Longitudinal adaptation of vastus lateralis muscle in response to eccentric exercise: Effects of magnitude, velocity and range of the applied eccentric stimulus

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A. Sharifnezhad

Zusammenfassung

In der Literatur wird die longitudinale Adaptation als eine Anpassungsstrategie der Muskulatur an Trainingsreize identifiziert (Williams & Goldspink, 1978). Aus Tierstudien ist bekannt, dass sowohl Immobilisation als auch exzentrische Trainingsreize longitudinale Muskeladaptation hervorgerufen können (Lynn & Morgan 1995). Derzeit existieren nur wenige Studien, die den Einfluss exzentrischen Trainings auf die longitudinale Adaptation beim Menschen untersucht haben. In der vorliegenden Studie wurde daher den Einfluss exzentrischen Trainings, mit unterschiedlicher Reizmagnitude, Bewegungsgeschwindigkeit und Muskellänge bei Reizapplikation, auf die Adaptation des M. vastus lateralis (VL) untersucht. Die 31 Studienteilnehmer wurden randomisiert in zwei Trainings- und eine Kontrollgruppe aufgeteilt. Die Trainingsgruppen führten 30 Trainingseinheiten (3x pro Woche, 10 Wochen) mit exzentrischem Training der Knieextensoren an einem Isokineten durch. Gruppe 1 (n=10) trainierte ein Bein mit 65% der maximalen willkürlichen isometrischen Kontraktion (MVC)(Protokoll 1) und das andere Bein mit 100% MVC (Protokoll 2), bei einer Winkelgeschwindigkeit von 90°/s und einem Kniewinkel von 25°-100°. Die zweite Gruppe (n=10) trainierte beide Beine mit 100% MVC, ein Bein aber mit einer Winkelgeschwindigkeit von 90°/s in einem Kniewinkel von 25°-65° (Protokoll 3) und das andere Bein mit 240°/s und 25°-100° (Protokoll 4). Die Kontrollgruppe (n=11) führte kein Training durch. In der Pre- und Postmessung wurde die VL Muskelfaserlänge mittels Ultraschall bestimmt und die Moment-Winkel- und Leistungs-Winkelgeschwindigkeitsrelation mit einem Dynamometer (Biodex 3) erfasst.

Die Ergebnisse zeigen nur für das Protokoll 4 eine signifikante ($p<0.05$) Zunahme der Faserlänge des VL gegenüber der Kontrollgruppe. Die größte Faserdehnungsgeschwindigkeit wurde in allen vier Protokollen bei absteigendem Knieextensionsmoment beobachtet. Die maximale Kraft der Knieextensoren hat sich in allen Trainingsprotokollen erhöht, wobei die größte Kraftsteigerung in Protokoll 2 zu verzeichnen war. Eine Zunahme der maximalen mechanischen Leistung war nur bei den Trainingsprotokollen mit einer Winkelgeschwindigkeit von 90°/s zu beobachten. Weder die Moment-Winkel- noch die Leistungs-Winkelgeschwindigkeitsrelation zeigten eine Rechtsverschiebung, was aus mechanischer Sicht auf eine longitudinale Adaptation hindeuten würde.

Die Ergebnisse belegen, dass (a) nicht jedes exzentrische Training eine Verlängerung der Muskelfasern hervorruft, und (b) die Faserdehnungsgeschwindigkeit ein entscheidender Faktor zum Auslösen longitudinaler Muskeladaptation zu sein scheint, was sich durch die „popping sarcomere“ Hypothese und spezifische Mechanotransduktionsmechanismen erklären lässt. Darüber hinaus (c) scheinen die Moment-Winkel und Leistung-Winkelgeschwindigkeitsrelation keine geeigneten Parameter zu sein um longitudinale Muskeladaptation zu untersuchen.

Abstract:

Longitudinal adaptation is a well-known strategy for muscle adaptation in response to exercise (Williams & Goldspink 1978). Animal studies have shown that immobilization as well as eccentric exercise can induce longitudinal adaptation of the muscle (Lynn & Morgan 1994). However, in humans there is little information about the effect of controlled eccentric training on the longitudinal adaptation of muscles. Therefore, the present study investigated the effects of magnitude, velocity and muscle length at which the eccentric stimulus is applied on the longitudinal adaptation of the vastus lateralis muscle (VL). The 31 participants were randomly assigned into two experimental groups to perform 30 sessions of eccentric training for the knee extensors (3 times/week for 10 weeks) and one control group. The first experimental group (n=10) exercised one leg (protocol 1) at 65% of maximum voluntary isometric contraction (MVC) and the second leg (protocol 2) at 100% MVC at 90°/s from 25° to 100° knee angle on an isokinetic device (Biodex 3). The second experimental group (n=10) exercised one leg at 100% MVC at 90°/s from 25° to 65° knee angle (protocol 3) and the other leg at 100% MVC at an angular velocity of 240°/s from 25° to 100° knee angle (protocol 4). The control group (n=11) did not perform specific eccentric training. In pre and post measurements the fascicle length of the VL was examined by ultrasonography and the moment-angle and power-angular velocity relationship of the knee extensors with a dynamometer.

The results showed an increase ($p<0.05$) in the fascicle length of vastus lateralis compared to the control group only in the leg trained with the higher angular velocity (protocol 4). The high strain velocity of the fascicles was located in the phase where the moment decreased. The strength of the knee extensor muscles increased in all protocols with the greatest improvement in a training protocol with high magnitude of loading (protocol 2). The mechanical power increased only in the protocols with lower angular velocities. However, the moment-angle and mechanical power-angular velocity curve did not show any rightward shift, which would have been an indirect indicator for longitudinal adaptation from a mechanical point of view.

In conclusion, the present findings give evidence that (a) not all types of eccentric exercise cause an increase in fascicle length and (b) the velocity of fascicle stretch seems to be important for the longitudinal adaptation of the muscle, which can be explained by the "popping sarcomere hypothesis" and/or mechanisms of mechanotransduction (i.e. role of titin). Lastly, it is concluded that (c) the shift in the moment-angle curve might not be an accurate measure of potential longitudinal muscle adaptation, since its assessment is affected by pre to post differences in tendon stiffness and activation level during the maximal contractions.

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1. Introduction

Meaningful movement is characteristic of humans, and requires the activity of skeletal muscle. The consequences of these activities vary from quiet breathing to more dramatic endeavors of an athlete running or throwing. Muscle is one of the few structures in which it is possible to understand how these very different actions take place and are regulated *(Jones, 2010)*. However, the major advance in understanding muscle contraction occurred in the 1950s and 1970s, largely from the work of A. F. Huxley, H. E. Huxley *(A. F. Huxley, 1957; H. E. Huxley, 1969)* and A. V. Hill *(Hill, 1953)*.

The mechanical properties of skeletal muscle verify its performance. Mechanical properties are defined here as those properties of skeletal muscle that may be measured by parameters derived from mechanics such as: force, length, velocity, work and power. The performance achieved in several sports depends on these parameters, for instance, on the power an athlete can produce, or the velocity (speed) he or she will be able to achieve or impart on an object *(Zatsiorsky et al., 2000)*.

On the other hand, adaptability may be regarded as a basic characteristic of life. There is an argument as to whether the mechanisms by which muscle enlargement occurs is due entirely to fiber hypertrophy or if there is also an associated increase in the number of sarcomeres in series within the muscle fibers (longitudinal adaptation). This question has been addressed by several investigators who have applied heavy resistance training techniques to a variety of large and small limb muscles. Most of these studies have focused on the radial plasticity of muscle so there is little information on longitudinal muscle adaptation.

According to the literature *(Goldspink, 1985)*, muscles undergo three strategies in response to exercise. These strategies allow adaptations and increased power production by changes (a) in the physiological cross-sectional area of the muscle (PCSA) (i.e. radial adaptation), (b) in lengthening of the muscle (i.e. longitudinal adaptation) and (c) in the specific muscle force (i.e. muscle force normalized to the PCSA) *(Goldspink, 1985)*. Radial plasticity is associated with changes in some factors, amongst the rate of synthesis and degradation of muscle proteins, which result in an increase of myofibrils. This process is repeated in order to increase the number of myofibrils (PSCA of the muscle) in parallel, very considerably. The longitudinal changes in muscle results from the modulation of the number of in-series sarcomeres, added along the length of the fiber. The third strategy, specific force, represents the intrinsic force-producing capability of a muscle (the maximum muscle force divided by muscle cross-sectional area, N/cm^2). A meaningful aspect of the muscle's intrinsic force-generating capability should involve isometric maximum muscle force and the muscle physiological cross-sectional area. The first one (isometric maximum muscle force) can be evaluated from the muscle joint moment, when the length of moment arm and muscle pennation angle are known. The second one (PSCA of the muscle) can be evaluated by dividing the muscle volume by the muscle fiber length *(Goldspink, 1985; Maganaris, et al., 2001; Reeves, et al., 2004)*.

These three structural changes in the muscle phenotype (i.e. radial adaptation, longitudinal adaptation and specific contractile force) directly affect the specific functional characteristics of muscle contraction and muscle force production, such as the force-length and force-velocity relationships.

Training-induced radial hypertrophy (increase in PSCA) of the muscle by functional loading has been examined in a variety of studies, both in humans *(MacDougall et al., 1979; Sale, et al., 1990)* and in animal experiments *(Timson, et al., 1985; Watt, et al., 1982)*. However, the ability of specific training stimuli to influence in series sarcomere growth within a muscle has been scarcely investigated. Only limited available research suggests clear indications of the plasticity of the longitudinal muscle fibers *(Lynn and Morgan, 1994)*.

A review of literature revealed that eccentric exercise may be the mechanical stimulus which initiates the longitudinal adaptation of the muscle *(Lynn and Morgan, 1994)*. Recent studies *(Duclay, et al., 2009; Potier, et al., 2009; Reeves, et al., 2009)* on different muscles of the lower extremities, provide information about a specific link between eccentric exercise and longitudinal muscle adaptation. Furthermore, specific contraction properties of muscle such as the force-length and force-velocity relationships, as well as mechanical power, may have a significant impact both for increasing athletic performance *(Abe, et al., 2001; Kumagai et al., 2000)* and reducing the risk of injury in athletes *(Askling, et al., 2003; Brockett, et al., 2004).*

It is generally believed that muscles which are capable of producing high forces in shorter muscle fibers, relative to the optimum length of muscle, are more prone to eccentric damage *(Brockett et al., 2004; Morgan and Proske, 2004; Proske, et al., 2004).* This means that at the optimum length of a muscle, where maximum moment is reached, muscle fibers are placed on a shorter position of the force–length curve and therefore this muscle have shorter muscle fibers length and consequently it is more prone to eccentric damage *(Morgan and Proske, 2004; Proske et al., 2004).* In this regard, Brocket et al. (2004) suggested that the knee angle where the maximum moment is reached is an important risk factor for muscle injuries due to imbalances between agonists and antagonists. The authors concluded that the risk for strain injuries on hamstring muscle is increased when the maximum moment is reached in a more flexed knee position (shorter than optimal length) *(Brockett et al., 2004)*. It is suggested that these kinds of injuries due to lengthening of muscle, would occur in the sarcomeres especially in the descending part of the force-length curve of a muscle *(Armstrong, 1984; Asmussen, 1956; Lieber and Fridén, 2002).* Also these injuries cause structural damage, due to the region of sarcomere length instability.

In this regard Morgan (1990) suggested a 'popping sarcomere' hypothesis, which states that stretch-induced muscle damage results from very irregular lengthening of sarcomeres when the active muscle is stretched over it's optimal length. In this situation, the longest sarcomeres are going to be the weakest and will be stretched more quickly than the others. Therefore they become fragile until falling active tension is compensated by rising passive tension. At least for some muscles, this corresponds to lengths beyond filament overlap. As the weakest sarcomeres don't seem to be at an identical point along every myofibril, irregular lengthening may result in huge deformations in shearing of myofibrils, and breaking of membranes, particularly T-tubules. This often seems to result in a loss of calcium ion homeostasis and consequently damage, either through tearing of membranes or opening of stretch-activated channels *(Morgan, 1990; Morgan and Proske, 2004)*. Morgan (1990) suggested three important roles of optimum muscle length in the popping sarcomere hypothesis. First of all, "optimum length becomes a main determinant of the susceptibility; damage is predicted to occur only when sarcomeres are used beyond optimum length. The second, the immediate shift in optimum length after eccentric exercise is a measure of damage. In its third role, the position of the optimum is a measure of adaptation. A muscle is expected to be protected from injury if the optimum length is near to or beyond the maximum length at which the muscle undergoes eccentric contraction" *(Morgan, 1990; Morgan and Proske, 2004).*

In respect of injury prevention of muscle after a period of eccentric contraction, Herring et al. (1984) and Proske (2001) suggested that, for a given muscle, serial sarcomere number is adjusted to attain optimal sarcomere length at the joint angle (or muscle length) where maximum force is made by the muscle *(Herring and Grimm, 1984; Proske and Morgan, 2001).* In other words, if eccentric training induces an appropriate increase in serial sarcomere number, sarcomere length will be optimal (thick and thin filament overlap will be maximal) at the joint angle where training force is maximal. Each sarcomere can then tolerate less instability than before *(Koh, 1995)*, leading to prevention of further injures.

Moreover, others studies have reported that an increase in the stability of the descending portion of the force-length relationship, due to eccentric contraction, can be achieved by an increase in the stiffness of inter-muscular connective tissue *(Lapier, et al., 1995; Williams, 1988)* and cytoskeletal proteins such as desmin and titin *(Peters et al., 2003)*. In this regard it is reported that microtrauma due to eccentric exercise can induce signal transduction pathways, which contribute to a strengthening of cytoskeletal proteins *(Barash, et al., 2004; Barash, et al., 2002)*. Barash et al. (2002) found an increase of desmin in rat muscle after seven days due to exercise-induced (eccentric training) muscle injury. The increase of the cytoskeletal protein content leads to an increase in the rigidity of muscle fibers and the entire muscle. Thus, through the development of training methods with a basis of eccentric contractions to yield longitudinal adaptation of muscle, a significant contribution to injury prevention could be made *(Lynn and Morgan, 1994; Proske and Morgan, 2001)*.

In another point of view, longer muscle fibers, resulting from an eccentric stimulus, have more sarcomeres in series and can therefore exhibit higher maximum shortening velocity and maximal mechanical power than shorter fascicles. It means each sarcomere can shorten at a given rate and the shortening rate of the fiber will be a direct function of the number of in series sarcomeres. Furthermore, longer muscle fascicles have a broader length-range of force development (force–length relationship) than shorter muscle fascicles, which may have an effect on muscle performance *(De Haan and Huijing, 2003; Stafilidis and Arampatzis, 2007; Bobbert and Harlaar, 1993; Reeves et al., 2009)*. Additionally, a shift to the right in the force-length relationship of muscle has been shown after a period of eccentric muscle contraction *(Brughelli and Cronin, 2007; Butterfield and Herzog, 2006a)* suggesting optimal muscle length is altered. Since human movement typically takes place when the muscles are on, or near to, their optimum lengths, *(Lieber, 2002; Lieber, et al., 2000)* performance may be altered if the optimum length is shifted. These outcomes strongly suggest that the training mode is a basic stimulus for fiber or fascicle length variation *(Blazevich et al., 2007a)*. Note that such a rise in force production can occur without a necessary increase in the cross-sectional area of the muscle *(Koh, 1995).*

However in most of the studies *(Lindstedt, et al. 2001; Lichtwark et al. 2006; Giannakou et al. 2011)* regarding eccentric exercise, the definition of the eccentric training stimulus is flawed. Eccentric contractions in exercise science are defined in relation to the dynamics of the whole muscle-tendon unit. According to literature, when there is lengthening of the muscle-tendon unit while the muscle is contracting, it is defined as an eccentric contraction. However, during active lengthening of the muscle-tendon unit due to tendon elasticity, the dynamics of muscle fibers are different compared to the dynamics of the muscle-tendon unit *(Butterfield and Herzog, 2006a; Butterfield and Herzog, 2005).* For example, it is possible that the muscle fibers shorten (concentric contraction), although the muscle-tendon unit is elongated (negative mechanical work) *(Giannakou et al., 2011; Lichtwark et al., 2006).*

Nevertheless, according to our search of relevant literature, it is evident that the magnitude of force and velocity as well as fascicle length at the point where the eccentric stimulus has been applied, would affect adaptation of fascicle length. Most of this knowledge comes from animal studies *(Butterfild et al. 2006).* Although there are some recent studies regarding the effects of eccentric exercise in human skeletal muscle, there is still limited information which highlights the interaction between concrete defined training stimuli and longitudinal muscle adaptation. Therefore it would be cautious the application of sport-specific to the athletes.

2. Literature review

2.1 Longitudinal plasticity of muscle during immobilization

Studies which have focused on immobilization of a muscle have reported that serial sarcomere number is greatly plastic in a number of species and muscles *(Goldspink, 1985; Goldspink et al., 1974; Tabary et al., 1972; 1976; Williams, 1990; Williams and Goldspink, 1978; Williams et al., 1986).* Immobilization of a muscle in different positions, such as shortening or lengthening results in rapid decreases or increases, respectively, in serial sarcomere number *(Koh, 1995)*.

There are various investigations, which aim to quantify the effects of stretch on muscle fiber length and sarcomere number. One of the most clear and direct explanations of longitudinal plasticity and the changes that occur to limb immobilization, is that of Williams and Goldspink *(Williams, 1988; Williams and Goldspink, 1973).* The study included hind limb immobilization of mice for three weeks by a plaster cast device. Two groups of animals, including old (more than 8 weeks) and young (1 week) mice were involved in the study. Ankle joints were immobilized and held in a lengthened or shortened position. The soleus muscle was analyzed after 3 weeks of immobilization. Another leg was used as a control. A single muscle fiber was then tested to determine the number of sarcomeres per fiber. As an outcome, the authors mentioned that sarcomere length for adult soleus muscles in the fully shortened position was 1.51 µm, and in the fully lengthened position was 3.23 µm. Sarcomere number was reduced following immobilization of the muscle in the shortened position and the corresponding sarcomere length was longer (2.08 µm) than sarcomeres from control muscles. The opposed was also true, sarcomere number was increased when muscles were immobilized in the lengthened position and the length of individual sarcomeres was shorter (2.43 µm) than sarcomeres from control muscles. The authors also concluded that the length–tension properties of muscle changed, regardless of the immobilization position, so that maximum isometric tension was detected at the muscle length identical to the angle of immobilization *(Williams and Goldspink, 1978).* Thus, the mouse soleus muscle had adjusted to its new mechanical environment by adding or removing the appropriate number of sarcomeres, such that optimal muscle length coincided with the length at which the muscle was immobilized. The results also demonstrated that the length-tension curves and sarcomere number return to normal within 4 weeks, in the absence of plaster casts. These results could imply that chronic lengthening changes the adaptation of muscles by readjusting sarcomere length to an optimum, or simply that muscles adapt by modifying the muscle to a specific sarcomere length. Muscles that were changed to a second immobilized position were found to quickly set to the second position with respect to their sarcomere number and passive length-tension properties *(Williams and Goldspink, 1978).* Consequently, immobilization seems to modify muscle strength via serial sarcomere number adaptation, along with adding sarcomeres in parallel (cross-sectional area).

Further research by Williams (1990) has indicated that immobilization in a shortened position, in addition to a period of stretching, can reduce the number of series sarcomeres. Thirty-six mice were used to identify the effect of short muscles of daily stretching, on joint range of motion and serial sarcomere number in immobilized mouse soleus muscles. Immobilized plaster casts were used on the right hind limbs of one group of mice for 2 weeks to keep the soleus muscle in a shortened position (ankle plantar flexion) and in another group, the cast was removed for 15 min, 30 min, 1 hour or 2 hours each day. Stretching of the soleus muscle (ankle dorsiflexion) occurred daily during this time.

After this period of lengthening the casts were re-applied. There was a reduction in muscle fiber length due to a loss of serial sarcomere number by almost 20% in the group that were immobilized in shortened positions. There was also a smaller significant reduction in sarcomere number and improvement in range of movement as a result of 15 min daily stretch over the period of immobilization. The author observed that dorsiflexion of the ankle maintained normal length of sarcomeres and a loss of sarcomeres was prevented when the soleus was stretched for more than 30 min daily. The study also illustrated that 2 hours of daily stretch increased serial sarcomere number by 10%. The stretch also provided a protection phenomenon of sarcomere loss, which occurs mostly during plaster cast immobilization. The author suggested that much of the atrophy linked with immobilization may be prevented by short periods of stretch *(Williams, 1990).*

Immobilized muscle at different lengths has been investigated in another study by Goldspink et al. (1974). Thirty-six adult cats were randomly separated into three experimental groups. One control group had muscles that were denervated but not immobilized. The second group had one denervated hind limb, which were then immediately immobilized in a fully dorsiflexed position to lengthen the muscle, and another hind limb not denervated. The third group was denervated and the muscle immobilized in a maximal plantar flexion position so that the soleus muscle was at a shortened length, and the other hind limb not denervated. After 4 weeks of immobilization by a plaster cast the muscles were examined physiologically and histologically. The results demonstrated that there were no significant differences between denervated immobilized muscles and non-denervated muscles on passive length-tension properties. Denervation had no effect on immobilization of the muscle, but there was a marked decrease and increase in sarcomere numbers in the shortened and lengthened position, respectively. Specifically, the number of sarcomeres increased by 25% when the denervated soleus muscle was immobilized in the lengthened position, whilst muscles immobilized in the shortened position lost 35% of sarcomeres. "This adaptation was essentially the same as in muscles that had been immobilized but not denervated. The authors suggested that adjustment of sarcomere number to the functional length of the muscles does not therefore seem to be directly under neuronal control. It appears to be a myogenic response to the amount of passive tension the muscle is subjected to" *(Goldspink et al., 1974).*

In another study by Matano et al. (1994), the length of sarcomeres *in situ* has been analyzed to quantify the adaptation of skeletal muscle during limb lengthening. Extensor digitorum lateralis muscles of the fifth digit in rabbits were measured *in situ*. A fixator device was used to lengthen the forearm of animals. After this operation, and at regular intervals, the extensor digitorum lateralis muscle was evaluated *in situ* and the length of the sarcomeres were measured by a laser diffraction technique. The left forearm was subjected to lengthening and the right forearm used as a control. The results indicated the increase in mean sarcomere length from 3.09 μm in the non-stretched muscle to 3.51 μm immediately after elongation. The sarcomere length then returned to a baseline value (3.10 μm) nearly equal to that of the control, 9 days after the operation. The authors suggested that muscle has the ability to adapt to a new length and "it appears that stretch-induced changes in sarcomere length are common in immobilized and non-immobilized muscles" *(Matano et al., 1994).*

Alteration of sarcomere length to a sustained stretch in an immobilized position has been studied by Tamai et al. (1989). The extensor digitorum longus muscle in mice was analyzed by the light diffraction method. Hind limb muscles were immobilized by plaster cast in a maximally stretched position (full plantar flexion of ankle). The results demonstrate that the length of sarcomeres increased immediately after stretch to 2.98 µm. In addition, sarcomere length decreased by 2.94, 2.86, 2.83, 2.77 and 2.78 µm after 1, 3, 5, 7, and 14 days of immobilization, respectively. The authors concluded that mice muscle can adapt to a new length under sustained stretch *(Tamai et al., 1989).*

Shah and Peters (2001) investigated changes in serial sarcomere number in skeletal muscle undergoing chronic length perturbation. The right hind limbs of wild-type mice were compared with those of homozygous desmin knockout mice after immobilization. Fascicle length of the tibialis anterior, extensor digitorum longus and soleus muscle was measured to identify the characteristics of intermediate filament desmin. The ankle joint of these animals was immobilized in a plantarflexed position, and with the knee in a flexed position for 28 days, by plaster casting. This caused lengthening of the tibialis anterior and extensor digitorum longus muscles, as well as shortening of the soleus muscle. The outcomes revealed that sarcomere number in the lengthened muscles increased 9% for the tibialis anterior and 17% for the extensor digitorum longus in wild-type mice. The results also showed a 10% increase in sarcomere number for the tibialis anterior and a 27% increase for the extensor digitorum longus in the desmin knockout mice. Serial sarcomere number decreased in the shortened soleus muscle for both groups (26% in wild-type and 12% in desmin knockout mice). Therefore, although desmin is not crucial for sarcomere addition in series in mouse hind limb muscles, the authors suggested that the results show indirect differences in the nature of sarcomere number adaptation. It is therefore possible that desmin may play a role in adjusting the optimal arrangement of in series sarcomeres within the muscle *(Shah et al., 2001).*

In another investigation by Coutinho et al. (2004), the effect of interval stretching was studied on shortened and immobilized muscle. Eighteen, 16-week-old rats were divided into three groups. The left soleus muscle of the first group was immobilized in a shortened position for 3 weeks. For the second group, the soleus was stretched for 40 min, every 3 days during immobilization, and the third group was stretched for the same volume but in the absence of any immobilization. Serial sarcomere length and number, as well as muscle fiber area, was measured before and after the stretch and immobilization period. There was a significant decrease in weight (44 ± 6%), length (19 ± 7%), serial sarcomere number (23 ± 15%), and fiber area (37 ± 31%) in the immobilized muscles (first group) compared to the contralateral muscles. The second group showed a similar reduction but milder muscle fiber atrophy compared to the other immobilized group (22 ± 40 vs. 37 ± 31%, respectively). Stretched muscles (third group) significantly increased the length (5 ± 2%), serial sarcomere number (4 ± 4%), and fiber area (16 ± 44%) compared to the contralateral muscles. The authors concluded that short periods of stretching to immobilized muscles did not prevent the muscle shortening significantly, but reduced muscle atrophy. It was concluded that muscle stretching influences the serial sarcomere number and cross-sectional area of muscle fibers differently *(Coutinho et al., 2004)*.

