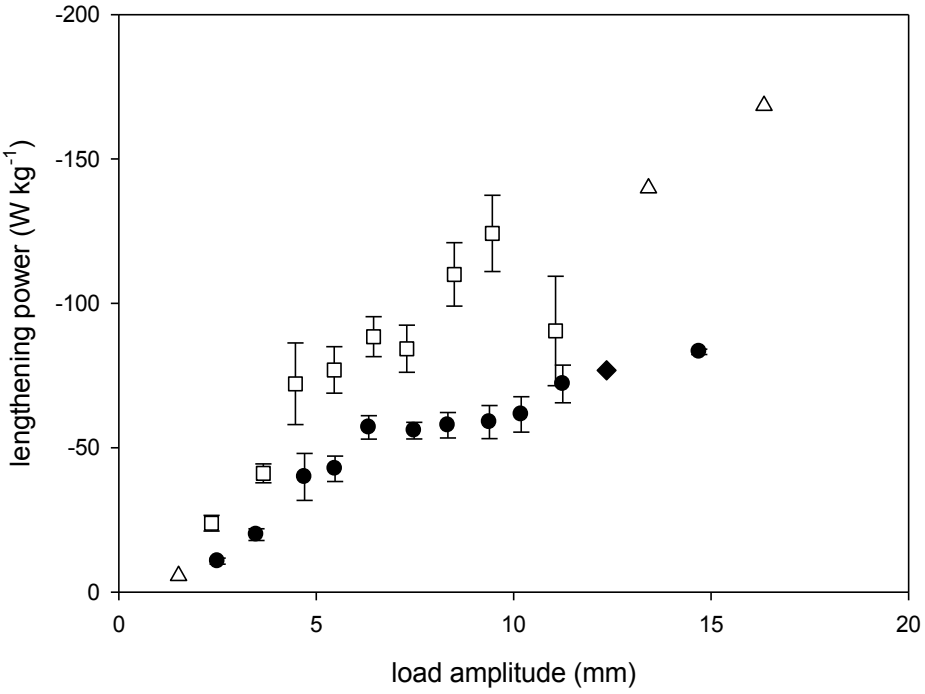


Figure 2.12. Lengthening power while lifting a load at different amplitudes with a non-compliant tendon (open symbols) and compliant tendon (black symbols). Open triangles (non-compliant) and black diamonds (compliant) show data points where only a single preparation is represented. Values are shown as mean +/- standard error. There was a significant difference between compliant and non-compliant (2-way ANOVA, $P<0.001$), with a significant increase seen for both tendons (1-way ANOVA, $P<0.001$) with increasing load amplitude.

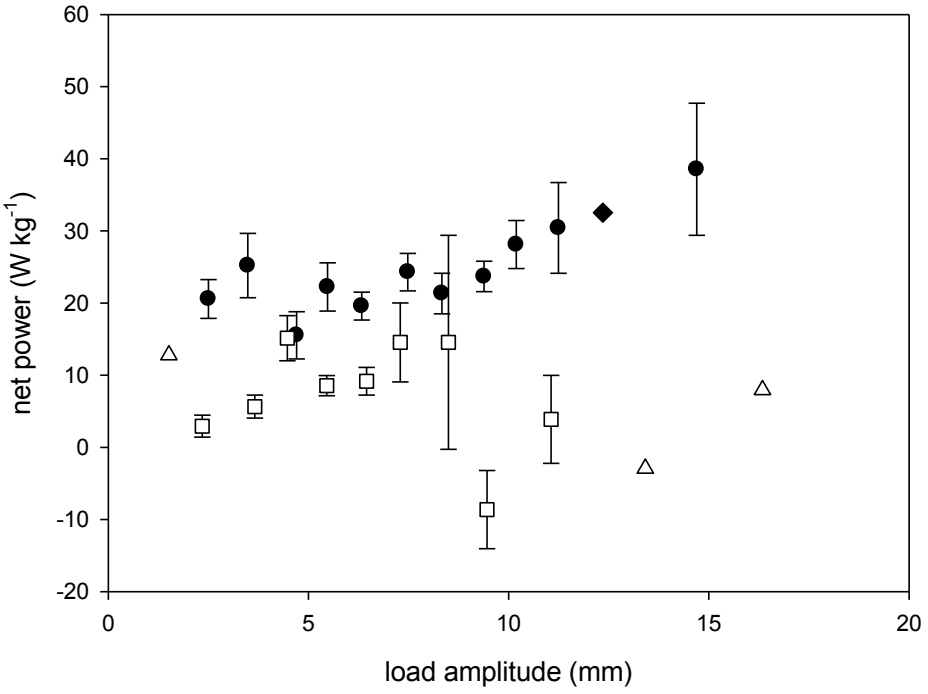


Figure 2.13. Net power while lifting a load at different amplitudes with a non-compliant tendon (open symbols) and compliant tendon (black symbols). Open triangles (non-compliant) and black diamonds (compliant) show data points where only a single preparation is represented. Values are shown as mean +/- standard error. There was a significant difference between compliant and non-compliant (2-way ANOVA, $P<0.001$), but no significant change seen for compliant (1-way ANOVA, $P=0.097$) or non-compliant net power (1-way ANOVA, $P<0.074$) with increasing load amplitude.

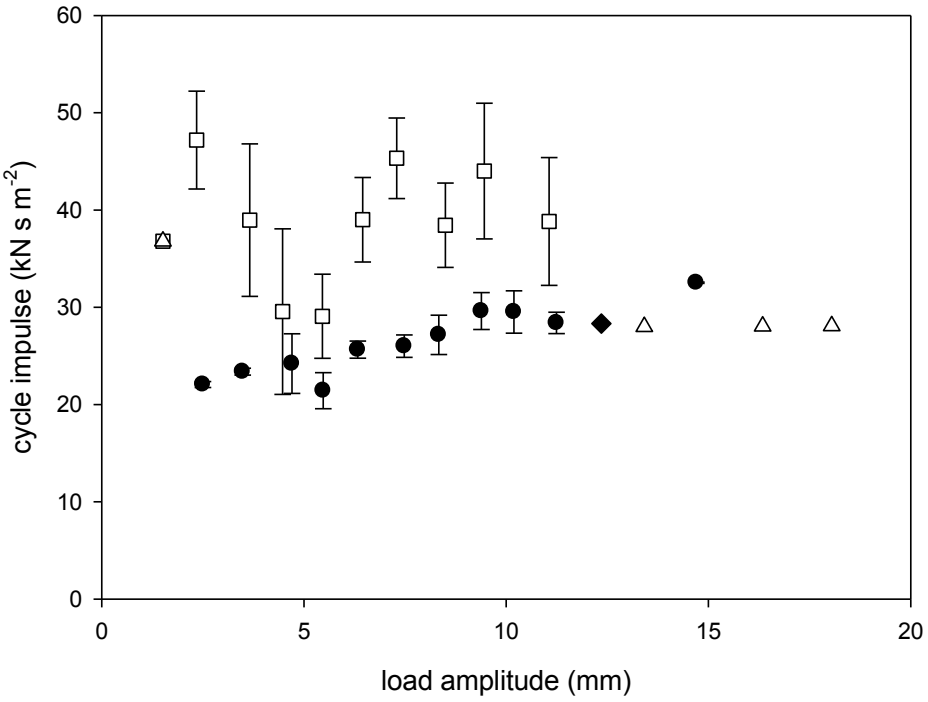


Figure 2.14. Cycle impulse while lifting a load at different amplitudes with a non-compliant tendon (open symbols) and compliant tendon (black symbols). Open triangles (non-compliant) and black diamonds (compliant) show data points where only a single preparation is represented. Values are shown as mean +/- standard error. There was a significant difference between the compliant and non-compliant values (2-way ANOVA, $P<0.001$) and a significant increase in the compliant values with increasing load amplitude (1-way ANOVA, $P=0.011$), but not in non-compliant values (1-way ANOVA; 0.216).

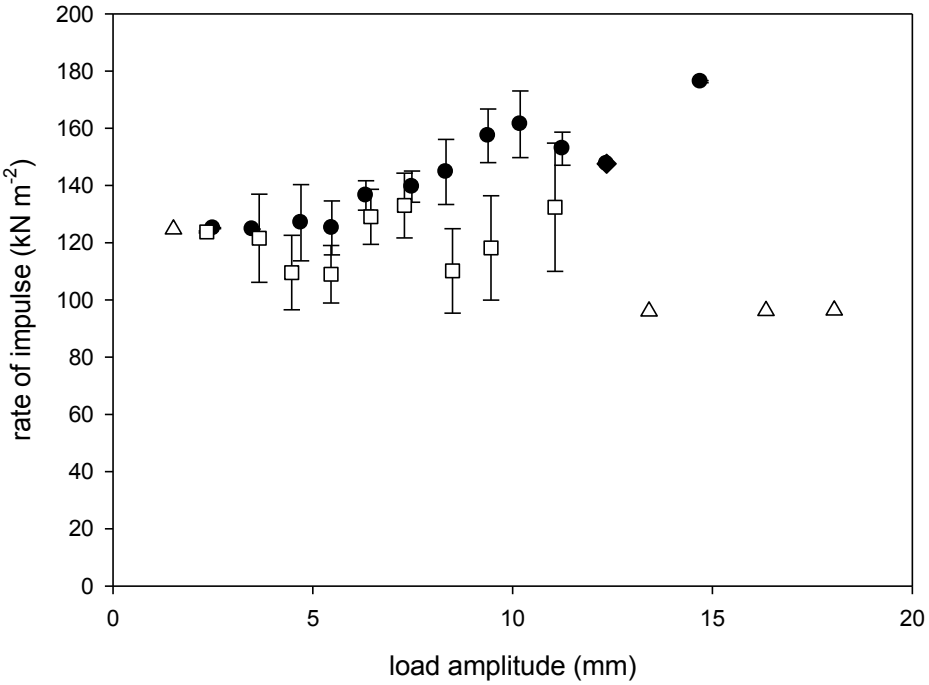


Figure 2.15. Rate of impulse while lifting a load at different amplitudes with a non-compliant tendon (open symbols) and compliant tendon (black symbols). Open triangles (non-compliant) and black diamonds (compliant) show data points where only a single preparation is represented. Values are shown as mean +/- standard error. There was a significant difference between the compliant and non-compliant values (2-way ANOVA, $P<0.001$) and a significant increase in the compliant values with increased load amplitude (1-way ANOVA, $P=0.034$), but not non-compliant values (1-way ANOVA, $P=0.739$).

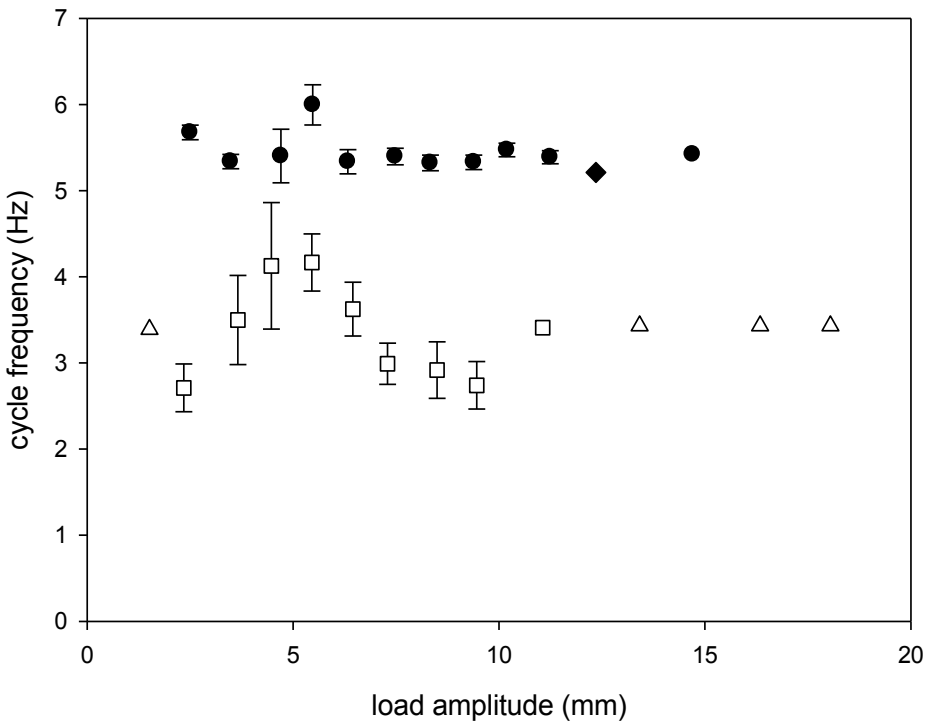


Figure 2.16. Cycle frequency that elicited cyclic contractions and oscillations of the load with a compliant (black symbols) and non-compliant (open symbols) tendon at different amplitudes of movement. Values are shown as mean +/- standard error. There was a significant difference between the compliant and non-compliant values (2-way ANOVA, $P<0.001$) however, a significant decrease was only seen in the compliant (1-way ANOVA, $P=0.042$) but not the non-compliant (1-way ANOVA, $P=0.056$) with increased load amplitude.

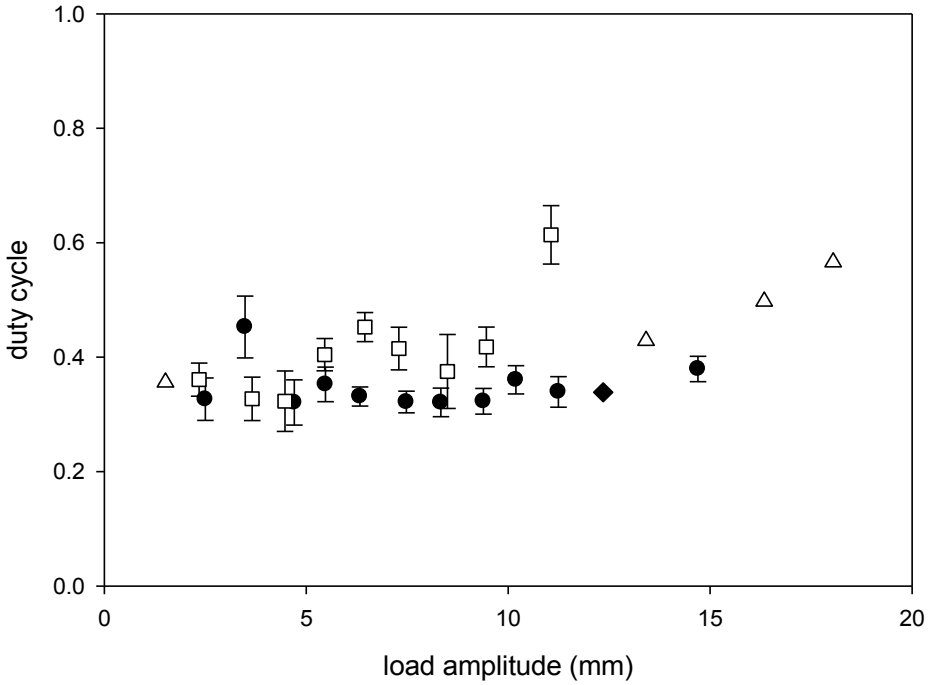


Figure 2.17. Stimulus duty cycle that elicited cyclic contractions and oscillations of the load with a compliant (black symbols) and non-compliant (open symbols) tendon at different amplitudes of movement. Values are shown as mean +/- standard error. There was a significant difference between the tendons (2-way ANOVA, $P<0.001$) but not within either the compliant (1-way ANOVA, $P=0.355$) or non-compliant (1-way ANOVA, $P=0.171$) with increased load amplitude.

