THE PHYSIOLOGICAL & BIOMECHANICAL BASES OF MUSCULAR HYPERTROPHY/ ATROPHY

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Last and not least to all the rest of the academic staff and technicians of the MMU Exercise & Sport Science department who helped me throughout my time in the university.

I dedicate this thesis to my late father, my mother and wife, for all they have done for me throughout my life and given all that they could to me – I love you all.

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ABSTRACT;

Introduction: The aims of the current investigations were to modulate muscle-tendon complex (MTC - *vastus lateralis* [VL] & patella tendon [PT]) adaptations through mechanical stress and strain. Groups performed resistance training (8 weeks) with the MTC placed in a shortened (SL) or a lengthened position (LL) with internal loading standardised. A third group trained over an entire ROM (LX) with the external loading matched to that in SL. MTC response to detraining (4 weeks) was also measured. A control, untrained group was measured during this 12-week period.

Methods: Measurements using ultrasonography, dynamometry, electromyography and dual energy absorptiometry were made at baseline (week 0), post-training (week 8), detraining 1 (week 10) and detaining 2 (week 12). VL measurements included volume, cross-sectional area (CSA), and architecture. PT properties included stiffness and Young's Modulus. Quadriceps MTC function was measured by isometric maximal voluntary contractions (MVC) over a range of joint –angles. Circulating levels of a growth factor (IGF-I) and cytokines (TGF-β1, TNF-α) were measured using enzyme-linked immuno-sorbant assay.

Main Results: VL volume, CSA, fascicle length, PT stiffness, modulus, quadriceps MVCs and IGF-I (LL only) were significantly greater ($p<0.05$) in both LL and LX groups compared to SL post-training. During detraining, CSA, fascicle length, stiffness, modulus, IGF-I (LL only) remained significantly elevated in the LL and LX groups compared to SL. There was no significant change in the control group in any measurement during the study period ($p>0.05$).

Conclusion: Training with the MTC in a lengthened position is more effective for inducing (and retaining) enhanced training MTC adaptations, owing to internal mechanical and physiological stress in this position. This loading method should therefore be incorporated into a structured resistance training program for a range of populations such as athletic, recreationally active, clinical or elderly individuals.

THESIS INTRODUCTION

The current body of work aims to describe the biomechanics and physiological impacts on the muscle and muscle-tendon complex, of altering biomechanical aspects of resistance training. Resistance training is frequently used in a variety of settings, such as athletic performance, health and fitness, injury prevention or rehabilitation and to offset the debilitating effects of illness/ disease and sarcopenia (Little and Phillips, 2009, McCartney et al., 1995, Mujika and Padilla, 2000, Thomas, 2007). However, due to the nature of the objectives of the resistance exercise protocol, each training regime should be optimised to bring about a desired set of results such as strength, muscle hypertrophy or power enhancement.

Indeed one particular consequence of resistance training, provided a sufficient stimulus is provided, is the phenomenon of muscle hypertrophy. Muscle hypertrophy is an increase in muscle fibre size, not fibre number (MacDougall et al., 1984). Despite the wealth of research accumulated in this area, what key factors need to be manipulated to optimise the effects of training, remains a matter for further research.

In the first chapter, the review of the literature aims to remind the reader with an in-depth insight into how skeletal muscle is structured, how muscles are arranged, how they functions, and how these factors (from architectural arrangement through to functional characteristics) can be altered by resistance training. Firstly, both the general structure and ultrastructure of human skeletal muscle is described – from the fibre to the fundamental contractile units; sarcomeres (sections 1.1.1-1.1.2). In turn, the complex theories from a range of disciplines over the last 120 years that have advanced our knowledge of how structures and molecules interact to produce muscular contraction are discussed (1.1.3). Following this, the architectural arrangement and its

implications for muscle function are outlined, proceeded by the intrinsic relationships such as the length-tension and force-velocity relationships of skeletal muscle, and the factors that influence the relationships (1.1.4). Muscle exhibit plasticity in its characteristics. Indeed, it responds acutely to the stresses that perturb homeostasis (such as those presented in section 1.2.1), and following regular and progressive stress (i.e. loading), and adapts to its environment by altering muscle phenotype (sections 1.2.2.1-5 in Chapter 1). In addition to skeletal muscle, the structure and function of the in-series elastic component (i.e. the tendon) is also described (sections 1.3.1 and 1.3.2 respectively). The tendon, once viewed as relatively inert material, has proved to be a rich source of investigation in the last decade, as *in vivo* data demonstrates tendon's ability to alter its mechanical and material properties in response to various stimuli, with such evidence being described in detail in section 1.3.3. Finally, the evidence from literature on the key cellular events that precede such changes in muscle-tendon properties is shown (section 1.3.3.2).

Chapter two of this thesis outlines in detail the research methodologies and design adopted in order to investigate the desired parameters. Initially, the rationale for the choice of muscle-tendon complex for measurement is presented (section 2.1), and also the anatomical location and function of the complex in humans (2.2.1). Muscle and tendon parameters are then systematically outlined, with the detail of how each measurement is made and analysed, being described (2.2.2-2.2.10). Also included is information regarding the participants, the resistance training regime details and the statistical analyses used to identify changes and relationships between the parameters and groups involved in the study (section 2.2.11-2.2.18).

Chapter 3 begins by discussing the rationale for investigating the effects of altering training muscle lengths in order to induce a combination of mechanical stretch and force- induced hypertrophy. Furthermore, the possible changes to muscle architecture, such as changes in

pennation angle and fascicle length, are outlined along with the detrimental effects of a period of subsequent detraining (section 3.1). The results of the resistance training intervention are shown and discussed, with the morphological, architectural and changes in subcutaneous fat the main parameters investigated (section 3.3). The key evidence from the results is then linked to existing research in order to provide possible links to the main stimuli involve in the observations from the study in section 3.4.

With regards tendon adaptation to resistance exercise, it would appear from previous evidence that in a similar fashion to muscle, that both mechanical force and stretch (or strain) are also key to inducing adaptation of tendinous structures and their properties. The mechanical properties of the tendon, such as tendon stiffness, have been shown to increase following resistance exercise, but as in the case of muscle hypertrophy, this process is not fully delineated (section 4.1). Chapter 4 aims to answer some of the questions regarding changes to the muscle-tendon complex following resistance training, whilst systematically trying to investigate the relative contribution of lengthening of the complex in combination with forces through the tendon as stimuli. Alterations to the muscle joint angle-torque relationship, and as such, muscle maximal force generating capacity over a range of joint-angles, are also demonstrated, as previous isometric evidence has demonstrated that this can be altered following length (or joint-angle) specific training. As with muscle, periods of detraining result in a loss of training-induced changes to tendon properties, and this study aims to provide further information on both the rate, and magnitude of such changes (sections 4.3 and 4.4).