In a recent investigation by Pontén et al. (2008) it was shown that muscle immobilization in a stretched position increased the number of sarcomeres in series in the tibialis anterior (TA) muscle in rabbits. The right ankle of fifteen adult rabbits were fixed in a maximal plantarflexion position, a mid range position and a maximal dorsiflexed position, by plastercast. The left leg of these animals was used as a control. A laser diffraction system was applied to measure sarcomere length in the soleus muscle and tibialis anterior muscle after 3 weeks of muscle immobilization. The authors found that sarcomere numbers increased in the maximal and mid range stretch position of the muscle. The observation from the laser diffraction *in vitro* of these muscles compared with the control, showed that for the same fiber length, sarcomere length decreased to adapt to a greater number of sarcomeres in series *(Pontén and Fridén, 2008).*

In summary, a review of this literature suggests that the number of sarcomeres and muscle fascicle lengths are highly adaptable during immobilization. In this regard several studies showed this effect following immobilization of different joints and different animal muscles such as the extensor digitorum lateralis, soleus, extensor digitorum longus and tibialis anterior muscle. Mostly in mentioned study, the numbers of sarcomeres as well as its length have been measured per muscle fascicle. The main results included: immobilization of muscle in a shortened position reduced the number of sarcomeres and increased sarcomere length, whilst immobilizing the muscle in a lengthened position caused an increase in the number of sarcomeres and a decrease in sarcomere length *(Goldspink, 1985; Goldspink et al., 1974; Tabary et al., 1972; 1976; Williams, 1990; Williams and Goldspink, 1978; Williams et al., 1986; Coutinho et al. 2004; Pontén and Fridén 2008).* Furthermore, neural control does not affected the adjustment of sarcomere number to the functional length of the muscle *(Goldspink et al., 1974)*. Finally, desmin may play a role in adapting the optimal arrangement of sarcomeres within the muscle *(Shah et al., 2001).*

2.2 Longitudinal plasticity of muscle during exercise

2.2.1 Animal experiments

Most of the previous studies reported that longitudinal plasticity resulted from a static stimulus (passive/inactive static stretching). However, there are some studies that have shown an increase in serial sarcomere number after eccentric exercise in animals. Lynn and Morgan were the first to report an increase in the number of sarcomeres in series after eccentric exercise. Rats were exercised on a small animal treadmill at either an incline or a decline of 16° for 7 days. This type of exercise has previously been shown to cause muscle damage in untrained rats, and thus may indicate a training effect. The exercise protocol involved running at 14 m/min followed by 1.5 minutes of rest for 15 minutes on the 1st day, and increased to 30 min on the 4th and subsequent days. The vastus intermedius muscle was fixed and digested in acid. A laser diffraction device was used to measure sarcomere length and the mean number of sarcomeres per fiber. The results showed the number of sarcomeres in fibers of the rat vastus intermedius muscle for both mature and young rats, within 1 week, were varied as a result of the angle of mild running exercise. The downhill running protocol resulted in eccentric contraction of the vastus intermedius muscle, due to the extreme knee flexion angles. This resulted in an increase in sarcomere number which was 12% greater than in the uphill group *(Lynn and Morgan, 1994).*

Another study with the same method was carried out by Lynn et al. (1998) to investigate the behavior of rat skeletal muscles after incline and decline running. Sixteen mature male rats were divided into two groups for incline training and decline training. The optimum knee angle for torque generation and sarcomere number were assessed for the vastus intermedius muscle from each rat. Animals were trained over 5 days with different slopes of the treadmill, either incline or decline training at 16° to horizontal. Training consisted of 15 minutes running on a treadmill for the first day, and was increased by 5 min per day, up to 35 minute on the final days, at a speed of 16 m/min. Computer imaging and laser diffraction techniques were used to measure fiber lengths and sarcomere lengths to produce an estimate of the average number of sarcomeres in series in the muscle fibers. The authors concluded that eccentric contraction leads to an addition of in series sarcomeres and results in a greater resistance to damage compared with concentric training *(Lynn et al., 1998)*.

Also of interest is the recent study of Peters et al. (2003). Forty-six rats completed 30 eccentric contractions. The dorsiflexor and plantar flexor muscles were stimulated by activating the peroneal nerve of the foot to around 40 deg. Sarcomere length of the tibialis anterior was altered over the range of 2.27–2.39 µm and 2.52–2.66 µm for the extensor digitorum longus, which would represent the plateau and descending limb of the length–tension curve for this species. Animals were allowed to recover for one of 10 time periods ranging from 30 minute to 10 days, at which time peak isometric torque was measured. The results revealed that peak isometric torque immediately after eccentric contraction, decreased significantly by ~40%, and then recovered in a linear mode to control levels 7 days later. Additionally, myogenic regulatory factors and desmin transcripts significantly increased 3 and 12 hours after eccentric contraction, respectively. The authors noted that none of the measured parameters could directly explain torque loss after eccentric contraction, or the linear recovery that occurred from that time point onwards, "but the time course of the cellular changes in the injured muscle suggests a dynamic remodeling of the cytoskeleton and gene expression profile that may lead to hypertrophy and provides mechanical protection from injury by increasing stability of the sarcomeres" *(Peters et al., 2003)*.

In another study, Butterfield et al. (2005) illustrated that increasing sarcomere number in series has an effect on muscle performance by increasing shortening velocity and power of the muscle. Forty-eight male rats aged 150 days walked on an animal treadmill with an incline or decline of 16° at a target speed of 16 m/min. Animals were divided randomly into six experimental groups. The first group designed to directly measure fiber dynamics in the VL during uphill or downhill walking. EMG, sonomicrometry signals and a video camera were used to record the kinematics of the walking rats. Sixteen rats were randomly divided to the second and third group, to determine the effects of incline or decline walking at 16°, for 5 days. These two groups exercised for 15 minute on the first day and gradually increased this by 5 min each day for 5 days in total (35 minute total). Exercise protocols for the fourth and fifth groups were identical to the second and third groups, however, the training period was doubled (10 days instead of 5), to assess the potential effects of time on sarcomere number adaptations. The final group was sedentary and served as a control group. Serial sarcomere numbers in the vastus lateralis and vastus intermedius muscle, as well as fiber dynamics of vastus lateralis, were measured after the incline or decline protocols. The results showed that the vastus lateralis and vastus intermedius lost in series sarcomere number as a result of concentric contraction due to uphill waking. Conversely, the vastus intermedius muscle gained a greater number of sarcomeres as result of downhill walking due to eccentric contraction. The authors found significant differences in sarcomere adaptation compared to the control group after only 10 days of either incline or decline training. The authors concluded that all four knee extensor muscles have been assumed to have similar fiber strains, although the vastus intermedius has a greater pennation angle and shorter fiber length as well as a different fiber type to the vastus lateralis. Consequently the slow-twitch vastus intermedius may be selectively recruited during unconstrained downhill walking and therefore it is possible that the magnitudes of fiber strain in the vastus intermedius and vastus laterais are different, as well as the different serial sarcomere number adaptations. In the other words, differences in muscle architecture could result in different adaptations in knee extensor muscles after uphill and downhill walking in rats. These different adaptations appear to be related to contraction type, and may influence the magnitude of fiber strain and/or injury *(Butterfield et al., 2005).*

Only one study to date has reported different results. Koh and Herzog (1998) studied the number of sarcomeres in the dorsiflexor rabbit muscle after eccentric contraction. Six mature rabbits were trained on a stepping motor device. The foot was fixed to a footplate and the cuff nerve was stimulated to super maximal intensity. Ankle joint range of motion was around 70 to 105 degrees, while full plantarflexion was 180°. Stimulation was applied during ankle plantarflexion at 70°/s. This caused an eccentric contraction of the dorsiflexor muscles. Animals were exercised by performing 50 maximal eccentric contractions for 12 weeks and 2 times per week. Each exercise session consisted of 5 sets of 10 repetitions, with two minutes rest between sets. The superficial and deep surfaces of the tibialis anterior fascicle, and proximal and distal parts of the extensor digitorum longus fascicle were tested. Fascicle length, sarcomere length and number of sarcomeres were measured using a laser diffraction system. Their results showed a 3% increase in sarcomere numbers on the superficial fascicles of the tibialis anterior muscle. Sarcomere number did not change in the deep fascicle of the tibialis anterior, and also no change was observed in the proximal or distal extensor digitorum longus muscle. The authors suggested that eccentric contraction has little or no effect on the addition of serial sarcomere number in these species. These results were not in line with previous assumptions that eccentric training produces increases in sarcomere number. The differences could be related to the insufficient training protocol used in this study, which did not induce an increase of serial sarcomeres. The authors concluded that artificial activation of the muscle may not have provided suitable stimuli for sarcomere adaptation, and neural input is not required for adaptation *(Koh and Herzog, 1998).*

As mentioned in the introduction, the dynamics of the muscle fiber is different to that of the whole muscle tendon unit. In this regard, Butterfield and Herzog (2005) suggested that the application of fiber strains estimated *in vitro* and *in situ* might not be suitable when applied *in vivo*. The authors mentioned that fiber strain is smaller than muscle-tendon unit strains and “great fiber strains can occur while the muscle-tendon unit is shortening” *(Butterfield and Herzog, 2005)*. According to these results, in another experimental study on animals, the effect of eccentric contraction on serial sarcomere adaptation has been directly examined by the same authors *(Butterfield and Herzog, 2006a)*. Forty-five rabbits were randomly assigned to 6 different groups. All rabbits were then sub-divided to one group undergoing an acute exercise protocol (n=24) and the other group undergoing a chronic exercise protocol (n=21). The acute protocol was designed to directly measure fiber strain, joint torque, and muscle injury. For the first group (n=11), plantar flexor muscles were stimulated at the onset of the stretch, with lengthening contractions starting at a short muscle length (70° tibiotarsal joint angle). The second group (n=7) involved stimulation, which preceded the stretch by 100 ms with lengthening contractions starting at a short muscle length (70° tibiotarsal joint angle). The third group (n=6) involved stimulation preceding the stretch by 100 ms with lengthening contractions starting at a long muscle length (95° tibiotarsal joint angle). In order to homogenize mechanical stimuli, three chronic exercise groups identical to the acute protocols described above were designed to subject the tibialis anterior of the rabbit to 18 exercise bouts in total. All rabbits were anaesthetized and placed with the knee joint at 90°. The foot was fixed to a servomotor footplate and ankle movement was controlled via motion planner software. For the acute groups, a post-exercise torque-angle relationship was achieved following the single exercise bout. For the chronic exercise groups, each rabbit was exercised for 6 weeks and 3 times per week. One week after the last exercise bout, serial sarcomere number and torque–joint angle relationships were determined for the 21-tibiotarsal joint angles, for direct comparison with the values of pre measurement. The results showed that the number of sarcomeres were not identical and not uniform in all regions of the tibialis anterior muscle in the chronic group, and between protocols. The authors concluded that, muscle tendon unit strain is not the primary cause for adding sarcomeres in series. Since four distinct regions of the tibialis anterior revealed different adaptations in sarcomere number and may be explained by the different architecture of the individual fibers. The authors concluded that, fiber strain is a more powerful stimulus than muscle tendon unit stress and strain for sarcomere adaptation, if the starting muscle length and activation timing prior to stretch are carefully adjusted. Additionally, the authors suggest that peak torque and stored elastic energy of the serial elastic elements are the best predictors of serial sarcomere number *(Butterfield and Herzog, 2006a)*.

It has also been documented that exercise involving lengthening of an activated muscle can cause injury. Extensor digitorum longus muscles of rabbits were used to quantify the cytoskeletal protein desmin after eccentric contractions *(Fridén and Lieber, 1998).* The animals completed an exercise protocol involving either an eccentric or isometric protocol for 30 minutes. Muscle function properties were measured before and 1 h, 1 day, 3, 7, and 28 days after repetitive eccentric contractions. The isometric group performed isometric contractions to estimate the muscle torque. In the eccentric exercise group, a dual-mode servomotor was used to move the ankle joints from 100° to 70° over a 400 ms period (stretch), and then returned them to the starting position (shortened). This intervention was repeated every 2 s for 30 minute, resulting in 900 eccentric contractions. After the experimental period, the animals were anesthetized and the extensor digitorum longus muscle was removed. The results showed a significant increase in fiber size, from 1–7 days after exercise. In addition, cytoskeletal disruptions, loss of myofibrillar registry, i.e., Z-disk streaming and A-band disorganization, and loss of cell integrity as manifested by intracellular plasma fibronectin stain, hypercontracted regions, and invasion of cells were shown. The authors concluded that, lengthening activity caused muscle injury due to a loss of the cytoskeletal protein desmin and cellular integrity. Muscle injury after eccentric exercise is different due to different muscle architecture, which can be fiber type-specific, particularly because of fiber strain in the acute phase *(Fridén and Lieber, 1998).*

Barash et al. (2002) studied the effects of eccentric contractions on desmin cytoskeletal content. The tibialis anterior muscles of 34 rats were analyzed after a bout of 30 eccentric contractions. Ankle isometric torque was measured before eccentric exercise, immediately after eccentric exercise, and after a specified recovery time of 1/4,1/2, 1, 2, 3, 5, or 7 days. The training intervention included 30 eccentric contractions on the dorsiflexors, by a dual-mode servomotor, at 2-minute intervals by stimulating the peroneal nerve for 650 ms at 100 Hz, with a small nerve cuff. The contralateral leg served as the control. The results showed that isometric torque decreased by around 50% immediately after a bout of eccentric exercise, and fully recovered 7 days later. Desmin staining disappeared fully 12 h after the eccentric contractions and recovered by 3 days. The authors concluded that this dynamic restructuring of the muscle's intermediate filament system may be involved in the mechanism of eccentric induced muscle injury, and may provide a structural explanation for the protective effects observed in muscle after a single eccentric bout. In other words, the increase in cytoskeletal protein content leads to an increase in the rigidity of muscle fibers and the entire muscle. The increased stiffness of muscle fibers caused an ability of the muscle to increase its resistance against muscle damage, without necessity an increase in the number of sarcomeres in series *(Barash et al., 2002; 2004).*

In summary, the review of literature with animal studies demonstrates the longitudinal plasticity of muscle could occur not only by immobilization, but also after a period of eccentric exercise. In most of these studies, animals were exercised on a small treadmill at either an incline or decline. Sarcomere length, sarcomere number and torque generated, were measured in different muscle groups (vastus intermedius, vastus lateralis, tibialis anterior and extensor digitroum longus muscle).

The main results of these studies included: (A) downhill running, designed to induce an eccentric contraction, increased the number of sarcomeres in the muscle fascicle *(Lynn and Morgan, 1994; Lynn et al. 1998)*. Peak isometric torque decreased after eccentric contraction. Moreover, myogenic regulatory factors and desmin transcripts increased after eccentric contraction, which increased the stability of the sarcomere *(Peters et al. 2003; Barash et al. 2002; Barash et al. 2004)*. (B) Differences in muscle architecture results in different adaptations in the knee extensor muscles following eccentric contraction. This appears to be related to the contraction type and the magnitude of fiber strain and/or injury *(Butterfield et al., 2005).* (C) Artificial activation of muscle may not provide a suitable stimuli for sarcomere adaptation, and neural input is not required for adaptation to occur *(Koh and Herzog, 1998)*. (D) Fiber strain is a more powerful stimulus than muscle-tendon unit stress and strain for sarcomere adaptation. Also peak torque and elastic energy storage of serial elastic elements are the best predictors of serial sarcomere number in the muscle *(Butterfield & Herzog, 2006a).*

2.2.2 Human experiments

Although studies using animal models provide information to determine the mechanical stimuli that most influence longitudinal plasticity of muscle after eccentric training *(Butterfield and Herzog, 2006a; Morgan and Proske, 2004),* they lack information with regards to human muscles.

In only one study, by Herzog et al. (1991), the authors found that the differences in the force-length relationship between professional runners and cyclists may be explained, in part, by differences in serial sarcomere number. Eight athletes took part in this study. The first group included 3 cyclists plus one speed skater and the second group contained 4 runners. Three testing sessions were designed to analyze the moment-length relationship of the rectus femoris muscle. Each participant performed 13 knee extension MVC maneuvers for each session (39 in total) on a Cybex dynamometer machine. Knee angles were 100, 135, 170° and hip angles 90-170° at 10° intervals. Full extension was defined for both joints as 180°. The results showed considerable differences between the moment-length (i.e., changes in moments revealed as a function of changes in muscle length) relationship of the rectus femoris muscles of cyclists and runners. Cyclists tended to be stronger at the short rectus femoris length compared with greater lengths, whereas the opposed was true for runners. The authors concluded that the moment-length relationship of skeletal muscles might adapt to their functional requirements over long periods. Moreover such an adaptation could have been due to stimulation, activation, or muscular factors (e.g. serial sarcomere number). The findings of this study may be explained, partially, by training stimuli which caused an adaptation of serial sarcomere number *(Herzog, et al., 1991).*

The effect of eccentric exercise on human hamstring muscle adaptation has been examined by Brockett et al. (2001). Ten participants consisting of 8 males and 2 females were analyzed before, immediately after and daily, up to 8 days post exercise. The training protocol involved 12 sets of 6 repetitions of eccentric exercise, using special equipment for the hamstring muscles. The device consisted of a 2-m long wooden board. The participants kneeled on the board and had their ankle stabilized with straps. Participants slowly lowered their body against the force of gravity towards the prone position, while maintaining an open and constant hip angle. To quantify hamstring angle-torque curves, maximum voluntary knee extension and flexion was measured using an isokinetic dynamometer system. Six subjects did a second bout of eccentric exercise after 8 days, and measurements were taken 10 days later. The results showed that torque generation had a significant shift in optimum angle towards longer muscle lengths immediately after exercise (7.7° ± 2.1°) as an indirect result of disruption to sarcomeres in muscle fibers. This change in optimum angle where maximum torque was applied indicated an increase in series compliance within some muscle fibers of the hamstring muscles. This shift remained even after other muscle injury parameters had returned to normal. The authors suggested this was a consequence of training. "The shift indicated a training effect, providing the muscle with protection against further damage from eccentric exercise" *(Brockett, et al., 2001).*

In another study by Blazevich et al. (2003), resistance training with a focus on eccentric contraction was investigated to see changes in muscle size, muscle architecture, strength, and sprint/jump performances on professional athletes. Twenty-three participants (8 female and 15 male) were divided into three training groups and they completed 5 weeks specific training phase. The first group performed squat lift training (SQ, n=8) two times per week plus one session of sprint/jump training, the second group performed forward hack squat training (FHS, n= 7) two times per week plus one session of sprint/jump training, and the third group performed sprint/jump training only (SJ, n= 8), four times per week. Participants in the SQ group used the barbell back squat at a 1.57 rad (90°) knee angle. The eccentric phase was performed in approximately 2 seconds, whereas the concentric phase of the squat was performed at maximum velocity such that the subjects' feet left the ground for six maximum repetitions (RM), approximately 85– 90% 1-RM. FHS participants used the one-legged FHS exercise, in contrast to SQ group, with 110° internal knee angle and 90° hip angle at the lowest point of the lift on their dominant leg during the specific training phase. Participants in the sprint/jump (SJ) group did not perform weight training during the 5-wk specific training phase. Muscle size, pennation angle, and fascicle length of the vastus lateralis (VL) and rectus femoris (RF) muscles (assessed by ultrasonography method) as well as 20-m sprint run, vertical jump, and strength performance changes were analyzed before and three days after the last training session. The results showed approximately a 15% increase in fascicle length, particularly in the VL for SJ subjects, who performed only high-velocity training, and a decrease in pennation angle in this group. In SQ and FHS groups, there were small, but consistent increases in VL and RF (except proximal of RF in SQ subjects) pennation angles, without changes in fascicle length. Muscle thickness of the VL and RF increased only at proximal sites, in all training groups. There were not any differences between groups in squat, forward hack squat, or isokinetic strength performances, or in sprint or jump performances. The authors concluded that muscle architecture adaptations occurred rapidly in response to the training programs imposed on the athletes, and these changes were related to the force and/or velocity characteristics, but not to the movement patterns of the exercises *(Blazevich, et al., 2003).*

In another study by Alegre et al. (2006) muscle architecture, isometric and dynamic strength changes after dynamic resistance training were studied. Thirty male participants volunteered in this study. They were divided into an experimental group (n = 16) and a control group (n = 14). The training protocol consisted of the free-weight half (90°) squat lift. The participants performed three to four sets of 6 – 12 repetitions, over 13 weeks. Training intensity during the first 2 weeks was 30% of 1-RM with three sets of 12 repetitions. It was 50 – 60% of 1-RM for weeks 3 to 13 and was progressively increased until week 9. The volume of training was modified by reducing the number of repetitions per set from 12 to 6 or 8. The concentric phase of the squat was performed explosively while the eccentric phase lasted approximately 1 second. Maximal dynamic strength, isometric strength and skeletal muscle architecture were analyzed in both groups, before and after the training period. The result showed significant increases in vastus lateralis muscle thickness (+6.9%), fascicle length (+10.3%), one-repetition maximum (+8.2%), rate of force development

(+23.8%), and average force produced in the first 500 ms (+11.7%), only in the training group. The authors concluded that, "dynamic resistance training with light loads leads to increases in muscle thickness and fascicle length, which might be related to a more efficient transmission of fibre force to the tendon" *(Alegre, et al., 2006).*

Blazevich, et al. (2007) examined the influence of different types of contraction on fascicle length in the quadriceps muscles of humans. Thirty-three participants volunteered for this study, with 12 men and 12 women, being divided to one of two training groups and 9 participants acting as a control group. Participants in the training groups performed four to six sets of six maximal concentric or eccentric knee extension exercises, 3 times per week for 10 weeks, on a Biodex isokinetic dynamometer. The training protocol for participants in the concentric group included performing knee extension exercise "as fast and hard as possible". The dynamometer lever arm was set to move at 30°/s, from maximum knee flexion to maximum knee extension, approximately 100° knee range of motion. The eccentric group applied a maximum upward force to stop the dynamometer lever arm at 30°/s from an extended (10 –15°) leg position, until the contractions were completed at a knee angle of 100°. Strength and quadriceps muscle size, in addition to vastus lateralis muscle architecture of the participants, were measured before training, 5 and 10 weeks after the training period and again after 14 weeks of detraining. Muscle architecture was measured *in vivo* by calculating anatomical and physiological cross-sectional area and muscle volume. This was measured by B-mode ultrasonography and MRI scanning. The results showed that fascicle length of the vastus lateralis increased after 5 weeks of training in both groups (Δ Concentric= +6.3 ± 3.0%, Δ Eccentric= +3.1 ± 1.6%, mean= +4.7 ± 1.7%; $p < 0.05$). It was also shown that, vastus lateralis fascicle length was not increased after 10 weeks, although a small increase (mean ~2.5%; not significant) was evident after detraining. Both training groups showed a significant increase in peak torque after 5 and 10 weeks of training. After 10 weeks of training, whole quadriceps muscle volume increased from 2,342.9 ± 111.2 cm^3 to 2,586 ± 114.8 cm^3. These data suggest a close association between vastus lateralis fascicle length and shifts in the torque-angle relationship through training and detraining. The torque-angle relationship shifted to the right (i.e. greater muscle length) in the first 5 weeks of training but did not shift from weeks 5 to 10, and these changes were not group-dependent. The results showed that, a 1% increase in fascicle length was related to approximately a 1% shift in the normalized torque-angle relationship. This may provide further evidence that fascicle length adaptations directly influence the force-length relationship in human muscle, and that the range of motion of the training exercises is the strongest influencing factor for fascicle length adaptations. The authors concluded that, eccentric and concentric resistance training caused rapid changes in muscle architecture, particularly changes in fascicle length. The contraction mode was shown not to influence the lengthening of muscle fascicles, but the training range of motion most likely has an effect on adaptations of fascicle length; whilst contraction velocity probably has little effect *(Blazevich et al., 2007a).*

In another study, Reeves, et al. (2009) studied the muscle structural and strength adaptations after eccentric contractions with higher loads compared with conventional resistance training. Nineteen volunteers were divided in two training groups. Five females and four males were assigned randomly to the first group as a conventional resistance training group (CONV), with the remaining ten participants (5 females and 5 males) assigned to the second group as an eccentric-only (ECC) training group. Both groups completed bilateral exercise training for 14 weeks and 3 times per week, using knee extension and leg press exercises. The CONV group trained with two sets of 10 repetitions, at 80% of five-repetition maximum (5RM). The ECC group trained with knee flexion exercise under eccentric contractions during 90° knee angle (full knee extension is equal to zero degree). They completed two sets of 10 repetitions at 80% of the 5 RM. Maximal knee extensor torque was evaluated during isometric, concentric and eccentric contractions. Ultrasonography was used to assess vastus lateralis fascicle length *in vivo* at rest. The results showed a significant increase in vastus lateralis fascicle length in both groups, but were greater in the ECC (20%) compared to the CONV group (8%). Eccentric knee extensor torque increased by 9–17% in the ECC group at all velocities, but concentric torque was unchanged. Therefore, the two training methods caused different adaptations in vastus lateralis fascicle length and muscle strength. The authors suggests that "the stimulus for adding sarcomeres in-series and in parallel may be different, which implies that different myogenic responses were induced by the two different training methods" *(Reeves et al., 2009).*

Potier, et al. (2009) investigated changes in fascicle length in the biceps femoris, and knee range of motion following eccentric strength training. Twenty-two participants were recruited voluntarily to an experimental group. They trained the hamstring muscles eccentrically. For this purpose, participants were instructed to lift a given weight with their non-dominant leg to end range of motion. The weight was then moved to the dominant (experimental) leg and lowered over a 5 second period. This was called the one repetition maximum (1 RM) of eccentric hamstring strength. The number of repetitions that could be performed at the 1 RM weight over the full knee range of motion then increased in the subsequent training. Following 8 weeks of the training, subjects aimed to complete three sets of eight repetitions each at a weight corresponding to the initial 1 RM. Hamstring muscle strength (1 RM), passive knee extension (knee joint angle at which the onset of passive tension occurs), fascicle length and pennation angle of the dominant leg were measured before and after the training protocol. An isokinetic machine (Cybex) combined with ultrasonography were used for the measurements. The results showed a 34% increase in hamstring strength, a 5% increase in passive knee joint range of motion, a 34% increase in vastus lateralis fascicle length and no changes in vastus lateralis pennation angle *(Potier et al., 2009).*

As previously stated, the dynamics of the muscle fiber is different to that of the whole muscle tendon unit. In this regard, the effect of eccentric strength training on both muscle fascicle length and tendinous structures was examined by Duclay et al., (2009). Eighteen male participants were assigned randomly to an eccentric training group (n = 10) or a control group (n = 8). The training protocol consisted of one training session each week on a calf machine, in addition to two other sessions on a leg press. This type of leg exercise was designed to induce adaptations in both the soleus and gastrocnemii muscle. The training load was 6 sets with 6 repetitions in each set, at 120% of one maximal concentric repetition, for 18 sessions over a 7 week period. For each session, participants performed a 3 second eccentric contraction, starting from the position of complete plantar flexion. The duration of exercise was controlled by a metronome. The ankle range of motion was from 50° to 60°, which corresponded to a mean angular velocity of 15°–20°/s. The fascicle length, pennation angle and muscle thickness of the medial gastrocnemius (MG) were analyzed at rest, at 50% of maximal voluntary contraction (MVC), and during MVC, using ultrasound imaging. All participants were measured before and 3–4 days after the last eccentric training session (during the eighth week). Ramped isometric contractions were used to measure tendon elongation by tracking the proximal displacement of the myotendinous junction of the medial gastrocnemius. The slope of the load–deformation relationship of the MG tendon above 50% MVC was defined as an index of stiffness, during ramped isometric contraction. The results showed an increase in maximal isometric plantar flexor strength (13%) following the period of eccentric training. Pennation angle and fascicle length were significantly increased at rest (average +7.6% and +6.8%, respectively), after the training period. The stiffness index was increased above 50% MVC, after eccentric training. These findings imply that eccentric training may change the cross-sectional area of the tendon and/or internal structures of the tendon. Further, shortening of the fascicles had a significant linear relationship to displacement of the myotendinous junction for both the control and training group. The authors concluded that the behavior of muscle architecture and the mechanical properties of the myotendinous junction are effected differently by eccentric training *(Duclay et al., 2009)*.