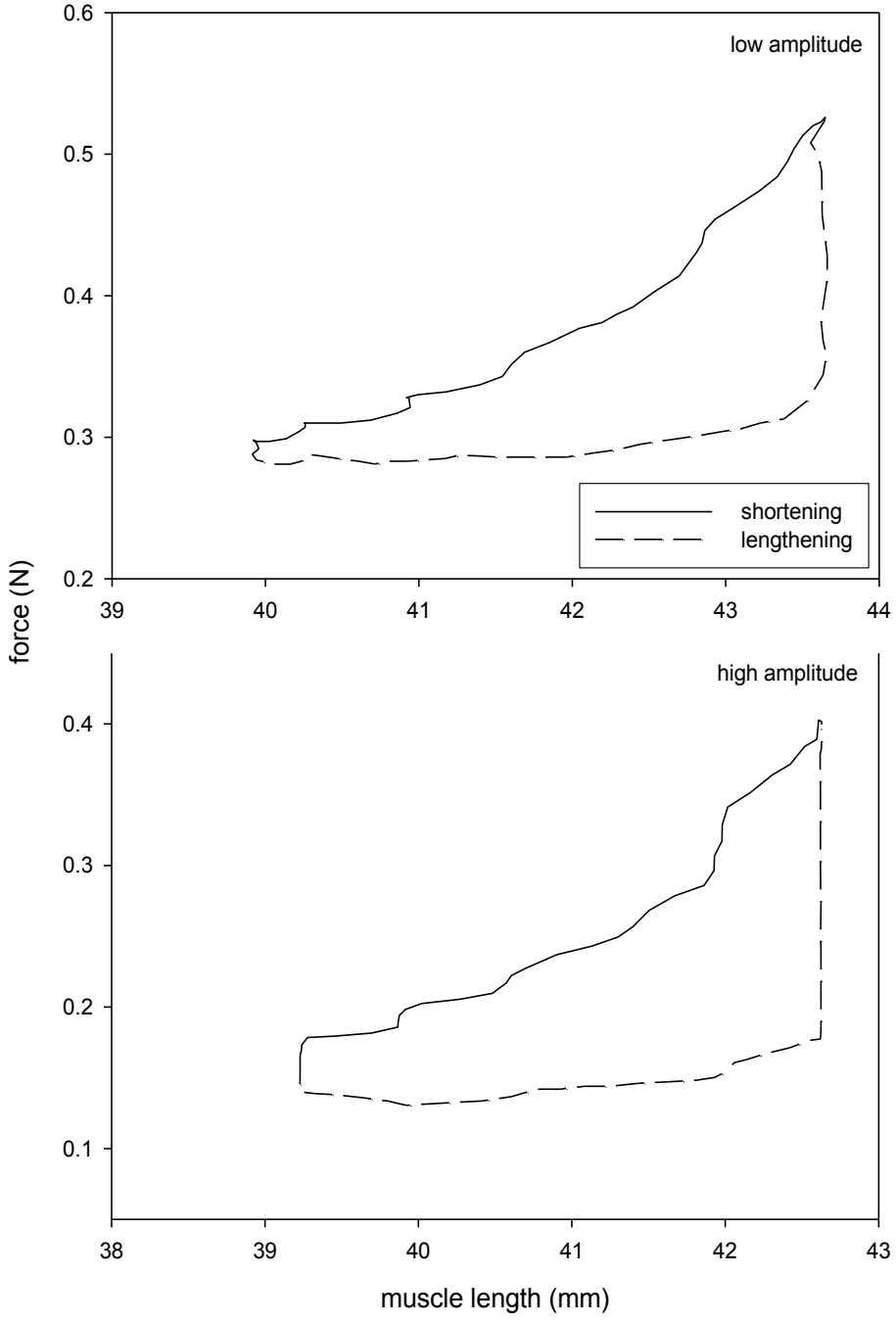

low amplitude
shortening
lengthening
high amplitude
force (N)
muscle length (mm)
0.6
0.5
0.4
0.3
0.2
0.1
39
40
41
42
43
44
38

Figure 2.18. Work loops for a single cycle of muscle contraction in the presence of a compliant tendon at a low (upper plot) or high (lower plot) amplitude of load movement. The cycles shown represent the change in muscle length and force seen when contracting at a steady state. For the low amplitude example, the muscle resting length was 45mm, and a cycle frequency of 5.47Hz and duty cycle of 0.36 resulted in a load amplitude of 6.11mm. The high amplitude example had a resting muscle length of 43.3mm and oscillated the load 10.29mm with a cycle frequency of 5.49Hz and a stimulus duty cycle of 0.37.

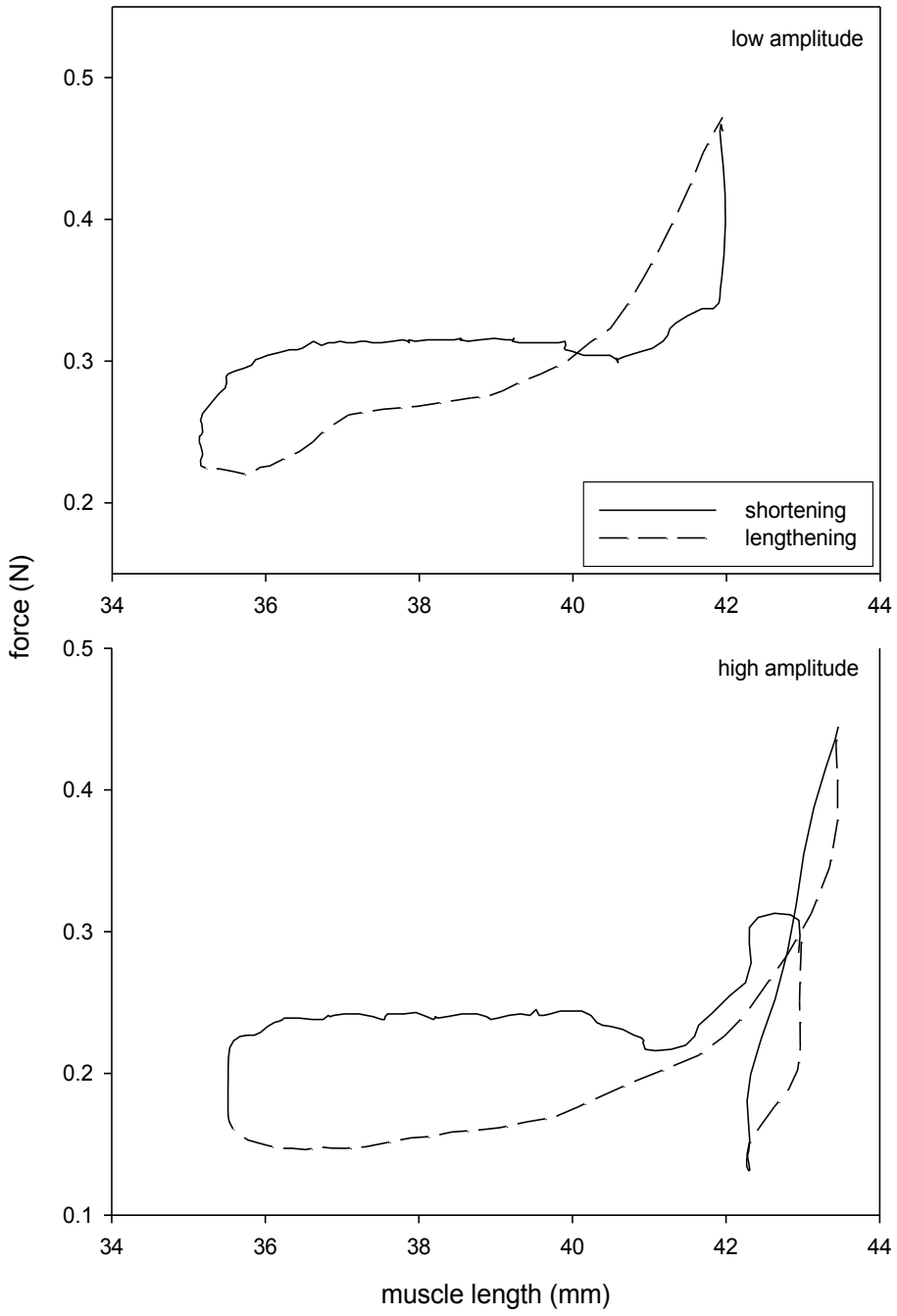

low amplitude
high amplitude
shortening
lengthening
force (N)
muscle length (mm)
0.5
0.4
0.3
0.2
0.1
34
36
38
40
42
44

Figure 2.19. Work loops for a single cycle of muscle contraction in the presence of a non-compliant tendon at a low (upper plot) or high (lower plot) amplitude of load movement. The cycles chosen represent the changes in force and length as the muscle was contracting at a steady state. For the low amplitude example, the muscle resting length was 42.27mm, and a cycle frequency of 3.76Hz and duty cycle of 0.47 resulted in a load amplitude of 5.59mm. The high amplitude example had a muscle resting length of 43.3mm and the load was oscillated 7.35mm with a cycle frequency of 2.74Hz and a stimulus duty cycle of 0.40.

2.4 Discussion

The activation conditions observed to oscillate a load with a compliant tendon required a faster cycle frequency and lesser stimulus duty cycle than with a non-compliant tendon. These conditions were dictated to an extent by the resonant properties of the compliant tendon (Figure 2.3), which were typically 20% greater than the cycle frequency used, likely due to the attachment of a load and timing the activation so that the muscle would begin contracting before the load reached its apogee. For greater amplitudes of load displacement there was a decrease in the cycle frequency, and as with the non-compliant tendon, the relationship between load amplitude and duty cycle was linear. Since any movement of the load in series with the non-compliant tendon was equal to the distance the muscle shortened, the cycle frequency had to decrease with increasing load amplitude to allow more time for a greater load displacement.

The amount of shortening work done during a single, steady-state contraction cycle for a muscle in series with a compliant tendon was less than the non-compliant shortening at both low and high amplitudes of load movement. The compliant lengthening work was also less than the non-compliant, however the disparity between the tendons was greater. As previous studies investigating terrestrial locomotion have suggested, the presence of a compliant tendon may result in storage of elastic strain energy during a stretch, and then release that energy upon tendon recoil, allowing the muscle to continue moving at a steady state while doing less work (Alexander, 1984; Cavagna et al., 1977). The greater amount of non-compliant work suggests a compliant tendon plays a role in decreasing the amount of shortening and lengthening work.

For both shortening and lengthening work, the presence of a compliant tendon resulted in increased savings at increasing amplitudes of load movement. The compliant shortening work decreased from approximately half that of the non-compliant shortening work at low amplitudes

to almost a third at higher amplitudes (where there were multiple measurements, e.g. 8-9mm), while the compliant lengthening work was less than half the non-compliant at lower amplitudes and decreased to nearly a quarter of the non-compliant lengthening work at the highest amplitudes. Since the set-up of this experiment is analogous to the cyclical movements an animal undergoes during locomotion, the load amplitude in this experiment is therefore analogous to the hop distance or stride length of a single cycle. Thus, the increased difference between the compliant and non-compliant work (for both shortening and lengthening) at increased load amplitudes suggests the effect of a compliant tendon on mechanical work output would be amplified at a greater hop distance/stride length for an animal moving in a cyclical manner. This trend is consistent with measurements of hopping wallabies by Biewener and colleagues (1995) who observed an increase in tendon strain energy storage at increasing speeds, which they showed positively correlated with an increase in stride length. Furthermore, Roberts et al. (1997) also observed a greater amount of shortening energy recovery with a tendon for turkeys running at increasing speeds, however he did not report lengthening work in his findings.

While the compliant tendon resulted in less shortening and lengthening work being done, the opposite was observed for the net work. The compliant net work was significantly greater than the non-compliant net work and increased at increasing load amplitudes (Figure 2.10). A potential cause for the greater compliant net work is found not by examining the muscle, but instead focusing on the tendon. The loss of energy as heat from an elastic is known as hysteresis, and studies examining the properties of naturally occurring tendons have found the hysteresis to range from 7-30% (Alexander, 2002; Pollock and Shadwick, 1994). Considering there is even a small amount of hysteresis in natural tendons, it seems reasonable to expect some degree of hysteresis occurred in the synthetic tendon used, especially at higher load amplitudes where the

tendon underwent a greater stretch. Therefore, oscillation of the load in a near-harmonic fashion with a compliant tendon likely required additional shortening work by the muscle. The non-compliant tendon could have potentially incurred a small degree of hysteresis, but the amount is likely negligible; heat lost during shortening due to viscosity within the parallel elastic component of muscle was more likely responsible for any change in the net work at increased load amplitudes. Unlike the compliant tendon where the stretch of the tendon accounted for a large amount of the load movement, the load movement that occurred in the presence of the non-compliant tendon was directly a result of the muscle shortening. At higher amplitudes of load movement where there was a greater amount of muscle shortening, it is possible viscosity could have accounted for increases in net work. However, at both low and high amplitudes of load movement the non-compliant net work was only slightly greater than zero and there was not a significant change as the load amplitude increased.

The compliant (Figure 2.18) and non-compliant work loops (Figure 2.19) illustrate the changes in force that occurred as the muscle shortened and lengthened during a single cycle. The compliant work loops resemble those described by Josephson (1985) as occurring at a stimulus phase that results in maximum work output, with the greatest forces encountered during steady shortening, and lesser forces occurring during the lengthening of the muscle. The non-compliant work loops observed resemble loops described by Josephson (1985) as some work having been done by the muscle, and some work having been done on the muscle. The non-compliant loops show the forces developed while the muscle lengthened back to its original length are greater than the initial forces exerted while shortening. This could be a result of a slight bounce of the load that occurred as it fell and slightly stretched the muscle after activation, an occurrence

exaggerated at higher amplitudes of load movement (Figure 2.19, lower panel). Regardless, the work loops are an effective depiction of the differences observed in the net work across tendons.

The differences observed between the compliant and non-compliant shortening and lengthening work were diminished when expressed as a rate (product of the cycle frequency and work). This suggests the mechanical energy conserved while shortening or lengthening in the presence of a compliant tendon is greater for a given distance than for a given time. For both tendons, an increase in the load amplitude resulted in an increase in the shortening and lengthening power. Thus, the difference in shortening and lengthening power across tendons is reduced relative to the differences in shortening and lengthening work done by a cyclically contracting muscle while the difference in net power was actually greater than seen for net work between the two tendons. As suggested for the net work, hysteresis likely plays a role in the compliant net power, since the value with a non-compliant tendon would have been expected to be near zero.

Perhaps due in part to the disparity in cycle frequencies, the non-compliant cycle impulse was greater than the compliant, while the compliant rate of impulse was greater (Figure 2.17). The higher compliant rate of impulse is in agreement with suggestions regarding the mechanism of locomotion with a tendon; the presence of a compliant tendon uncouples muscle movement from limb or body movement, allowing the muscle to shorten less, and according to the force-velocity relationship (Hill, 1938), generate greater forces (Roberts et al., 1997; Taylor, 1985). Roberts (1997) mentions that with muscles acting essentially as active struts, the economy of locomotion in the presence of a compliant tendon is improved. Since the greatest disparity in the rate of impulse is seen at the highest load amplitude, this suggests the rate of force production increases as the amplitude of load movement/stride length/hop distance increases. Similar to the

results observed in this investigation, Biewener and Baudinette (1995) observed muscle forces increased at greater hopping speeds for three different distal limb muscles. As mentioned previously, a positive correlation between hopping speed and stride length was also observed, which translates to an observation of greater forces at increasing load amplitudes, supporting the results seen in this investigation.

2.4.1 Conclusion

The reduction in work and power outputs observed in addition to the greater rate of impulse for a compliant tendon, demonstrates the influence of compliance on the mechanical work production of a cyclically contracting muscle. The compliant tendon is suggested to allow the muscle to shorten less while accomplishing the same limb/body movements, therefore exerting enough force to counteract gravity during a cyclical contraction (Roberts, 1997) but also performing less mechanical work. By contracting at a higher force on the force-velocity curve, a muscle expends less heat during a contraction (Fenn, 1924). A reduction in the amount of heat loss suggests less metabolic energy is being used, indicating a compliant tendon may be responsible for energy savings.

Chapter Three: Energy Use of a Cyclically Contracting Muscle

3.1 Introduction

For studies of animal locomotion, the impact of a compliant tendon on energy use in muscles has only been assumed through indirect measurements such as mechanical work or whole-animal oxygen consumption. A study of the mechanical work output in running turkeys conducted by Roberts and colleagues (1997) showed tendon was responsible for a reduction in the work done by the gastrocnemius, allowing the muscle to generate the force necessary for locomotion with fewer activated fibres and suggested this may result in a reduction of the metabolic energy consumed. Likewise, studies involving kangaroos (Dawson and Taylor, 1973) and tammar wallabies (Biewener and Baudinette, 1995) have demonstrated a decrease in the oxygen consumption at increased hopping speeds, a finding in opposition to the linear relationship proposed by Taylor et al. (1970). In both studies, the authors suggested the presence of a compliant tendon was responsible for the reduction in oxygen consumption and perhaps a reduction in metabolic energy consumption during locomotion.

To fully understand the effect of a tendon on metabolic energy use during locomotion, the properties of a tendon would need to be altered in a moving animal. Instead, *in vitro* studies have been undertaken as they allow for more controls during measurements. Specifically, a recent study by Lichtwark and Barclay (2010) varied the compliance of artificial tendons in series with a cyclically contracting mouse soleus and found that more compliant tendons allow muscle to use energy more efficiently and generate more power. While these findings suggest more compliant tendons affect energy use of a muscle, the activation conditions were not chosen to simulate the movements a muscle might undergo *in vivo* and thus are not necessarily

representative of the metabolic energy use of a muscle during animal locomotion. In this study, my aim was to activate a muscle in a fashion that simulated the movement of a hopping animal while quantifying the energy consumed by a cyclically contracting muscle.

The jumping trials allowed for the measurement of the cycle frequency, stimulus phase, duty cycle, muscle movements and strain necessary to replicate a muscle contracting in the presence of a compliant/non-compliant tendon. The oxygen consumption trials detailed within this chapter replicated the aforementioned activation conditions and muscle movements in an effort to quantify the work, impulse, power and oxygen consumption of a muscle cyclically contracting within the oxygen chamber. This chapter addresses the hypothesis that replication of the muscle movements in series with a compliant tendon would result in less work being done and less metabolic energy being consumed than with a non-compliant tendon.

3.2 Materials and Methods

3.2.1 Oxygen consumption apparatus

The chamber used to measure work and oxygen consumption was custom-built based on a previous chamber design (Trinh and Syme, 2007). The current design maintained the general structure used previously by suspending the muscle within a sealed chamber and attaching the muscle to a force transducer and servomotor at either end. The muscle was also stimulated via magnet wires attached externally to the chamber and the solution was kept well stirred by a miniscule stir-bar within the chamber. There were also some key differences to account for the use of a larger frog muscle and improved resolution of force (see Figures 3.1 and 3.2).

The chamber was a square made from aluminum with side lengths of 8.95cm and a depth of 1.9cm. The chamber contained a shallow depression surrounding an inner chamber, where the

muscle resided during experiments. This inner chamber measured 1.4cm in width, 6.0cm in length and had a depth of 1.5cm. The chamber was designed to have a volume great enough to accommodate a muscle, but small enough to allow resolution of the drop in the partial pressure of oxygen (P_{O_2}) in the saline within the inner chamber due to oxygen consumption of that same muscle. To facilitate mixing of the saline within the chamber, a shallow circular depression was milled into the centre of the chamber floor to accommodate a small, glass-encapsulated, magnetic stir bar, driven by a magnetic stirrer situated below the chamber.