As mentioned above, acute responses to changes in loading pattern form the basis for the chronic adaptations described in the previous chapters. In chapter 5, the acute responses to the training stimulus provided by exercising at different muscle lengths are presented (section 5.1). These

include the electrical activity of the muscle, oxygen consumption and cardiovascular changes. From the evidence in sections 5.3.1 and 5.3.2, one can begin to make an informed hypothesis on how these responses contribute to not only the chronic responses in the aforementioned variables presented later in chapter 5, but also how these have contributed to the results observed earlier in chapters 3 and 4.

The adaptive response of muscle and tendon has been purported to be mediated by growth factors that have been shown to result in changes in the production of the tissue specific cells (section 6.1). Both IGF-I and TGF-β1 are two such examples that have been implicated in increasing protein and collagen synthesis in muscle and tendon respectively. In chapter 6 the results from the current investigation can ultimately provide further indication of their contributions to adaptations outlined in chapters 3 and 4 (section 6.3). Additionally, TNF-α is a cytokine that has been shown to be intimately linked with protein degradation in skeletal muscle models. TNF-α levels were also measured in order to provide information on how effective resistance training is in altering the circulating levels, and also how a bout of detraining also affects the level of circulating cytokine (sections 6.3 and 6.4).

Finally, chapter 7 takes a retrospective look at all the results observed in the each of the chapters and attempts to combine them to provide the reader with a clear and concise take-home message of how the training regimes have affected the muscle-tendon complex (sections 7.1 and 7.3) as a whole unit. This is done through the use of composite z-score analyses. This chapter also provides the opportunity to use the results of the current study to produce a predictive tool for use in further investigations (section 7.5). This is done through the use of correlations, bivariate and multiple linear regressions. Crucially, the information from the thesis generates further

research questions that remain to be elucidated and therefore outlines possible directions and implications for further research (see sections 7.2 and 7.4.

GLOSSARY OF TERMS

Aponeurosis; broad, flat tendon like structures found in pennate skeletal muscles to which fascicles or fibres are attached

Collagen; Protein that makes up to ~95% of the dry weight of tendon, found in both muscle and tendon

Electromyography; a technique for evaluating and recording the electrical activity produced by skeletal muscles

Fascicle; bundles of muscle fibres, used as a surrogate in estimating fibre length

Force-Velocity relationship; The relationship describing the changes in force developed at various contraction speeds from static to maximal shortening velocity characterised by a concave shaped curve

Hypertrophy; Increase in the size of individual muscle fibres

IGF-I (Insulin Like Growth Factor 1); Hormone involved in myoblast and fibroblast proliferation and differentiation

Length-tension relationship; The relationship describing the changes in tension developed in sarcomeres due to changes in its length characterised by an ascending limb, plateau region and elongated descending limb.

Mechanical stress; the physical forces experienced by components of a system which can be generated internally or externally

Moment Arm; The perpendicular distance from the joint centre (axis of rotation) to the line of force application

Muscle architecture; the arrangements of fibres within a muscle relative to the axis of force generation

Muscle length; The distance from the origin of the most proximal muscle fibres to the insertion of the most distal muscle fibres

MVC; maximal voluntary contraction (isometric)

Myofilaments; Protein filaments (actin & myosin) that form the structural basis for skeletal muscle contraction

Pennation angle; The realtive arrangement of the fibre (or fascicle) to the force generating axis

Plasticity; The ability to adapt phenotypical characteristics in response to the environment

Sarcomere; basic individual contractile unit of skeletal muscle

Tendon Stiffness; Describes the change in tendon length (ΔL) (deformation) in relation to the force applied to the tendon (ΔFt).

Tendon strain; describes the elongation/deformation of the tendon (ΔL) relative to the normal length (L_0)

Tendon stress; describes the tendon force (F_t) relative to the tendon CSA

TGFβ-1 (Transforming Growth Factor Beta 1); Pleiotropic growth factor involved in various cell functions, which are dependent on cell type.

TNF-α (Tumor Necrosis Factor Alpha); Pro-inflammatory cytokine associated with proteolysis

Young's modulus; describes the relation between tendon stress and tendon strain. Modulus represents the properties of the actual tendon material independently of the CSA.

Author's Contribution to Work:

The author was the main contributor to the training protocol design (sets, reps, intensity, recovery, exercise order) and duration (8 weeks resistance training, 4 weeks detraining). The author also recruited the target population, and verified adherence to inclusion/exclusion criteria. In addition, the author actively contributed to the decisions on ranges of motion as well as outcome measures.

During data collection, the author performed all of the ultrasound, electromyographical, gross anatomical, strength, cardiovascular measurements made during the studies on each of the training groups and control groups at all time points. The author was also responsible for the collection of venous blood samples. Furthermore, as a fully qualified Strength & Conditioning coach and professional fitness instructor, the author supervised 94% of the training sessions. The remaining sessions were supervised by a member of the research team in conjunction with a qualified gym instructor.

The author also assisted in all laboratory sessions related to the biochemical analyses, which were carried out in conjunction with the Director of Studies (Dr. Gladys Pearson). The vascular ultrasound measures were made by Georgina Stebbings, an MSc student, with acute measures (heart rate, blood pressure and oxygen consumption) made by three BSc students (Ross Parkin, Mike Hewitt, Liam McArthur) under the direct day-to-day supervision of the author.

Following data collection, data processing and statistical analyses were also carried out by the author.

CHAPTER 1:

LITERATURE REVIEW

1.1 THE HUMAN SKELETAL MUSCLE:

1.1.1. Skeletal muscle function:

In humans, skeletal muscle carries out several key primary functions, and also has other properties that allow contribution to secondary functions as by-products of their main function. The main functions associated with skeletal muscle are listed below:

- Force generation
- Execute movements of the skeletal component
- Assist with thermoregulation
- Maintain posture and support
- Circulation (particularly venous return)

Each of these plays an important role depending on the requirements of the internal and external environments.

1.1.2. Skeletal Muscle Structure:

The structure of human skeletal muscle tissue reflects its function, which is primarily to generate force in order to produce movement of the skeletal system. Skeletal muscle is often referred to as 'striated' muscle due to its striped appearance under the microscope, which is the result of the arrangements of the dark anisotropic A-bands and light isotropic I-bands within the muscle fibres when viewed longitudinally (Macintosh et al., 2006).