In summary, only few studies have reported the longitudinal plasticity of muscle during exercise in human muscle. Mechanical properties (force-velocity-length relationship) as well as morphological properties (fascicle length, muscle thickness and pennation angle) of the muscle have been measured by dynamometer, ultrasound and MRI scanning techniques. These variables have been tested on different muscle groups particularly the vastus lateralis, biceps femoris and rectus femoris. The main results of these studies include: (A) force-length relationships of skeletal muscle may adapt to the functional requests that are chronically asked of them. Training stimuli can in part, cause an adaptation of serial sarcomere number in the muscle *(Herzog et al., 1991)*. (B) There is a shift in the optimum angle at which maximum torque is produced, towards longer muscle lengths, as an indirect result of disruption to sarcomeres in the muscle fascicle *(Brockett et al., 2001).* (C) Muscle architecture adaptations occur rapidly in response to training imposed on athletes. These changes are related to the force and/or velocity characteristics of the muscle, but not to the movement patterns of the exercises *(Blazevich et al., 2003)*. (D) Resistance training with light loads increases muscle thickness and fascicle length of the muscle, which might be related to a more efficient transmission of fiber force to the tendon *(Alegre et al., 2006)*. (E) Eccentric and concentric resistance training causes rapid changes in muscle architecture, and in particular they modify fascicle length. Additionally, contraction mode does not influence the lengthening of muscle fascicles, but the training range of motion most likely effects the adaptation of fascicle length, whilst contraction velocity probably has little effect *(Blazevich et al., 2007a)*. (F) “The stimulus for adding sarcomeres in-series and in parallel may be different, which implies that different myogenic responses were induced by the two different training methods” *(Reeves et al., 2009).*

3. The goal of study

Longitudinal plasticity of muscle is one well described phenomena of muscle strategy to increase force and power production *(Morgan, 1990; Williams and Goldspink, 1978)*. Several studies have addressed the morphological and mechanical adaptations of muscle after a period of eccentric training *(Blazevich et al., 2003; Ishikawa and Komi, 2008; Potier et al., 2009; Reeves, et al., 2006).*

According to the literature and the popping sarcomere hypothesis, eccentric contraction leads to an increase in the number of sarcomeres in series and may prevent injury to muscle fibers *(Morgan and Proske, 2004),* by optimizing muscle length.

There are several investigations that report the effects of immobilization in a lengthened or shortened position, on muscle fiber length and sarcomere numbers in animals, either after a static or dynamic stimulus *(Goldspink et al., 1974; Lynn and Morgan, 1994; Williams and Goldspink, 1978).* The results demonstrate that limb muscles immobilized and held in a lengthened position, lead to an increase their number of sarcomeres as well as decrease the corresponding sarcomere length. The opposed adaptations were also true when the muscle was immobilized in a shortened position *(Coutinho et al., 2004; Goldspink et al., 1974; Sayers et al., 2003; Williams and Goldspink, 1978).* In relation to eccentric contractions on animal subjects, Lynn and Morgan (1994) were the first investigators to study serial sarcomere number in muscle with dynamic movement. Animal hind limb muscles which were trained with downhill or uphill running, illustrated a significant adaptation by increasing or decreasing serial sarcomere number *(Lynn et al., 1998; Lynn and Morgan, 1994).* More recently, another study by Butterfield et al. (2006) showed an increase in series sarcomere number, on animals' muscles, which had a positive effect on muscle performance. This implies that longer muscles, which may result from an eccentric stimuli, can exhibit more maximal mechanical power and higher maximum shortening velocities. The idea that adaptation to eccentric exercise consists of adding sarcomeres in series, has been examined *in vivo* and a few studies have reported changes in fascicle length in human muscle *(Blazevich et al. 2007; Reeves and Narici 2003).*

According to literature, different contraction types *(Blazevich et al., 2007a; Brockett et al., 2001; Reeves and Narici, 2003),* load of eccentric contractions *(Reeves et al., 2009)*, muscle range of motion during eccentric strength training *(Potier et al., 2009)*, and force as well as velocity characteristics of the muscle *(Blazevich et al., 2007a)* provide evidence for longitudinal adaptation. However, it is not clear which of the eccentric stimuli can affect the mechanical properties of human muscle. Furthermore, there is still limited data reporting the effects of controlled loading of eccentric stimuli in human muscle.

In light of the above considerations and limitations of previous studies, the present study aims to assess the effect of controlled eccentric loading on the longitudinal adaptation of muscles in humans. Therefore the purpose of this study was to investigate the effect of magnitude, velocity and the range of the applied eccentric stimulus on the longitudinal adaptation of the vastus lateralis muscle.

4. Hypothesis

The hypothesis suggests that with an equal volume of stimuli:

1- A greater magnitude of eccentric contraction of the vastus lateralis muscle will have an inferior influence on the longitudinal adaptation of the VL muscle.

2- A greater lengthening velocity of the vastus lateralis muscle will have an inferior influence on the longitudinal adaptation of the VL muscle.

3- A higher range of motion of vastus lateralis muscle which eccentric stimulus is applied will have an inferior influence on the longitudinal adaptation of the VL muscle.

5. Methodology

5.1 Experimental design

Fifty-three sport students volunteered to participate in this study. All participants were free from abnormality and none had suffered from neuromuscular or musculoskeletal injury within one year prior to the experiment. Participants also took part in no more than four vigorous exercise sessions per week. Participants were randomly assigned to one of two experimental groups or one control group. The experimental groups performed eccentric exercise on the knee extensors for 10 weeks, 3 times per week. The control group did not take part in any specific training. Twenty-two participants withdrew from the study whilst thirty-one participants (10 in each experimental group and 11 in control group) finished the intervention successfully, and performed all pre and post measurements. Based on the data of Blazevich et al. (2007), Reeves et al. (2009) and Potier et al. (2009) regarding exercised induced changes in fascicle length due to eccentric training, we achieved a statistical power of ~0.90. All participants provided written consent regarding their participation after being informed of all risks, discomforts and benefits of being involved in the study.

For analyses of longitudinal adaptation and its effect on fascicle length in response to eccentric training, we investigated four different exercise protocols. For this purpose, we modified the magnitude of the eccentric stimulus, the lengthening velocity of the muscle and the muscle length at which the eccentric stimulus was applied. Figure 5-1 shows the experimental design of the current study and the number of participants. Each training session was implemented on an isokinetic dynamometer (Biodex 3).

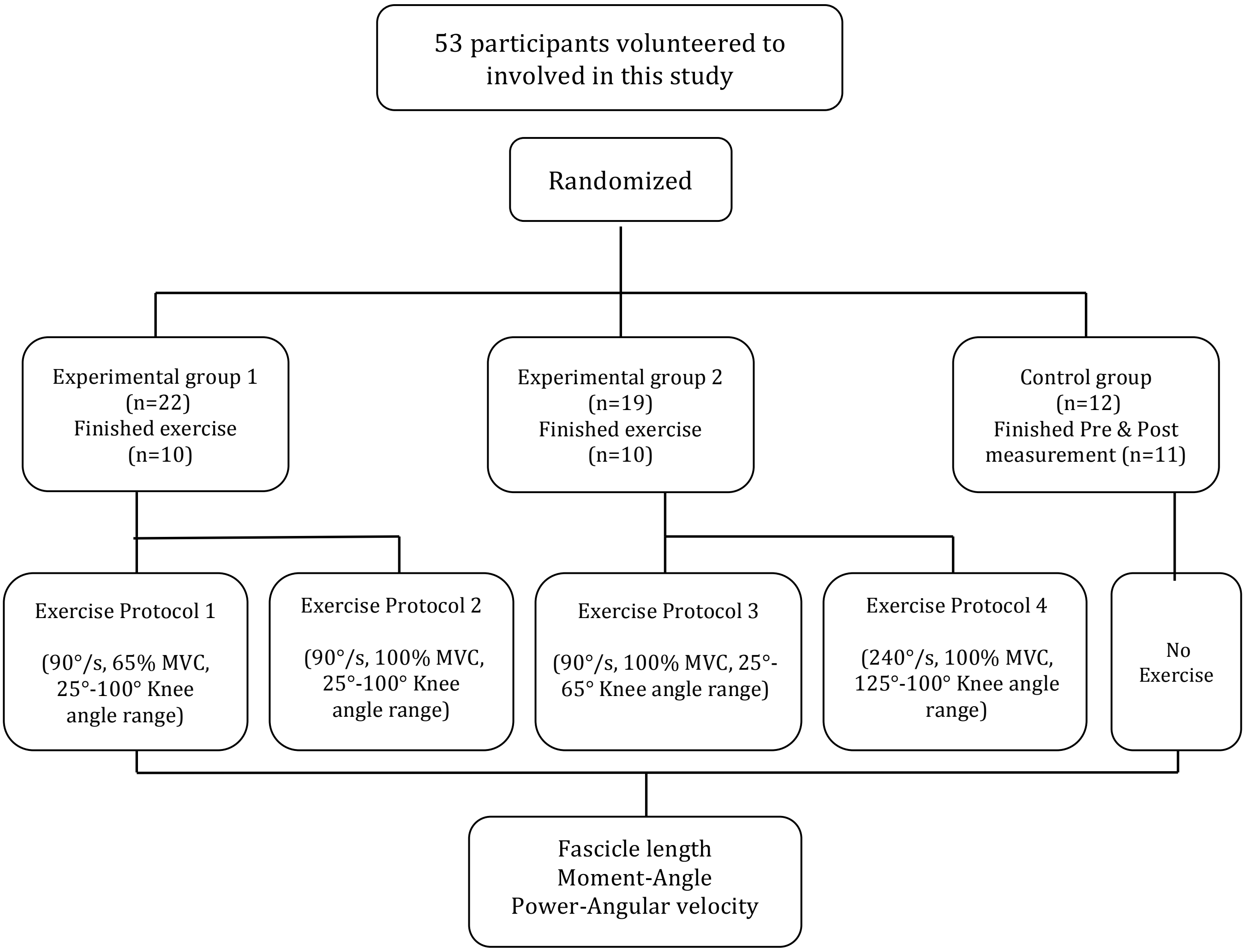


Figure 5-1: Experimental design

As shown in figure 5-1, the magnitude of the eccentric stimuli has been modified in exercise groups 1 and 2, whilst muscle length at which the eccentric stimuli is applied, and also the lengthening velocity of muscle, have been modified in exercise groups 3 and 4, respectively.

The participants in the first experimental group (age: 24.9 ± 4.3 y, body mass: 77.1 ± 7.4 kg, height: 183.5 ± 7.3 cm) exercised one leg at 65% of the maximum knee joint moment, as examined during a maximal isometric voluntary contraction (MVC) maneuver (exercise protocol 1: small magnitude of muscle force) and the other leg at 100% of the MVC (exercise protocol 2: high magnitude of muscle force). The training in both legs was conducted at a knee angular velocity of 90°/s and a knee angle range of 25° to 100° (full knee extension is equal to zero degrees). This knee joint range of motion used during the eccentric training is greater than that which commonly occurs during walking (around 25°; *(Biewener et al., 2004))* and jogging (around 55°; *(Biewener et al., 2004))* and slightly lower than the countermovement jump (around 80°; *(Bobbert et al., 1986))* and stair climbing (around 88° for 25.5 cm stair; *(Andriacchi et al., 1980))*. Consequently, the loading stimulus can be considered greater than the normal range of motion *(Blazevich et al., 2007a)*.

The total training volume (integral of the knee joint moment over the time) was equal in both protocols. Therefore in exercise protocol 1 (65% MVC), participants completed 5 sets per training day with 10 repetitions, and in exercise protocol 2 (100% MVC), 5 sets per training day with 6 repetitions (figure 5-2) and three minutes rest between each set. At the outset of every seventh training session during the intervention period, the MVC's of all participants were re-measured, and the relative values were updated.

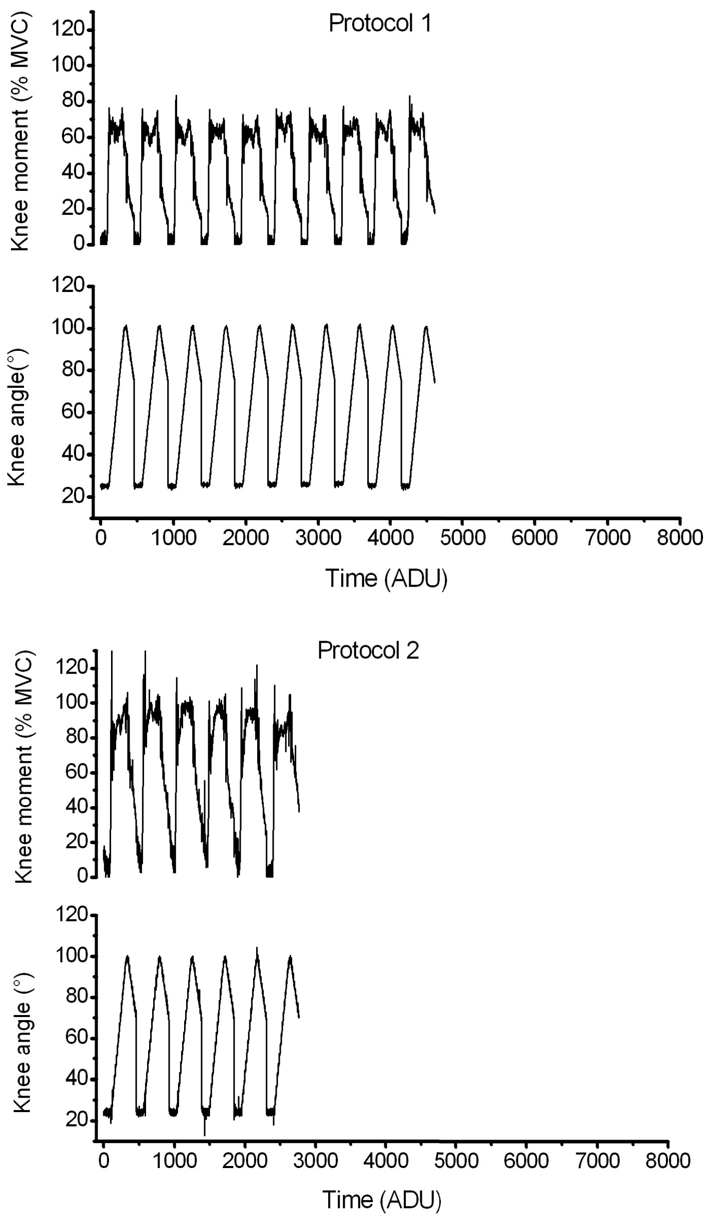


Figure 5-2: Knee joint moment and knee angle during exercise protocol 1 and 2 on each training day. Participants completed 10 repetitions of eccentric contractions in each set, in exercise protocol 1 (top graph), and 6 repetitions in exercise protocol 2 (bottom graph). One leg was trained with a low magnitude of muscle force (65% MVC, 90°/s, 25-100° knee angle amplitude) (top graph) and the other leg with a high magnitude of muscle force (100% MVC, 90°/s, 25-100° knee angle amplitude) (bottom graph).

The participants of the second experimental group (age: 29.3 ± 3.1 y, body mass: 77.1 ± 9 kg, height: 181.7 ± 7.4 cm) also performed eccentric training for 10 weeks, 3 times per week, 5 sets per training day. One leg exercised at a knee angle of 25° to 65°, in the ascending limb of the force-length curve of the vastus lateralis *(Herzog et al. 1991)* (exercise protocol 3: short muscle length). The training was performed at 100% MVC and at a knee angular velocity of 90°/s. The second leg trained at a higher knee angular velocity (240°/s), at 100% MVC and at a knee angle of 25° to 100° (exercise protocol 4: high lengthening velocity of muscle). Again, both legs were trained at the same exercise volume (integral of the knee joint moment over the time). In protocols 3 and 4, the participants performed 12 and 16 repetitions per set, respectively (figure 5-3), and repetitions were continuously performed within each set. Furthermore the exercise volume was similar to protocols 1 and 2, allowing a direct comparison of the results from all four protocols. The participants of the control group (n=11, age: 28.6 ± 4.7 y, body mass: 77 ± 7.7 kg, height: 180.2 ± 5.7 cm) did not execute any specific training during the 10 weeks.

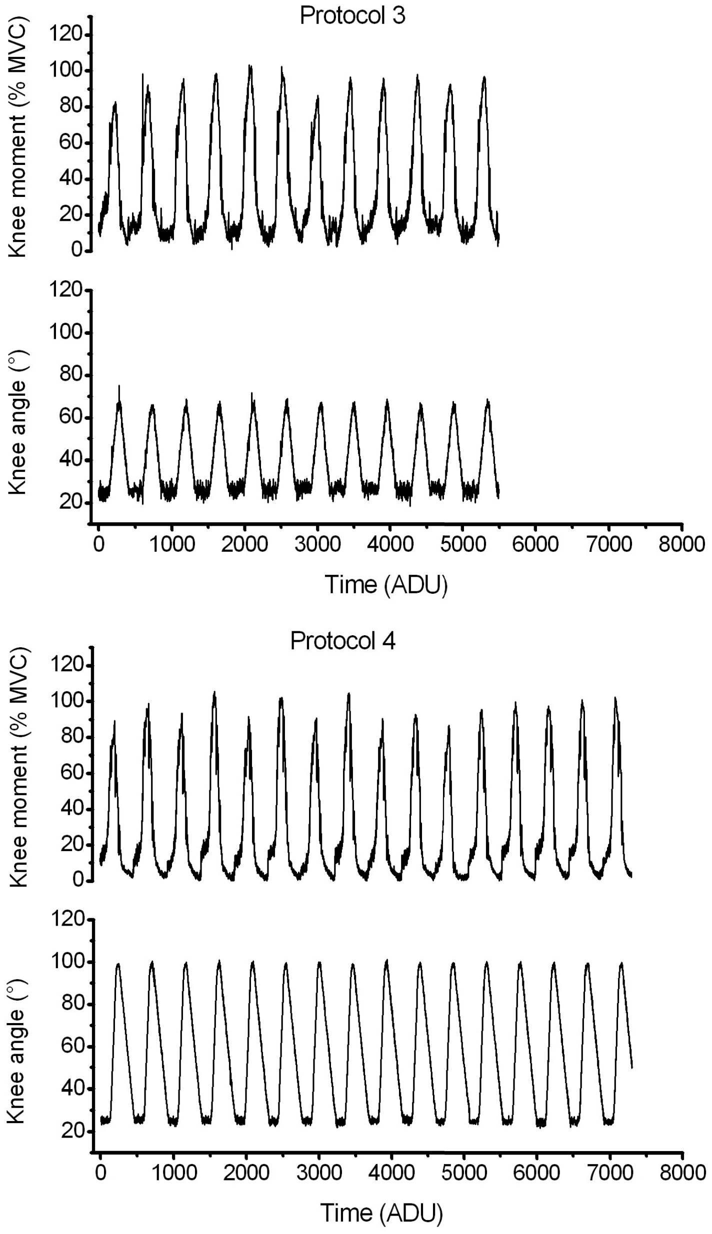


Figure 5-3: Knee joint moment and knee angle during exercise protocols 3 and 4, on each training day. Participants completed five sets of eccentric contractions. One leg was trained in a short muscle length position (90°/s, 100% MVC, 25-65° knee angle amplitude) (top graph) and the other leg with a high lengthening velocity (240°/s, 100% MVC, 25-100° knee angle amplitude) (bottom graph).

The participants' task was to resist against the lever arm of the dynamometer, by applying an upward force, whilst the dynamometer lever arm continued to move downwards for the whole defined knee range of motion. In each experimental group, the participant performed a knee extension contraction to achieve the trigger level (start point of dynamometer lever arm was around 35% of MVC). Following this, the downward movement of the dynamometer lever arm began (figure 5-4). This meant that the moment of the knee extensors was activated to the required pressure against the lever arm of the dynamometer, and then flexion of the knee joint was initiated in each repetition. In support of this, Butterfield and Herzog (2006) found that a pre-activation of the muscle leads to an increased stretch of the muscle fibers during eccentric contractions *(Butterfield and Herzog, 2006a)*. The participants had already completed this training procedure over the whole period of 30 sessions via a dynamometer, for which they received online feedback regarding the duration and magnitude of moment (figure 5-4).

The participants completed three isometric extension MVC maneuvers at 25, 65 and 100 degrees knee angle, before and after each of the seven training sessions to find the required target for eccentric training. Their maximum isokinetic moment was set as a target and displayed as a blue marked area on the screen. They were instructed to match the displayed lines on the monitor, representing their own target moment. This ensured that participants achieved the same intensity of eccentric contraction over the entire training period (10 weeks)(figure 5-4).

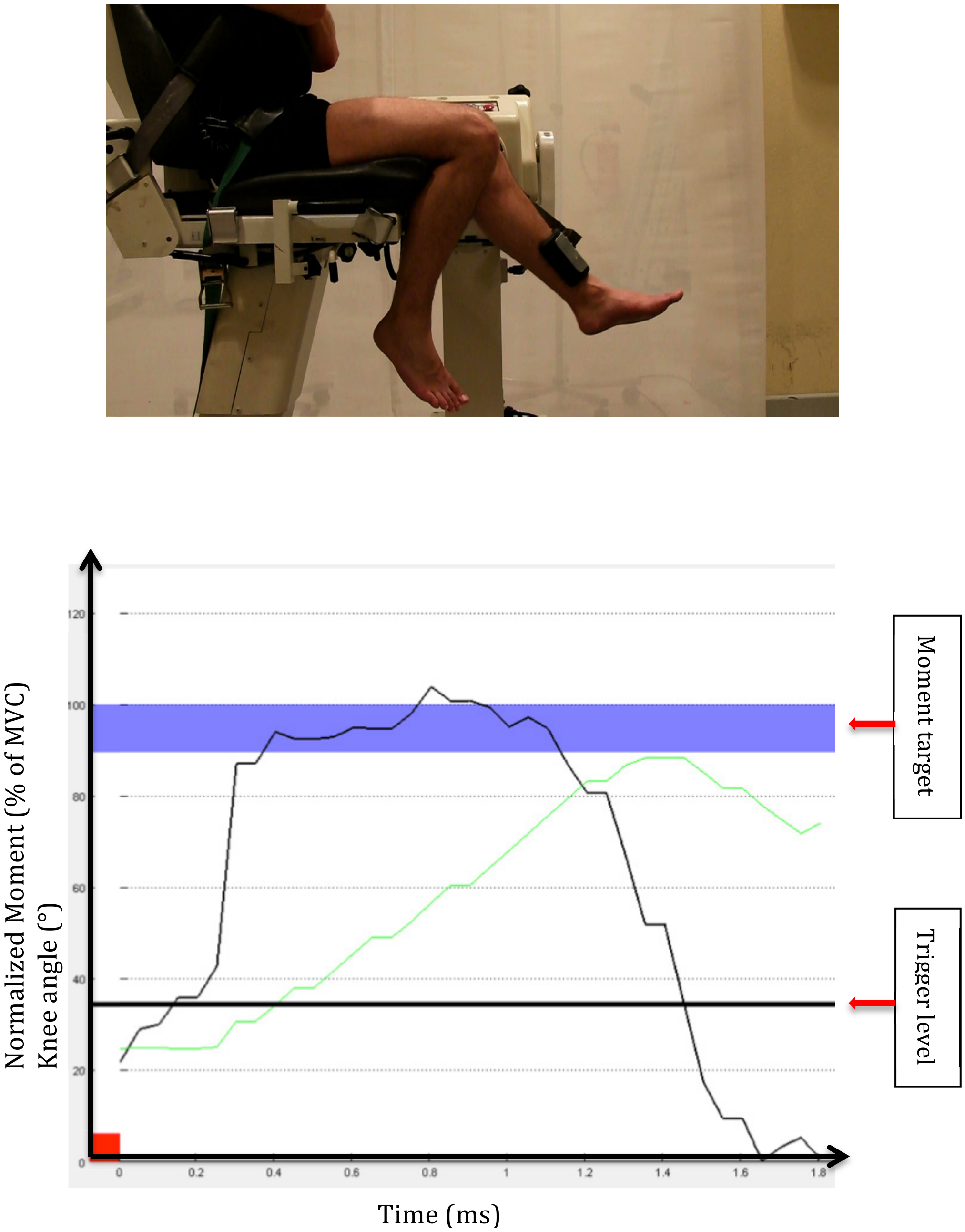
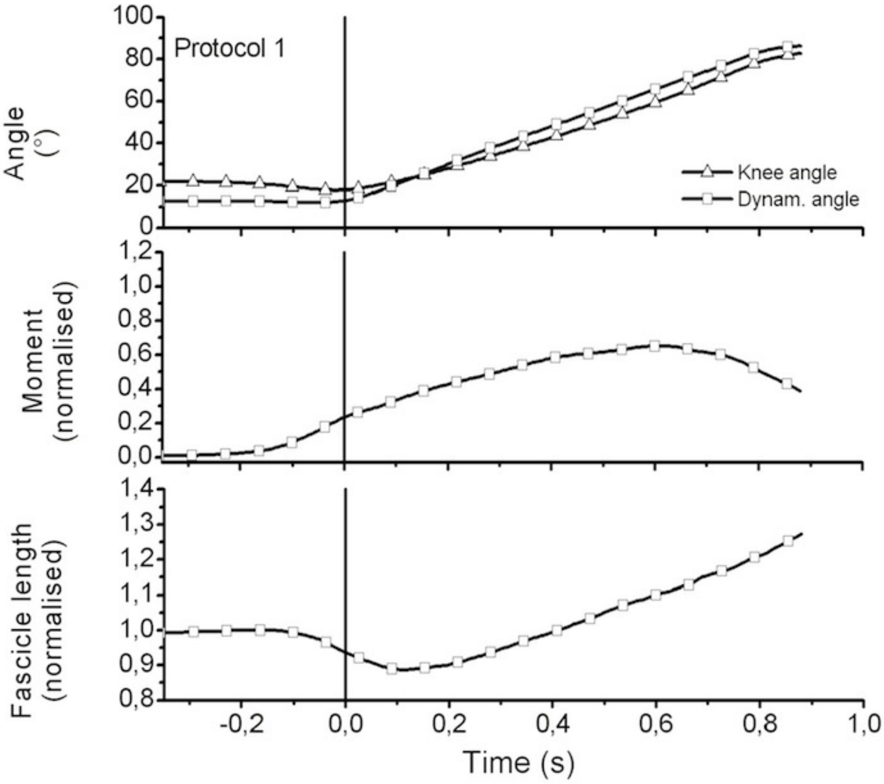


Figure 5-4: Seated position of the participant on the dynamometer (Biodex) during eccentric training (top picture) and its feedback monitor information (bottom graph). Here, the black line represents normalized moment (% of MVC) during one eccentric contraction over a defined knee range of motion. The green line shows knee angle (°) during the corresponding eccentric contraction. The blue area corresponds to the moment target that participants were instructed to reach during each eccentric contraction. The black horizontal line shows the trigger level

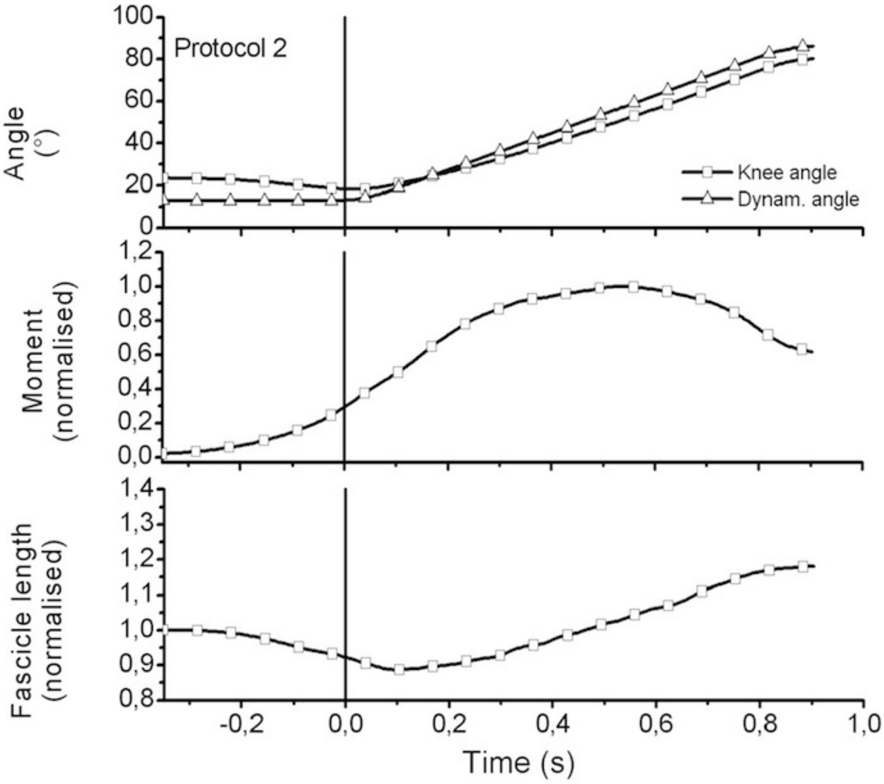
(35% of MVC), which is the starting point of the dynamometer lever arm. Note that the moment curve of the knee muscle was individually adjusted for the best resolution.