Above the inner chamber, a 0.3cm depression measuring 5.0cm wide and 7.8cm long allowed for saline overflow out of the inner chamber and supported a glass microscope slide that was placed over the inner chamber to form a functionally sealed chamber that housed the muscle. This depression is greater in depth than the slide, allowing a small amount of saline to cover the edges of the glass slide and prevent air bubbles from seeping in at the edges. The glass slide was practical to use as a cover since it could seal the chamber's upper surface with at least one aspect of the chamber being transparent so the muscle movements and attachments could be easily observed and accessed if the need arose, and it was transparent allowing use of a fibre-optic oxygen measurement system to monitor PO_2 in the saline surrounding the muscle (Fibox 3 using a PSt3 oxygen-sensitive foil probe, PreSens GmBH, Regensburg, Germany).

A stainless steel 18-gauge needle penetrated the floor of the inner chamber and allowed for influx of fresh saline from an external reservoir to replenish the chamber between experiments. The oxygenated saline drained from an elevated flask through a thin tube into the chamber. A small pinchclamp, located just before the tubing connected to the needle, was used to control the flow of saline to the chamber.

Small openings at either end of the chamber allowed passage of metal pins for attachment to the muscle segment. The opening in one end of the chamber was sealed with a thin, pliable membrane, through which a piece of platinum wire encased in a segment of glass capillary tubing passed. The platinum wire was hooked at each end such that the end within the chamber could be attached to one end of the muscle and the end outside the chamber was attached to a force transducer (model 407A, Aurora Instruments, Aurora, ON, Canada). A segment of 40 gauge magnet wire was soldered onto the external portion of the platinum wire so that the electrical impulses from the stimulator could be delivered to the muscle. The glass capillary tubing that encased the platinum wire restricted the area of exposed platinum wire within the chamber to only the attachment site of the muscle. This was done to reduce the possibility of hydrolysis in the saline when current was passed through the wire to activate the muscle. Similarly, the ends of the inner chamber were coated in a matte black enamel for the same purpose.

The opening at the other end of the chamber was lined with a short segment of polyethylene tubing and a 00 stainless steel insect pin passed through this tubing into the chamber. The polyethylene tubing was just large enough to allow uninhibited movement of the pin while preventing saline from leaking out or presenting a large diffusion pathway for oxygen into the chamber. Like the platinum wire, this pin was bent into hooks at each end so that it could linked with one end of the muscle within the chamber and connected to a servomotor outside the chamber (model 300, Cambridge Technology Inc., Boston, MA, USA). Likewise, a second segment of magnet wire was soldered onto the external portion of the steel insect pin to stimulate the muscle.

The servomotor and stimulator were controlled by software custom-written in Labview 6.1 and a PCI-MIO-16-E4 data acquisition and control card (National Instruments, Austin, TX, USA) with a 5 kHz time resolution. The muscle force and length and stimulus were likewise measured at 1 kHz.

Six chamber-length holes located within the walls of the chamber (three to each side of the inner chamber) were continuously flushed with temperature-controlled water to maintain the saline and muscle at 22°C, the same temperature as the muscle was maintained during the jumping trials. A hole was placed in the side of the chamber, adjacent to the well that housed the muscle, for the insertion of a temperature probe as part of the oxygen sensing system (PreSens GmBH, Regensburg, Germany).

The chamber was held in place above the magnetic stirrer by aluminum struts anchored onto a large aluminum base plate. The servomotor and transducer were also anchored to this aluminum plate, and were adjustable in three dimensions to accommodate different muscle lengths and to precisely align the pins attached to the muscle with the motor and transducer.

3.2.2 Measurement of oxygen consumption

Once dissected, the muscle preparation was tied onto the platinum wire within the chamber and hooked onto the steel pin at the other end of the chamber. The muscle was fully submerged in saline within the chamber. The servomotor position was adjusted to remove any visible slack from the muscle. As performed in the jumping trials, the stimulus current was increased until further increases did not result in an increase in force, and then increased an additional 50%. The muscle was then stimulated tetanically (100Hz for 200ms) to cause it to pull forcefully on the pins and ties to ensure it was oriented parallel to the pins before force-length

measurements were performed. The muscle was then lengthened in small increments and isometric force measured at each length until the force no longer increased. That point was judged to be the optimal length of the muscle, as done during the jumping trials, and was measured using a ruler with the aid of a microscope suspended over the muscle. The chamber was then flushed with fresh, oxygenated saline and sealed, free of air bubbles, by the glass microscope slide. Excess saline in the depression above the chamber was then removed by suction.

The fibre-optic probe was positioned over the oxygen sensing spot on the microscope slide covering the chamber and connected to the oxygen meter. The meter measured the P_{O_2} every second and logged it to the computer.

A two-point calibration of the oxygen meter with saline at 0% and 100% air saturation was performed once a week, or between every six experiments, whichever came first. For 100% air saturated solution, 100 ml distilled water was bubbled with room air for 20 minutes, while 0% air saturation was achieved by dissolving 1 g of sodium sulfite (Na_2SO_3) in 100 ml of distilled water.

The initial air saturation after setting the muscle in the chamber was approximately 250%, but decreased steadily as the muscle consumed oxygen. Each experiment began with 20 minutes of baseline P_{O_2} measurements with the muscle at rest. The muscle was then activated to contract, using the activation parameters and length changes as described above, in 6 sets of 10 cycles performed at 45 s intervals, for a total of 60 contractions over a period of about 4 minutes. After such a series of contractions, the muscle was given 15 minutes to rest and allow the rate of oxygen consumption to return to baseline levels before conducting further trials using different activation and length change parameters. The saline was replaced after every set of trials (60

cycles) to remove any minor bubbling/hydrolysis that occurred around the platinum wire after an extended period of stimulation and air saturation was restored to the level preceding contraction. Trials were performed alternating compliant and non-compliant conditions, pairing amplitude and duty cycle (e.g. performing low amplitude, medium duty cycle compliant trials followed by low amplitude, medium duty cycle non-compliant trials, etc.).

To gauge the viability of the muscle, a 25ms isometric contraction was performed at the outset of the experiment and then routinely before each set of trials. Once isometric force dropped below 60% of the initial value the muscle was deemed no longer viable and experiments were concluded. At that point, the muscle was removed from the chamber and the mass obtained as described for the jumping trials.

3.2.3 Oxygen consumption analysis

The amount of oxygen consumed by the muscle was assessed using the change in P_{O_2} that resulted from the muscle contracting repeatedly within the sealed chamber (Figure 3.3). This analysis was performed using a custom program written in Labview 6.1. Regression lines were fit to the resting P_{O_2} slope before and after a series of working contractions, and the vertical displacement between these lines was taken to equal the change in P_{O_2} as a result of the muscle contractions. This change in P_{O_2} between the resting periods before and after activity was measured at a point equidistant to the starting and endpoint of the series of contractions, which should minimize error in the measurement associated with small differences between the resting rates of oxygen consumption before and after activity.

The change in P_{O_2} measured was then multiplied by the oxygen compliance of the saline used in the chamber (as taken from a standard table; 8.45 mg O_2 l^{-1}, 101.36 kPa with 20.95%

oxygen at 22°C) and the chamber volume (12.35 ml) to yield the mass of O_2 used by the muscle. This value was converted to moles O_2 (32.00 g mol^{-1}) and multiplied by an energetic equivalent (450 kJ mol O_2 $^{-1}$; Nelson and Cox, 2005), assuming a mixed diet, to determine the energy used by the muscle while performing working contractions.

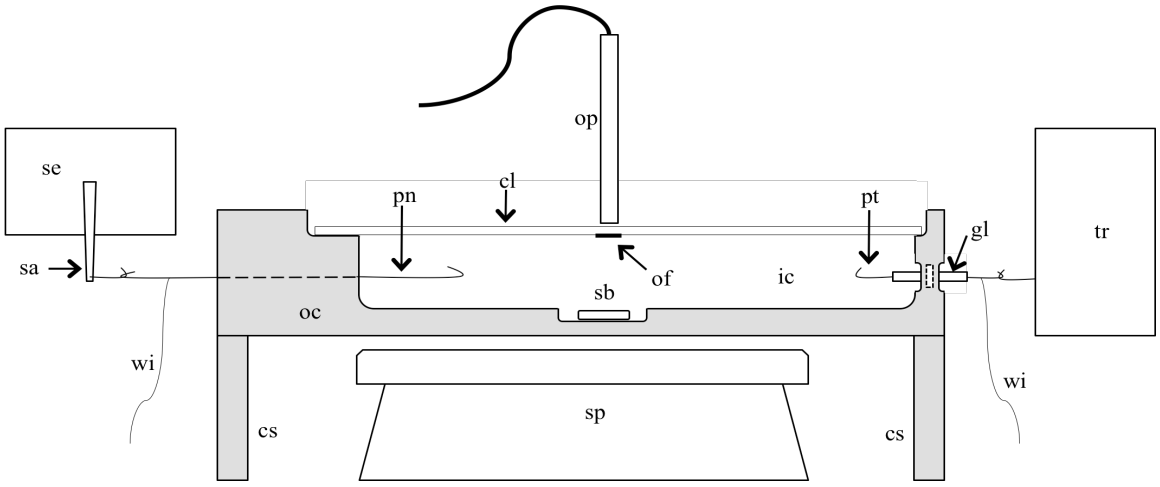


Figure 3.1. Side view of the experimental apparatus used during oxygen consumption trials. The muscle (not pictured) was suspended in the inner chamber (ic) between a stainless-steel pin (pn) and a hooked platinum wire (pt). The pin extended through a hole (depicted by broken line) in the oxygen chamber (oc) to the outside where it was linked with the servomotor arm (sa) controlled by the servomotor (se). The pin was insulated within the chamber walls by polyethylene tubing to prevent stimulus current from passing from the wire into the chamber. The platinum wire was encased along most of its length by a small glass tube (gl) to reduce hydrolysis. The glass tube passed through a flexible membrane (dashed rectangle in chamber wall) at the edge of the oxygen chamber. Outside the chamber, the platinum exited the glass tube and connected to the force transducer (tr). Magnet wires (wi) were soldered onto the platinum and stainless steel externally as a means of stimulating the muscle within the inner chamber. Centrally located in the floor of the inner chamber was a shallow depression to allow a small, glass-encapsulated stir bar (sb) to mix the saline once the chamber was sealed. The stir bar was controlled by a magnetic stirrer (sp) located just below the oxygen chamber in the space provided by the chamber supports (cs). Once filled with saline, the inner chamber was sealed by the chamber lid (cl), a microscope slide with an oxygen sensing foil (of) located centrally on its underside. The chamber lid fit within a depression above the inner chamber. The oxygen foil allowed for measurement of the oxygen partial pressure within the chamber by the fibre-optic oxygen probe (op).

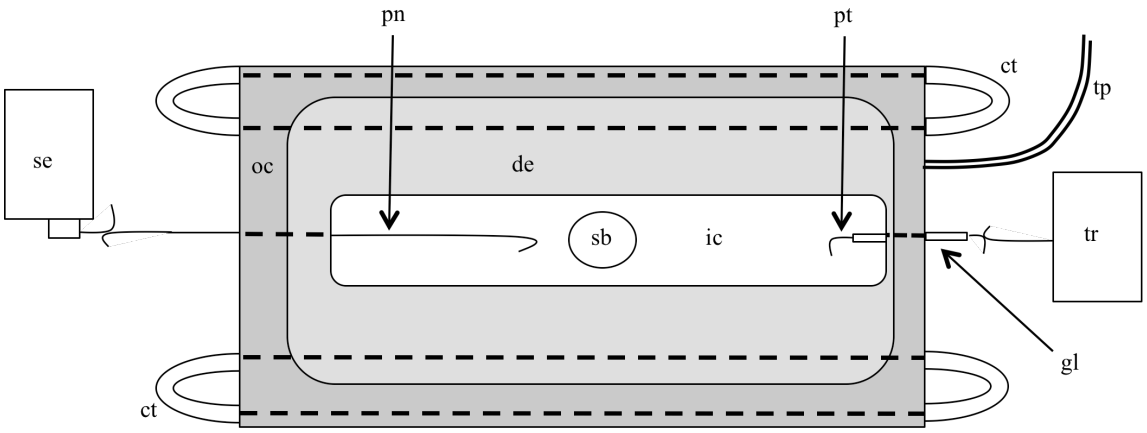
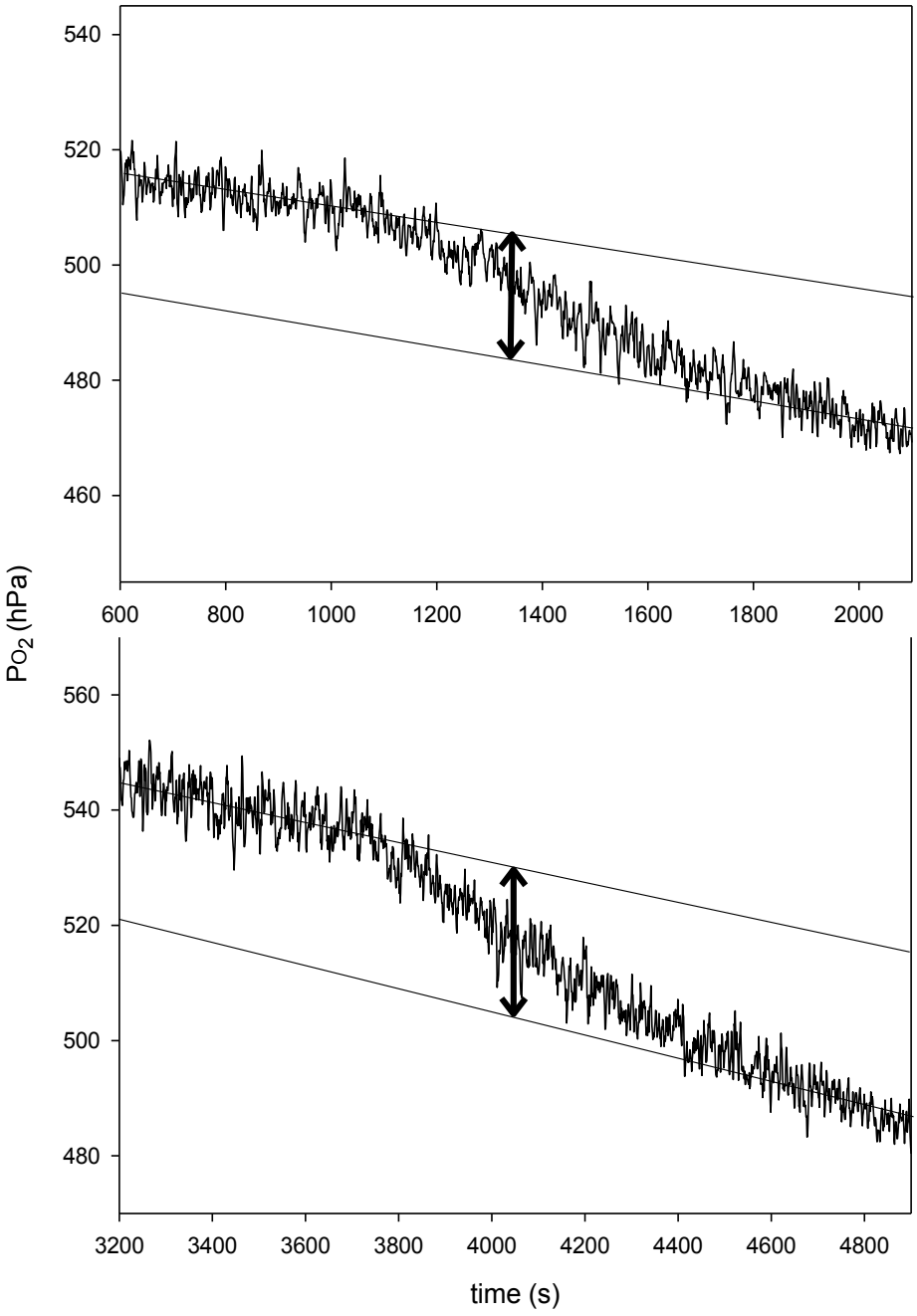


Figure 3.2. Overhead view of the experimental apparatus used during oxygen consumption trials. This angle shows the depression (de) in the outer oxygen chamber (oc) as it relates to the inner chamber (ic) where the muscle (not pictured) would be suspended between a platinum wire hook (pt) and a stainless steel pin (pn). Also within the inner chamber was a central depression where the stir bar (sb) would rotate. Six interconnected cooling tubes (ct) ran within the wall of the chamber (broken lines; only four shown for clarity) to maintain a constant chamber temperature (22°C). A temperature probe (tp) was located within the chamber wall, extending half the length and in close proximity to the inner chamber. All other acronyms are as stated in Figure 5.


540
520
500
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460
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1200
1400
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1800
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560
540
520
500
480
3200
3400
3600
3800
4000
4200
4400
4600
4800
P_{O_2} (hPa)
time (s)

Figure 3.3. Example oxygen partial pressure recordings (P_{O_2}) for a muscle contracting under low amplitude, compliant conditions (upper) and low amplitude, non-compliant conditions (lower). The initial decline in P_{O_2} is due to the resting metabolism of the muscle before doing work. Regressions represent the slope of the change in P_{O_2} before and after contractions, with the change in P_{O_2} between these regressions representing the additional oxygen consumed by the muscle while working (indicated by vertical lines with arrowheads), and being measured approximately half-way between the initiation and termination of work.