1.1.2.1 General structure

The structure of skeletal muscle is shown in Figure 1.1. The bundles of fascicles are made up by bundles of muscle fibres, which are in turn composed of the smaller myofibrils. Each muscle fibre, enclosed by the sarcolemma, contains sarcoplasm in which organelles, such as mitochondrion are present. The myofibril is a long threadlike structure approximately 1-3 μm in diameter and contains a number of repeating units called *sarcomeres* (Figure 1.2). It is the resultant contraction of these individual units that brings about whole muscle contraction (discussed in 1.1.4).

1.1.2.2 Sarcomeres

Each individual sarcomere is mainly composed of two types of protein filaments, or myofilaments. Actin (thin) myofilaments are ~8nm in diameter and 1000nm in length, whereas myosin (thick) myofilaments are ~12nm in diameter and 1800nm in length (Seeley et al., 1992).

When a muscle fibre is at rest, there is a partial overlap between the myofilaments in an interdigitating manner, which gives the characteristic 'dark' appearance within the A-band. The H-zone occurs in the central region of the sarcomere, and has a relatively paler appearance due to the lack of filament overlap in this area. Lying centrally within the H-

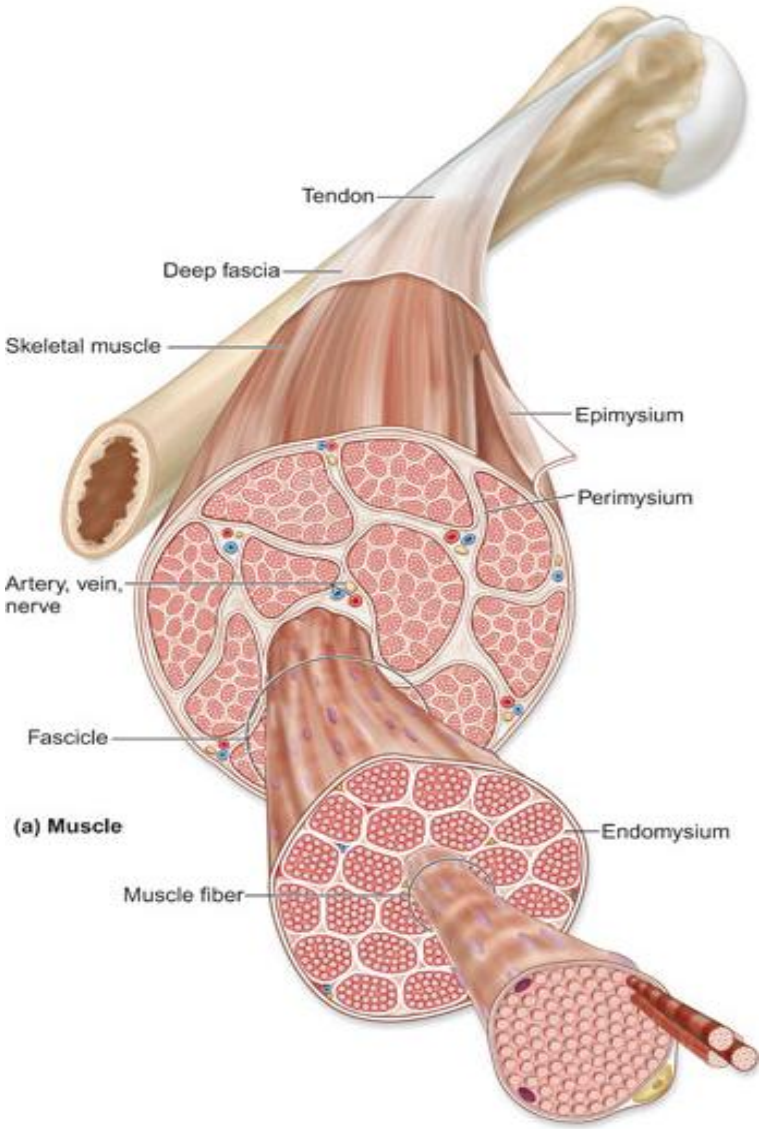


Figure 1.1. *Structure of skeletal muscle, showing connective tissue components and arrangement of muscle fascicles, fibres and myofilaments.* (Taken from http://academic.kellogg.edu)

zone is the darker M-region, which is made up of proteins (such as myomesin, M-protein and creatine kinase) that label the myosin filaments, some of which cross-link adjacent myosin filaments to form M-bridges (Squire, 1997). Each sarcomere is defined at either side by the Z-band (or disc), where antiparallel actin filaments are cross-linked by a structure mainly comprised of α-actinin. The largest protein discovered, named titin (or connectin) extends from the Z-band to the M-region in one half sarcomere. Its primary

roles appear to be to act as a longitudinal stabiliser for myosin, contribute to elasticity when stretched, and to serve as a template or organiser for myofibril assembly (Granzier and Labeit, 2007). Finally, the 'light' I band is the region where only the thin filamenets, actins, appear. The arrangement, interaction and dynamics of sarcomeres when undergoing contraction will be discussed in more detail in section 1.1.4.