It is well known in the literature that the kinetics of the muscle fascicle is different to that of the whole muscle-tendon unit during eccentric contractions *(Butterfield and Herzog, 2006a; Butterfield and Herzog, 2005).* In this respect, the kinetics of fascicle length of the vastus lateralis during the four designed protocols were examined before the exercise interventions began, as a pilot study. As expected the fascicle length of the vastus lateralis exhibited different kinetics compared to the entire muscle-tendon unit, during four training protocols (figure 5-5). The pilot study highlighted four important findings across all protocols: (A) shortening of the vastus lateralis fascicle occurs before the movement of the dynamometer, (B) continuous shortening of the vastus lateralis fascicle despite knee flexion (i.e. lengthening of the MTU), (C) lengthening of the vastus lateralis fascicle with an increase in knee joint moment and (D) continuous lengthening of the vastus lateralis fascicle despite a reduction of knee joint moment (figure 5-5). In other words, flexion of the knee joint and associated lengthening of muscle-tendon unit results in concentric shortening of the vastus lateralis fibers at the start of dynamometer lever arm movement. This can be explained by the simultaneous elongation of the tendon.

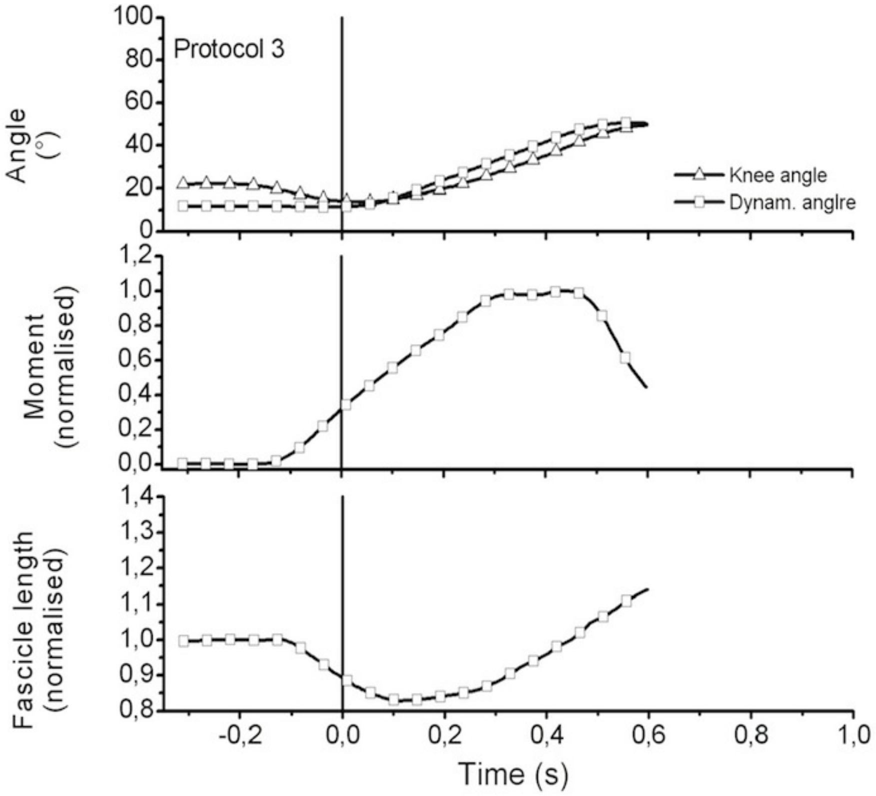
(A)


Protocol 1
Angle (°)
100
80
60
40
20
0
Knee angle
Dynam. angle
Moment (normalised)
1,2
1,0
0,8
0,6
0,4
0,2
0,0
Fascicle length (normalised)
1,4
1,3
1,2
1,1
1,0
0,9
0,8
-0,2
0,0
0,2
0,4
0,6
0,8
1,0
Time (s)

(B)


Protocol 2
Angle (°)
100
80
60
40
20
0
Knee angle
Dynam. angle
Moment (normalised)
1,2
1,0
0,8
0,6
0,4
0,2
0,0
Fascicle length (normalised)
1,4
1,3
1,2
1,1
1,0
0,9
0,8
-0,2
0,0
0,2
0,4
0,6
0,8
1,0
Time (s)

(C)


Protocol 3
Angle (°)
Knee angle
Dynam. anglre
Moment (normalised)
Fascicle length (normalised)
Time (s)
-0,2
0,0
0,2
0,4
0,6
0,8
1,0

(D)

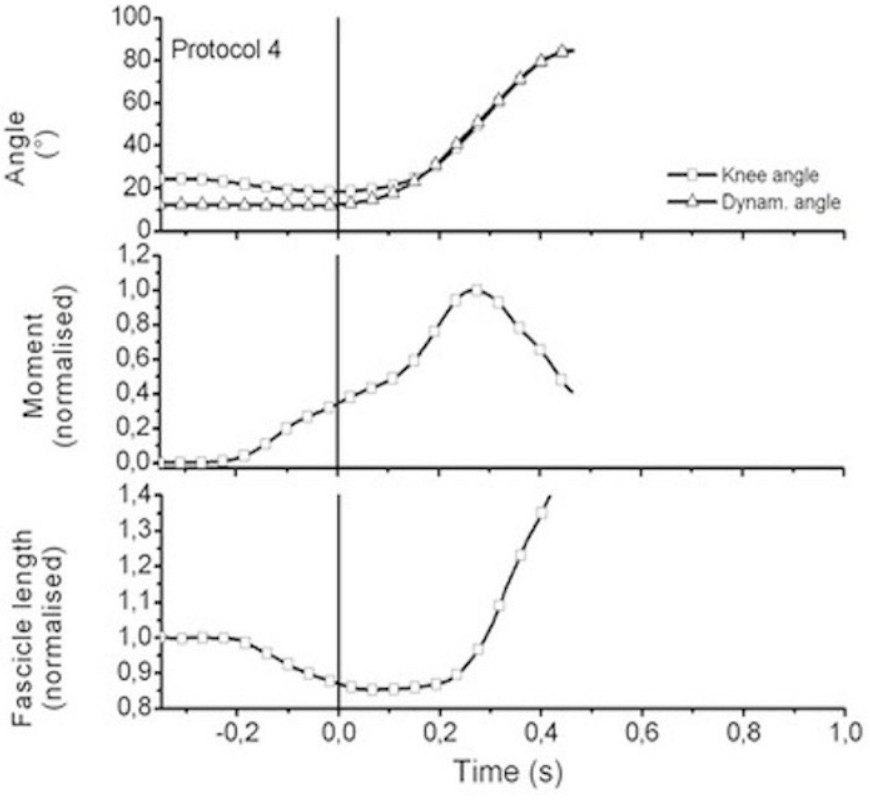

Protocol 4
Angle (°)
Knee angle
Dynam. angle
Moment (normalised)
Fascicle length (normalised)
Time (s)
-0,2
0,0
0,2
0,4
0,6
0,8
1,0

Figure 5-5: Time course of the knee angle and dynamometer arm angle. The normalized knee moment (moment normalized to the maximum isometric contraction) and the normalized fascicle length of the vastus lateralis muscle (fascicle length normalized to the inactive fascicle length of muscle) during four training protocols. (A) is related to protocol 1, with 90 °/ s, 65% MVC, from 25-100 ° knee angle amplitude; (B) is related to protocol 2, with 90 °/ s, 100% MVC, from 25-100 ° knee angle amplitude; (C) is related to protocol 3, with 90 °/ s, 100% MVC, from 25-65° knee angle amplitude and (D) is related to protocol 4, with 240 °/ s, 100% MVC, from 25-100° knee angle amplitude. The vertical line indicates the onset of movement of the dynamometer lever arm.

For investigating the longitudinal adaptation of the vastus lateralis fiber, the direct counting of sarcomeres in-series in the vastus lateralis fiber in humans is not practical and ethical. Increasing the number of in-series sarcomeres leads to an increase of inactive vastus lateralis fascicle length at a given knee angle. Therefore, the fascicle length examined before and after the four training protocols during an inactive knee flexion by synchronous registration the knee joint angle.

Furthermore, functional parameters (indirect indicators) are used to investigate the potential longitudinal adaptation of the muscle fibers. Mechanically, the increase in-series sarcomere number on the quadriceps muscle leads to a shift in the moment-angle curve towards a greater knee angle (i.e. greater muscle length). This functional result is observed and accepted in animal studies *(Butterfield and Herzog, 2006a; Butterfield and Herzog, 2005).* Therefore, the moment-angle relationship of the knee extensors, for all involved participants, was examined before and after training.

Another functional achievement of increasing the number of sarcomeres in series on the quadriceps femoris, is the increase in the maximum mechanical power, as well as a shift to greater knee angular velocities during concentric contractions. The increase in maximum power can result from an increase in muscle volume that can be due to a longitudinal, as well as radial, hypertrophy. It is assumed that the shift in maximum mechanical power towards greater knee angular velocities is due solely to the longitudinal adaptation of the muscle. Therefore, the mechanical power-angular velocity relationship of the knee extensor muscles was measured as a further functional assessment for longitudinal muscle adaptation, before and after the training period.

Two measurement sessions were arranged separately to assess changes in the mechanical properties of the vastus lateralis muscle (moment-angle and power-angular velocity relationship) before and after eccentric training interventions. Similarly to Butterfield and Herzog (2006), post measurements were taken one week after the last training session to ensure full recovery of the muscles *(Butterfield and Herzog, 2006a).*

5.2 Measurement of vastus lateralis fascicle length

One linear array B-mode ultrasound transducer (7.5 MHz, 10 cm wide, Esaote MyLab 60) was used to visualize the fascicle length of the vastus lateralis muscle *in vivo* before and after the four exercise interventions. This non-invasive method has been used to measure variations in muscle fascicle length in two-dimensions before and after 10 weeks of training. The vastus lateralis muscle was chosen as other knee extensors muscles have shown unacceptably low reliability in terms of lengths, due to their complex architecture *(Blazevich, et al., 2006; Blazevich, et al., 2007b).* For this reason, the ultrasound transducer was placed into a foam cast and secured over the belly of VL muscle, midway between the trochanter major and epicondylus lateralis using elastic strapping (figure 5-6). To aid acoustic coupling and remove the need to contact the skin, adequate water-soluble gel was applied to the transducer. The echoes reflected from the fascicles and deep aponeurosis were observed by the transducer (figure 5-6). The echoes from interspaces of the VL were sometimes imaged more clearly when the plane of probe was changed slightly, in which case the recreated image was used for the measurement. The same experienced observer carried out all measurements.

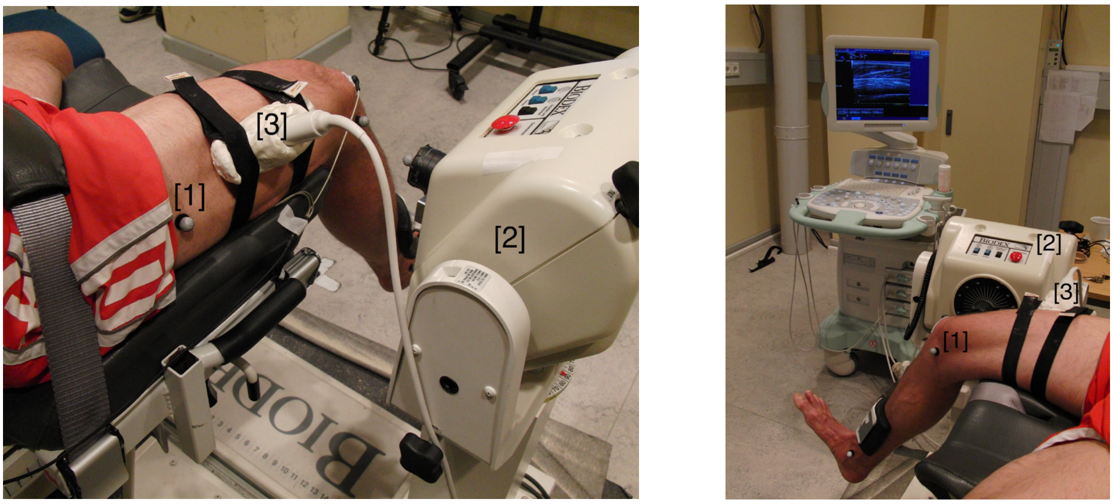


Figure 5-6: Test environment with (1) Vicon markers, (2) Biodex system and (3) Ultrasound probe.

To synchronize the video data (ultrasound images) with the respective data from the Vicon System, a manual trigger was used. The experiment leader manually triggered an analogue signal (0–5 V), which was displayed on the video images and simultaneously captured by the Vicon System. As a detail, in the ultrasound system the heart rate curve was used to visualize the analogue trigger signal and in the Vicon System the trigger signal was captured as a separate channel (beside EMG, moment and joint position). The trigger signals were activated manually immediately prior to the start of the movement, until the end of the movement. The ultrasound video and analogue data, which captured by the Vicon system were trimmed from trigger to trigger. For further analysis, both recorded data were interpolated into the same number of data points (200 data points).

A series of 301 images (43-Hz sampling frequency) were taken during each passive knee extension, for each subject. The fascicle length of the vastus lateralis was determined during the whole examined range of motion from 20°-90° knee flexion (figure 5-7). Fascicle length was defined here as the length of the fascicular path between the upper and deep aponeurosis.

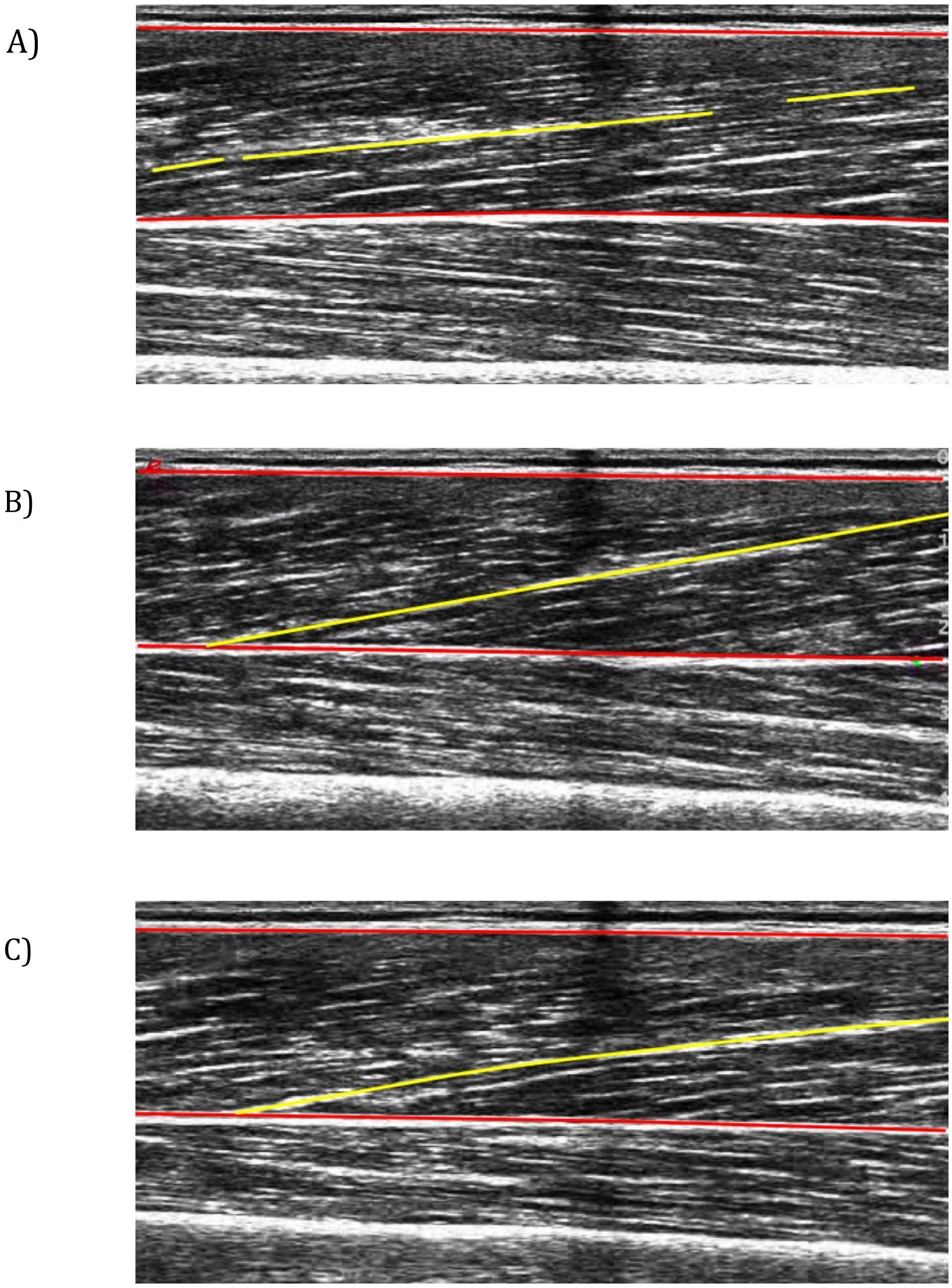


Figure 5-7: Upper and deep aponeurosis (red lines) and visible vastus lateralis fascicle (yellow lines) during analysis and before the calculation of fascicle length. (A) At the start (~20° knee angle), (B) middle (~ 55 knee angle) and (C) end of the measured knee angle (~90° knee angle).

For the measurements of the vastus lateralis fascicle length we used an ultrasound probe of 10 cm. However, often the fascicles were longer than the attained image, therefore the length of the missing portion was estimated by linear extrapolation. The measurement of fascicle length has been previously performed using a semi-automatic tracking algorithm. The proposed method consists of three steps, which were implemented in a custom made MATLAB software program (R2010, The Math works): First, three points over the upper and deep aponeurosis were tracked manually during the whole video sequence. From these points the “digital“ aponeurosis was calculated by linear regression. Aponeurosis extrapolation beyond the field of view was performed in a linear manner. The second step was to determine the initial fascicle orientation. The ultrasound frame (Key Frame) in which fascicle fragments were most visible was digitized (figure 5-8). From the mean slope of the identified fragments, an initial fascicle orientation was calculated. The initial fascicle length was then calculated as the line integral between the deep and upper aponeurosis. For the third step, fascicle length was automatically tracked, frame-by-frame, over the entire ultrasound video by a self-developed MATLAB script (developed at the department of training and movement science of the Humboldt-Universität zu Berlin). To analyze the fascicle movement in the ultrasound video, the changes in brightness contours between two subsequent frames on certain levels between the upper and deep aponeurosis were compared. It was predicted, that a shift in the brightness contours is related to a change in the initial fascicle orientation in subsequent frames. Again the fascicle length in each frame was calculated as the line integral between the deep and upper aponeurosis *(Kugler and Arampatzis, 2010).*

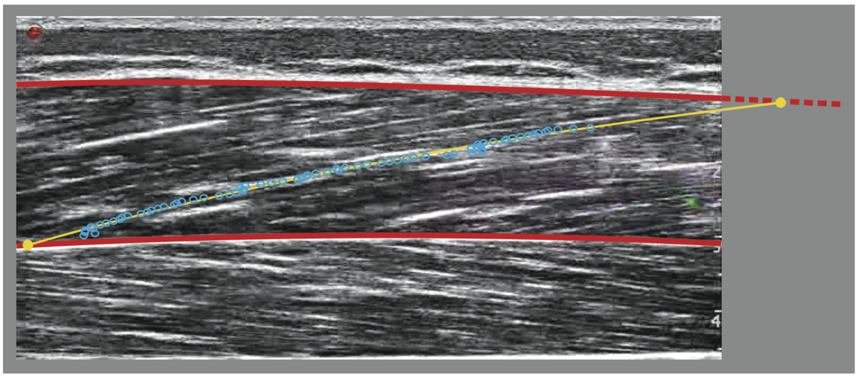


Figure 5-8: Ultrasound image of the vastus lateralis muscle in the inactive state. The red curves represent the upper and deep aponeurosis and the yellow path curve shows the fascicle length.

The evaluation of the ultrasound images was performed by three independent observers and the average of these three image analyses were calculated. For the statistical analysis the average values of the fascicle length from 7 data ranges (20°-30°, 30°-40°, 40°-50°, 50°-60°, 60°-70°, 70°-80° and 80°-90°) were used.

5.3 Measurement of moment-angle relationship on knee extensors

To determine moment-angle relationships of the knee extensors before and after training, our participants were seated on a dynamometer (Biodex - System3, Biodex Medical Systems Inc., USA) with hip angle of 85°. We chose the 85° hip angle because in this position the contribution of the bi-articular rectus femoris, to the resultant knee moment is minimal *(Herzog and Keurs, 1988; Herzog et al., 1990).* The knee joint angles varied during the maximal isometric contractions from 25° to 70° because in this range, the knee joint is expected to achieve the maximal isometric resultant moment *(Meijer et al., 1998)*. Knee angle was defined as the angle between the mid-point of the lateral and medial condyles of the femur to the mid-point of the lateral and medial malleoli, and the mid-point of the lateral and medial condyles of the femur to the greater trochanter. The lever arm of the Biodex was securely attached to the shank at around 5 cm above the lateral malleolus. Velcro straps were placed firmly across the hip and trunk to restrict the participants' movement during the knee extension contraction. Individual positioning of the participants in response to backrest inclination, lever arm length and seat height was held constant between test sessions. For the warm-up, participants exerted five submaximal and two maximum voluntary isometric concentric contractions within 2–3 minutes. Following this the experimental protocol began. All participants were instructed to perform the maximal (100% MVC) isometric extension contraction in a ramped fashion, gradually increasing the knee extension effort over 2 s (loading), and to hold the achieved moment for a further 2–3 s. To assist the subjects in holding the moment-plateau, real time visual feedback of the exerted moment was given along with verbal encouragement to achieve maximum force. These maximum isometric knee extension contractions were performed at 7 - 10 different knee joint positions (from 25° to 70° knee angle) in a randomized order in 5° intervals for each leg. The subjects had a mandatory rest period of at least 3 minutes between each of the isometric contractions. Absolute and normalized moment variations (particularly relative to body mass) were specified at each angle.