3.3 Results

Six muscles from 6 frogs were used during the oxygen consumption trials. The mean mass of the muscles was 128.4 ± 12.7 mg and the mean muscle length was 46.67 ± 0.77 mm. All work and power values were normalized for muscle mass and expressed as Joules or Watts per kilogram (J kg^{-1} and W kg^{-1} respectively) to facilitate comparisons with values from other muscle preparations. All cycle impulse and rate of impulse values were normalized for muscle cross-sectional area and expressed as kNs m^{-2} and kN m^{-2} respectively.

The length trajectories imposed on the muscle while working in the oxygen chamber were the average of those measured during jumping trials grouped according to an intermediate stimulus duty cycle and either a low or high load amplitude in the presence of either a compliant or non-compliant tendon (see Chapter 2: Methods: *Determination of oxygen consumption parameters* for further information). The length traces were initially scaled so the longest muscle length had a value of 1 and the shortest a value of -1, and so the cycle period was equal to the average of the trials under consideration. These individual traces were then averaged to create single, representative waveforms of length change, one for compliant tendons (Figure 3.4) and one for non-compliant tendons (Figure 3.5), that would be imposed on the muscle in the oxygen chamber. During actual oxygen measurement trials these waveforms were scaled to absolute values based on the measured resting length of the muscle under study and a value of strain calculated from the jumping trials. This was then used to impose movement on the muscle that was equivalent to what the muscle experienced during the jumping trials.

The high amplitude, non-compliant trace (Figure 3.5, lower panel) has two small peaks at the longest length rather than a single peak, again due to the slight stretch and bounce experienced by the muscle as the load fell after the muscle relaxed. Even after the high

amplitude, non-compliant movements were averaged across several preparations this characteristic was still prevalent in the length trace.

3.3.1 Work production

Work and power production for each amplitude (low/high) and tendon type (compliant/non-compliant) were averaged for statistical analysis. A 1-way repeated measures (RM) ANOVA was used to test for significance of differences for each variable with $P<0.05$ deemed significant. Repeated measures were used for the oxygen consumption trials because all activation conditions were performed on each muscle, unlike the jumping trials which required variances in the activation conditions used for each muscle. Comparisons were made between different tendon types at the same amplitude of load oscillation (e.g. compliant versus non-compliant values at low amplitude, etc.) and within tendon types between different amplitudes (e.g. low versus high amplitude with non-compliant tendon, etc.). Differences between values of work using a different tendon and amplitude were not analyzed as such comparisons are not relevant to this study.

Within the oxygen chamber, the non-compliant shortening work done at a high amplitude of load movement was more than twice the compliant shortening work done (15.99 vs. 7.79 J kg^{-1};1-way RM ANOVA, $P<0.05$; Figure 3.6) while at a low amplitude, the non-compliant shortening work was a little less than twice the compliant value (14.42 vs. 7.51 J kg^{-1}; 1-way RM ANOVA, $P<0.05$). Within each tendon type (compliant or non-compliant) the difference in shortening work at low versus high load amplitude was minimal and therefore a significant difference was not seen for compliant (1-way RM ANOVA, $P=0.249$) or non-compliant (1-way RM ANOVA, $P=0.101$).

An even larger discrepancy was seen for the lengthening work, as at a low amplitude of load movement, the non-compliant value was five times greater than the compliant value (-11.44 vs. -2.23 J kg^{-1}; 1-way RM ANOVA, $P<0.05$; Figure 3.7), while at a high amplitude the non-compliant value was just under five times the compliant value (-13.80 vs. -2.84 J kg^{-1}; 1-way RM ANOVA, $P<0.05$). However, as with shortening work, the differences at a low versus high load amplitude were small for both compliant (1-way RM ANOVA, $P=0.275$) and non-compliant (1-way RM ANOVA, $P=0.237$) lengthening work.

Unlike what was seen for shortening and lengthening work, the values of compliant net work were greater than the non-compliant values. The difference in net work between tendons was significant at a high amplitude (4.95 vs. 2.19 J kg^{-1}; 1-way RM ANOVA, $P<0.05$; Figure 3.8) but not at a low amplitude (5.28 vs. 2.98 J kg^{-1}; 1-way RM ANOVA, $P=0.078$). The differences across load amplitudes for compliant (1-way RM ANOVA, $P=0.342$) and non-compliant (1-way RM ANOVA, $P=0.482$) net work were marginal and thus neither was statistically significant.

The product of work and cycle frequency is power, and it is an especially interesting measurement in this investigation as it accounts for differences seen in work that might be due to differences in the cycle frequencies. Therefore, just as with the work, the shortening, lengthening and net power were all measured and compared between tendons and across amplitudes. The non-compliant shortening power was 40% greater than the compliant (59.55 W kg^{-1} vs. 42.36 W kg^{-1}; 1-way ANOVA, $P<0.05$; Figure 3.9) at a low amplitude and while only 17% greater (49.26 W kg^{-1} vs. 42.05 W kg^{-1}; 1-way ANOVA, $P<0.05$) at a high amplitude of load movement, it was still a significant difference. A significant difference in shortening power between load amplitudes was also seen within the non-compliant values (1-way ANOVA, $P<0.05$), as the high

amplitude shortening power was less than at low amplitude. However, the difference in the compliant shortening power between load amplitudes was insignificant (1-way ANOVA, $P=0.80$).

The non-compliant lengthening power was nearly four times greater than the compliant at a low amplitude of load movement (47.24 W kg^{-1} vs. 12.57 W kg^{-1}; 1-way ANOVA, $P<0.05$; Figure 3.10), while at a high amplitude the non-compliant was also significantly greater, at 2.5 times the compliant lengthening power (42.51 W kg^{-1} vs. 15.34 W kg^{-1}; 1-way ANOVA, $P<0.05$). A significant difference was not seen within the non-compliant (1-way ANOVA, $P=0.478$) or compliant lengthening power values at different amplitudes (1-way ANOVA, $P=0.359$).

The compliant net power was significantly greater than non-compliant for both low and high amplitudes of load movement (1-way RM ANOVA, $P<0.05$; Figure 3.11). At a low amplitude, the compliant value was nearly 2.5 times greater than the non-compliant value (29.79 vs. 12.31 W kg^{-1}), while at a high amplitude the compliant value was nearly four times the non-compliant value (26.72 vs. 6.75 W kg^{-1}). Within tendons, there was not a significant change in the net power from a low to high amplitude for the compliant (1-way RM ANOVA, $P=0.140$) or non-compliant tendon (1-way RM ANOVA, $P=0.235$).

3.3.2 Impulse measurements

In the oxygen consumption trials, as in the jumping trials, impulse was measured over a complete cycle (Figure 3.12), and as a rate after accounting for the cycle frequency (Figure 3.13). For a single cycle, the non-compliant cycle impulse was significantly greater than compliant at both low and high amplitudes of load movement (1-way RM ANOVA, $P<0.05$;

Figure 3.12), with non-compliant more than twice the impulse seen for compliant (29.34 vs. 13.92 kN s m^{-2} respectively, low amplitude; 30.40 vs. 13.76 kN s m^{-2} respectively, high amplitude). There was very little difference seen within tendon types at different amplitudes of load movement, with less than a 5% change seen for either compliant (1-way RM ANOVA; P=0.871) or non-compliant values (1-way RM ANOVA; P=0.761).

The non-compliant rate of impulse was significantly greater than the compliant at a low amplitude of load movement (1-way RM ANOVA, P<0.05; Figure 3.13), at about 55% greater than the compliant value (121.16 vs. 78.50 kN m^{-2}). The non-compliant rate of impulse at a high amplitude of load movement was also significantly greater (26%) than the compliant (93.62 kN m^{-2} vs. 74.29 kN m^{-2}; 1-way RM ANOVA, P<0.05). Within the compliant tendon there was no significant difference in rate of impulse between load amplitudes (1-way RM ANOVA, P=0.451), however, there was a significant difference between the non-compliant values (1-way RM ANOVA, P<0.05), with the low amplitude value being nearly 30% greater than the high amplitude value.

3.3.3 Energy consumption

The amount of metabolic energy used by a muscle replicating selected movements from the jumping trials was measured using an oxygen chamber. Energy consumption values are expressed for a single cycle as Joules per kilogram and as rates by multiplying the consumption for a single cycle by the cycle frequency. All values are the averages of the total energy consumed divided by the number of cycles performed. To test for significance, a 1-way repeated measures (RM) ANOVA was used with P<0.05 deemed significant.

The non-compliant energy consumption for a single cycle was over 40% greater than the compliant at a high amplitude of load movement (28.23 J kg^{-1} vs. 19.70 J kg^{-1}), which was a significant difference (1-way RM ANOVA, $P<0.05$; Figure 3.14). At a low amplitude of load movement, the non-compliant energy consumption was about 20% greater than the compliant (22.54 J kg^{-1} vs. 18.67 J kg^{-1}), which was nearly a significant difference (1-way RM ANOVA, $P=0.05$). There was only a small and statistically insignificant change (5%) in energy consumption at the different amplitudes for the compliant tendon (1-way RM ANOVA, $P=0.446$), however the non-compliant differed significantly across amplitudes of load movement, as the high amplitude energy consumption was 25% greater than at a low amplitude (1-way RM ANOVA, $P<0.05$).

After accounting for the different cycle frequencies and expressing the energy consumption values as rates (Figure 3.15), a reversal of the single cycle trends was seen as the compliant values were greater than the non-compliant by 13% at a low amplitude (105.31 W kg^{-1} vs. 93.07 W kg^{-1}; 1-way RM ANOVA, $P=0.065$; Figure 3.15) and 22% at a high amplitude of load movement (106.37 W kg^{-1} vs. 86.94 W kg^{-1}; 1-way RM ANOVA, $P<0.05$). Within tendons at different amplitudes of load movement, there was nearly no difference for compliant (1-way RM ANOVA, $P=0.882$) and only a marginal difference of 7% for the non-compliant (1-way RM ANOVA, $P=0.123$).

3.3.4 Work loops

Example work loops, depicting the relationship between force and muscle length for a single cycle, are shown for a muscle contracting at low and high load amplitudes in the presence

of a compliant (Figure 3.16) and non-compliant tendon (Figure 3.17). Activation conditions and muscle optimal lengths are shown in the figure captions.

Table 3.1. Activation conditions used within the oxygen chamber to replicate the movements of a cyclically contracting muscle in series with a compliant tendon (c) or non-compliant tendon (n) oscillating a load at a low amplitude (1) or high amplitude (2) (e.g. c2 represents high amplitude compliant). Cycle frequency is expressed in Hertz, Hz, stimulus duty cycle is without units, the muscle strain is expressed as a percentage of the original muscle length and stimulus phase occurs at a percentage of the period, T.

Movement	number of trials (n)	cycle frequency (Hz)	stimulus duty cycle	muscle strain (% of l_o)	stimulus phase (% T)
c1	15	5.64	0.31	7.31	7.8
n1	8	4.13	0.47	13.94	3.0
c2	15	5.40	0.35	8.65	11.0
n2	6	3.08	0.39	17.34	25.0

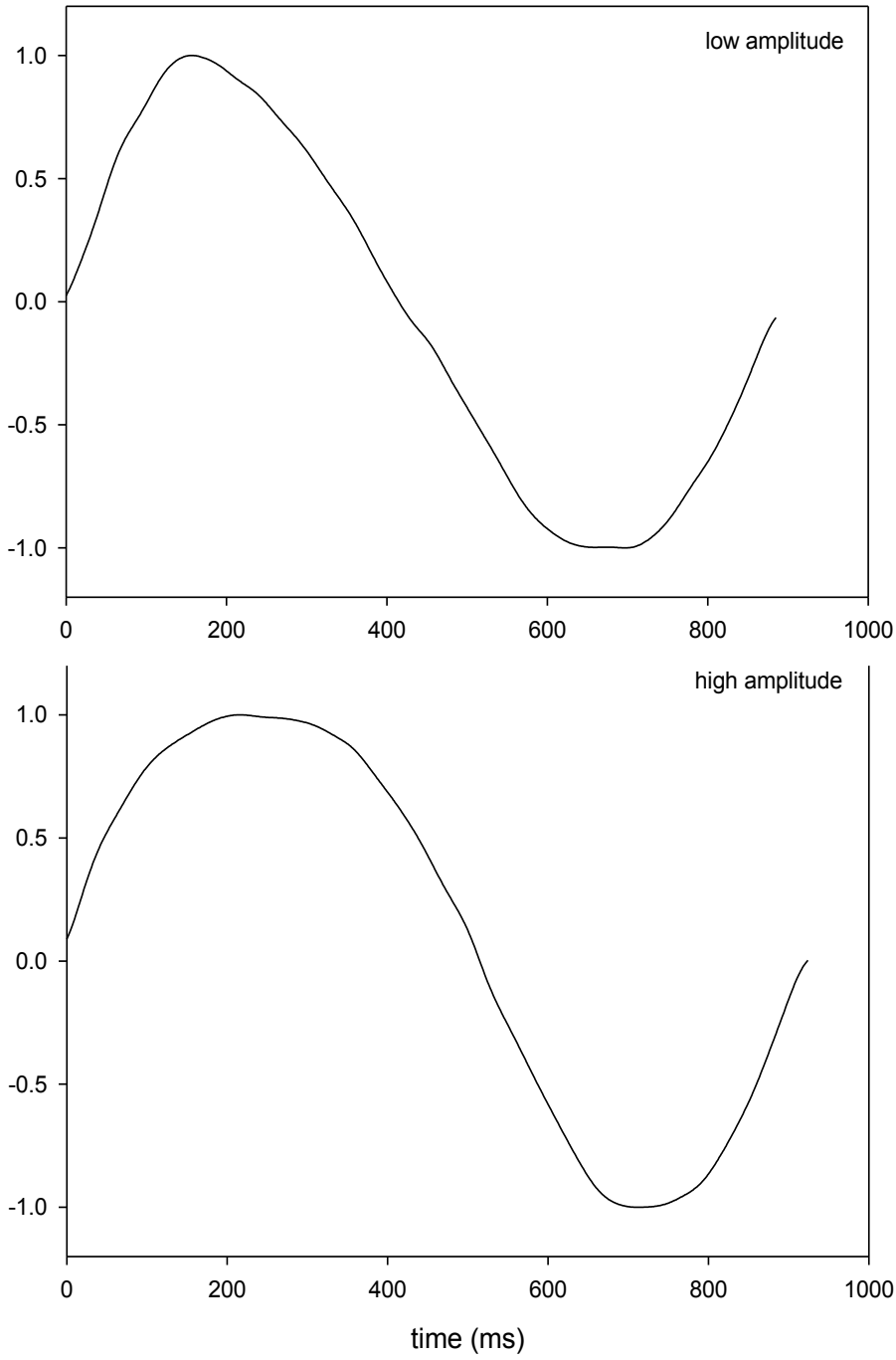

Figure 3.4. Muscle length trajectories used in the oxygen chamber in the presence of a compliant tendon. The traces represent the averaged movements of a muscle lifting a load at a low (upper) or high (lower) amplitude during the jumping trials. Positive values indicate lengthening of the muscle while negative values indicate shortening. This trajectory was later scaled using the strain values calculated based on jumping trials.

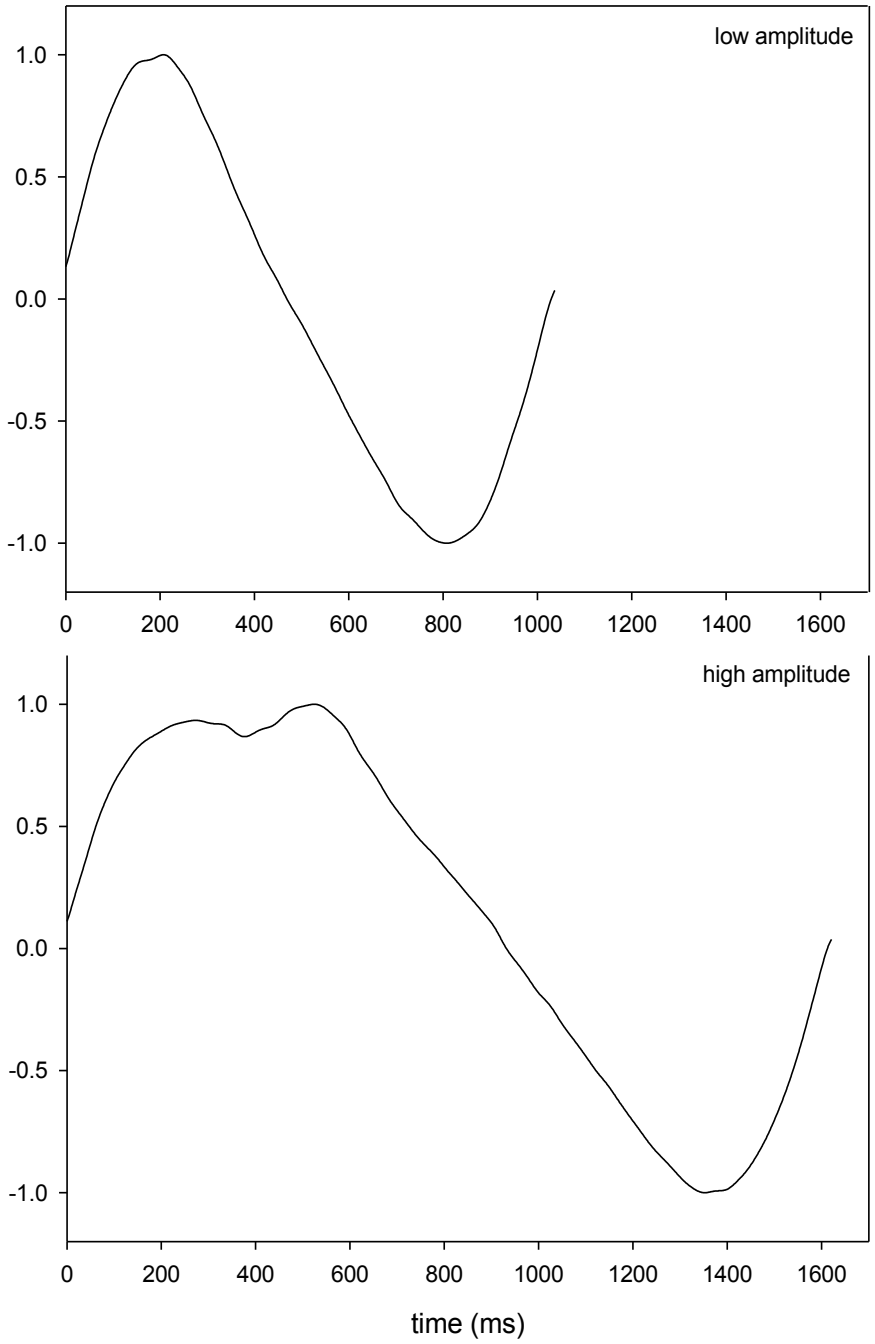

Figure 3.5. Muscle length trajectories used in the oxygen chamber in the presence of a non-compliant tendon. The traces represent the averaged movements of a muscle lifting a load at a low (upper) or high (lower) amplitude during the jumping trials. Positive values indicate lengthening of the muscle while negative values indicate shortening. This trajectory was later scaled using the strain values calculated based on jumping trials.