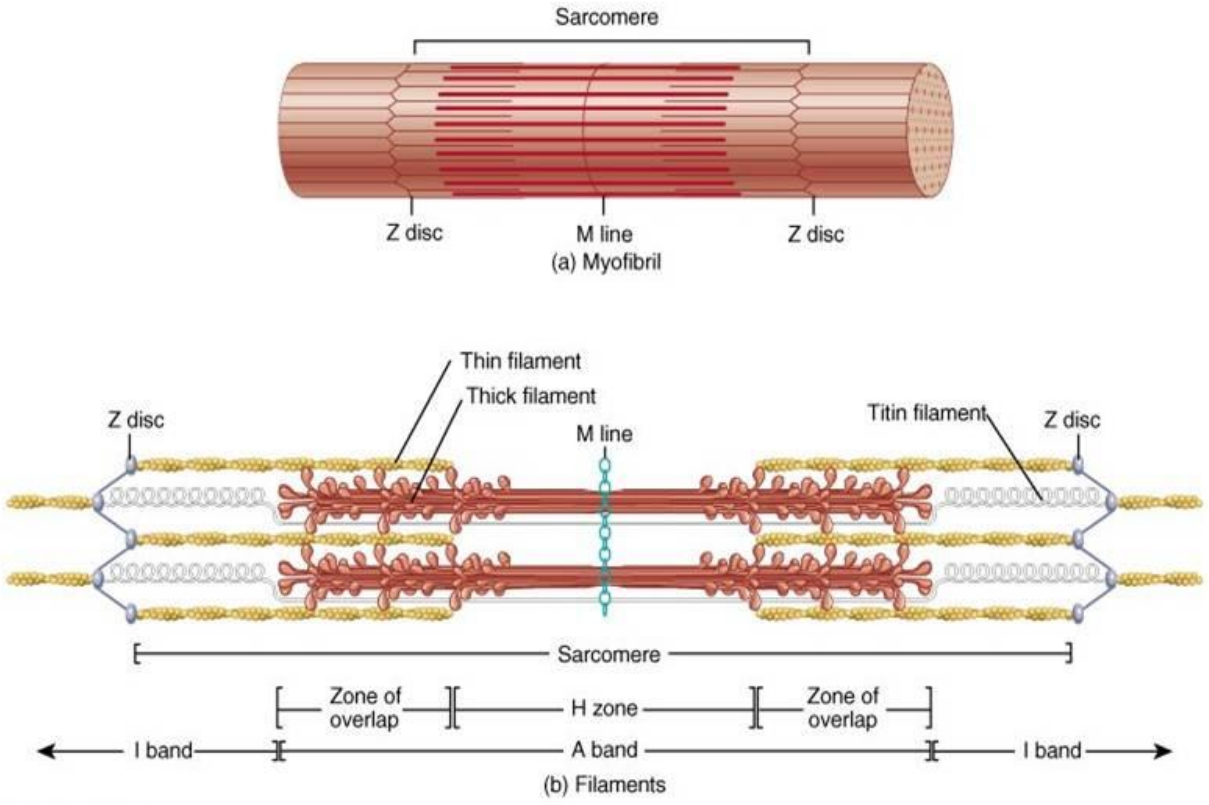


Figure 1.2. *Structure of a single sarcomere, showing myofilament and structural components, and the charateristic I and A-bands, H-Zone, and Z and M-regions.* (Taken from http://bailey.bio.com)

1.1.3. Contraction of skeletal muscle

Excitation-Contraction Coupling

In order for muscular contraction to proceed, the muscle must be first stimulated to do so. A difference in electrical potential exists between the inside of a cell and its external fluid environment. Inside the muscle fibre are potassium cations (K^+), whereas outside the muscle fibre, the predominant cation is sodium (Na^+) and anion is chloride (Cl^-). When the motor neurone membrane becomes depolarised beyond a certain threshold (by electrical, chemical or mechanical means), the membrane becomes permeable and Na^+ ions move into the cell via concentration gradient and under the attraction of the internal negativity, resulting in the inside of the cell becoming more positively charged. Influx of Na^+ ions into the cell decreases with a simultaneous efflux of K^+ ions (via the Na^+-K^+ pump), which restores the resting potential and polarisation. Once this has been initiated, excitatory changes automatically take place in neighbouring regions of the membrane, allowing transmission along the length of the fibre (in humans about 50-65m/s and 40-55m/s in the upper and lower limbs respectively). During the action potential, the interior of the fibre is some 30mV positive relative to the exterior, while further along the fibre a resting potential is maintained (-85mV relative to the outside), providing a sink for the current to pass along, until the potential arrives at the terminal branches of the axon and the motor end-plate.

The terminal portion of the axon forms the smaller axon branches, whose endings, the pre-synaptic terminals, lie close to, but do not contact the sarcolemma (i.e. fibre membrane). The release of acetylcholine (ACh) changes the electrical impulse to a chemical stimulus, causing the opening of ion channels in the fibre membrane which depolarises and spreads the impulse to the transverse or T-tubules. Voltage sensors (i.e. dihydropyridine receptors) detect the depolarisation and trigger the opening of calcium

(Ca^{2+}) channels (ryanodine receptors) in the terminal cisternae of the sarcoplasmic reticulum (SR).

The cytoplasmic concentration of Ca^{2+} ions increases to ~10^{-5}M, and rapidly bind to the troponin C molecules (located on the actin filament) causing a conformational change that lifts the tropomyosin molecule (attached by troponin T) away from the myosin-binding sites, allowing the myosin heads to attach to the actin filament, and thus form cross-bridges.

The myosin-ATP complex can now bind to actin, which results in the partial hydrolysis of ATP by ATPase to produce ADP + Pi + E. This exergonic reaction produces ~40-55kJ.mol^{-1}, although this can vary depending on pH and temperature. The myosin head is now able to move the actin filament along by alteration of the angle of the head, it can then break, and provided there is a sufficient source of ATP, can reattach to another site further along the actin filament, generating muscle tension. ATP can be regenerated simultaneous to its breakdown by the reaction of phosphocreatine (PCr + ADP + E = ATP + Cr), catalyzed by the enzyme creatine kinase. The net free molar energy (available for mechanical work) as a result of the two aforementioned reactions is ~26-36kJ/mol^{-1}. A molecule of ATP can produce 2-4 cross-bridge formations, with the exact number still not fully known, and can be dependent on activation history (Gordon et al., 2000). With intracellular concentrations of ATP/ PCr being limited (55-95mmol.Kg^{-1}.dm^{-1}), contribution to energy provision is increasingly made by the glycolytic and aerobic systems.

Sliding Filament Theory

The sliding filament theory was devised originally and independently by Andrew Huxley and Rolf Neidergerke and by Hugh Huxley and Jean Hansen in 1954 (Huxley and Niedergerke, 1954, Huxley and Hanson, 1954). With the use of the electron microscope, Hugh Huxley studied the arrangement of the thick and thin filaments within myofibrils, and noticed each thick filament was surrounded by a hexagonal array of thin filaments (Figure 1.3A), which were then confirmed to be the previously known contractile proteins of actin and myosin. Using the interference microscope, Andrew Huxley was studying the striations of frog muscle fibres undergoing contraction and relaxation, and observed that when undergoing contraction, the bands of the sarcomere changed (depicted in Figure 1.3B). The light I-band became shorter, whilst the darker A-band remained the same length; although within the dark A-band, the paler H-zone narrowed or disappeared completely. The two groups independently published their findings, and proposed that they could be attributed to the relative sliding of the actin and myosin filaments past one another, and this idea came from the apparent constant lengths of the two filaments.

However there are several variations since this theory was developed, although the general features and framework of the model still remain in textbooks. The success of the theory is supported by several pieces of evidence:

1) The theory's structural underpinning is supported by x-ray diffraction evidence (Squire, 1983).