The axis of rotation of the knee joint was set to be parallel to the axis of the rotation of the dynamometer, and passing through the mid-point of the line connecting the lateral and medial femoral condyles. During the contraction these two axes clearly shifted away from one another, which can significantly influence the resultant joint moments *(Arampatzis et al., 2004).* Moreover, the fluctuation of the dynamometer lever arm and the soft tissue deformity of the lower extremities demonstrate a typical hysteresis phenomenon during the contraction, which may also result in significant differences between the knee joint angle recorded from the dynamometer, and the actual knee joint angle *(Arampatzis et al., 2004).* For both these reasons (axis deviation and non rigidity of the dynamometer-body system), a correction of measured dynamometer moment and knee angle was necessary. To do this, kinematic data were recorded using the Vicon 624 system (Vicon Motion Systems, United Kingdom) with eight cameras operating at 250 Hz. The resultant moment at the knee joint was calculated through inverse dynamics *(Arampatzis et al., 2004)*. During the knee extension efforts, 8 reflective markers (radius 7mm) which were fixed and captured on the following body landmark positions: crista iliaca on the tuberclum iliacum, trochanter major, most prominent points of the lateral and medial femoral condyles, lateral and medial malleoli, axis of the dynamometer and the lever arm of the dynamometer (figure 5-9).

The influence of the simultaneous activation of the hamstrings, working as antagonists during the knee extension contraction, on the resultant joint moment was taken into account by establishing a relationship between hamstrings EMG-amplitude and exerted moment, whilst working as agonists (Described in following with more details).

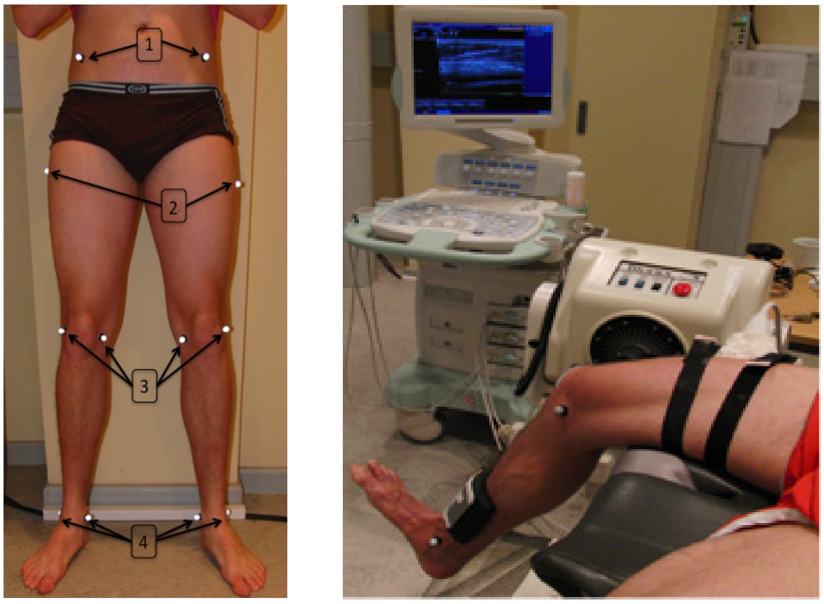


Figure 5-9: Seat position of participant on Biodex machine (Right) and experimental setup for Vicon marker position (Left).

(1) Vicon markers on crista iliaca on the tuberclum iliacum.
(2) Vicon markers on trochanter major.
(3) Vicon markers on most prominent points of the lateral and medial femoral condyles.
(4) Vicon markers on lateral and medial malleoli.

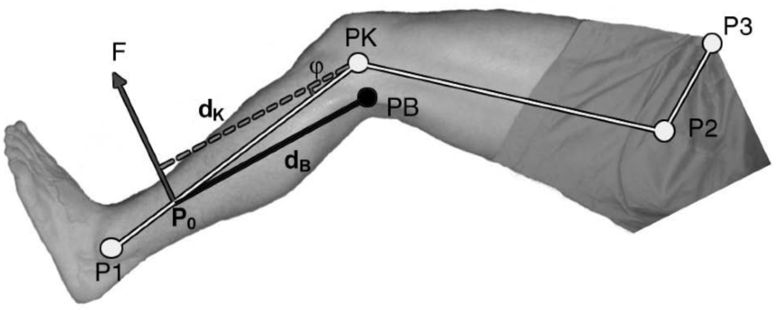


Figure 5-10: Experimental setup for the calculation of the resultant knee joint moment during knee isometric extension. This figure is reprinted from *(Arampatzis et al., 2004).*

F: Applied force of the participant on the lever arm of the dynamometer (by definition orthogonal to the line between P_B and P_0)

P_B: Markers on the dynamometer

P_0: Marker on the lever arm of the dynamometer at the point representing the application of force.

d_K: Lever arm of force F to the knee joint (point P_K is the center of the line between the two femoral condyles)

d_B: Lever arm of force F to the axis of dynamometer (P_B marker)

The exerted moment measured by the dynamometer was synchronized with kinematics from the Vicon system. The correction of the measured moment due to gravitational forces was determined for all participants by obtaining a passive knee joint rotation before the knee extension contraction. It has been assumed that the reaction force is perpendicular to the dynamometer lever arm. However, according to Kaufman et al. (1995), the force component perpendicular to the lever arm, even during isokinetic contractions (60°/s and 180°/s), is approximately 91–93% of the total force during knee flexion and 95–98%

during knee extension. It is assumed that the introduced error should be small in this part of the measurement *(Arampatzis et al., 2004)*. The detailed method for calculating the resultant joint moment has been reported previously *(Arampatzis et al., 2004)* (figure 5-10 and 5-11).

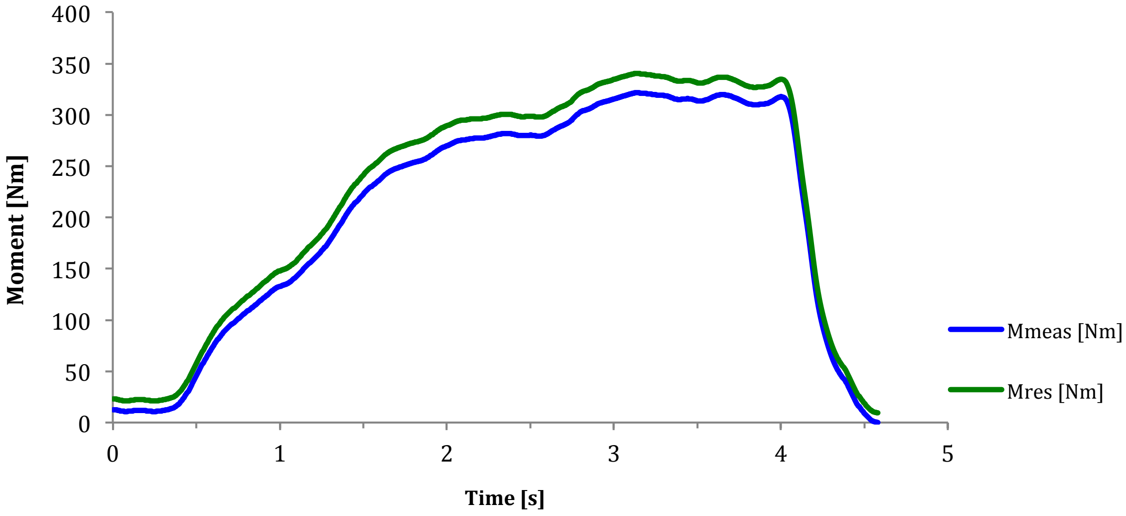


Figure 5-11: Measured (M_{meas}) and resultant (M_{res}) moment generated during one isometric knee extension (Nm). Measured moment from the dynamometer system (Blue line), moment corrected according to gravity and axis deviation of knee joint angle and axis of rotation of the dynamometer (Green line).

For each trial, the maximal resultant knee joint moment and its knee angle was used for data analysis. The maximal resultant moment values were calculated and corresponded to the mean value of 500 ms (±250 ms) around the maximum point for all 7-10 knee joint angles for each participant. The resultant moment values and corresponding knee angles were plotted for each individual participant at pre and post-exercise. Data from the MVCs (i.e. maximum resultant knee joint moment vs. real knee joint angle) were analyzed using a second order polynomial, to identify the resultant knee joint moments from 25° to 70° knee angle at every 5° (figure 5-12). Therefore, for statistical analysis the resultant moment data from the polynomial were analyzed every 5° in the aforementioned range. Individual shifts in the moment–joint angle relationships were calculated by comparing the angle of peak isometric resultant moment post-exercise, to the angle of peak isometric resultant moment pre-exercise. The ratio of post maximum resultant moment angle to pre maximum resultant moment angle ($r = \alpha_{\text{at max post}} / \alpha_{\text{at max pre}}$) indicates the shift in maximum resultant moment angle. A ratio of $r>1$ indicates a shift in maximum resultant moment towards a greater knee angle (i.e. longer muscle length) and $r<1$ indicates a shift in maximum resultant moment towards a smaller knee angle (i.e. shorter muscle length) after the intervention. In addition, for evaluating the effectiveness of the exercise protocols, the ratio of post measurement to pre measurement values of the maximal resultant knee moments were examined. These resultant moments were calculated for each experimental group and control group, and used for the subsequent statistical analyses.

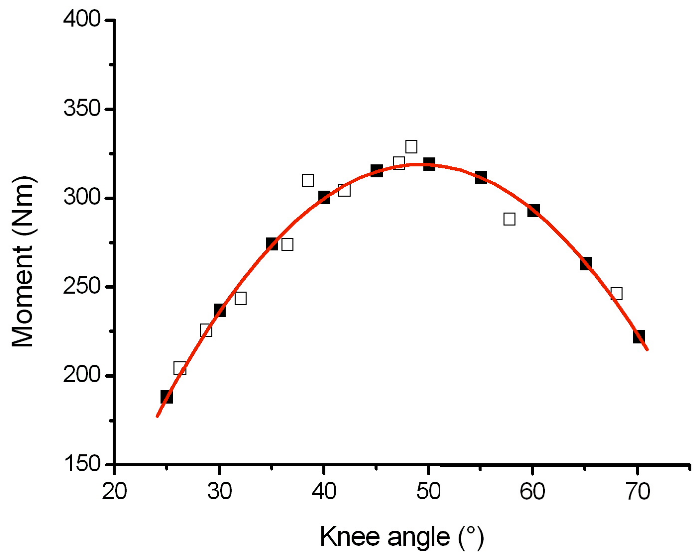


Figure 5-12: Resultant (open) and interpolated (dark) knee joint moments as a function of knee angle.

5.4 Measurement of antagonist coactivation moment

During maximal isometric efforts, contraction of agonist muscles (knee extensors) may be associated with simultaneous contraction of their antagonist (knee flexors) muscles, referred to as co-activation *(Baratta et al. 1988; Komi and Medicine, 2003)*. The moment produced by antagonistic muscle contraction during the maximal isometric knee extension contractions can be estimated by assuming a linear relationship between surface EMG amplitude of the antagonist (knee flexor) muscles, while working as agonists *(Baratta et al., 1988)*. This approach is valid only under specific conditions, particularly when the force generation is dependent only on the neuromuscular activation *(Mademli et al., 2004)*.

In voluntary movement, force is associated with motor unit recruitment and variations in motor unit firing frequency *(Moritani and Muro, 1987)*. At the same muscle length, and during isometric conditions, a greater number of recruited motor units with greater discharge frequencies (i.e. muscle activation) lead to a greater generation of force. Therefore, a linear relationship between EMG and muscle force is assumed (figure 5-13). On the other hand, the force generated by contractile elements is dependent not only the neuromuscular activation, but also the force potential due to the force–length relationship, and the force potential because of the force–velocity relationship *(Mademli et al. 2004; Kaufman, et al., 1991).* During isometric measurements (designed to measure the moment-angle relationship), muscle length is considered to be constant and muscle velocity is zero. Therefore, the contribution of the antagonistic muscle to the resultant moment seems to be dependent only upon neuromuscular activation *(Mademli et al. 2004).*

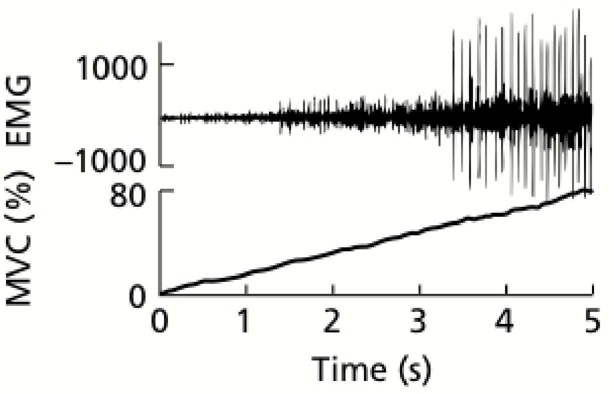


Figure 5-13: A typical set of computer outcomes showing the variation in raw EMG signals recorded from the biceps brachii muscle and the corresponding frequency power spectra during linearly force-varying isometric muscle contraction. This figure is reprinted from *(Moritani & Muro, 1987).*

The EMG signals were registered by the Vicon System at a sampling rate of 3000 Hz during the MVC's and submaximal contractions. The root mean square (RMS) was calculated from the raw signal over a 1 s window, at the plateau of the calculated knee extension force *(Ullrich et al. , 2009b)*.

For the experiment, two sticky surface electrodes (Ag/AgCl) with an electrolytic gel interface were positioned above the midpoint of the muscle belly (with 2 cm distance on inter-electrodes) of the biceps femoris (figure 5-14). Moreover, one-reference electrode was located on the patella bone. The position of all electrodes with regards to muscle length and anatomical landmarks were documented to ensure identical placement post measurement. Care was taken to avoid interference with the electrodes by the fixator straps of the ultrasound probe above the vastus lateralis muscle belly. The skin was carefully shaved and cleaned with alcohol to reduce skin impedance. To reduce motion artifacts of the electrodes they were further secured to the skin with an elastic tape, together with the preamplifier. Prior to the experiment, the leg was passively shaken to check mechanical artifacts of EMG signals from each muscle. Several tests (e.g. contractions against manual resistance in knee flexion and extension) were performed to visualize whether a good signal was produced from each muscle. When artifacts or a poor signal were observed, the preparation procedure was repeated. All knee extension and flexion (regarding to coactivation) contractions were performed within one testing session and the electrodes were not replaced during the measurements.

Figure 5-14: EMG electrodes position for the biceps femoris muscle.

The participants were seated on a Biodex chair with their knee angle set at 45° and hip angle set at 85° (according to Vicon data). The participants were instructed to execute an isometric knee extension MVC and hold it for approximately 4 seconds, while the EMG signal and knee moment were recorded. Following this, the EMG activity and the corresponding moment were measured in three additional trials: (a) in a relaxed condition, (b) producing a knee flexion moment displaying an EMG amplitude of the biceps femoris muscle below the maximal amplitude achieved during the knee extension MVC and (c) a second knee flexion maneuver with an EMG amplitude slightly higher than the maximal amplitude registered during the knee extension MVC (figure 5-15) *(Mademli et al., 2004).* The EMG amplitude of these three knee flexion trials were recorded for ~4-5 seconds in each condition. Throughout the submaximal knee flexion contractions, the co-activation of the vastus medialis, vastus lateralis and rectus femoris were within the noise level of the EMG signal, but revealed no co-activation during the knee flexion contractions.

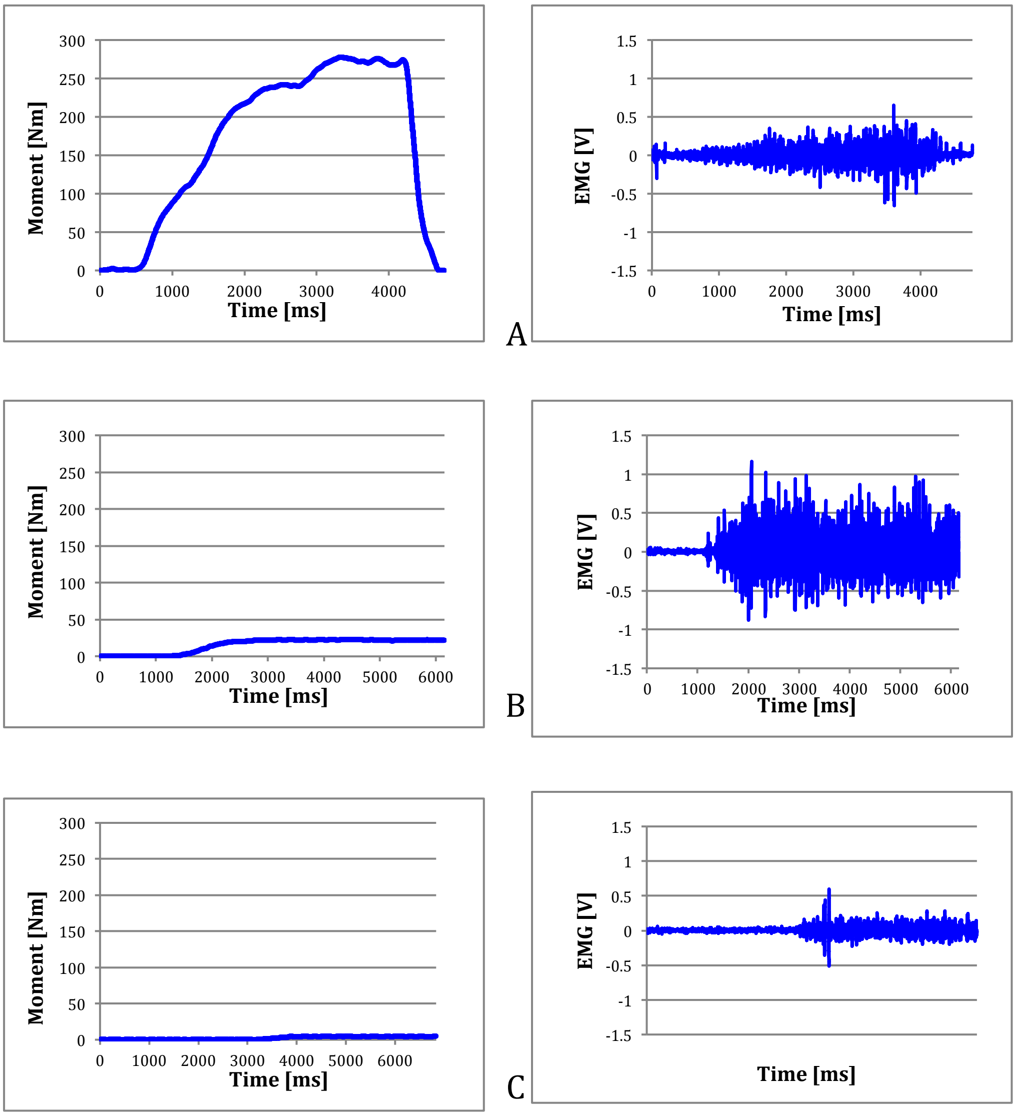


Figure 5-15: Measured moments of knee extensors during one isometric knee extension exercise (A, Left) and the corresponding EMG signals from the biceps femoris muscle (A, Right). First and second submaximal isometric knee flexion moments at the knee angle nearest to the real angle (B and C, Left), and the corresponding EMG signals from the biceps femoris muscle (B and C, Right). Data is from one subject.

The EMG signals recorded from the electrode over the biceps femoris muscle were initially high pass filtered with a 2nd order Butterworth filter, with a cutoff frequency of 6 Hz. Following this the data was rectified and filtered again with a 2nd order low pass Butterworth filter, with a cutoff frequency of 3 Hz.

The moments captured during the relaxed experimental and submaximal knee flexion contractions were fitted with a linear regression curve, as a function of the corresponding rectified and smoothed EMG-values of the biceps femoris muscle. This allowed the estimation of antagonistic moment during the MVC *(Mademli et al., 2004)* (figure 5-16).

Therefore, in this study, maximal knee joint moment refers to the maximal joint moment values where the effect of antagonist moment on the measured moment of the dynamometer, the effect of the joint axis variations relative to the dynamometer axis and the effect of gravitational forces are taken into account (figure 5-16).

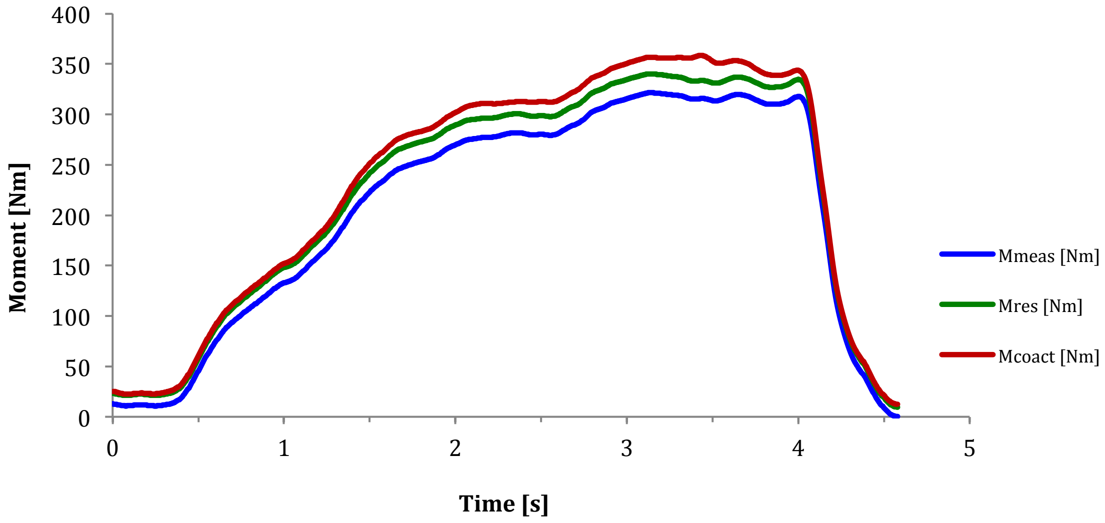


Figure 5-16: Measured (M_{meas}), resultant (M_{res}) and co-activation (M_{coact}) moment generated during one isometric knee extension (Nm). Measured moment from the dynamometer system (blue line), corrected moment according to gravity, axis deviation of knee joint angle and rotation of the

axis of the dynamometer (green line), and corrected moment according to muscle co-activation (red line).

5.5 Measurement of power-angular velocity relationship on knee extensors

A dynamometer device (Biodex-System3) was used to measure the power-angular velocity relationships of knee extensors at different angular velocities. The participants were secured with straps placed around the hips and shoulders whilst in a seated position. Once in this position, the backrest was adjusted so the participant was able to push against it with the lower back. The arms were positioned across the chest, and the hip angle was set to 85 degrees. This angle was chosen to minimize the influence of bi-articular muscles such as the rectus femoris, on knee joint moment. The pad of the dynamometer lever arm, was placed at the axis of rotation, and was positioned approximately 5 cm above the malleolus *(Iossifidou and Baltzopoulos, 1998a).* Full knee extension was defined as zero degrees.

Previous literature *(Arampatzis et al., 2004; Arampatzis et al. 2007)* has highlighted the error which can occur due to the misalignment of the knee joint axis and dynamometer rotation axis, deformation of the dynamometer arm and the soft tissues of the shank, and the effect of inertia and gravity during isokinetic movement *(Iossifidou and Baltzopoulos, 1998b).* Therefore, correction of measured dynamometer moment and knee angle was necessary *(Arampatzis et al., 2004).* For this purpose, kinematics data were recorded by a Vicon 624 system (Vicon Motion Systems, United Kingdom) with eight cameras operating at 250 Hz. Eight reflective markers were placed on the participant's skin at the crista iliaca on the tuberclum iliacum, trochanter major, most prominent points of the lateral and medial femoral condyles, the lateral and medial malleoli, axis of the dynamometer and the lever arm of the dynamometer. Since the moment of inertial and gravitational forces (e.g. of the dynamometer and the lower leg) are important for calculating the resultant moment *(Iossifidou and Baltzopoulos, 1998b)*, the resultant knee joint moment was calculated according to Arampatzis et al. (2007) as follows (figure 5-17):

Equation 1:

$$M_{res} = \underbrace{M_B \frac{dk}{dB}}_{\text{Part 1}} + \underbrace{(W_B\, d_{WB} + I_B\, \alpha_B)}_{\text{Part 2}} \frac{dk}{dB} + \underbrace{(W_s\, d_{Ws} + I_s \alpha_s)}_{\text{Part 3}}$$

The resultant moment (M_{res}) and the moment measured from the dynamometer (Biodex) (M_B), lever arm of the reaction force to the knee joint (assumed to be perpendicular to the dynamometer pad) (d_K), the lever arm of the reaction force to the axis of the dynamometer (d_B), the weight of the dynamometer lever arm (W_B) and its distance to the axis of the dynamometer (d_{WB}), the weight of the shank (W_s) and its distance to the knee angle (d_{ws}), the moment of inertia of the dynamometer lever arm about its axis of rotation (I_B), the angular acceleration of the dynamometer lever arm (α_B), the moment of inertia of the shank about a transverse axis through the knee joint (I_s), and the angular acceleration of the shank (α_s) were all analyzed to evaluate the effects of gravitational and inertial forces of the dynamometer and lower leg, and also to calculate the resultant and measured moments *(Arampatzis et al., 2007a)* (figure 5-17).

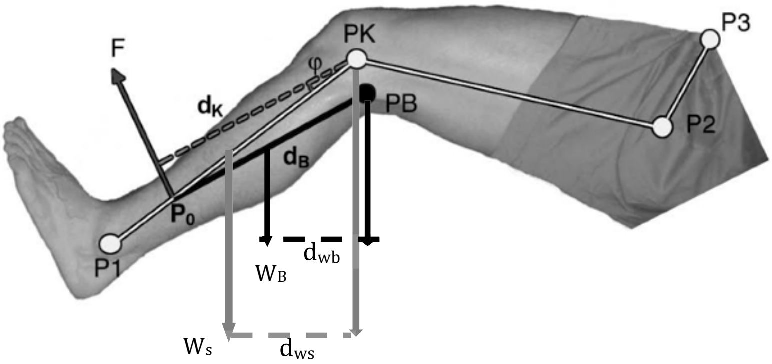


Figure 5-17: Experimental setup for the calculation of the resultant knee joint moment during isokinetic knee extension. This figure is reprinted from *(Arampatzis et al. 2004)*.

F: Applied force of the participant on the lever arm of the dynamometer (by definition orthogonal to the line between P_B and P_0)

P_B: Markers on the dynamometer

P_0: Marker in the lever arm of the dynamometer on the force application point.

d_K: Lever arm of force F to the knee joint (point P_K is the center of the line between the two femoral condyles)

d_B: Lever arm of force F to the axis of dynamometer (P_B marker)

W_B: weight of the dynamometer lever arm

d_{WB}: distance to axis of dynamometer

W_S: weight of the shank

d_{WS}: distance to the knee angle,

Part 2 of equation 1 represents the effects of gravitational and inertial forces of the dynamometer lever arm. Part 3 represents the effects of gravitational and inertial forces of the lower leg. To calculate part 2, the dynamometer lever arm was estimated as the point at which the centre of mass acts. Therefore the lever arm's moment of inertia was calculated as the mass of the lever arm multiplied by the square of the length of the lever arm. The length of the lever arm was previously calculated from the distance of vicon markers on the axis of the dynamometer, and the point at which the lever arm is connected to leg.