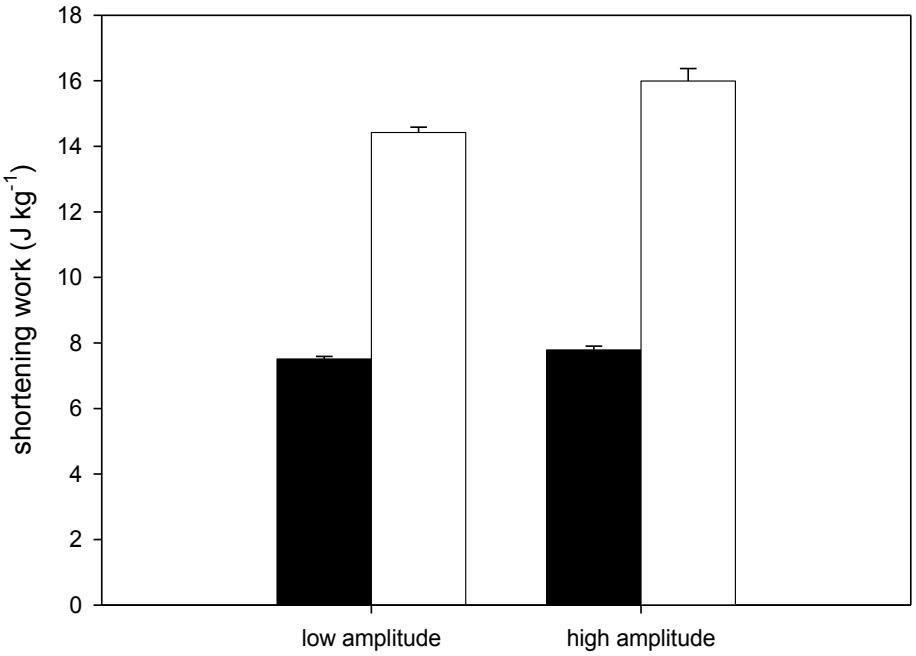

Figure 3.6. Shortening work done by muscle when replicating the movements and activation as during jumping trials in the presence of a non-compliant tendon (open bars) and compliant tendon (filled bars). Trials are classified by the amplitude of load movement which the muscle movements replicate. Values are shown as mean +/- standard error. A significant difference was seen between the compliant and non-compliant values at both low and high amplitudes (1-way RM ANOVA, $P<0.05$).

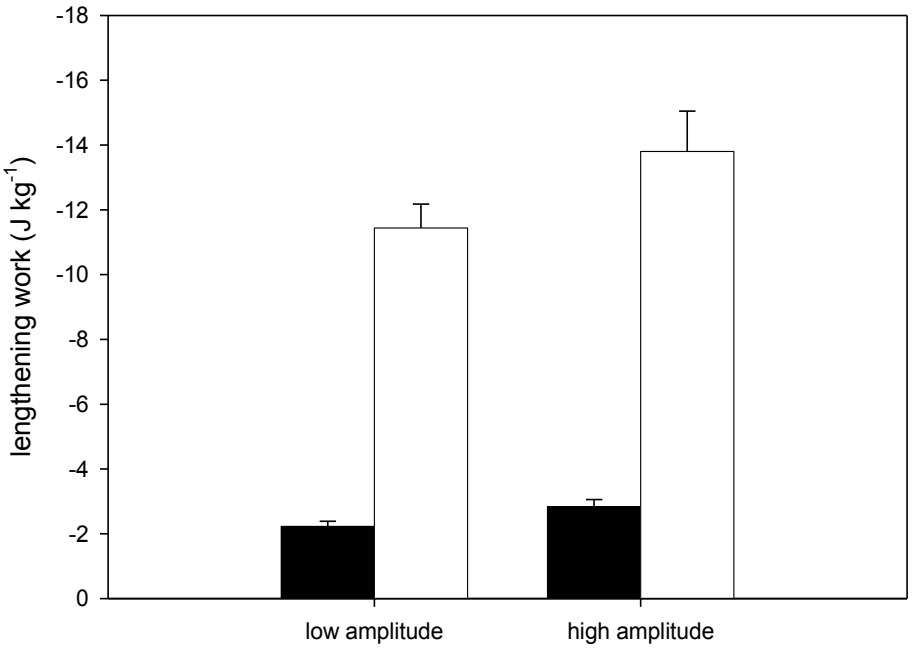

Figure 3.7. Lengthening work done by muscle when replicating the movements and activation as during jumping trials in the presence of a non-compliant tendon (open bars) and compliant tendon (filled bars). Trials are classified by the amplitude of load movement which the muscle movements replicate. Values are shown as mean +/- standard error. A significant difference was seen between the compliant and non-compliant values at both low and high amplitudes (1-way RM ANOVA, $P<0.05$).

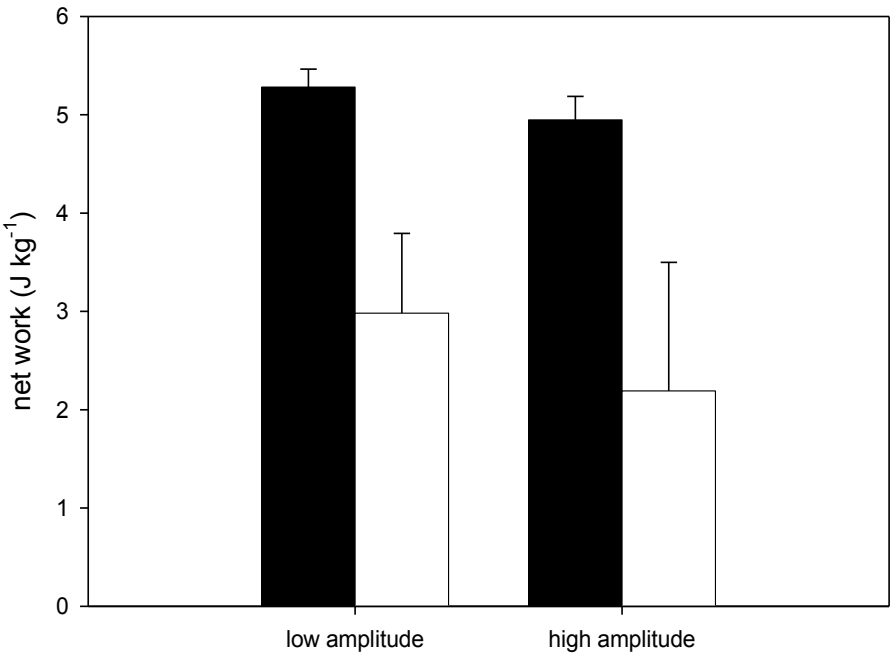

Figure 3.8. Net work done by muscle when replicating the movements and activation as during jumping trials in the presence of a non-compliant tendon (open bars) and compliant tendon (filled bars). Trials are classified by the amplitude of load movement which the muscle movements replicate. Values are shown as mean +/- standard error. A significant difference was seen between the compliant and non-compliant values at a high amplitude (1-way RM ANOVA, $P<0.05$) but not at a low amplitude (1-way RM ANOVA, $P=0.078$).

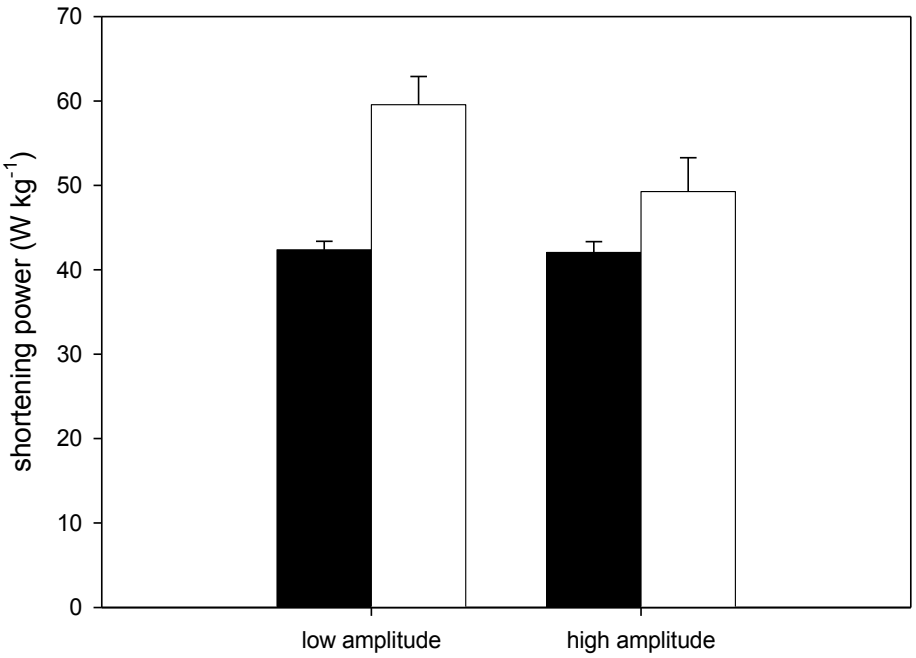

Figure 3.9. Shortening power of a muscle when replicating the movements and activation as during jumping trials in the presence of a non-compliant tendon (open bars) and compliant tendon (filled bars). Trials are classified by the amplitude of load movement which the muscle movements replicate. Values are shown as mean +/- standard error. A significant difference was seen between the compliant and non-compliant values at low and high amplitudes (1-way RM ANOVA, $P<0.05$).

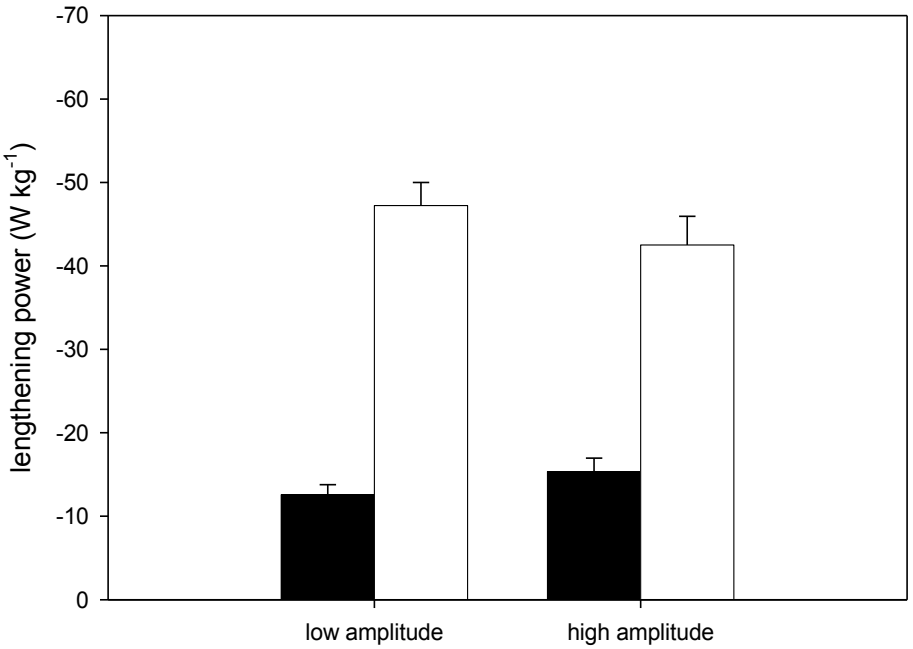

Figure 3.10. Lengthening power of a muscle when replicating the movements and activation as during jumping trials in the presence of a non-compliant tendon (open bars) and compliant tendon (filled bars). Trials are classified by the amplitude of load movement which the muscle movements replicate. Values are shown as mean +/- standard error. A significant difference was seen between the compliant and non-compliant values at low and high amplitudes (1-way RM ANOVA, $P<0.05$).

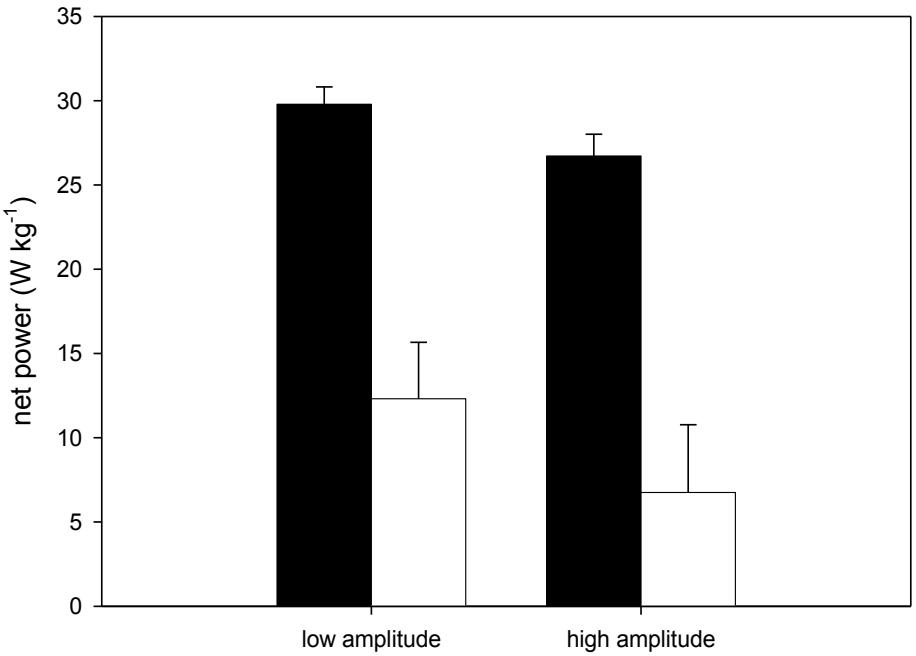

Figure 3.11. Net power of a muscle when replicating the movements and activation as during jumping trials in the presence of a non-compliant tendon (open bars) and compliant tendon (filled bars). Trials are classified by the amplitude of load movement which the muscle movements replicate. Values are shown as mean +/- standard error. A significant difference was seen between the compliant and non-compliant values at low and high amplitudes (1-way RM ANOVA, $P<0.05$).

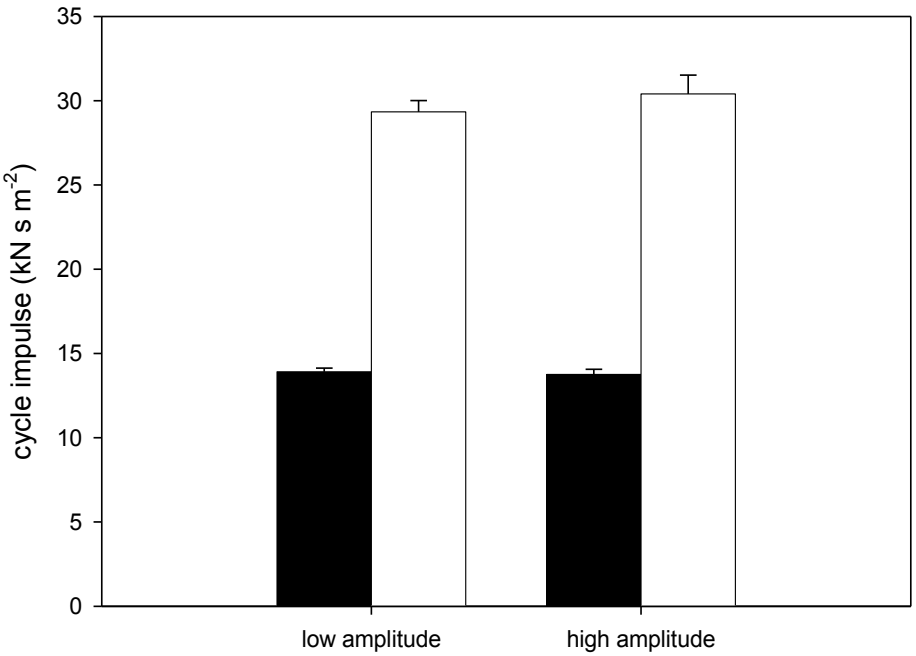

Figure 3.12. Cycle impulse of a muscle when replicating the movements and activation as during jumping trials in the presence of a non-compliant tendon (open bars) and compliant tendon (filled bars).Trials are classified by the amplitude of load movement which the muscle movements replicate. Values are shown as mean +/- standard error. A significant difference was seen between the compliant and non-compliant values at low and high amplitudes (1-way RM ANOVA, $P<0.05$).