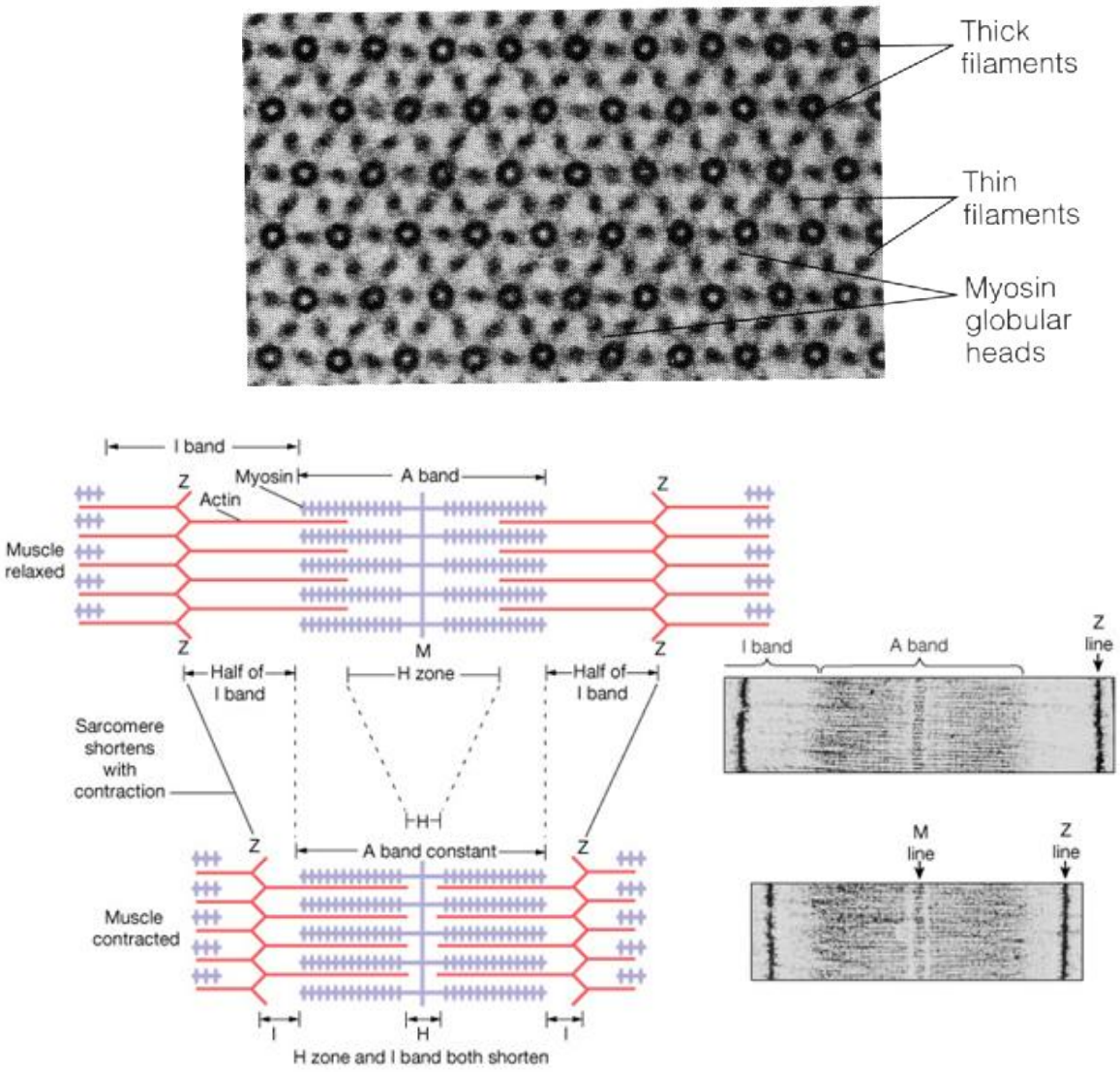


Figure1.3. *Cross-sectional view of the hexagonal arrangement of thin filaments around the thick filaments along the length of the sarcomere (A) and the change in the band widths following contraction – sliding of filaments (B).* *(Taken from physioweb.uvm.edu)*

2) The myosin head contains the expected actomyosin ATPase site (Eisenberg and Greene, 1980). It should be noted here that there is still uncertainty about the exact nature of the molecular states at this step (Gordon et al., 2000, Pollack, 1995)
3) A scheme coupling actomyosin-ATPase with a plausible mechanical cycle can be formulated from biochemical kinetic evidence (Bagshaw, 1982)

4) A structural basis for realisation of the cross-bridge stroke is implicit in crystallographic evidence for the structure of actin and myosin (Rayment et al., 1993b, Rayment et al., 1993a)
5) Mechanical measurements are in good agreement with theoretical expectations (Finer et al., 1994), with the exception of eccentric contractions, where more work is currently taking place on delineating the relationship between experimental observation and prediction.

However, the focus will now turn to the more contemporary theories of the sliding filament and cross-bridge theories, due to the limitations of the original models and technological advances.

Modifications to the Theory

Overview of Huxley's 1957 model: The first of Huxley's cross-bridge theories (Huxley, 1957) stated muscles generate tension or force (***F***) by myosin filaments forming cross-bridges with actin filaments, and these cross-bridges act as independent force generators and move the actin filaments along the myosin filaments. The actin filament is displaced by a variable distance ***x***, so that ***x*** would be small as the sarcomere shortened. ***F*** was assumed to be proportional to ***x***, and could be positive, negative or zero during shortening, lengthening or isometric conditions respectively.

However, as the relative movement of actin along the myosin filament is many times larger than the length of any individual myosin molecule, it was proposed that during muscle shortening/ lengthening, each cross-bridge has a limited displacement capacity ***h***,

over which it can attach (i.e. a step size of 10-20nm). Thus, the cross-bridges need to attach, stroke, break and then reattach further along the actin filament. The stroke produces mechanical work and the involvement of energy liberation generates heat. The rates (***f*** and ***g***) of the attach-break-reattach cycle were proposed to be both a function of ATP utilisation and also the ***x*** distance. This implies that when ***x*** is positive, such as during shortening, cross-bridges will attach more rapidly than when negative, such as during lengthening. So when a cross-bridge is in a position to exert force, and ***f*** > ***g***, then it will (Figure 1.4).

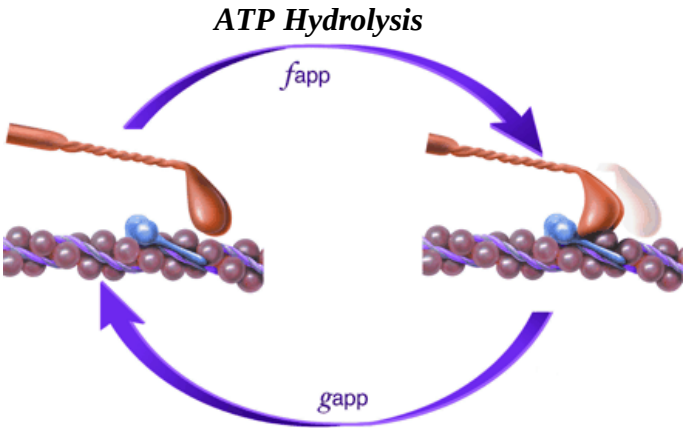


Figure1.4. Huxley (1957) *Cross-bridge in its two functional states – attached (exerting force); detached (not exerting force). The change in states was dependent on the energy derived from ATP hydrolysis, while the rate itself was determined by displacement 'x'. Attachment rate fapp, and detachment rate gap*

However, further research highlighted shortcomings of the theory (Pollack, 1995) such as:

1. The theory predicts that the active tension should vary directly with the number of available cross-bridges (see 'length-tension relationship'). However, this is not the case when the contractile history of the muscle is included, where following lengthening for example, tension can remain unaltered, or even can be greater than an

immediately preceding isometric contraction force, despite a 50% reduction in filament overlap.