The kinematic data and the exerted moments were synchronously captured during the rotation. This allowed the calculation of gravitational and inertial forces from the dynamometer lever arm. For the calculation of part 3 in the equation, the mass and moment of inertia of the lower leg were estimated by using the data provided by *(Zatsiorsky and Seluyanov, 1983).*

To determine the power-angular velocity relationship, 7 angular velocities were selected (270, 300, 330, 360, 400, 450 and 500°/s). Within this range the maximum power in the knee joint is attainable (figure 5-18). In this method, maximum voluntarily activated muscle is required to contract concentric knee extensors at a constant angular velocity.

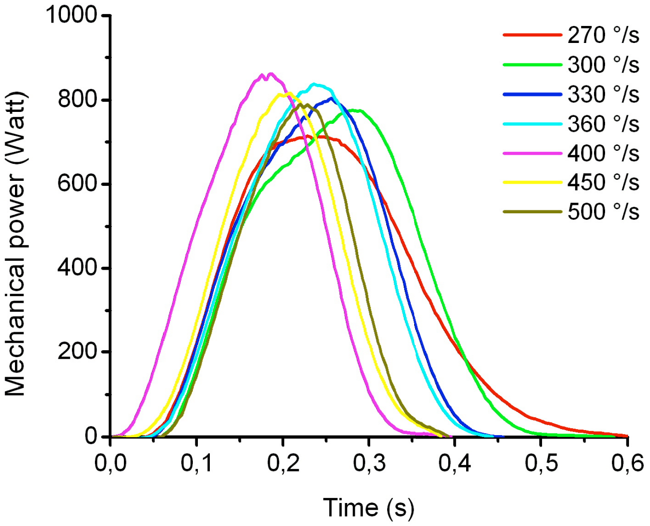


Figure 5-18: Time dependence of the mechanical power of knee extensors during the investigated angular velocities for one participant.

The measurement included concentric extensions of the knee joint. Ten submaximal repetitions of concentric knee extension maneuvers at different angular velocities (from 270 to 500°/s) were performed as a warm up and familiarization with the isokinetic movement. The main test included one set of 3 maximum repetitions of knee concentric contractions, and this was repeated at the 7 velocities, over the whole knee range of motion. The order of angular velocity sets was chosen at random for all participants. A rest period of approximately 3 minutes was allowed between each set and in this way fatigue was found to be negligible *(Taylor et al., 1991).* The participants initiated a concentric knee extension without pre activation in each repetition. They received appropriate guidelines prior to the test and before each set. Verbal and visual feedback was given to the participants during the test, and they were instructed to produce a contraction as hard and as fast as possible. Participants were made aware of the Biodex emergency button to stop the test in case of discomfort or pain. The same examiner administered all tests, in order to ensure consistent testing procedures.

The mechanical power of the knee extensors at different angular velocities was comparable only when knee extensions were performed within the same angular range (i.e. the same muscle length). Otherwise the different force potential of the muscle at different muscle lengths (i.e. knee angles) would affect the power-velocity relationship. Consequently, the mechanical power of each participant was determined at a plateau in the same angular range (50 to 60° knee angle) at each angular velocity (figure 5-19). From these data, similar to those of the isometric contractions, the mechanical power as a function of angular velocity was fitted by a second order polynomial and calculated in 50°/s intervals from 250 to 550 °/ s. These values were used for subsequent statistical analysis.

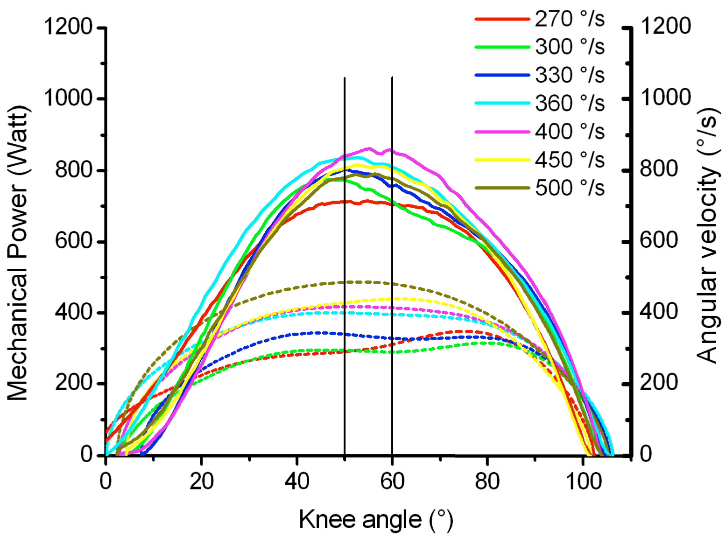


Figure 5-19: Mechanical power (solid line) and angular velocity of the knee joint (dashed line) as a function of knee joint angle during the investigated isokinetic contractions for one participant. The two vertical lines indicate the angular range in which the mechanical power and the angular velocity of the knee joint were analyzed.

5.6 Statistics

In this study a two-factor analysis of variance for repeated measures (exercise protocol × pre-post condition) was applied to determine the effects of eccentric training in each experimental group to the control group, separately. The parameters included the moment-angle relationship, power-angular velocity relationship and fiber length of the vastus lateralis muscle. In case of a significant interaction effect, post hoc tests (Bonferroni) were conducted in order to determine where differences lie between protocols, and in each experimental group. To assess the homogeneity of variance across groups, a one-way ANOVA was conducted in pre measurements. In this regard, the Levene test was used. The analyzed parameters were fascicle length, moment-angle relationship and power-angular velocity relationship. For significance a level of α=0.05 was used. In the tables, the data are presented as means and SD (standard deviation) of the anthropometric data of the test groups, which were calculated by descriptive statistics. In figures, data is presented as mean and standard errors. The statistical analysis was performed using SPSS for Windows (SPSS Inc., version 17.0, Chicago, USA).

6. Results

6.1 Anthropometric parameters

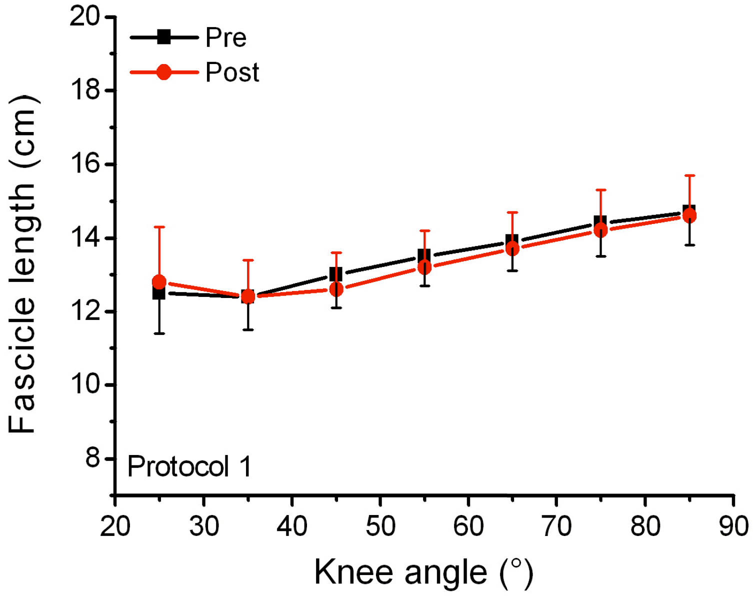
Thirty-one participants were randomly assigned to one of two experimental groups or a control group. The first experimental group included 10 participants (age: 24 ± 4 y, body mass: 77.1 ± 7.4 kg, height: 183 ± 7 cm). The second experimental group included another 10 participants (age: 29 ± 3 y, body mass: 77.1 ± 9.1 kg, height: 181 ± 7 cm), and the control group included 11 participants (age: 28 ± 4 y, body mass: 77.0 ± 7.7 kg, height: 180 ± 5 cm). Table 6-1 shows the mean and SD (standard deviation) of body mass, body height and age of participants in experimental groups 1 and 2, and the control group.

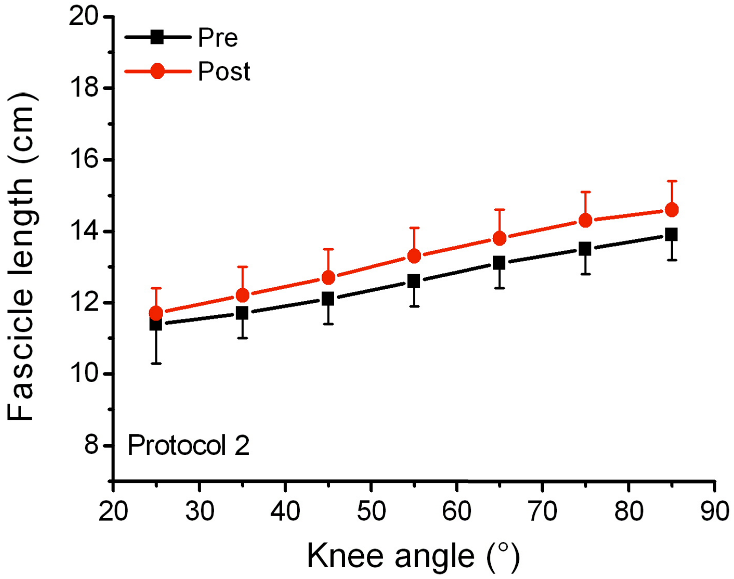
Table 6-1: The mean and SD (standard deviation) of the body mass, body height and age of participants in experimental groups 1 and 2 and the control group.

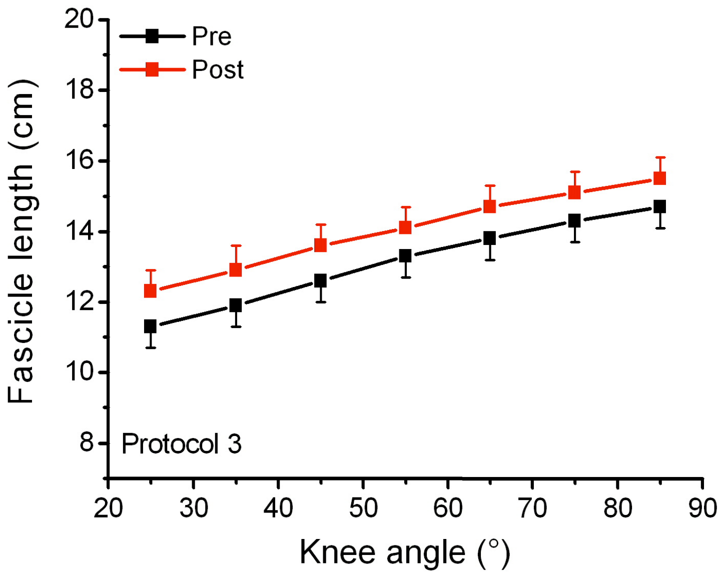
	Experimental group 1 (n=10)	Experimental group 2 (n=10)	Control group (n=11)
Body mass	77.1 ± 7.4	77.1 ± 9.1	77.0 ± 7.7
Body height	183 ± 7	181 ± 7	180 ± 5
Age	24 ± 4	29 ± 3	28 ± 4

6.2 Fascicle length of the Vastus lateralis muscle

Figure 6-1 shows the relationship between vastus lateralis fascicle length (cm) and knee angle (°), from 25° to 90° at 10 degree intervals. This relationship is inclusive of data from four training protocols and one control group taken pre and post intervention. It shows an increase in vastus lateralis fascicle length from approximately 12 cm at a knee angle of 25° to approximately 15 cm with a knee angle of 85° in four different protocols. The pre measurements showed no significant ($p > 0.05$) difference in fascicle length between the groups. The graph also shows a significant ($p > 0.05$) difference in fascicle length between pre and post measurements, however this was evident only after exercise protocol 4 (240 °/s, 100% MVC, 25°-100° knee angle amplitude). Muscle fascicle length in exercise protocol 4 showed a statistically significant ($p<0.05$) increase of approximately 14%, across the whole measured knee angle range (25°-100 ° knee angle). All other exercise protocols showed no significant ($p > 0.05$) differences in muscle fascicle length before and after the training intervention (figure 6-1).


Pre
Post
Fascicle length (cm)
20
18
16
14
12
10
8
Protocol 1
20
30
40
50
60
70
80
90
Knee angle (°)


Pre
Post
Fascicle length (cm)
20
18
16
14
12
10
8
Protocol 2
20
30
40
50
60
70
80
90
Knee angle (°)


Pre
Post
Fascicle length (cm)
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18
16
14
12
10
8
Protocol 3
20
30
40
50
60
70
80
90
Knee angle (°)

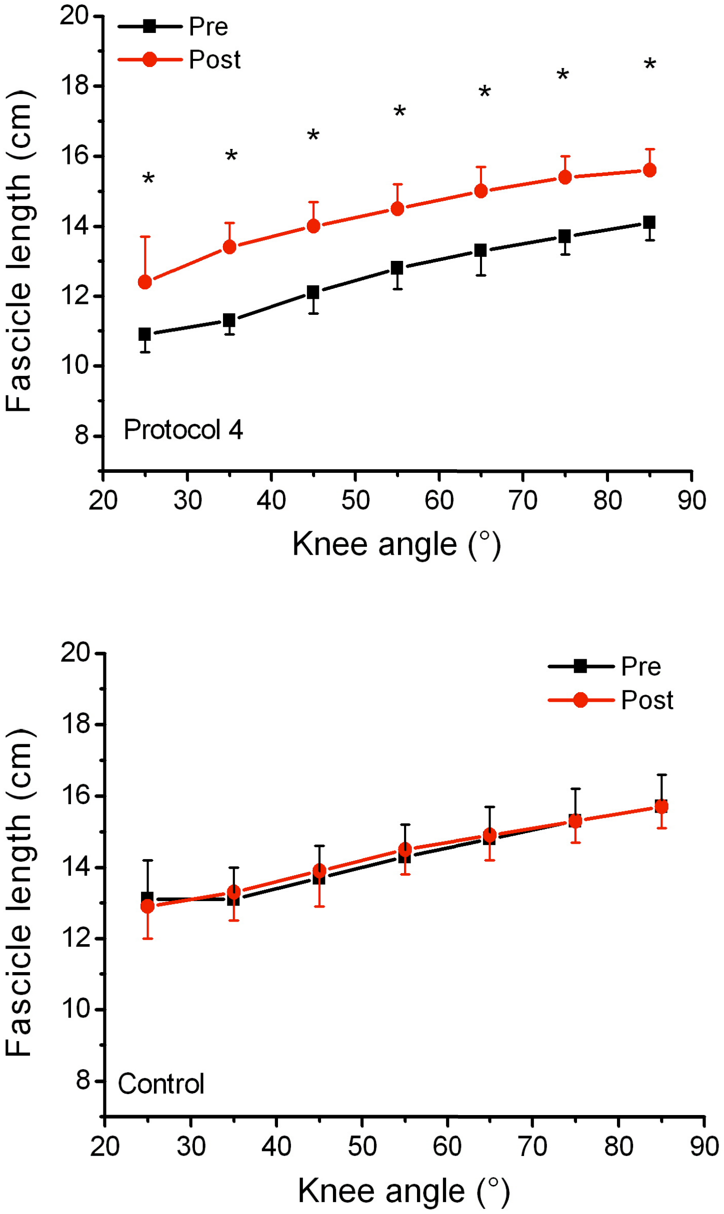


Figure 6-1: Average and standard errors of the fascicle length of the vastus lateralis muscle as a function of knee angle before (pre) and after (post) the exercise intervention protocols.

Protocol 1: 90°/s, 65%MVC, 25-100° knee angle amplitude;

Protocol 2: 90°/s, 100% MVC, 25-100° knee angle amplitude;

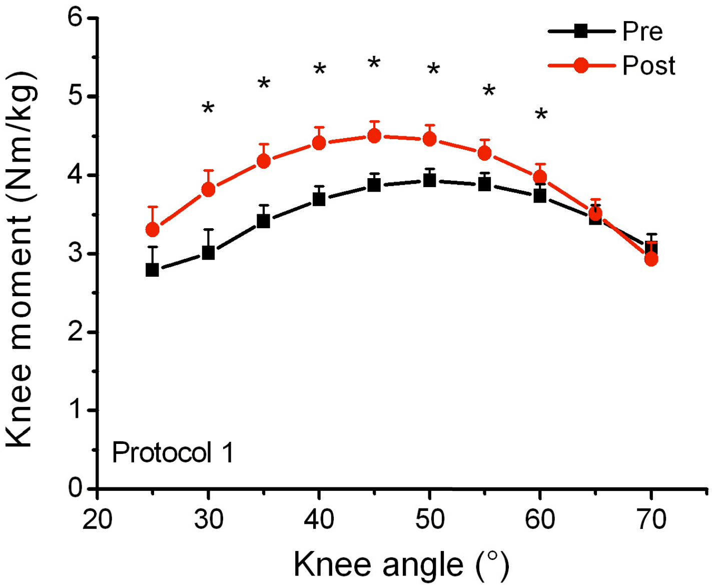
Protocol 3: 90°/s, 100% MVC, 25-65° knee angle amplitude;

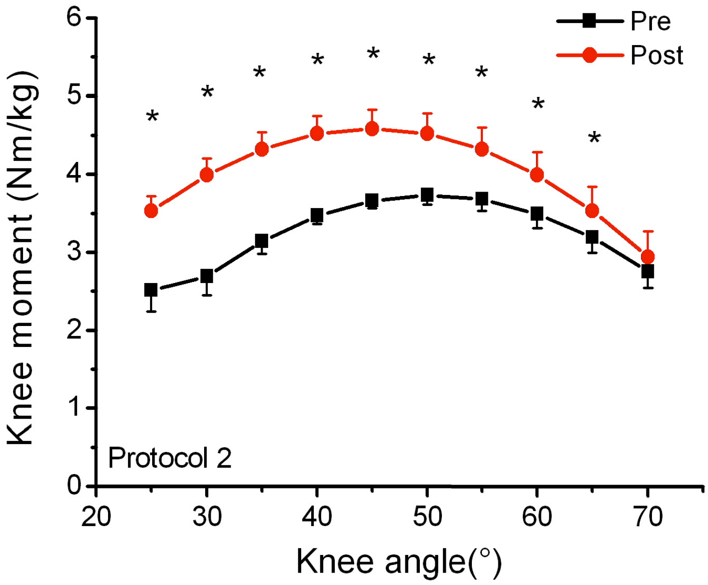
Protocol 4: 240°/s, 100% MVC, 25-100° knee angle amplitude.

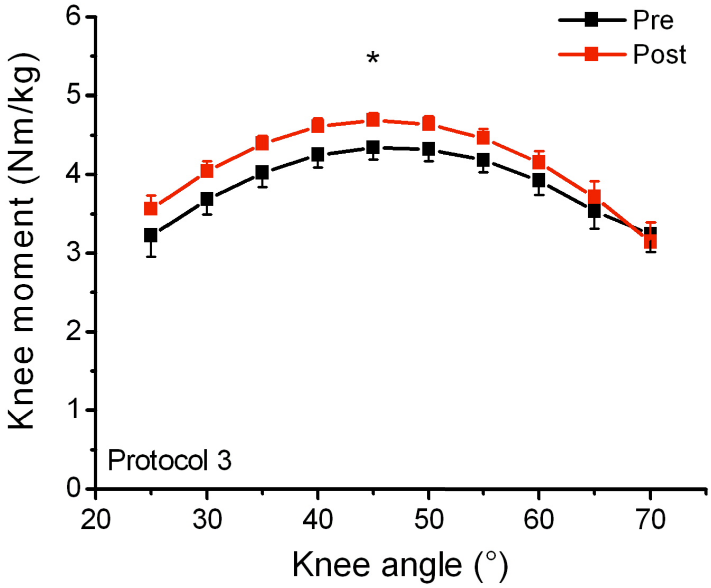
* Statistically significant difference ($p < 0.05$)

6.3 Moment-angle relationship of the knee extensors

Figure 6-2 shows the normalized resultant knee moment (Nm/kg) in relation to knee angle (°) from 25° to 70° at 5° intervals. This graph is inclusive of four exercise protocols and one control group, taken pre and post intervention. The knee moment-angle figure shows an ascending curve until 45-50°, after which comes the descending curve (figure 6-2). The maximal knee extension moments were on average 315.11 Nm/kg pre intervention, and 356.41 Nm/kg post intervention. The maximum resultant knee joint moments in the pre-measurement showed no statistically significant differences ($p>0.05$) between each exercise protocol and the control group. The graph shows no differences in the moment-angle relationship, pre to post for the control group. However, the post-measurement data did show that the participants who trained with exercise protocols 1, 2 and 4 had significantly ($p <0.05$) higher resultant moment values in almost all measured knee angles, than before the exercise intervention. In addition, exercise protocol 3 (ascending part of the moment-angle relation) showed a significant ($p<0.05$) increase in resultant knee joint moment at a joint angle of 45° (figure 6-2).


Pre
Post
Knee moment (Nm/kg)
0
1
2
3
4
5
6
Knee angle (°)
20
30
40
50
60
70
Protocol 1
*


Pre
Post
Knee moment (Nm/kg)
0
1
2
3
4
5
6
Knee angle(°)
20
30
40
50
60
70
Protocol 2
*


Pre
Post
Knee moment (Nm/kg)
0
1
2
3
4
5
6
Knee angle (°)
20
30
40
50
60
70
Protocol 3
*

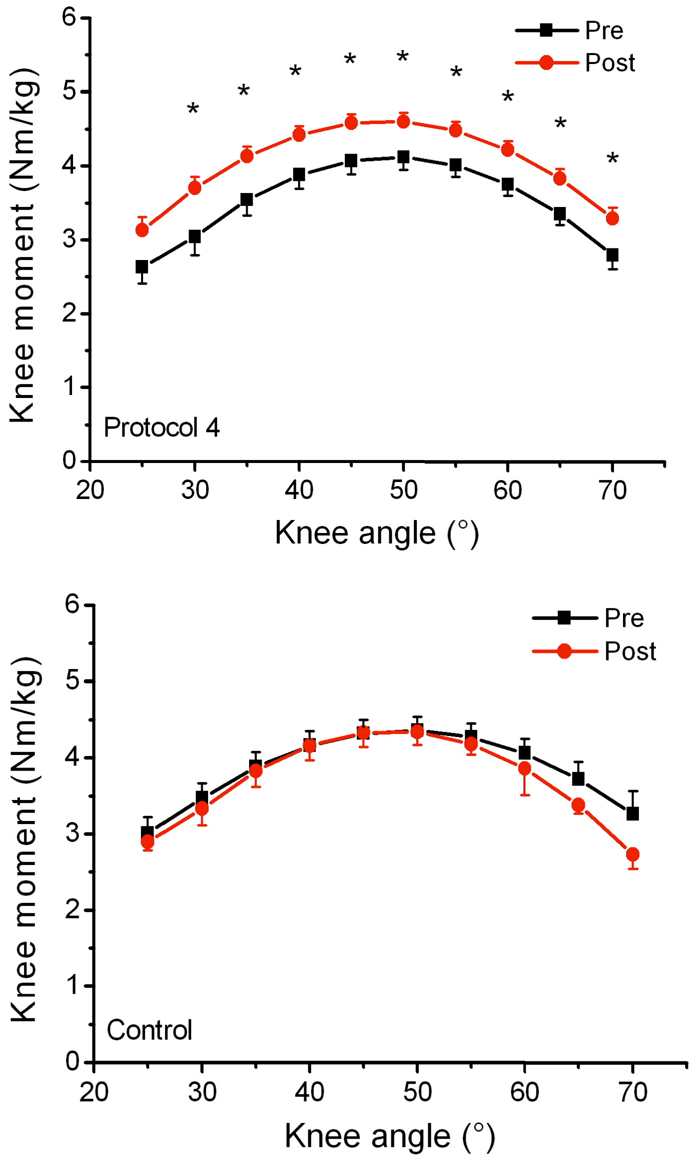


Figure 6-2: Average and standard errors of the moment-angle relationship of the knee extensors before (pre) and after (post) the exercise intervention protocols. This data derived from a second order polynomial of the resultant knee joint moments from 25° to 70° knee angle at every 5° intervals.

Protocol 1: 90 °/s, 65% MVC, 25-100° knee angle amplitude;

Protocol 2: 90 °/s, 100% MVC, 25-100° knee angle amplitude;

Protocol 3: 90 °/s, 100% MVC, 25-65° knee angle amplitude;

Protocol 4: 240 °/s, 100% MVC, 25-100° knee angle amplitude.

*: Statistically significant difference ($p < 0.05$).

To evaluate the effectiveness of the exercise protocols, the ratio (i.e. post to pre values) of maximal resultant knee moments were examined (10 values, see figure 5-12). Figure 6-3 shows the ratio of maximal resultant knee moment for the four exercise protocols and for the control group. The ratio of each exercise protocol showed significantly ($p < 0.05$) higher values in comparison to the control group (figure 6-3). Furthermore, the increase in average resultant knee joint moment in exercise protocol 2 (90 °/s, 100% MVC, 25-100° knee joint amplitude) was significantly ($p < 0.05$) higher than in protocols 1 and 3, and also tended ($p = 0.055$) to be higher than exercise protocol 4 (figure 6-3). The increase in maximum resultant knee joint moment was on average 15%, 27%, 9% and 17% for the exercise protocols 1, 2, 3 and 4, respectively.

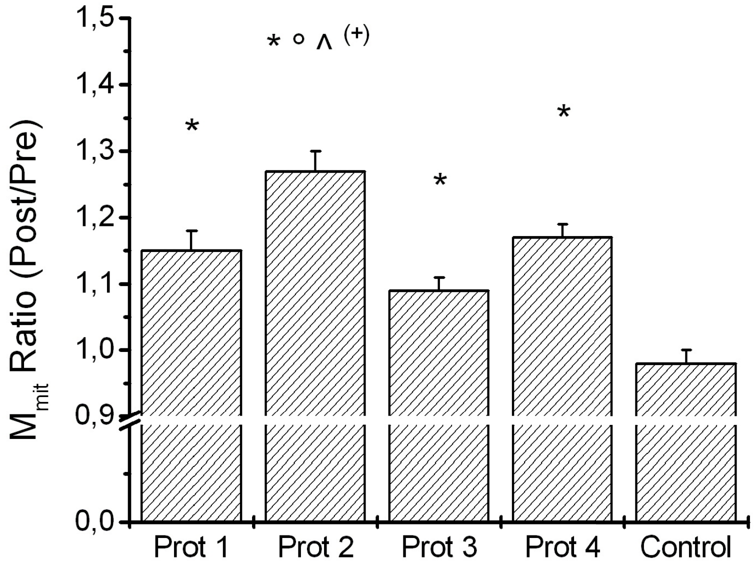


Figure 6-3: The ratio (i.e. post to pre values) of maximal resultant knee joint moment in the different exercise protocols and control group.