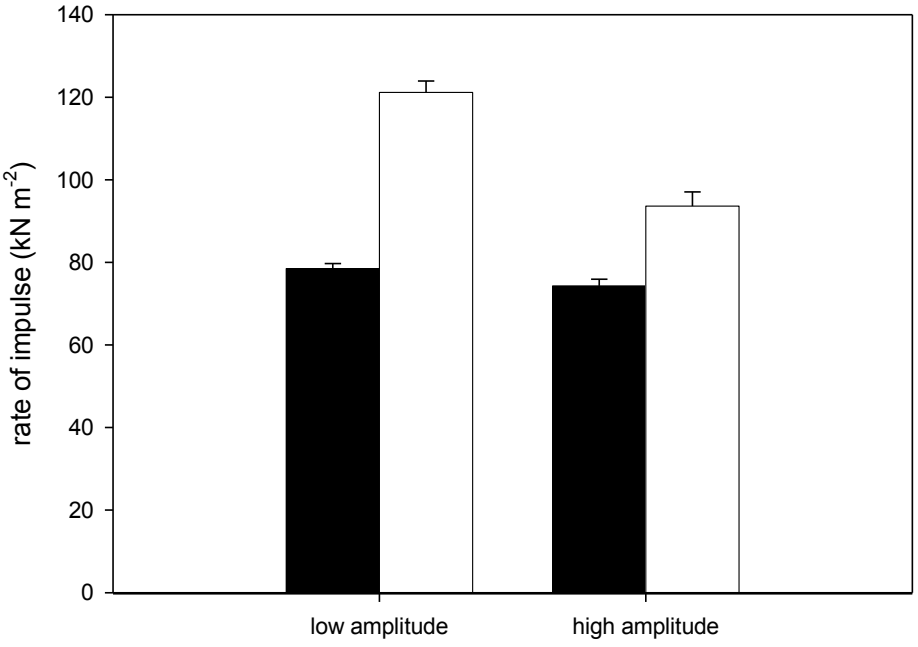

Figure 3.13. Rate of impulse for a muscle when replicating the movements and activation as during jumping trials in the presence of a non-compliant tendon (open bars) and compliant tendon (filled bars). Trials are classified by the amplitude of load movement which the muscle movements replicate. Values are shown as mean +/- standard error. A significant difference was seen between the compliant and non-compliant values at low and high amplitudes (1-way RM ANOVA, $P<0.05$).

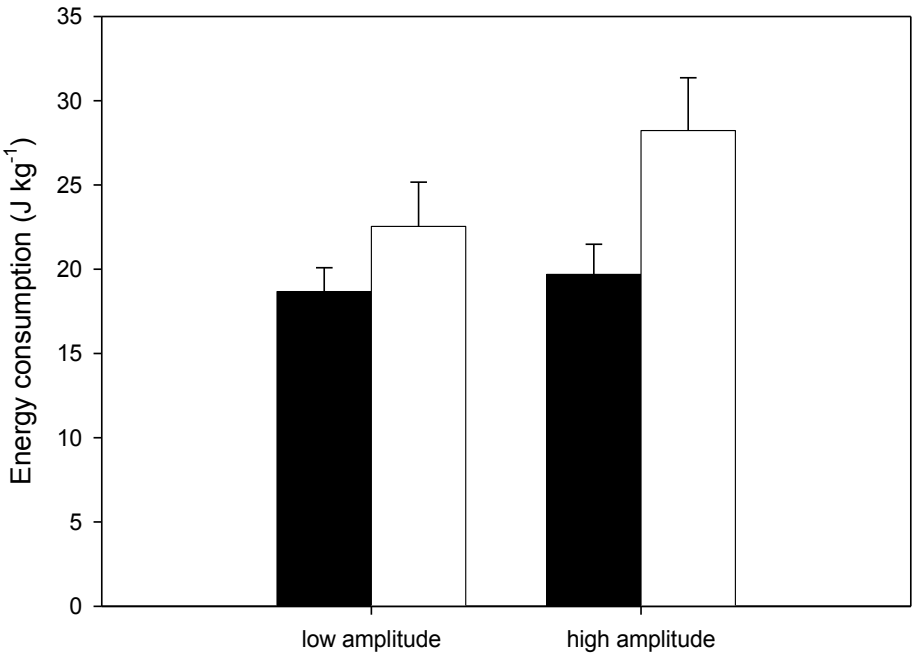

Figure 3.14. Energy consumption during a single cycle for muscle when replicating the movements and activation as during jumping trials in the presence of a non-compliant tendon (open bars) and compliant tendon (filled bars). Trials are classified by the amplitude of load movement which the muscle movements replicate. Values are shown as mean +/- standard error. A significant difference was seen between the compliant and non-compliant values at a high amplitude (1-way RM ANOVA, $P<0.05$) and low amplitude (1-way RM ANOVA, $P=0.05$). There was a significant difference between the non-compliant values at different amplitudes (1-way RM ANOVA, $P<0.05$).

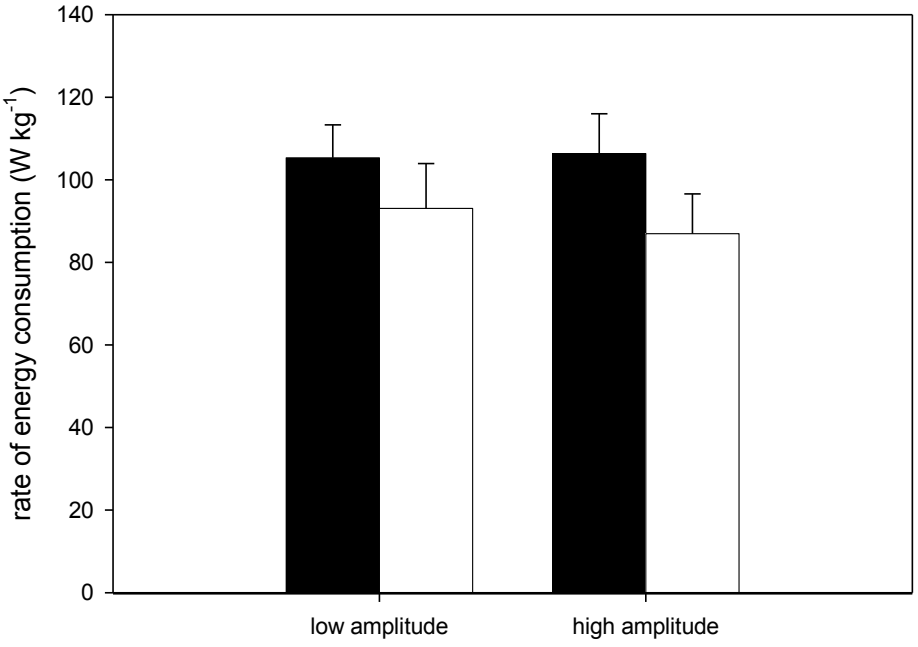

Figure 3.15. Rate of energy consumption for muscle when replicating the movements and activation as during jumping trials in the presence of a non-compliant tendon (open bars) and compliant tendon (filled bars). Trials are classified by the amplitude of load movement which the muscle movements replicate. Values are shown as mean +/- standard error. A significant difference was seen between the compliant and non-compliant values at a high amplitude (1-way RM ANOVA, $P<0.05$) but not at a low amplitude (1-way RM ANOVA, $P=0.065$).

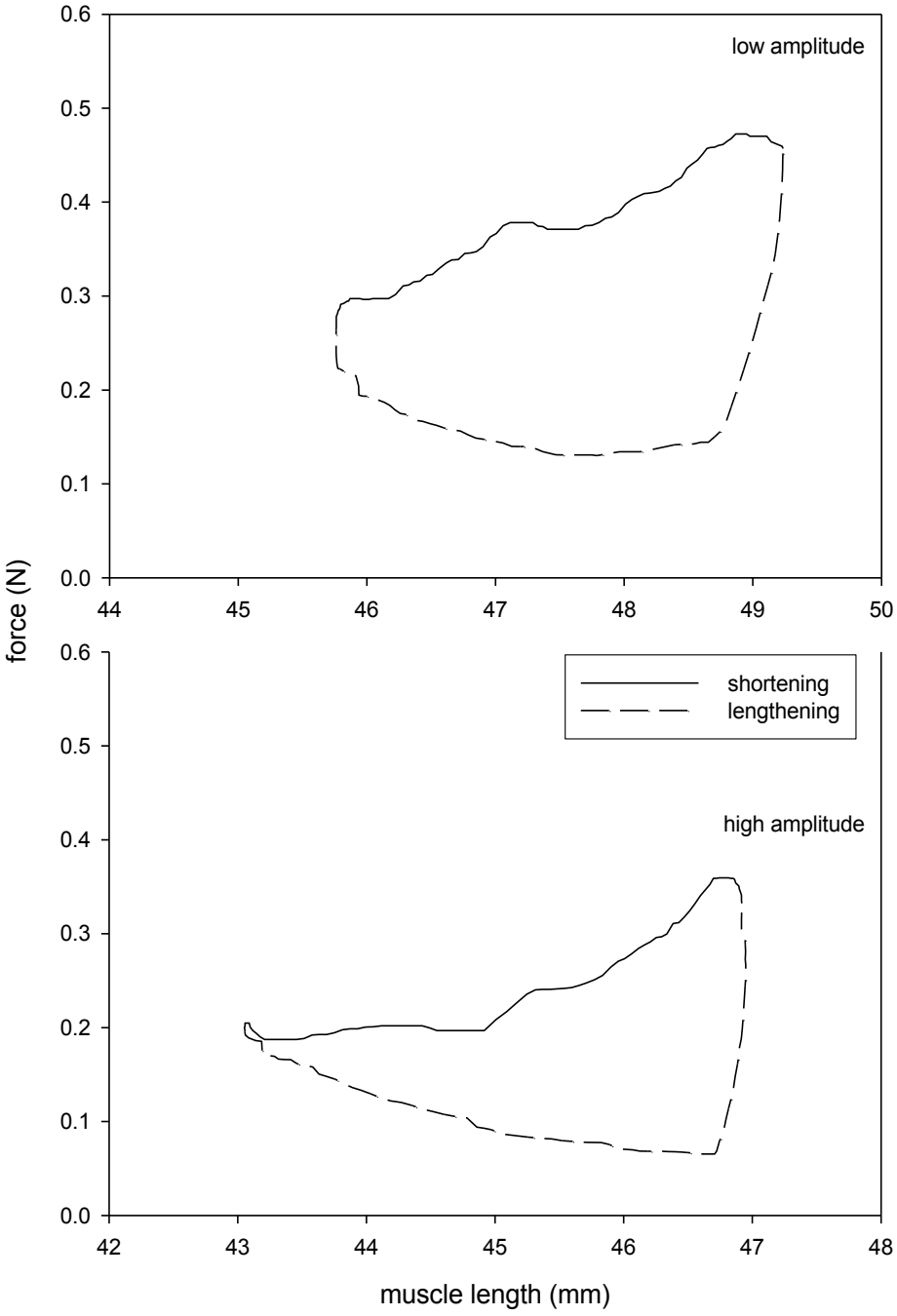
low amplitude
high amplitude
shortening
lengthening
force (N)
muscle length (mm)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
42
43
44
45
46
47
48
49
50

Figure 3.16. Work loops for a single cycle of muscle contraction when replicating the steady-state movements and activation as during jumping trials in the presence of a compliant tendon at a low (upper plot) or high (lower plot) amplitude of load movement. For the low amplitude example, the muscle length was 47.5mm and a cycle frequency of 5.64Hz and duty cycle of 0.31 were used for activation. The high amplitude example had a muscle length of 45mm and was activated by a cycle frequency of 5.40Hz and a stimulus duty cycle of 0.35.

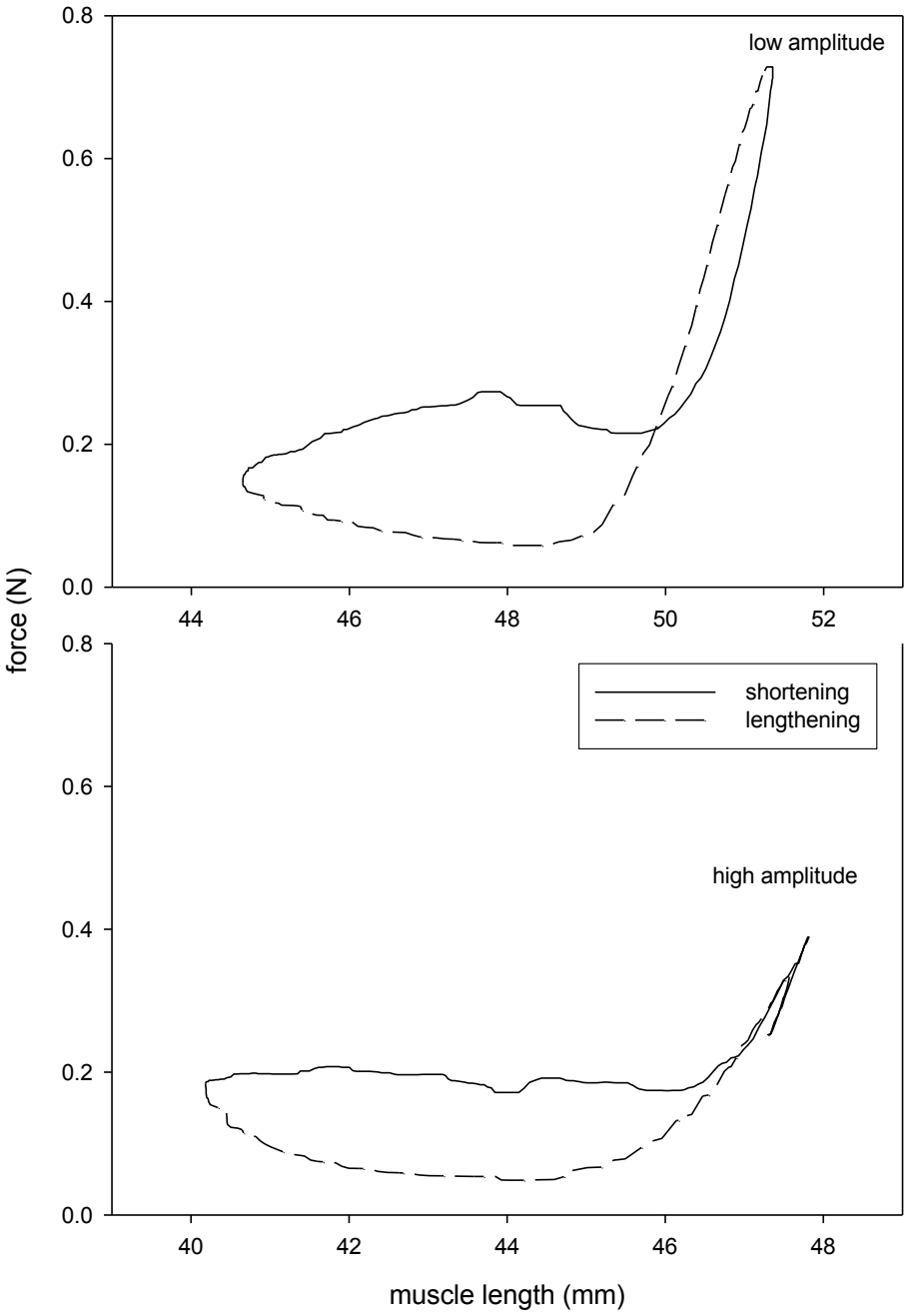
low amplitude
high amplitude
shortening
lengthening
force (N)
muscle length (mm)
0.0
0.2
0.4
0.6
0.8
40
42
44
46
48
50
52

Figure 3.17. Work loops for a single cycle of muscle contraction when replicating the steady-state movements and activation as during jumping trials in the presence of a non-compliant tendon at a low (upper plot) or high (lower plot) amplitude of load movement. For the low amplitude example, the muscle length was 48mm and a cycle frequency of 4.13Hz and duty cycle of 0.47 were used for activation. The high amplitude example had a muscle length of 44mm and was activated by a cycle frequency of 3.08Hz and a stimulus duty cycle of 0.39.

3.4 Discussion

Replicating the conditions of muscle activation and movement during the jumping trials required tracing the muscle movements and averaging the cycle frequency, stimulus duty cycle, muscle strain and stimulus phase (Table 3.1). This was done using a custom-written program (Chapter 2: *Determination of activation conditions for oxygen consumption*) that allowed the length traces to be scaled to a maximum and minimum length over an average cycle period. The results of this method were deemed representative of the muscle movements seen during the jumping trials since minor details such as the small bounce that occurred as the falling load stretched the muscle in series with a non-compliant tendon were conserved and then visible in the length traces used for the oxygen consumption trials (Figure 3.4). The activation values used in the oxygen consumption trials were calculated by averaging the values from the jumping trials within a group binned according to their tendon, load amplitude and stimulus duty cycle and were very consistent within the individual group (e.g. c1 for low amplitude, compliant and n2 for high amplitude, non-compliant; see Figure 3.5). Only two suspected outliers used during the jumping trials were omitted (for trial n1) because of large differences in the activation conditions.