2. The process of cross-bridge formation was very simple, involving one ATP molecule, one myosin head and one actin attachment site per cross-bridge cycle, and the physiological conditions that will determine myosin and actin number.
3. The nature of the cross-bridge was poorly understood, and for example where the force-generating power-stroke occurs.
4. It does not account for the varying rates of ATP hydrolysis as a function of shortening velocity, wrongly suggesting that the rate of energy liberation was a function of velocity.

Huxley & Simmons (1971, 1973) *Modifications*

Based on the observation of Hill (1964) that maximal heat and work production was highest at $0.5V_{max}$ (maximum shortening velocity), this suggests that the number and rate of cross-bridge formation was a function of velocity, and not a as a function of the proportion of cross-bridges formed in the region ***x***. The authors performed length perturbation experiments that investigate the rapid transient behaviour of muscle fibres. Changes in muscle tension were measured before and after a length step, which is a fast, small amplitude step in muscle length which results in rapid recovery of tension (Barclay, 2002, Onambele et al., 2004).

Huxley & Simmons (1973) developed the idea of the myosin head containing two spring-containing 'hinge regions'. Hinge 1 was located on the myosin molecule tail,

with hinge 2 located on the myosin head itself. Furthermore, they suggested that the myosin head had multiple attachment sites so that like the previous model's displacement ***x***, the angle of attachment would also determine the amount of tension developed. The vertical displacement created by hinge 2 on the myosin head (z) the authors suggested that the greater the vertical displacement, the greater the potential for larger force generation.

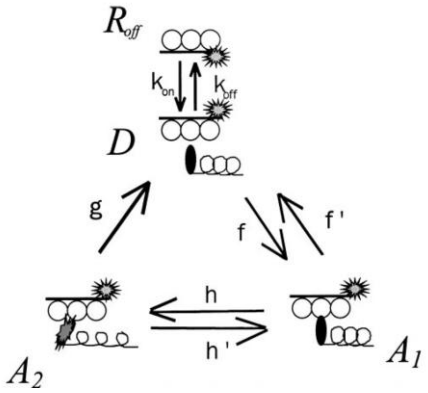


Figure 1.5. *Huxley & Simmons (1973) Cross-bridge model showing the possible states. Where D is detached, A1 is attached but with no force, and A2 is the power stroke.*

In relation to velocity, if hinge 2 did not have 'time' to rotate, tension (F1) would be linearly related to the tension on hinge 1. However if hinge 2 could rotate, the relationship would be non-linear (F2). This also meant that there was no need for movement in relation to actin, and that the myosin heads would not need to detach from actin, when the angle of the head changed.

Modern Views & models on cross-bridge Theory

As time has gone by, there have been several additions and restructuring of the two aforementioned models. To comment on and provide all of the biochemical and

biological evidence provided is beyond the scope of this literature review, although contemporary reviews are available (Gordon et al., 2000, Cooke, 1986, Pollack, 1995). Instead, key features and updates on the theory will be combined and presented.

Although by the early 1990s, many of the limitations of the model had been addressed and improved upon, a major breakthrough for theorists came with the publication of two papers on the atomic structure of the myosin heads, which provided novel insights into the possible implications for function during contraction (Rayment et al., 1993a, Rayment et al., 1993b). The structure of a myosin head is shown in Figure 1.6A. The two globular heads that compose the myosin head are referred to as sub-unit 1 (S1) fragments, consisting of a heavy chain and two light chains attached to it. These globular heads and the necks form the cross-bridges with actin. The major finding of Rayment et al. (1993b) was a cleft through the 50kD segment. The width of the cleft is controlled by the interaction of ATP with the pocket; an open cleft weakens the binding of the 50kD segment to actin, while the closure strengthens the binding.

Prior to activation (i.e. Ca^{2+} release), each myosin S1 unit is in a dissociated state, with ATP bound in the pocket (Figure 1.6B.B). The rapid (and reversible) cleavage of ATP to ADP+Pi causes the head to move into a 'cocked' position, ready to bind to actin (Figure 1.6B.C). When the actin site for myosin binding has been exposed by Ca^{2+} binding to troponin C, there is a rapid re-association of actin to myosin (+ADP.Pi) that forms a weakly bound state (Figure 1.6B.D). This form then isomerizes to produce the

more strongly bound state corresponding to lever arm movement of the myosin head (i.e. force producing power stroke; Figure 1.6B.E), as outlined in Rayment et al. (1993a) .

The cross-bridge is stabilised in the strong bound state by the release of Pi. Finally following further isomerisation, ADP is also released (Figure 6B.E-A). This view of the cross-bridge mechanism is the result of successful incorporation of models such as that of Eisenberg et al. (1980), Piazzesi and Lombardi (1995), and also Woledge et al. (1985), and also dozens of other individual research papers.