Protocol 1: 90 °/s, 65% MVC, 25-100° knee angle amplitude;

Protocol 2: 90 °/s, 100% MVC, 25-100° knee angle amplitude;

Protocol 3: 90 °/s, 100% MVC, 25-65° knee angle amplitude;

Protocol 4: 240 °/s, 100% MVC, 25-100° knee angle amplitude, Control: control group.

*: Statistically significant difference from the control group ($p < 0.05$);

°: Statistically significant difference from Protocol 1 ($p < 0.05$);

^: Statistically significant difference from Protocol 3 ($p < 0.05$),

(+): tendency to a statistically significant unlike Protocol 4 ($p = 0.055$).

To determine the shift in maximum resultant knee joint moment, we calculated the ratio of post/pre knee angle values, where the maximum resultant knee joint moment was achieved. A ratio of $r > 1$ indicates a shift in the maximum resultant moment towards a greater knee angle (i.e. longer muscle length) and $r < 1$ indicates a shift of maximum resultant moment towards a smaller knee angle (i.e. shorter muscle length) following the intervention. Figure 6-4 shows the results for each training protocol and for the control group. In comparison to the first two protocols (protocols 1 and 2) with the control group, the values in exercise protocol 1 were smaller on average than protocol 2. However, there were no significant differences ($p > 0.05$) in exercise protocols 1 and 2 when compared to the control group, as well as the other exercise groups.

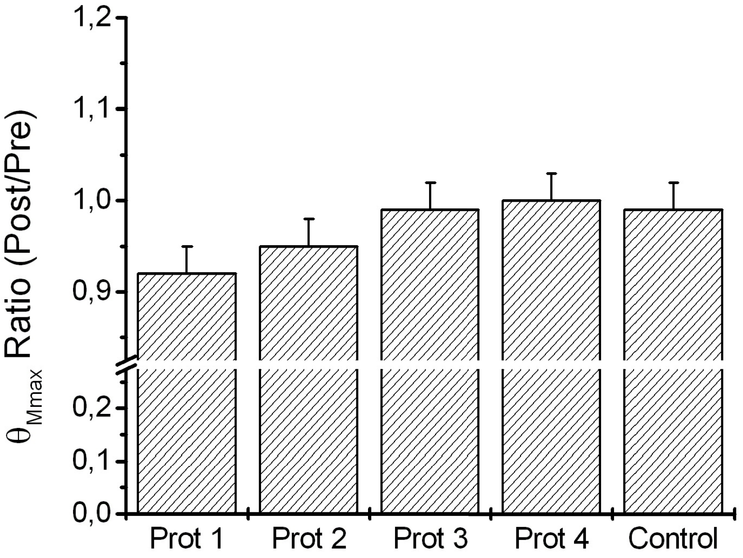
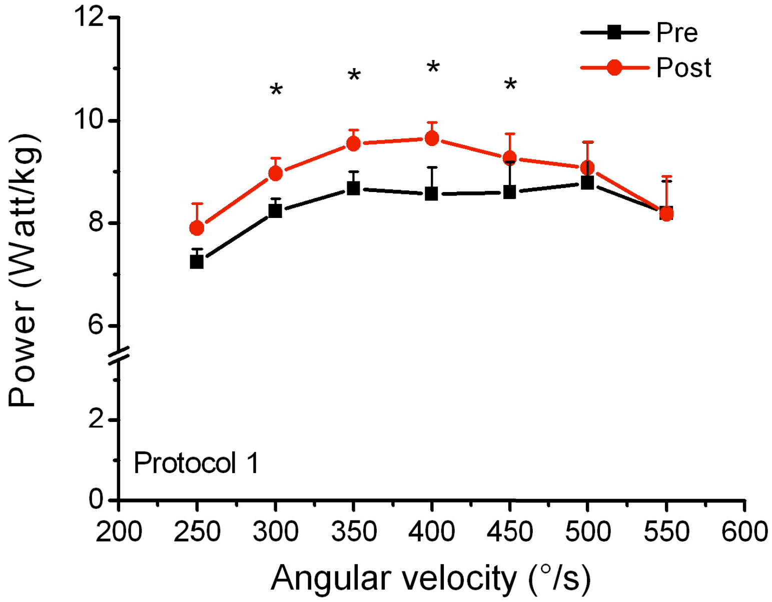


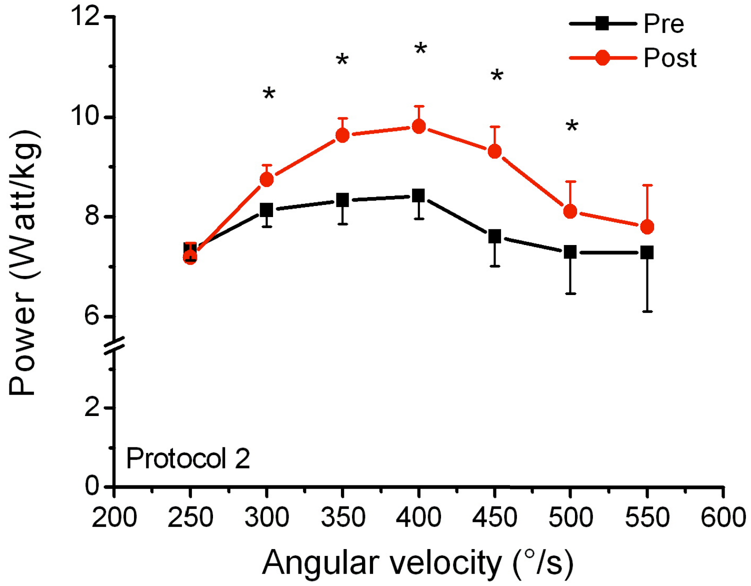
Figure 6-4: The ratio of knee angle values (θ Mmax) in which the maximum resultant knee moment was reached, post to pre training intervention, for each exercise protocol.

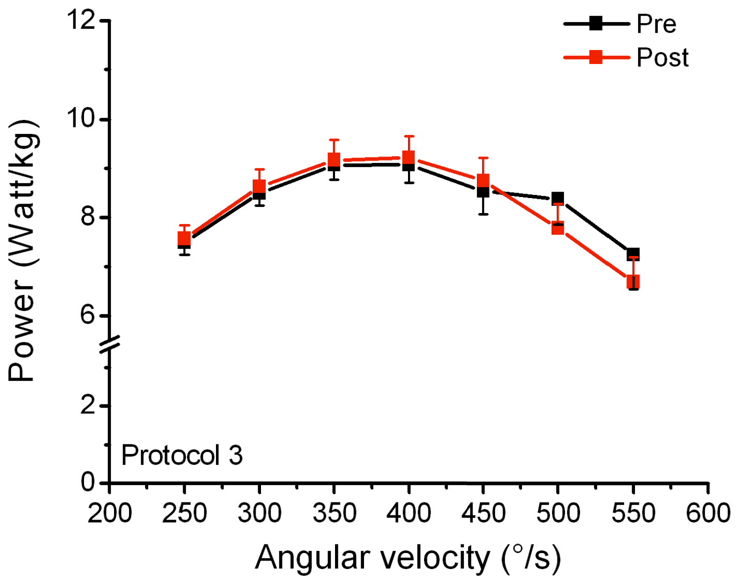
Protocol 1: 90 °/s, 65% MVC, 25-100° knee angle amplitude;
Protocol 2: 90 °/s, 100% MVC, 25-100° knee angle amplitude;
Protocol 3: 90 °/s, 100% MVC, 25-65° knee angle amplitude;
Protocol 4: 240 °/s, 100% MVC, 25-100° knee angle amplitude,
Control: control group.

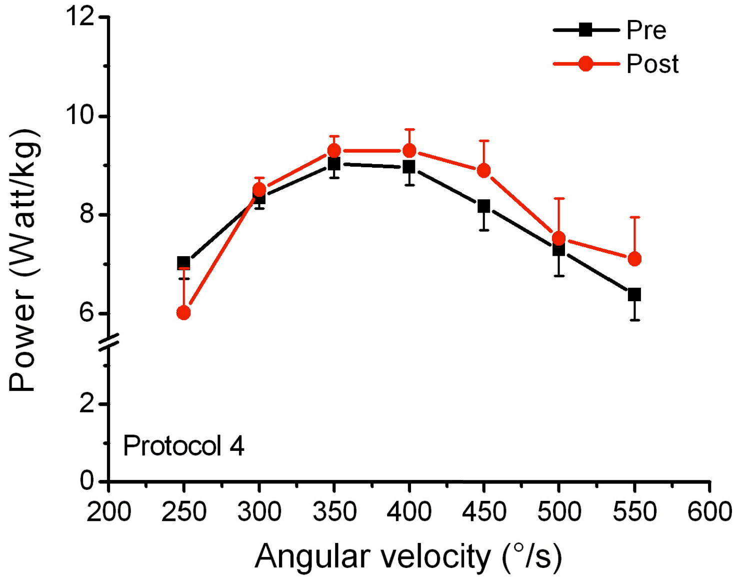
6.4 Mechanical power-angular velocity relation of knee extensors

Figure 6-5 shows the relationship between normalized mechanical power (watt/kg) and knee angular velocity (°/s) from 250°/s to 550°/s at 50°/s intervals. It also includes the values pre and post intervention for the four training protocols and the control group. On average, the maximal mechanical power was 699.16 Watt/kg before and 751.47 Watt/kg after the intervention (figure 6-5). The maximal mechanical power was achieved at a knee angular velocity of approximately 400 °/s. Similar to the maximum resultant knee joint moments (section 6.3), in compare to the control group, the maximum mechanical power of the knee extensors was not significantly ($p > 0.05$) different in the pre training protocols. This graph shows the participants who trained with protocols 1 and 2, had significantly ($p < 0.05$) higher values for the mechanical power of the knee extensors after ten weeks of training. Furthermore, there were no statistically significant ($p > 0.05$) differences between pre and post measurement values for the participants in the control group, as well as the participants who trained according to training protocols 3 and 4 (figure 6-5).






Pre
Post
Power (Watt/kg)
12
10
8
6
2
0
Protocol 3
200
250
300
350
400
450
500
550
600
Angular velocity (°/s)


Pre
Post
Power (Watt/kg)
12
10
8
6
2
0
Protocol 4
200
250
300
350
400
450
500
550
600
Angular velocity (°/s)

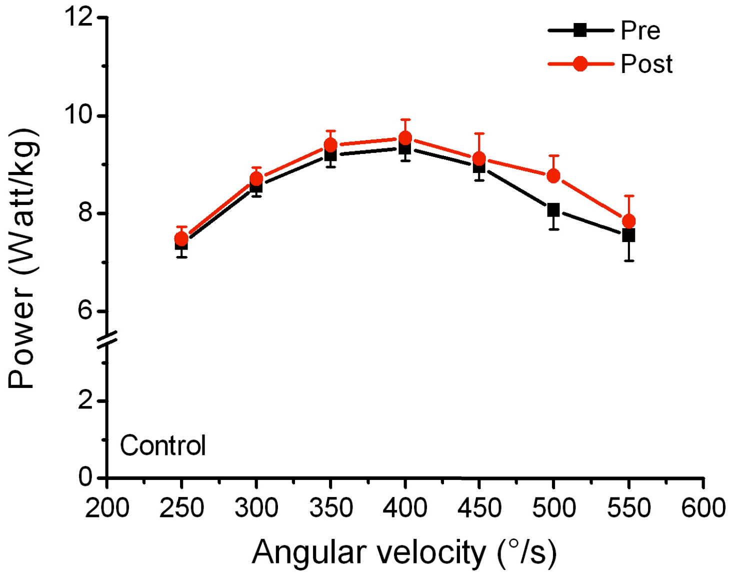

Pre
Post
Power (Watt/kg)
12
10
8
6
2
0
Control
200
250
300
350
400
450
500
550
600
Angular velocity (°/s)

Figure 6-5: The average and standard errors of the mechanical power-angular velocity relationship of the knee extensors before (pre) and after (post) the particular exercise protocol.
Protocol 1: 90 °/s, 65% MVC, 25-100° knee angle amplitude;
Protocol 2: 90 °/s, 100% MVC, 25-100° knee angle amplitude;
Protocol 3: 90 °/s, 100% MVC, 25-65° knee angle amplitude;
Protocol 4: 240 °/s, 100% MVC, 25-100° knee angle amplitude.
*: Statistically significant difference ($p < 0.05$).

As mentioned in methodology, it is assumed that the shift in maximum mechanical power towards a greater knee angular velocity is due only to the longitudinal adaptation of the muscle. Therefore, to determine the shift in maximum mechanical power, we calculated the ratio of the post/pre values for knee angular velocity, where the maximum mechanical power was achieved. A ratio of $r>1$ indicates a shift of maximum mechanical power towards a greater knee angular velocity and $r<1$ indicates a shift of maximum mechanical power towards a slower angular velocity. Figure 6-6 shows these results for each training protocol and for the control group. This ratio showed no significant ($p> 0.05$) difference between each training protocol and the control group (figure 6-6) indicating there was not a shift in the maximal mechanical power at a higher angular knee velocity, following the interventions.

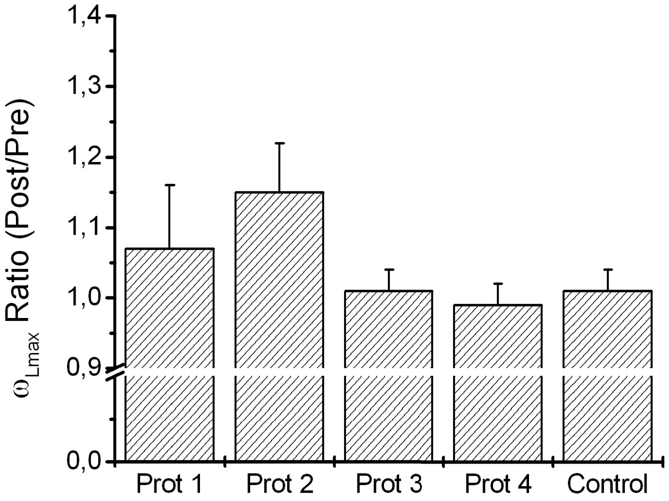


Figure 6-6: Ratio of the knee angular velocity (ωLmax) values (post/pre), in which the maximum mechanical power of the knee extensors was reached, from different exercise protocols.

Protocol 1: 90 °/s, 65%MVC, 25-100° knee angle amplitude;

Protocol 2: 90 °/s, 100% MVC, 25-100°knee angle amplitude;

Protocol 3: 90 °/s, 100% MVC, 25-65° knee angle amplitude;

Protocol 4: 240 °/s, 100% MVC, 25-100° knee angle amplitude,

Control: control group.

7. Discussion

The aim of present study was to investigate the effect of controlled eccentric loading on longitudinal adaptation of vastus lateralis muscle. The longitudinal plasticity of muscle is a well-known phenomenon in the literature, which can increase the maximum shortening velocity of the muscle *(Sacks and Roy, 1982)*, increase the maximum mechanical power of the muscle *(Huijing 2003)* and can decrease the risk of strain injuries *(Askling 2003).* According to the literature, based largely on animal studies, there is evidence that load magnitude (muscle force, fascicle strain) *(Butterfield and Herzog, 2006a)* and fascicle length at which the eccentric stimulus has been applied *(Butterfield and Herzog, 2006b)* may be important factors for longitudinal adaptation of muscle. Although there are some recent studies regarding the effects of eccentric exercise on the longitudinal adaptation in human muscle *(Brockett et al., 2001; Blazevich et al., 2007; Reeves et al., 2009),* it is not clear what the eccentric loading stimulus must be to regulate the longitudinal adaptation of human muscles. For this purpose, used four different trainings protocols where the magnitude of the eccentric stimulus, the lengthening velocity of the muscle fascicle and muscle length at which the eccentric stimulus is applied were modified. It has been hypothesized that different intensities of these three factors would result in the adaptation of vastus lateralis fascicle length after 10 weeks of exercise. To test this hypothesis, four different training protocols with the same volume of eccentric loading were designed in two experimental groups. Furthermore, a control group was also included who performed no specific eccentric exercise, to allow comparisons with the experimental groups. To modify the magnitude of the eccentric stimulus the participants of the first experimental group exercised one leg with protocol 1 according to 65% of their isometric MVC, 90°/s, from 25° to 100° knee angle, with 5 sets and 10 repetitions in each set. The second leg of these participants was trained according to protocol 2, which was carried out at 100% of their MVC, 90°/s, from 25° to 100° knee angle with 5 sets, but 6 repetitions in each set, to attain the same loading volume. We changed the muscle length at which the eccentric stimulus was applied in protocol 3. Therefore, the participants of the second experimental group trained one leg according to 100% of their MVC, 90°/s, but only from 25° to 65° knee angle with 5 sets and 12 repetitions in each set. To modify the lengthening velocity of the muscles the other leg of these participants were trained at 240°/s angular velocity, at 100% of MVC and from 25° to 100° knee angle with 5 sets but 16 repetitions in each set, to attain the same loading volume. Although muscle strength of the knee extensors was improved in all four exercise protocols; morphological changes (i.e. increase of fascicle length of vastus lateralis) was detected only after the intervention with the high lengthening velocity of the muscle.

7.1 Morphological adaptation

The main hypothesis of the study was that the three factors (i.e. magnitude of eccentric stimulus, lengthening velocity of the muscle fascicles and muscle length at which the eccentric stimulus has been applied) would show differences in the longitudinal adaptation of vastus lateralis fascicle, in an intensity-dependent manner. However, only the high lengthening velocity protocol caused a significant increase (~14%) in the fascicle length of vastus lateralis muscle. Therefore, our hypothesis has been rejected. The results of this study showed that not all types of eccentric training causes an increase in fascicle length and that the velocity of fascicle stretch seems to be the most important factor for the longitudinal adaptation of fascicle. Although the underling mechanisms regarding the longitudinal adaptation of the muscle (i.e. increase of sarcomeres in series) are not well known, we can argue that a high lengthening velocity of the fascicles might be necessary to trigger a homeostatic perturbation in muscles which affects their longitudinal plasticity.

Morgan (1990) suggested that non-uniform lengthening of sarcomeres, when active muscle is stretched beyond optimum length increases muscle damage. One proposed explanation for the observed muscle damage during eccentric contractions beyond optimum length has been provided from Morgan (1990) and it is known as the "popping sarcomere hypothesis" *(Morgan 1990)*. It is reported that during the lengthening of the muscle fiber and particularly beyond the optimum length, the "weakest" sarcomeres will be stretched faster compare to the "stronger" ones achieving higher passive tensions *(Morgan and Proske, 1994).* This leads to large deformations in fiber membranes, particularly T-tubules *(Lieber and Fridén, 2002; Takekura, et al., 2001)*, causing muscle damage. It has been assumed that these disconformities in the sarcomeres due to eccentric loading can trigger the increase sarcomere number in series within the muscle fiber *(Lynn and Morgan 1994),* reducing its susceptibility to injury. The proposed mechanism is that by increasing the number of sarcomeres in series at a given muscle (fascicle) length, the length of each sarcomere would be reduced *(Morgan 1990).* The consequence is a decrease of injury risk because sarcomere 'instabilities' are associated with the descending limb of the force—length curve *(Morgan 1990).*

In the literature, it is reported that high lengthening velocity of the muscle fiber is a powerful function, which causes muscle damage in comparison to lower lengthening velocities. Chapman et al. (2006) found significant increases in the level of plasma creatine kinase (CK) and a decrease in maximum isometric torque as an indicator for muscle damage, after a period of eccentric loading on elbow joint. The authors suggested that muscle fibers are more prone to damage at higher lengthening velocity *(Chapman, et al, 2006).*

Also in another study, Shepstone (2005) investigated the effect of fast (210 °/s) and slow isokinetic (20 °/s) muscle lengthening contractions on biceps brachii muscle fiber hypertrophy. The muscle fiber type and muscle cross-sectional area of the elbow flexors were measured before and after 8 weeks of training (3 days/week). The authors found that muscle fiber cross-sectional area from types I and II of the elbow flexors was increased in both fast and slow lengthening protocols. However, the increase of type II muscle fiber cross-sectional area was greater in the fast lengthening velocity training protocol. Furthermore, it has been found that lengthening velocity affects the degree of structural damage on the Z-band of the muscle fiber *(Shepstone, 2005)*. These reports indicate that a fast lengthening velocity of muscle fascicles during eccentric loading increases muscle fiber damage compared to slow lengthening velocities and therefore this type of loading might be advantageous for the longitudinal muscle adaptation, as was demonstrated in the current work. It might be assumed that a higher lengthening velocity increases the damage at the sarcomere level of muscle fiber and this kind of damage can trigger the muscle fiber to adapt to the new situation. Conversely, muscle adaptation can occur without muscle damage if training intensity gradually increases *(Flann, et al., 2011; LaStayo, et al., 1999).* Flann et al. (2011) stated that muscle rebuilding after 8 weeks of eccentric training could be initiated, independent of any discernible damage to the muscle. They found that quadriceps muscle alteration- for example radial hypertrophy, occurred as a result of increased muscle strength and myogenic growth factor insulin (IGF-1Ea), independent of any muscle damage. Specifically, the authors reported that a gradual ramped design was successful to avoid detectable muscle damage *(Flann et al., 2011).* In a similar way, LaStayo et al. (1999) reported that gradually increasing frequency and duration of training had an effect to decrease muscle injury, caused by eccentric contraction. They found improvements in muscle strength after 6 weeks of eccentric exercise *(LaStayo et al., 1999).*

However, all of these previously cited studies investigated the effect of eccentric loading on muscle hypertrophy (i.e. radial muscle adaptation) or on muscle strength, but not on the effects of eccentric exercise to longitudinal muscle adaptation. Therefore, it is difficult to argue with their findings about a possible longitudinal muscle adaptation during eccentric loading without muscle damage.

7.2 Functional adaptation

The results of our study show an increase in quadriceps muscle strength as well as maximal mechanical power, after 10 weeks eccentric training. This demonstrates that the present training intervention had an effect on muscle adaptation, increasing muscle strength and maximal mechanical power.

The overall gain in maximal knee moment generation was significantly greater in all four training protocols ($p < 0.05$) compared to the control group. Although the increase in maximal knee joint moment in protocol 3 (i.e. loading only in the ascending part of the moment-angle curve) occurred only in one joint position, the significant differences in maximal knee joint moment to the control group was evidence of a training effect. In contrast, the maximal mechanical power was significantly greater only in protocols 1 and 2 (i.e. exercise on the lower angular velocity) compared to control group.

Several studies reported muscle strength increases after 5 to 12 weeks of regular eccentric training *(Hortobágyi, et al. 1996a; Pavone and Moffat, 1985)*. Eccentric training may provide greater muscle strength improvements compared to concentric or isometric exercise *(Hortobágyi, et al. 1996a; Hortobágyi, et al. 1996b)* because the magnitude of muscle force can be higher during eccentric contractions due to the force-velocity relationship. Therefore, it may be argued that eccentric loading provides a powerful stimulus to promote muscle strength.

The ratio of knee moment values from post to pre measurement was the highest (~1.27) in protocol 2, with the highest muscle loading (i.e. 100% MVC) and the lowest angular velocity (i.e. 90 °/s). The superior improvement in muscle strength in protocol 2 compared to protocol 1 (i.e. 65% MVC, 90 °/s) can be explained by the different magnitudes of loading between these two training protocols. There are several studies, which report different magnitudes of loading, and their varied affects on muscle strength *(Holm et al., 2008; Hortobágyi, et al., 2001; McBride, et al,. 2002; Moss et al., 1997)*. A higher magnitude of muscle loading causes a higher degree of muscle strength improvements. The association between the magnitude of muscle loading and muscle strength improvements has been found during combined (i.e. concentric-eccentric) resistance training *(Hortobágyi et al. 2001; McBride et al. 2002; Moss et al. 1997)* as well during isolated eccentric training *(Holm et al., 2008).*

In the present study, the training protocol which caused muscle loading in the ascending limb only (i.e. low range of motion) showed the lowest muscle strength improvements and a non-significant increase in mechanical power. There is evidence that the improvement in muscle strength gains after exercise is probably limited by the range of motion during the muscle loading *(Pinto et al., 2012; Bloomquist et al., 2013).* Pinto et al. (2012) reported 10 weeks resistance training on the elbow flexors at various range of motion increased muscle strength. However, the result of Pinto's et al. (2012) study depicted that muscle strength was significantly higher in muscle which performed the training through the full range of motion in comparison to training in only a partial range of motion *(Pinto et al., 2012).* The authors argued that the tension gained from the training is related to the number of cross bridge attachments within the sarcomere and the maximum tension is generated on the optimum range of sarcomere length. The tension produced by a muscle is related to the fiber length, which is related to joint position. Thus, the force generated would be different when the intended muscle is trained at a different range of motion.

This argument is in accordance with the result of our study, since the knee extensor muscles, which trained only in ascending part, shows the lowest muscle strength improvements compared with the training through full range of motion. Therefore, training in different range of motion could be a possibility to see different results in our study too. Another explanation for this outcome would be the shorter duration of muscle contraction in a given repetition during intervention, which is performed in the ascending part of the force-length curve, in comparison to other training protocols. Since muscle force production is mainly influenced by muscle length, also depends on angular velocity. Then it could be assumed that reducing the contraction duration by shortening the eccentric range of motion would impair muscle strength.