Within tendons, the compliant activation conditions were consistent across high and low amplitudes while the non-compliant activation conditions were different from low to high amplitudes. Given that any movement of the load with a non-compliant tendon was directly correlated with and equal to a change in the muscle length, higher amplitudes of load movement required a lower frequency and duty cycle as well as a greater phase and strain than was required for low amplitudes of load movement. Across tendons, there was a great amount of difference in the activation and movement conditions required to elicit near-harmonic oscillation of the load,

which emphasizes the ability of a compliant tendon to allow a muscle to activate and shorten independently of load movement as previously suggested (Roberts et al., 1997; Taylor et al., 1985). This is demonstrated by the differences in strain for a compliant and non-compliant tendon that result in the same amplitude of load movement, as the non-compliant strain was greater than the compliant, a difference that increased with higher amplitudes of load movement. Since the compliant tendon resulted in less muscle shortening irrespective of the amplitude of load movement, the muscles are likely consuming less metabolic energy to displace a load an equal distance given a contraction that requires less shortening has been shown to use less metabolic energy (Fenn, 1924).

The compliant tendon was responsible for reductions in shortening and lengthening work relative to the work done with a non-compliant tendon for a cyclically contracting muscle. Previously, Roberts et al. (1997) demonstrated a compliant tendon was responsible for a reduction in the shortening work done by a muscle, as measured in this study, however, he did not include measurements for lengthening work. The non-compliant shortening work was twice the compliant at both high and low amplitudes, while the difference was even greater for the lengthening work, with non-compliant values approximately five times greater than the compliant. These observations are not surprising since the movement and activation conditions used in the oxygen consumption trials are based on those observed during the jumping trials where a similar pattern in work was observed, but they do further support the suggestion that the muscle movements and activation conditions chosen for replication within the chamber are indicative of the jumping trials. Also, shortening work measurements taken within an oxygen chamber for a sartorius muscle that was activated under similar conditions by Trinh and Syme (2007) and Heglund and Cavagna (1987) showed nearly identical values to the non-compliant

shortening work observed in this experiment, providing further support for measurements obtained within the oxygen chamber. As observed for the work, the non-compliant power output was greater than compliant for both shortening and lengthening, indicating the muscle does work at a faster rate with a non-compliant tendon than with a compliant tendon.

The compliant net work was greater than the non-compliant net work across both amplitudes of load movement, however, the difference was not significant at a lower amplitude ($P=0.078$). The compliant net power was also significantly greater at both amplitudes and increased at greater amplitudes of load movement. The compliant work loops reflect the greater amount of compliant work done as both high and low amplitude loops have a greater area than either of the non-compliant loops. These results show a greater amount of net work is being done in the presence of a compliant tendon, which could be the result of heat lost from the elastic tendon, an occurrence known as hysteresis. In naturally occurring tendons, the amount of hysteresis has been measured between 7-30% (Pollock and Shadwick, 1994), an occurrence that would likely result in an animal having to use more metabolic energy to sustain steady-state locomotion, but only if the tendons did not also result in significant energy savings through other mechanisms. Also, since the synthetic elastic used in this study is not a perfect elastic, meaning it would have a hysteresis of 0%, it is reasonable to assume some energy was lost during the stretch and recoil of a single cycle. If hysteresis is indeed responsible for an increase in the compliant net work and power, the energy loss appears to increase with increasing load amplitudes. This could be due to an increase in the strain of the tendon experienced as the tendon stretches a greater distance in order to oscillate a load a greater distance.

Replicating the muscle movements within the oxygen chamber resulted in the same relationships between tendons as observed in the jumping trials for rate of impulse, where the

non-compliant impulse was greater than the compliant for a single cycle and when expressed as a rate. The impulse, which is the product of force and time, was unaffected by an increase in amplitude over a single cycle, but experienced a slight decrease with increased amplitude when measured as a rate. This suggests the rate of force production would decrease as stride amplitude increases for a non-compliant tendon, a suggestion at odds with measurements from the jumping trials. One possible cause for the change in relationship for the rate of impulse between experiments could involve the averaging of trials for replication within the oxygen chamber (see Chapter 4: *Conclusions*).

A compliant tendon resulted in a lower metabolic energy consumption than a non-compliant tendon during a single cycle of contraction, and also used energy at a faster rate. At a low amplitude of load movement, the savings from using a compliant tendon were found to be 17% and increased to 30% at a higher amplitude of load movement. The reduction in energy consumption for a muscle contracting with a compliant tendon supports a widely held belief suggested many times, but never explored in a manner that attempted to simulate animal locomotion. The savings appear to be robust, suggesting the compliant tendon has an impressive influence on energy consumption of a cyclically contracting muscle and is very likely responsible for depressions in oxygen consumption of hopping kangaroos (Dawson and Taylor, 1973) and wallabies (Baudinette et al., 1992). Interestingly, the increase in energy savings at greater amplitudes suggest locomotion is more economical at greater stride lengths for an animal with a compliant tendon. Similarly, the observation that the muscle consumes energy at a greater rate in the presence of a compliant tendon, and that this difference is increased at greater load amplitudes, suggests animals use energy faster while also conserving their metabolic energy consumption.

3.4.1 Conclusion

The influence of a compliant tendon on mechanical performance of a cyclically contracting muscle was similar to the results observed for the jumping trials. There were some minor differences in the measurements from the jumping trials to the oxygen consumption trials, however, replicating the muscle movements and activation conditions resulted in similar work loops and measurements, suggesting the methods, and thus values, were satisfactory. Given the reproducibility of the results in the oxygen chamber, the compliant tendon appeared to be responsible for a reduction in the shortening and lengthening work done by a cyclically contracting muscle, and while it resulted in an increase in the net work done by the muscle, it reduced the amount of metabolic energy consumption. These measurements suggest the presence of a compliant tendon does improve the economy of movement during cyclic contractions.

Chapter Four: Synthesis and Conclusions

4.1 Introduction

Some animals are better equipped anatomically to carry out locomotion in an economic fashion. An animal that is an economical locomotor can be likened to an economical car in that both use less energy or fuel to travel the same distance as another animal/car. The physical characteristics that allow an animal to use energy more economically vary, but one that has been suggested for a number of species is the presence of a compliant tendon. A compliant tendon is suggested to store and release elastic strain energy as it stretches and recoils, resulting in a lower mechanical energy output required for the subsequent contractions of the muscle in series. This reduction in work done by the muscle has led to the proposal that compliant tendons may also be responsible for lowering the metabolic energy consumption during cyclical contractions. While increasing tendon compliance under certain activation conditions has been shown to result in more efficient energy use (Lichtwark and Barclay, 2010), the activation conditions and movements of the muscle during that investigation were not chosen to be representative of a moving animal and thus may not reflect what occurs during locomotion. This thesis attempted to simulate the movements of a muscle, tendon and load/limb within a hopping animal and through comparison to a muscle contracting without a tendon, measure the metabolic energy savings afforded by the presence of a compliant tendon during cyclical contractions.

This synthesis chapter aims to integrate the results of the jumping trials and oxygen consumption trials into a cohesive overview that addresses the central hypothesis, that the presence of a compliant tendon would result in a muscle doing less work and consuming less metabolic energy to move a load an equal distance.

4.2 Summary

The presence of a compliant tendon was shown to allow a cyclically contracting muscle to do less than half the shortening and lengthening work necessary to oscillate a load an equal distance and reduced the metabolic energy consumption per cycle by more than 15% at low amplitudes of load movement and 30% at high amplitudes, compared to what occurs with a non-compliant tendon. The rate of energy consumption with a compliant tendon was 10% faster than with a non-compliant tendon at low amplitudes of load movement and over 20% faster at high amplitudes of load movement, suggesting that not only did the compliant tendon allow for more economical use of metabolic energy, it also used that energy quicker. In accordance with the faster rate of energy consumption the muscle contracted faster as it required a greater cycle frequency to oscillate a load in the presence of a compliant tendon. The muscle also required a lesser duty cycle with a compliant tendon, indicating it needed to be stimulated for a smaller fraction of the cycle than with a non-compliant tendon. The impulse produced with a compliant tendon for a single cycle was less than that observed in the presence of a non-compliant tendon, however, when expressed as a rate, the compliant impulse was greater than the non-compliant. The compliant shortening and lengthening power was less than the non-compliant, however, as with the net work, the net power for a cyclically contracting muscle was greater in the presence of a compliant tendon. Nearly all parameter relationships between tendons were identical across the jumping and oxygen consumption trials, with the only difference being a higher compliant rate of impulse during the jumping trials and a lower value seen in the oxygen trials. The difference in the rates of impulse were minor however, suggesting the values measured in the oxygen consumption trials are an accurate replication of the measures from the jumping trials.

It has been shown that tendons are able to store and release elastic strain energy (Alexander, 1984; Biewener and Baudinette, 1995) resulting in a lower mechanical work output during subsequent cycles of locomotion. It was suggested this reduction in work output could potentially be correlated with the reductions in oxygen consumption observed at greater speeds for hopping kangaroos (Dawson and Taylor, 1973) and tammar wallabies (Biewener and Baudinette, 1995), however, the link had never been investigated directly. The findings presented in this thesis show the presence of a compliant tendon allows a cyclically contracting muscle to reduce its work output and consume less energy when moving a load an equal distance and thus suggest a compliant tendon is responsible for more economical locomotion in animals.

4.3 Conclusions

As an analog for the experiments undertaken in this study, the movements of the muscles and tendons occurring during animal during locomotion were envisioned. By this analogy, the muscle used was non-specific and representative of any muscle used in locomotion, while the synthetic compliant tendon represented the accompanying tendon that might be found in series with that muscle, but was non-specific to any one muscle. The non-compliant tendon represented the absence of a tendon as though the muscle connected directly to the bone. Furthermore, the load oscillated by the collective movements of the tendon and muscle could be thought of as the bone onto which a tendon might insert, resulting in movement of that limb. The load amplitude would therefore represent the distance the limb was moved, a measurement that would be considered the stride length during a single cycle of movement. Based on the results of this study, an animal with a compliant tendon would be expected to do less shortening and lengthening work but more net work in a locomotory muscle for a single stride than if there was

no tendon present. That muscle would also use less energy for a single, equal stride length with a compliant tendon than the same animal moving without a tendon in series with the muscle, and the energy would be consumed at a faster rate, allowing the animal to move more economically and faster. Furthermore, the differences in work and energy use between an animal with a compliant tendon and without a tendon would be significantly increased at greater stride lengths.

The compliant properties of tendons allowed the muscle to shorten at velocities which reduced mechanical work output and reduced energy use. During each cycle of locomotion, the tendon stretches and recoils, transferring kinetic and potential energy into elastic strain energy and allowing the muscle to contract at velocities that reduce the mechanical work done but still support the animal against gravity and allow it to sustain movement (Roberts et al., 1997; Taylor et al., 1985). Given that contractions at lower shortening velocities use less energy (Fenn, 1924) and higher force contractions occur at lower shortening velocities (Hill, 1938), it follows that a contraction that produces more force should use less energy in addition to doing less work. In the jumping trials it was seen that any movement of the load was a direct result of the muscle shortening while the stretch and recoil of the compliant tendon was responsible for most of the load movement, allowing the muscle to shorten less and oscillate the load an equal distance. The reductions in compliant shortening work (almost 66% at high amplitudes) and lengthening work (nearly 75% at high amplitudes) are suggestive of mechanical energy savings perhaps brought on by the uncoupling of muscle and tendon. As Roberts (1997) observed in running turkeys, the presence of a compliant tendon allowed the muscle in series to contract at higher forces, thereby requiring fewer muscle fibres and potentially using less metabolic energy to sustain a steady speed of running. Strain energy is stored within the tendon while it is stretched, but as the recoil occurs the strain energy is converted back into kinetic and potential energy with only a small

portion lost to hysteresis (Cavagna et al., 1977). Hysteresis was suggested to be responsible for the greater net work and net power seen in the presence of a compliant tendon. Similarly, the muscle force and strain energy storage increased in the hindlimbs of tammar wallabies as hopping speed increased and oxygen consumption decreased (Biewener and Baudinette, 1995; Biewener et al., 1998), which, given the correlation suggested between speed and stride lengths/load amplitude, the increase in the elastic energy savings could indicate not only potential metabolic energy savings, but if present, greater savings with an increase in load amplitude.

Plots contrasting the results of the work and impulse obtained within an oxygen chamber with the average values from the jumping trials (that comprised their muscle movements and activation conditions selected for use during measures of oxygen consumption) show that while the jumping trials resulted in slightly greater measurements, the overall relationships between tendons at the same amplitude (e.g. c1 and n1) and within tendons at different amplitudes (e.g. c1 and c2) remain the same (see Figure 4.1-4.8). The cause of the disparity across experiments is not readily apparent, however, given the slight differences it is my belief that a combination of two factors led to the deviation. The first suggestion is that the disparity in work and impulse measurements between the jumping trials and oxygen consumption trials is a result of the minor stretch that occurred after dropping the load in the presence of a non-compliant tendon. The stretch of the muscle by an external force would affect the non-compliant force recordings while not necessarily having a great influence on the muscle length trace. While there was a slight ripple in the non-compliant, high amplitude length trace, it may not be indicative of the force exerted on the muscle. Additionally, the lengthening work values (Figure 4.2) from the jumping trials are seen to exhibit the greatest divergence of any measurement relative to the oxygen trials.

Since the cycle frequencies, stimulus duty cycles and muscle strains from the jumping trials were all replicated during the oxygen consumption measurements, and the lengthening power (Figure 4.5) measured during the jumping trials had the same relationship between tendons as seen in the oxygen consumption values, the force is the only remaining variable. This suggestion alone is not sufficient since there was also a slight increase seen in the shortening work values (Figure 4.1). My second suggestion is that the muscle movements were perhaps altered slightly in their conversion for use within the oxygen-chamber, specifically, that they may no longer represent the movement of a particular trial. This might explain depressed values for shortening work and power (Figure 4.4), as well as the cycle impulse (Figure 4.7) and rate of impulse (Figure 4.8). Even though care was taken to avoid damping the traces, the diminished returns could be the result of minor smoothing and averaging that was unavoidable in altering traces with different frequencies to one average cycle frequency. In consideration of the net work (Figure 4.3) and net power (Figure 4.6) did not allow for much insight into why the there might be differences between results from the jumping trials versus oxygen measurements as they represent the difference between shortening and lengthening work and thus information has been lost but the work loops are very informative as they depict the entire cycle of shortening and stretching.

For a muscle contracting cyclically in an oxygen chamber, movements replicating the presence of a compliant tendon were observed to result in energy consumption savings compared with a non-compliant tendon at low amplitudes of load movement and increased savings as the amplitude of load movement increased. The mechanical work measured while a muscle shortened and lengthened was likewise reduced in the presence of a compliant tendon versus non-compliant, while the net work increased, perhaps due to heat lost from the tendon. The cycle and rate of impulse were also reduced in the presence of a compliant tendon. Collectively, these

measurements from the jumping trials and the oxygen consumption trials demonstrate the impact a compliant tendon has on metabolic and mechanical energy conservation.

The results of this study suggest my hypothesis is correct and therefore a compliant tendon allows a cyclically contracting muscle to do less work and consume less energy than a cyclically contracting muscle in series with a non-compliant tendon when load displacement is equal.

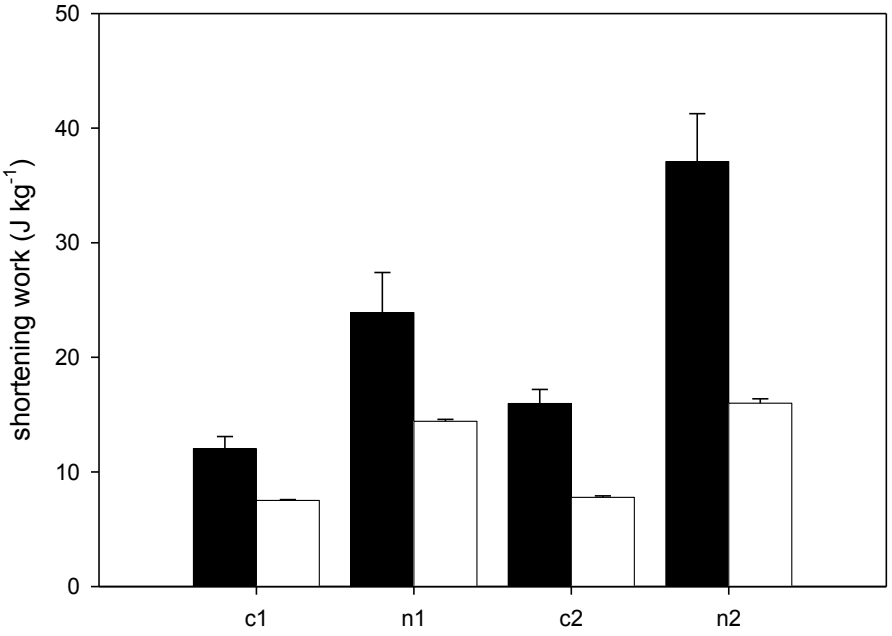

Figure 4.1. Shortening work done during a single cycle comparing the jumping trials (filled bars) and oxygen consumption trials (open bars). The categories along the x-axis refer to the tendon present (e.g. c for compliant, n for non-compliant) and amplitude of load movement (e.g. 1 for low amplitude, 2 for high amplitude). Values are shown as mean +/- standard error. The activation conditions and muscle movements that resulted in the oxygen consumption values seen were based on averages calculated for the jumping trials. There was a significant difference between the jumping trials and oxygen trials for all activation conditions (2-way ANOVA, $P<0.001$) except c1 (2-way ANOVA, $P=0.068$).