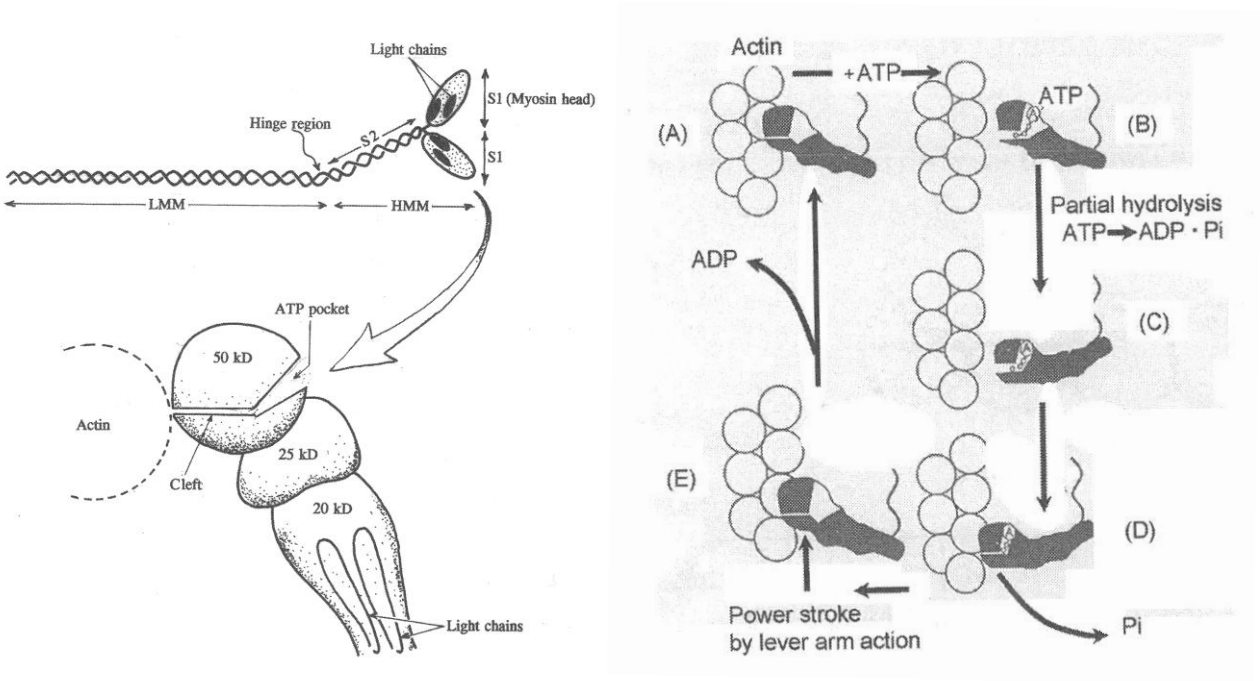


Figure 1.6. *(A) Diagram showing the components of the myosin molecule. HMM; heavy meromyosin, LMM; light meromyosin, S2; α-helical rod, S1; globular head. Enlargement of the globular head shows the three segments, of which the 50kD is divided into upper and lower domains by the ATP-interacting cleft. (B) Hypothetical scheme of cross-bridge cycling proposed by Rayment et al. (1993a).*

Not specifically denoted in this theory is the importance of the light chains of myosin in providing stability to the cross-bridge. Removal of these regulatory light chains increases Ca^{2+} sensitivity of the contractile proteins (Metzger and Moss, 1992), suggesting that these light chains decreases Ca^{2+} sensitivity. When Ca^{2+} concentration increases during muscle contraction, some of the Ca^{2+} will bind to calmodulin, and this activates myosin light chain kinase, resulting in phosphorylation of the light chains. This is thought to cause the cross-bridge to swing out from a position close to the thick filament to as position that places the actin binding portion of the myosin head in close proximity to actin (Levine et al., 1996). The phosphorylation of the light chains is thought to contribute to activity-dependent potentiation, influence the maximal shortening velocity, and also their removal reduces the force per cross-bridge by 50% (VanBuren et al., 1994).

There are however several areas that are still in need of addressing due to some recent findings, such as activation of tropomyosin and actin units, various actions and regulatory behaviour of different isoforms of some molecules, the number of stages involved in strong binding, force development and Pi release and also the number of working strokes generated by the hydrolysis of one ATP molecule (Gordon et al., 2000, Pollack, 1995).

1.1.4. Skeletal Muscle Architecture

Muscle architecture can be defined as "the arrangements of fibres within a muscle relative to the axis of force generation"(Lieber, 1992). Although there are other factors influencing muscle function, such as fibre type, muscle architecture is a major determinant of muscle function (Burkholder et al., 1994). Thus, gaining an appreciation of the structure-function relationship has many inherrent advantages for practice, providing not only a physiological basis for force production and movement, but also provides insights that can be used in surgical procedures, clinical monitoring and injury pervention (Lieber and Friden, 2000, Gans and de Vree, 1987).

It is also vital to understand, not only how architecture affects the force producing characteristics of muscle, but due to muscle's plasticity, to also investigate how the nature of changes to the architecture following peroids of variation in physical activity impact on these charateristics (Blazevich, 2006).

At the microscopic level, skeletal muscle organisation and structure has been well researched, however the particular arrangement of the fibres within the muscle, with few exceptions, is only just beginning to receive the level of attention that would further our understanding of skeletal muscle function *in vivo*. It is often assumed that the force generating capacity of muscle is determined by fibre size, and although there is a positive relationship between fibre size and force (Frontera et al., 1988), muscle fibre size actually varies very little across muscles in the same organism. It is therefore the architectural

arrangement, which displays much more variation, that will be a more accurate predictor of muscle force generation.

1.1.4.1 Muscle Fibre Arrangements

Muscle fibres are often depicted as enclosed in fasicles emanating from a proximal tendon plate to an insertion distal to it (similar to that in Figure 1.1). Although this form of muscle (known as fusiform) is probably the most frequently occuring fibre arrangement in humans, many of the larger, major force-generating muscles (such as the *Vastus Lateralis*) are often pennate (or pinnate). Figure 1.7 shows some of the common types of architectural arrangement found in human skeletal muscle. Figure 7A shows the arrangement of muscle fibres in a fusiform muscle (*biceps brachii*). Here, the fibres run parallel to the force-generating axis and are said to have a paralell or longtitudinal architecture, and although the fibres run parallel, it has been demonstrated that some muscle fibres do not actually run the entire length of the muscle or indeed the whole fascicle (Loeb et al., 1987, Ounjian et al., 1991). In Figure 1.7B, the fibres are orientated at a single angle relative to the force generating axis, known as a unipennate architecture such as in the *vastus lateralis* or *gastrocnemius* muscles. Finally, Figure 1.7C shows the arrangement of a multipennate muscle such as the *gluteus medius* or deltoid muscles, in which the muscle fibres are arranged at several angles relative to the force-generating axis (N.B. bipennate arrangements such as in *rectus femoris* exist but have not been discussed).