It is reported by the researchers, that resistance training with maximum or near maximum load can increase maximum mechanical power *(Moss et al., 1997)*. The improvement in muscle strength and mechanical power of the knee extensors induced by training protocol 2 may seem surprising in the light of the much lower angular velocity (90°/s) in comparison with protocol 4 with a higher angular velocity (240°/s). It is reported that strength and power gains after isokinetic eccentric and concentric training are highly characteristic to the velocity of movement *(Farthing and Chilibeck, 2003; Seger et al., 1998; Roig et al., 2009).* A small number of investigations have attempted to draw the velocity-specific nature of only chronic eccentric contractions by subjecting individuals to different eccentric training velocities *(Paddon-Jones et al., 2001).* Paddon-Jones et al. (2001) and Shepstone (2005) reported that 8 to 10 weeks of eccentric training with a higher lengthening velocity is more effective to increase muscle strength in comparison to lower lengthening velocities. It is notable to mention that these two studies were performed on elbow flexors and the highest lengthening velocity was 180°/s. The authors concluded that a greater quantity of protein remodeling (Z-band streaming), caused improvements in motor unit recruitment and/or greater damage on contractile tissues would be the result of increasing muscle strength after interventions with higher lengthening velocities *(Paddon-Jones et al., 2001)*. However, the result of our study shows the eccentric contraction with higher lengthening velocity (240°/s) does not significantly increase muscle strength in comparison with training at a lower angular velocity. Since differences in the methodology of the study, such as a trained muscle group, exercise device and training protocols, it is difficult to directly compare the results of the previously mentioned studies.

An explanation for the higher strength value during the training with loading at a low angular velocity could be the duration of contraction in each repetition. In order to compare the protocols, the volume of training (integral of the moment over the time) was the same during each of the interventions. This means that the participants in protocol 2 performed 6 eccentric contractions with 90°/s in each set, in contrast to 16 eccentric contractions with 240°/s in training protocol 4. The velocity that muscle contracts at during each repetition with lower lengthening velocities was ~2.6 time slower than the training with higher lengthening velocities. Therefore during a given repetition, eccentric contraction was performed for 0.83 s in the protocol with lower lengthening velocities, but it was only 0.31 s in the protocol with a higher velocity. Consequently, it may seem specious that a training stimulus with maximum load and greater duration of eccentric contraction would cause greater muscle strength.

The functional consequence of an increase in muscle fiber length is a shift of the moment-angle relationship in the knee extensors, to greater knee angle (i.e. to the higher length of the muscle-tendon unit) *(Butterfield and Herzog 2006a).* Further, with a longitudinal adaptation in the muscle fiber, it is expected that the maximal mechanical power would also be increased and would be achieved at a higher angular velocity, because of the expected increase in the maximal shortening velocity of the muscle. By the same muscle fiber composition, longer muscle fibers show higher maximal shortening velocity due to the increased number of sarcomeres in series. The aforementioned possible shift in the joint moment-angle relationship has been used often in the literature as an indirect indicator to examine the effects of eccentric training with regards to longitudinal adaptation of the muscle *(Brockett et al., 2001; Proske and Morgan, 2001).* In the applied training protocol with the highest angular velocity (i.e. 240°/s) the fascicle length of the vastus lateralis was increased ~14% on average. Therefore, due to this adaptive response it would be expected to shift the moment-angle and mechanical power-angular velocity curve to the right after the intervention. No shift was found in the moment-angle curve or in the maximum mechanical power-angular velocity relationship of the knee extensors in the current work, as a consequence of the observed increase in fascicle length in protocol 4. This questions the accuracy of this functional criterion regarding longitudinal muscle adaptation.

Several biological factors within the muscle tendon-unit may affect the moment-angle or mechanical power-angular velocity relationship of the knee extensors muscles. Beside the longitudinal adaptation, tendon stiffness and activation level during the maximal isometric or isokinetic contractions can affect the characteristic of the moment-angle and mechanical power-angular velocity curve before and after an exercise intervention. Since tendon is in series with the muscle, the magnitude of fascicle shortening may be affected by the elongation of the tendon. During isometric contraction used in this research, to calculate the moment-angle relationship, the length of the muscle-tendon unit remained constant. Therefore the tendon elongation during the isometric contractions and thus the shortening of the muscle fibers until the plateau of the achieved maximum moment was dependent on tendon stiffness. Recent studies depicted that resistance training increases the tensile stiffness of the tendon *(Reeves et al. 2004; Arampatzis, et al., 2007b; Arampatzis, et al., 2010).* With a stiffer tendon for a given tendon force and a given muscle-tendon unit length, the elongation of tendon will be shorter and in this case the fascicle length shorter as well. This means that an isolated increase in tendon stiffness without any changes in muscle strength or fascicle length would cause a shift of the moment-angle relationship to the lower knee angles (i.e. in the opposite direction compared to an isolated increase in fascicle length). In a similar way, an isolated increase in muscle strength without any changes in tendon stiffness and fascicle length would introduce a shift of the moment-angle curve to the greater knee angles. Therefore due to the fact that the shift of the moment-angle curve cannot be affected only by changes in fascicle length we can conclude that this criterion is not accurate to assess possible longitudinal muscle adaptation after an exercise intervention.

After the 10-weeks of training with protocol 4, an increase in muscle strength during maximum voluntary isometric knee contractions and an increase in fascicle length of the vastus lateralis were present. Both parameters can affect the maximum mechanical power of the knee extensors. However the mechanical power-angular velocity relationship examined during the maximum voluntary isokinetic contractions did not change after the training. The absence of an increase in maximum mechanical power in protocol 4 is difficult to explain. During the maximum voluntary isokinetic contractions we supposed the maximum activation level of the knee extensors as well. However, we did not measure the activation level of the knee extensors during our isokinetic as well as isometric tests. During maximum isometric knee extension contractions the activation level in healthy participants is quite high (~93-97%) *(Babault, et al., 2001)* and therefore the contribution to the findings should be marginal. During maximum voluntary isokinetic contractions the activation level can be lower and more variable compared to the isometric trials. This means an uncontrolled effect on the estimation of the mechanical power-angular velocity relationship before and after the training. Possible differences in the activation level in the pre and post measurements can considerably affect the findings and could be a reason why we saw no increase in maximum mechanical power after the intervention in protocol 4.

7.3 Possible explanation of the findings

According to the results of our study and implicated reports, the main question is whether there is a possible explanation for increased vastus lateralis fascicle length after 10 weeks of eccentric exercise only with the high lengthening velocity? To answer this question we conceived two possibilities:

7.3.1 Popping sarcomere hypothesis

It is well known that unaccustomed eccentric contraction leads to subsequent muscle soreness. It has been proposed that there is a relationship between muscle soreness and muscle damage, which provide evidence that mechanical factors can be an initial event *(Morgan 1990; Wood et al. 1993).* Morgan (1990) was among the first to present evidence of non-uniform distribution of sarcomere length during eccentric contraction. Morgan at 1990 proposed the popping sarcomere hypothesis. He predicted by using a simulation approach that lengthening the active muscle on the plateau of the length-tension curve or beyond that would cause non-uniformly with some sarcomeres lengthening much more than others. This means that when active muscle extends at long lengths, all sarcomeres do not show constant lengthening, but rapid extension of sarcomeres results in the development of a non-uniform distribution of sarcomere length from the weakest toward the strongest one. Lengthening of the sarcomere beyond filament overlap occurs randomly throughout the muscle fiber. After stretching of a muscle fiber and during the relaxation period, most sarcomeres recover to a normal length, while a few of them do not reinterdigitate to their myofilaments length and may become disrupted. This makes a weak point in the filament lattice to be pre-disposed for overstretching during the next eccentric contraction. Whereas the most fragile sarcomeres attained their “yield tension, the corner at the end of the steep phase of rising tension during stretch” *(Morgan 1990),* their length would quickly and uncontrollably increase, until there was no filament overlap. At this point, internal or passive force raises and prevents them from further lengthening. By repeating the contraction, the membrane would be damaged which leads to a release of calcium into the sarcoplasm and distribution of the damage *(Morgan 1990; Wood et al. 1993).*

In our study, all protocols have been designed to induce eccentric contractions in different loading magnitudes, range of motion and angular velocity. However, the results show an increase in fascicle length after the intervention, only in the protocol with a higher angular velocity. Therefore, in the light of popping sarcomere hypothesis, it raises a question why the other eccentric training protocols did not cause an increase in fascicle length?

The participants in training protocol 3 performed eccentric contractions only in the ascending limb (i.e. low range of motion). We showed that subjecting muscle to a lower range of eccentric contraction improved the muscle strength, although only in one knee angle, and no increase in fascicle length. Whereas the sarcomere disruption due to the active lengthening of the muscle would be expected to occur in the descending part of force length relationship, as is the main assumption of non-uniform lengthening of sarcomere in popping sarcomere hypothesis *(Morgan 1990).* Therefore it could be suggested that muscle fibers during eccentric training on the lower range produced force on the ascending and/or plateau region of force length relationship, and then does not cause instable lengthening of the sarcomere across the fascicle, which leads to muscle damage. This argument could be an explanation for the lack of increase in fascicle length after the training in protocol 3.

However, in concern to the lengthening velocity, no lengthening of the fascicle after eccentric training with lower angular velocity on protocol 1 and 2 could be controversial. The results show the lengthening of the fascicle only after eccentric training with higher angular velocity (protocol 4). Morgan (1990) reported that when a muscle fiber is lengthened at a constant velocity, the tension initially rise steeply. However, at a certain level, further increase in lengthening velocity does not increase tension. This demonstrates the tension is independent from lengthening velocity at higher rate and has been justified by the instability of the sarcomere on the descending limb of length tension curve. Since the weakest sarcomeres on the descending part of length tension curve are unable to hold the existing tension at any velocity. Therefore at this point, rapid lengthening causes stretching of the whole muscle fiber, and even induces a decline in the tension for a time, as other parts of the fiber shorten. Later on Talbot and Morgan at 1996 provided direct evidence to support this hypothesis also *(Talbot and Morgan, 1996).*

If we assume that the muscle damage due to instability of sarcomere initiates the longitudinal muscle adaptation, it seems that the lower angular velocity which was included in training protocols 1 and 2, would not cause any damage due to the instability of sarcomeres in the muscle fascicle. In the exercise protocol with the high angular velocity the maximum lengthening of the muscle fascicle happened while the joint moment decreased. This finding provided evidence that the lengthening of the fascicle in the descending part of the force-length relationship occurred whiles the muscle decreased its activation level. Lengthening of the fascicle in the descending limb of the force-length curve at high stretch velocity, and deactivation of the muscle in the same time, might increase the instability of the sarcomeres and thus initiate longitudinal muscle adaptation. Butterfield and Herzog (2006) reported that lengthening of the fascicles by the deactivation of the muscle was one of the most important prediction factors for inducing longitudinal adaptation in rabbits. It can be assumed that the higher lengthening velocity in combination with a decrease in the activation level during protocol 4, favoured an uncontrolled lengthening of the muscle fascicle, which leads to higher muscle damage compared to lengthening contractions on lower angular velocities (protocols 1 and 2). Although we did not examine any biomarkers for muscle damage in our experiments and therefore there is no direct evidence regarding the amount of muscle damage between the investigated exercise protocols, we can argue a high lengthening velocity in the ascending limb of the force length relationship and at the same time a deactivation of the muscle may be an important mechanical stimulus for longitudinal muscle adaptation.

7.3.2 Mechanotransduction mechanism (Role of titin)

It is generally accepted that resistance training perturbs the muscles tensional entirety. This perturbation is mechanochemically transduced into a muscle's cells *(Tidball, 2005),* and finally results in specific structural adaptations *(Toigo and Boutellier, 2006).* One of the main proteins responsible for detecting mechanical strain in a particular position along the sarcoma is titin *(Lange, 2005).* It assumes that titin kinase has the ability to transmit the mechanical load from the sarcomere to the respective nucleus, and consequently regulating protein synthesis and turnover *(Tskhovrebova and Trinick, 2008).*

From a structural point of view, the titin molecule is a giant (around 3–3.7 MDa) sarcomeric protein, more than one micrometer long that involves a series of spring components within its I-band area *(Prado et al., 2005; Toigo and Boutellier, 2006).* Titin extension is through half sarcomere from M-line (C-terminus) to Z-disks (N-terminus). It is attached to the thick filament in the A-band region of the sarcomere. Titin is elastic in the I-band region of the sarcomere and it extends when the sarcomere is stretched, this may therefore contribute to increasing passive muscle force. Its arrangement depicts that titin involves about 300 domains similar to immunoglobulins (Ig, I-set) and fibronectins (Fn, type-3). The I-band region involves primarily Ig domains, N2A domain and PEVK fragments which are arranged in tandem in skeletal muscle. N2A is known as an isoform property of skeletal muscle and the PEVK named according to its most common amino acids (P, proline; E ,glutamic acid; V, valine; and K, lysine). In the I-band area and near the N2-line, this array is divided by a single chain that is connected to the distal and proximal (to the Z-line) tandem-Ig segments. On the other side, which is connected to the thick part of the sarcomere, titin is composed of both Ig and Fn3 domains *(Tskhovrebova and Trinick, 2010)*. In the center of the sarcomere in situ, close to the M line, titin is involved in the kinase domain. The kinase domain could be a trigger for the signaling pathway, which is evident in the feedback control mechanisms, and behaves as a receptor and transmitter of mechanical signals to muscle. Titin kinase has a link to scaffolding proteins and RING-finger, such MURFs (novel titin binding protein *(McElhinny et al., 2002)*) act in muscle gene expression *(Tskhovrebova and Trinick, 2008)*.

During passive lengthening of the sarcomere over a short range, the folded tandem Ig domains elongate and passive tension changes slightly. However, when sarcomeres lengthen further, the PEVK part stretches and passive tension increases rapidly. Elasticity of skeletal muscle fibers during passive stretch is highly characterized by extension of the PEVK segment *(Monroy et al., 2012)*. Furthermore, atomic force spectroscopy methods demonstrate two series of peak force during pulling sarcomeres. The first low peak force results from an unfolding of the kinase domain and the second large peak, illustrates unfolding of the Ig and Fn domains *(Tskhovrebova and Trinick, 2008).*

From a functional perspective, maintaining sarcomere integrity *(Horowits and Podolsky, 1987)*, contribution to passive tension *(Linke et al., 1998),* regulating myofibrillar assembly *(Gregorio et al., 1999)*, cell signaling *(Krüger and Linke, 2011)* as well as sarcomere elasticity; are the major functions of titin *(Granzier and Irving, 1995; Maruyama et al., 1985).*

In an experiment by Leonard and Herzog (2010), myofibrils were stretched actively and passively, beyond the overlap of the thick and thin filaments (i.e., sarcomere lengths more than 4 μm). The magnitude of force increased in both conditions and the differences were significant at the longest lengths. This study provides evidence that increases in passive force requires not only Ca^{2+} influx but also active force production. The authors concluded that titin adjusts the muscle force relay on cross-bridge force-dependent titin-actin interactions. The results show the role of titin in stabilizing sarcomeres in the descending limb of the force length relationship to protect the muscle against stretch induced muscle injuries *(Leonard and Herzog, 2010).*

It has been reported that the sequence of the titin kinas domain can rupture as a consequence of stretching velocity. This tear is known as an essential property of the force induced activation mechanism. The N terminal sheet in the titin domain (close to Z discs) is significantly more stable in slow pulling velocities than C terminal sheet (close to M line) (figure 7-1). In contrast, at higher stretching velocities, both N and C terminals would rupture in a similar way. The different sheets orientations to the applied stretching velocities expected to cause this result. Whereas the N terminal sheet are placed in parallel to the pulling direction and the C terminal sheets positioned in a vertical orientation *(Gräter et al., 2005).*

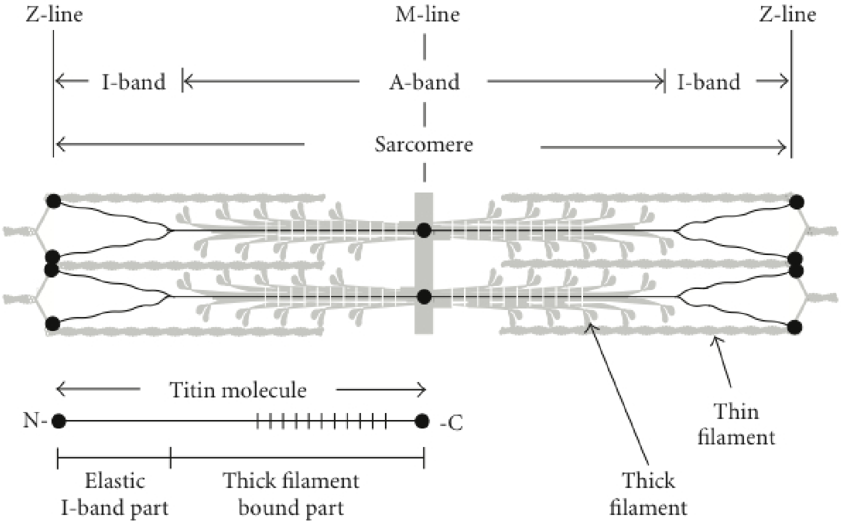


Figure 7-1: Layout of titin in the skeletal sarcomere and domain periodicity in the thick filament bound part. Schematic representation N- and C-terminal regions in titin which are accommodated in the Z-disc and M-line, respectively. This figure is reprinted from *(Tskhovrebova and Trinick, 2010).*

According to this argument and different lengthening velocities, which were performed during our training interventions, it could be expected that eccentric contractions under higher lengthening velocities (protocol 4) present more instability on titin constructions and hence makes more rupture in the titin domain. In this scenario, we suspicious that the titin kinas domain under higher lengthening velocities interacts to produce a mechanically modulated regulation of protein turnover and gene transcription in muscle and may contribute to longitudinal adaptation of the muscle.

8. Strengths and weakness

In this part of the work we would like to present the strengths and weaknesses of the current PhD study. A critical approximation of the methodology employed and the reported results would help the readers to identify the advantages and limitations of the scientific knowledge.

8.1 Strengths of the study

The dynamometer system used for training and testing purposes allows the control of magnitude of muscle loading, velocity of movement and contracting designated muscle(s) through a specific range of motion. Eccentric contraction has been investigated in several studies. However controlled loading, velocity and muscle length where the eccentric stimuli is applied were a significant limitation among previous investigations. For instant, Brockett et al. (2001) used specialized self-made equipment to perform an eccentric intervention on the hamstring muscles *(Brockett et al., 2001)* which is not accurate enough in compare with the dynamometer device. Therefore, one of the most advantageous points in our study was performing eccentric contractions throughout the intervention using the dynamometer device to control the intended parameters (i.e. load magnitude, angular velocity and range of motion).

Another advantage in the present study was being able to compare the output data of different protocols, which were performed with the same training volume. As mentioned in previous chapters, the volume of the training (integral of the knee joint moment over the time) was equal in all protocols. In this respect we made a direct comparison between different training protocols.

An additional strength of the current work is the quantification of fascicle length during the different isokinetic contractions. As mentioned in details in the methodology, the kinetics of vastus lateralis fascicle length have been assessed during the four designed protocols. In this way the elongation and strain of the fascicle is measured during eccentric contractions with different magnitude of loading and different lengthening velocities. This is done here for the first time compared to previous literature.

The accuracy of isokinetic parameters during maximum activation exertions would be affected with the presence of visual feedback. When the participants received biofeedback, the real time display of the moment production during their activation effort, the maximum moment was significantly higher *(Kellis and Baltzopoulos, 1996).* In this respect we designed a custom Matlab script so participants could visualize the duration as well as moment exertion of the knee extensors during training and testing sessions. According to this online feedback, we could be sure that participants achieved the same intensity of eccentric contraction over the entire training period.

The analysis of muscle fascicle length was a great concern in several studies using different methods. The ten cm ultrasound probe, which was used during fascicle length measurements, made fascicle length measures in this study more accurate. Since the length of vastus lateralis fascicle is often longer than the ultrasound probe, the longer probe results in a more enhanced fascicle image. In addition, we designed and developed a semi-automatic tracking algorithm in our group to analyze the vastus lateralis fascicle length (R2010, The Math works), based on the changes in brightness contours between two subsequent ultrasound frames on certain levels between the upper and deep aponeurosis. This Matlab script was developed at the department of training and movement science of the Humboldt-Universität zu Berlin.

8.2 Limitations

The 53 participants were randomly assigned into different experimental groups and one control group. From these participants, only 31 could finish the designed training interventions. Twenty two participants could not finish the training because of the knee pain. Most of the participants suffered from the pain under the knee patella after some training period.

According to the hypothesis of the study, it could be better to design four experimental groups and one control group to evaluate the variations of magnitude, velocity and muscle length where the eccentric stimuli was applied. However, because of restrictions to time and budget, we used both legs of the participants and applied four different training protocols. In this respect, we adjusted the experimental groups into two experimental groups and one control group.

Additionally, the impact of a cross-education effect led us to speculate that different training protocols on the contralateral limbs might be a limitation for the results of the study. The cross-education effect is an effect of one training protocol impacting on the opposite limb *(Colliander and Tesch, 1992; Shima et al., 2002; Tesch et al., 2004)* and is mainly evident with maximum voluntary unilateral contractions which can have a potential effect on the neural components which control the contralateral limb *(Lee and Carroll, 2006).* From the aspect of functional parameters (moment and mechanical power) it is reported that muscle strength was variably modified in an untrained limb *(Shepstone, 2005; Shima et al., 2002)* or remained unchanged *(Tesch et al., 2004)*. However, when both limbs trained, it appears that strength gains that transfer from one limb to other would be minimal, compared with the transfer of strength to a non-trained limb *(Shepstone, 2005)*. This argument has been reported also by Holm et al. (2008) that no cross-education effect was seen after resistant training in different legs by different magnitudes of loading *(Holm et al.,* *2008)*. The results of our study provide evidence that different training modes cause different gains in muscle strength even between two legs of the same individual. Therefore, it makes clear the different effect of the trainings protocols on muscle strength and demonstrates using contralateral limbs was probably not a limitation in this study. Further, it is reported in the literature that muscle architectural adaptation does not contribute to contralateral limb *(Kawakami et al., 1995).* Kawakami et al. (1995) reported, unilateral elbow extensor training for sixteen weeks did not cause significant differences in the thickness and fascicle angles as well as estimated fascicle length of the untrained arm *(Kawakami et al., 1995)*. Moreover, the study by Helge et al. (2007) revealed that one-leg knee extension training for 4 weeks had no effect on physiological parameters such as nuclear hormone receptor peroxisome proliferator-activated receptor α (PPARα) protein or mRNA expression in skeletal muscle *(Helge et al., 2007).*

Therefore we can argue the effect of cross-education training on muscle morphology, if any, would be to small to show significant effects on the current findings. Since the results partly support the hypothesis and demonstrate the ability to detect significant increases in fascicle length after 30 sessions of eccentric contraction with high lengthening velocity. Moreover, the sample size was limited to those who came forward to participate in the study, which may limit the degree to which inferences can be made to a larger population.

9. Summery of the study

Three strategies have been assumed for adaptation of the muscle in response to exercise and increasing power production. The longitudinal plasticity of muscle is one well-known phenomenon in this regard *(Williams and Goldspink 1978).* Animal studies describe that not only immobilization of muscle in longer position, but also eccentric exercise can mechanically stimulate and initiate longitudinal adaptation of the muscle *(Lynn and Morgan, 1994)*. Butterfield and Herzog (2006) reported that the best predictors for longitudinal adaptation in the rabbit tibialis anterior muscle, were maximal moment and the magnitude of fiber strain. In humans, there is little information about the effect of controlled eccentric loading on the longitudinal adaptation of muscles. The purpose of this study was to investigate the effects of magnitude, velocity and muscle length at which the eccentric stimulus was applied on the longitudinal adaptation of the vastus lateralis muscle. The 31 participants were randomly assigned into 2 experimental groups (n=20) to perform 30 sessions of eccentric training (3 times per week for 10 weeks) and one control group (n=11). The first experimental group (n=10) exercised the knee extensors of one leg (protocol 1) **at 65% of MVC** and the second leg (protocol 2) **at 100% MVC** at 90°/s and from 25° to 100° knee angle on a dynamometer device (Biodex 3). The second experimental group (n=10) exercised one leg at 100% MVC at 90°/s and **from 25° to 65° knee angle** (protocol 3) and the other leg exercised at 100% MVC at an **angular velocity of 240°/s** and from 25° to 100° knee angle (protocol 4). The control group included 11 participants without specific eccentric training. In pre and post measurements we examined the fascicle length of the VL from a 20° to 90° knee angle by ultrasonography, and also the moment-angle (from 55° to 85° for every 5° interval) and power-angular velocity relationship (from 270°/s to 500°/s angular velocity) of the knee extensors.

We found an increase ($p<0.05$) in the fascicle length of vastus lateralis compared to the control group only in the leg trained with the higher angular velocity protocol (protocol 4). The increase in fascicle length (~14%) was similar across the whole knee angle range. The high strain velocity of the fascicles has been located in the phase where the moment decreased. Furthermore, the strength of the knee extensor muscles was improved in all protocols with the greatest improvement in the training protocol with the higher magnitude of loading (protocol 2). The results showed an increase in mechanical power only in the protocol with lower angular velocities (protocols 1 and 2). Also did not depict any shift in the mechanical properties of muscle toward the right on the moment-angle and mechanical power-angular velocity curve.

The findings give evidence that (a) not all types of eccentric exercise cause an increase in fascicle length and (b) the velocity of fascicle stretch seems to be important for the longitudinal adaptation of the muscle. We concluded that the greatest improvement in muscle strength in protocol 2, could be explained by the higher magnitude of loading and greater duration of contraction in a given repetition. In contrast, the restricted muscle range of motion and the reduced contraction duration during the training intervention at which the eccentric contraction was applied on ascending part (protocol 3), leads to the lowest improvement in muscle strength. Furthermore, we suspect that tendon stiffness and activation level during the maximal contractions could affect the properties of the moment-angle and mechanical power-angular velocity curve after an exercise intervention. Therefore, the shift in the moment-angle curve could not be an accurate assessment for possible longitudinal muscle adaptation after an exercise intervention.

Finally, we speculate that the "popping sarcomere hypothesis" and/or mechanotransduction mechanism (i.e. role of titin) could be a possible answer for the lengthening of muscle fascicle after long-term eccentric contractions at a high angular velocity. According to the "Popping sarcomere" hypothesis, the instability of the sarcomeres takes place on the descending portion of force-length curve. The results of our study show the maximum lengthening of the muscle fascicle in the training protocol with the highest angular velocity, placed on deactivation of the muscle. Therefore, we suspected that a higher lengthening velocity might increase the instability of the sarcomeres and thus initiate a longitudinal muscle adaptation. Furthermore, sarcomere integrity *(Horowits and Podolsky, 1987),* regulating myofibrillar assembly *(Gregorio et al., 1999)*, as well as sarcomere elasticity *(Granzier and Irving, 1995; Maruyama et al., 1985)* are defined as the major functional role of titin. We speculate that the titin kinas domain under higher lengthening velocities may interact in the mechanically modulated regulation of protein turnover and gene transcription in muscle, and may contribute to its longitudinal adaptation.

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