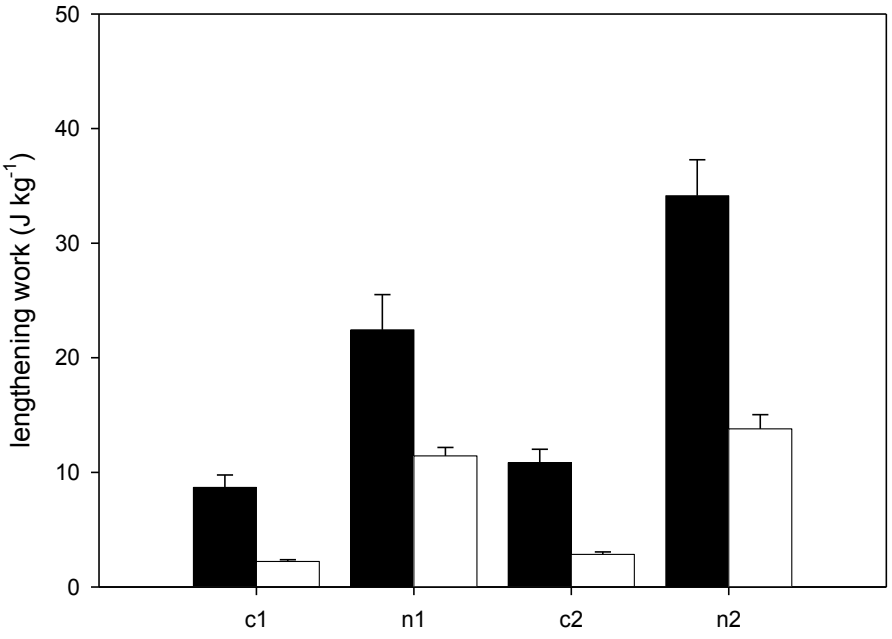

Figure 4.2. Lengthening work done during a single cycle comparing the jumping trials (filled bars) and oxygen consumption trials (open bars). The categories along the x-axis refer to the tendon present (e.g. c for compliant, n for non-compliant) and amplitude of load movement (e.g. 1 for low amplitude, 2 for high amplitude). Values are shown as mean +/- standard error. The activation conditions and muscle movements that resulted in the oxygen consumption values seen were based on averages calculated for the jumping trials. There was a significant difference between the jumping trials and oxygen trials for all activation conditions (2-way ANOVA, $P<0.001$).

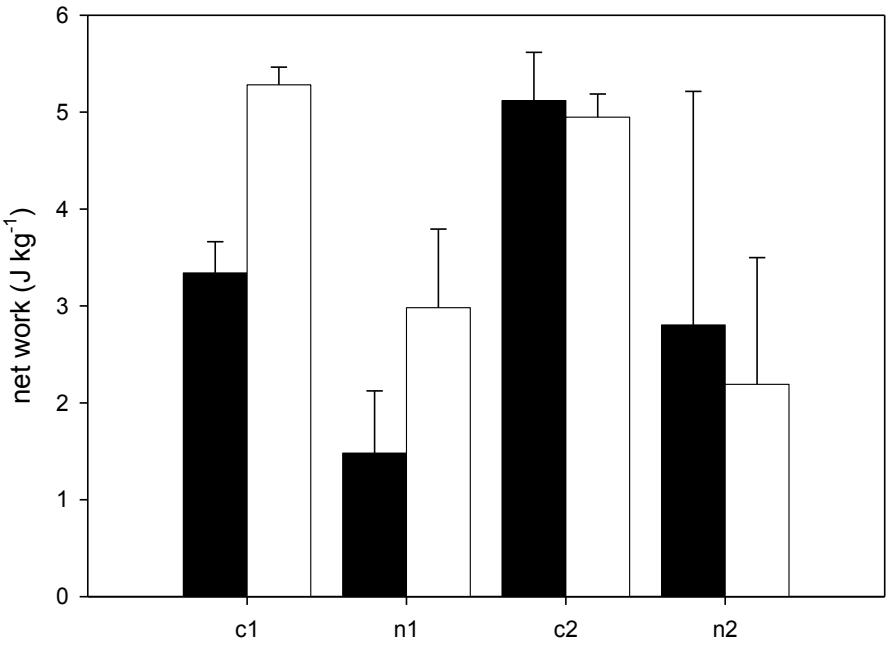

Figure 4.3. Net work done during a single cycle comparing the jumping trials (filled bars) and oxygen consumption trials (open bars). The categories along the x-axis refer to the tendon present (e.g. c for compliant, n for non-compliant) and amplitude of load movement (e.g. 1 for low amplitude, 2 for high amplitude). Values are shown as mean +/- standard error. The activation conditions and muscle movements that resulted in the oxygen consumption values seen were based on averages calculated for the jumping trials. There was not a significant difference between the jumping trials and oxygen trials (2-way ANOVA, P=0.406).

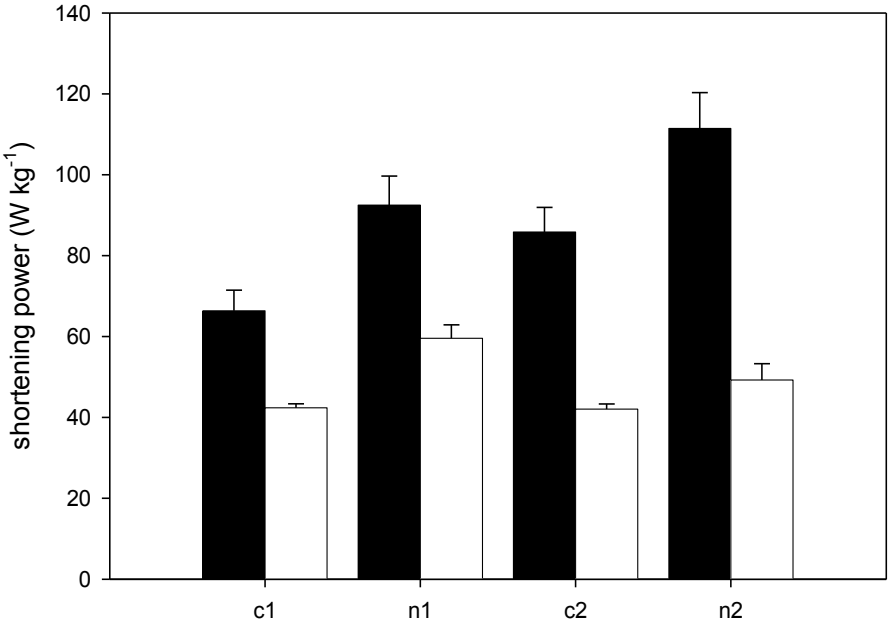

Figure 4.4. Shortening power comparing the jumping trials (filled bars) and oxygen consumption trials (open bars). The categories along the x-axis refer to the tendon present (e.g. c for compliant, n for non-compliant) and amplitude of load movement (e.g. 1 for low amplitude, 2 for high amplitude). Values are shown as mean +/- standard error. The activation conditions and muscle movements that resulted in the oxygen consumption values seen were based on averages calculated for the jumping trials. There was a significant difference between the jumping trials and oxygen trials for all activation conditions (2-way ANOVA, $P<0.001$).

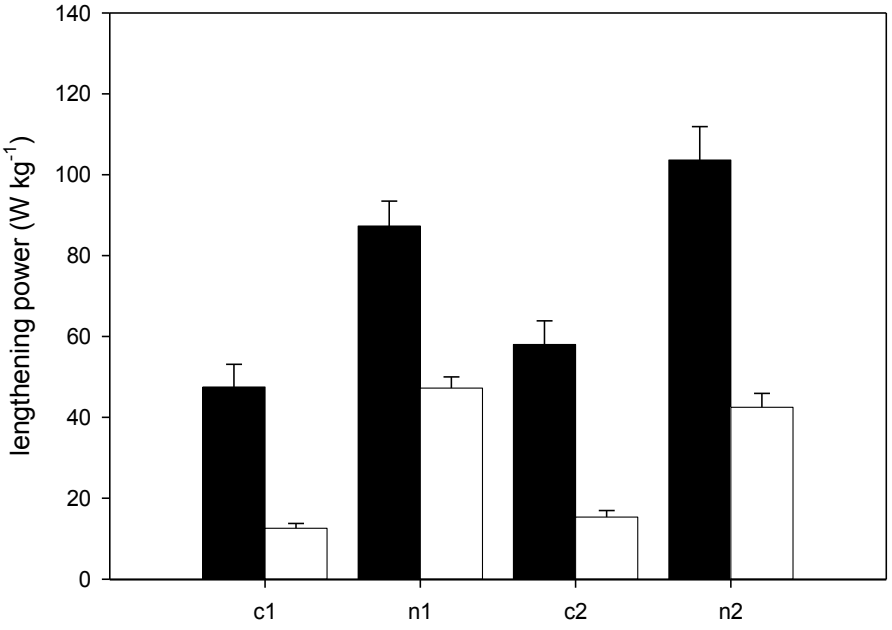

Figure 4.5. Lengthening power comparing the jumping trials (filled bars) and oxygen consumption trials (open bars). The categories along the x-axis refer to the tendon present (e.g. c for compliant, n for non-compliant) and amplitude of load movement (e.g. 1 for low amplitude, 2 for high amplitude). Values are shown as mean +/- standard error. The activation conditions and muscle movements that resulted in the oxygen consumption values seen were based on averages calculated for the jumping trials. There was not a significant difference between the jumping trials and oxygen trials (2-way ANOVA, P=0.227).

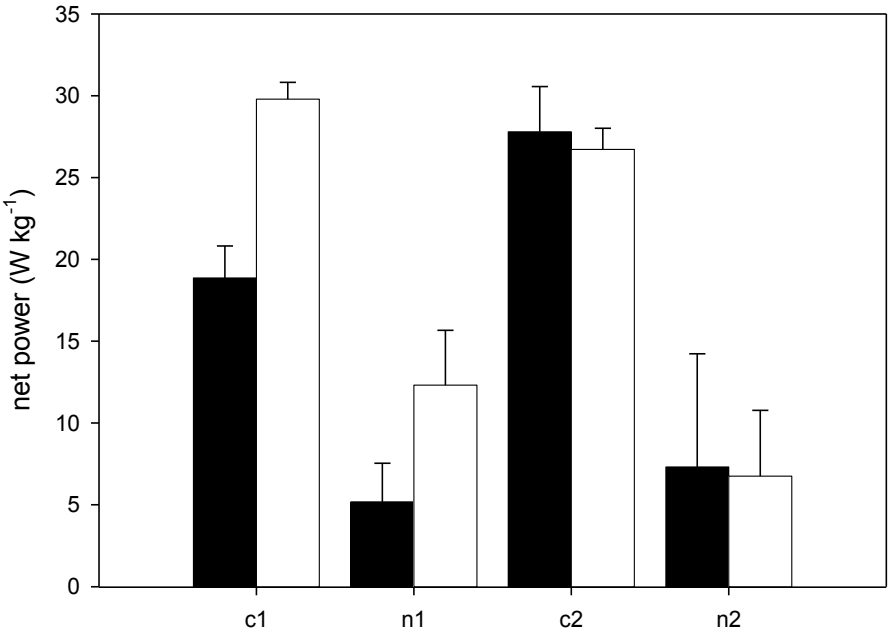

Figure 4.6. Net power comparing the jumping trials (filled bars) and oxygen consumption trials (open bars). The categories along the x-axis refer to the tendon present (e.g. c for compliant, n for non-compliant) and amplitude of load movement (e.g. 1 for low amplitude, 2 for high amplitude). Values are shown as mean +/- standard error. The activation conditions and muscle movements that resulted in the oxygen consumption values seen were based on averages calculated for the jumping trials. There was not a significant difference between the jumping trials and oxygen trials (2-way ANOVA, P=0.196).

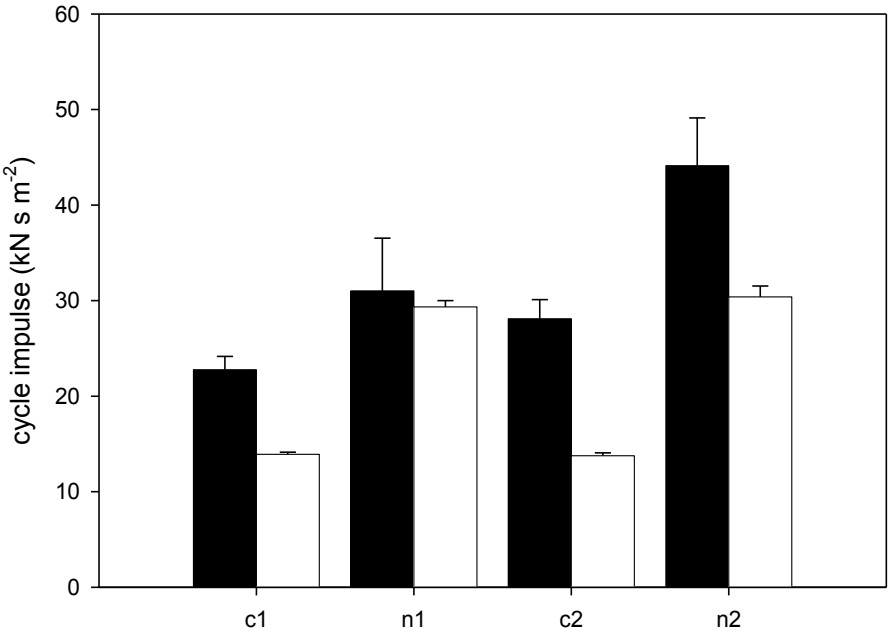

Figure 4.7. Cycle impulse from a single cycle comparing the jumping trials (filled bars) and oxygen consumption trials (open bars). The categories along the x-axis refer to the tendon present (e.g. c for compliant, n for non-compliant) and amplitude of load movement (e.g. 1 for low amplitude, 2 for high amplitude). Values are shown as mean +/- standard error. The activation conditions and muscle movements that resulted in the oxygen consumption values seen were based on averages calculated for the jumping trials. There was not a significant difference between the jumping trials and oxygen trials (2-way ANOVA, P=0.113).

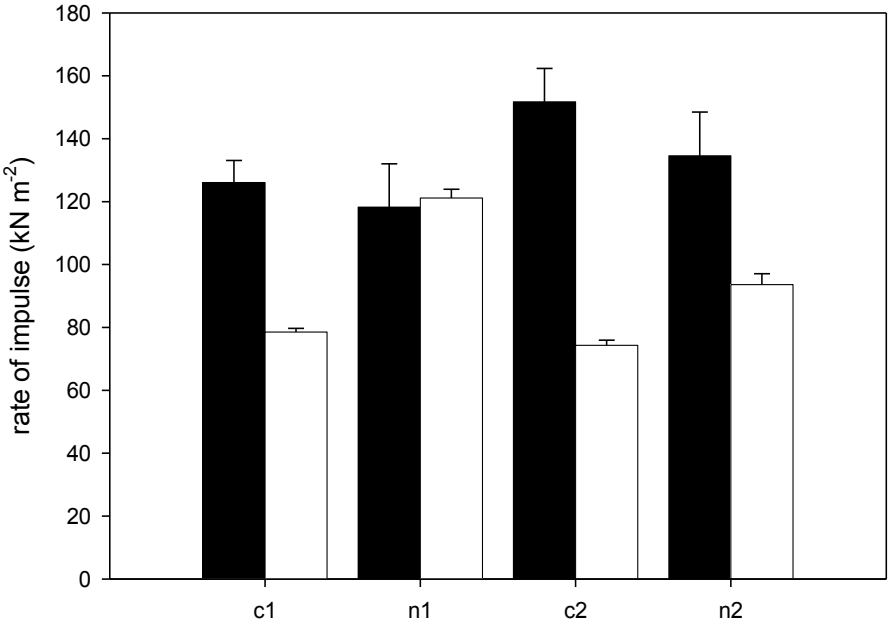

Figure 4.8. Rate of cycle impulse comparing the jumping trials (filled bars) and oxygen consumption trials (open bars). The categories along the x-axis refer to the tendon present (e.g. c for compliant, n for non-compliant) and amplitude of load movement (e.g. 1 for low amplitude, 2 for high amplitude). Values are shown as mean +/- standard error. The activation conditions and muscle movements that resulted in the oxygen consumption values seen were based on averages calculated for the jumping trials. There was a significant difference between the jumping trials and oxygen trials for all activation conditions (2-way ANOVA, $P<0.001$) except n1 (2-way ANOVA, $P=0.855$).

4.4 Significance and prospectus

While past research has suggested a correlation between tendon elastic energy storage and reduced levels of energy consumption in animals during locomotion, there has been limited research into demonstrating that correlation, as it is not ethically or physically feasible to simply adjust the compliance of an animal's tendon during locomotion, nor is animal locomotion easily reproduced in a laboratory setting to measure energy consumption. Previous research has quantified the ability of tendons to store elastic energy (Alexander, 1984; Biewener et al., 1981; Roberts et al., 1997), demonstrated reduced levels of oxygen consumption at increasing speeds (Dawson and Taylor, 1973; Baudinette and Biewener, 1992), quantified the abilities of a tendon for energy storage while measuring oxygen consumption (Biewener and Baudinette, 1995; Roberts et al., 1998) and measured the effects of tendon compliance on energy use, although not under conditions attempting to simulate animal locomotion (Lichtwark and Barclay, 2010). Thus, the results presented in this study are suggestive of the effects a compliant tendon have on an animal performing locomotion and indicate a compliant tendon would reduce the amount of work done and energy used by a cyclically contracting muscle. While related research has previously focused on the mechanics of locomotion or locomotion energetics, the hope is that this study will incite further discussion into both fields and encourage cross-over between the two. Although this study has important implications for multiple fields, it represents only a fraction of the overall picture that describes how animals move, how they use their muscles and tendons, and what constitutes an economical form of locomotion.

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