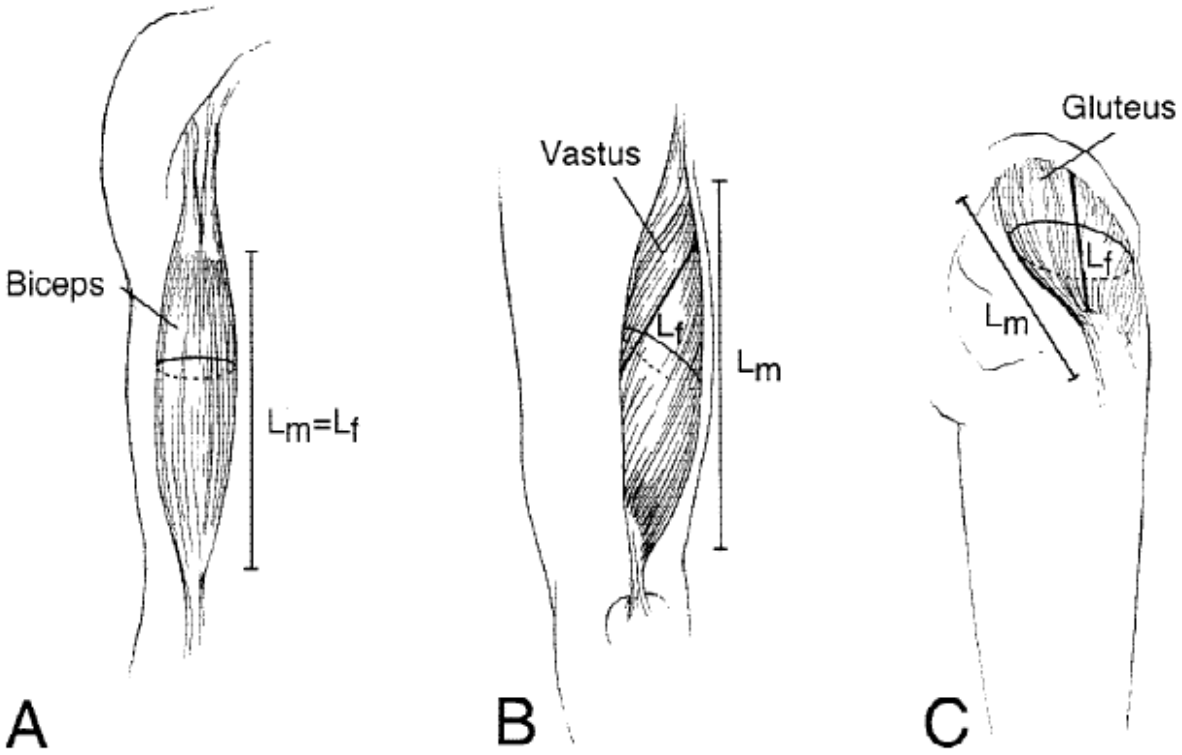


Figure 1.7. *Three general types of skeletal muscle architecture. (A) Longitudinal architecture in which muscle fibres run parallel to the muscle's force-generating axis, as in the biceps brachii. (B) Unipennate architecture in which muscle fibres run at a fixed angle relative to the muscle's force-generating axis, as in the vastus lateralis muscle. (C) Multipennate architecture in which muscle fibres run at several angles relative to the muscle's force generating axis (gluteus medius muscle). L_f, muscle fibre length; L_m, muscle length* (Figure taken from (Lieber and Friden, 2000).

1.1.4.2 Muscle Architectural Parameters

When investigating muscle architecture, previous work was often done on animal or cadaveric models. Most quantitative architectural parameters were deterimed through the pioneering work of Gans and Bock (1965), and later Gans and de Vries (1987). From

this, the parameters defined when describing muscle architecture arose, including muscle length (L_m), fibre length (L_f), pennation angle (i.e. the realtive arrangement of the fibre to the force generating axis – θ) and physiological cross-sectional area (PCSA).

Muscle length is defined as "the distance from the origin of the most proximal muscle fibres to the insertion of the most distal muscle fibres"(Lieber, 1992). This however, is not equal to L_f as previously mentioned; muscle fibres do not necessarily extend the whole length of the muscle. The only way that fibre length can be accurately determined is by either microdissection of individual fibres from fixed tissues or from glycogen depletion on serial sections along the length of the muscle (Ounjian et al., 1991). Therefore investigators use the more accurate term of muscle 'fascicle' length, as it describes the bundles of fibres that are actually measured. Figure 1.8 depicts the classical

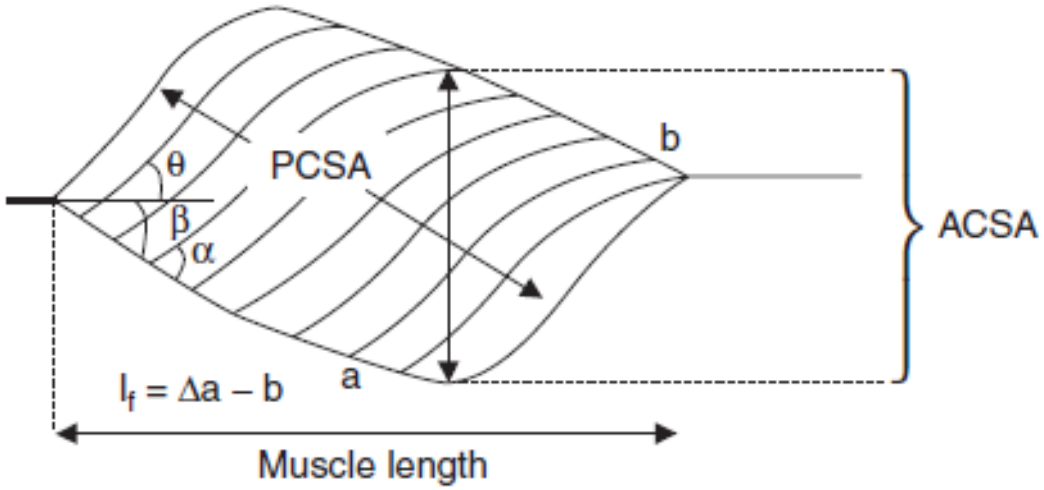


Figure 1.8. *Diagram showing muscle architectural parameters. Fibre length = distance a – b; pennation angle (θ) = fascicle angle (relative to the aponeurosis [α] minus the aponeurosis angle (relative to the tendon [β]); muscle length; and anatomical (ACSA) or physiological (PCSA) cross-sectional area.*

Figure taken from (Blazevich, 2006).

muscle architectural parameters, and how they are defined for measurement. More recently, the advancement in technology has allowed researchers to investigate muscle architecture *in vivo*, with the use of magnetic resonance imaging (MRI) and 2D B-mode ultrasonography (Scott et al., 1993, Kawakami et al., 1993, Narici et al., 1996).

Once most of the architectural parameters are measured, including fascicle length and pennation angle, the PCSA can be calculated. The PCSA represents the contractile area available for force production. In contrast the anatomical CSA (the area at right angles to the long axis of the muscle), underestimates the contractile area in proportion to the angle of pennation (Lieber and Friden, 2000, Aagaard et al., 2001). In essence the PCSA is the magnitude of all the muscle fibres CSA, and is calculated using the equation below, which was experimentally verified by Powell and colleagues (Powell et al., 1984):

$$\text{PCSA}\,(\text{mm}^2) = \frac{\text{muscle mass}\,(\text{g}) \cdot \text{cosine}\,\theta}{\rho\,(\text{g/mm}^3) \cdot \text{fiber length}\,(\text{mm})}$$

Where p represents muscle density (1.056 g/cm3 in mammalian muscle) and θ represents deep aponeurosis surface pennation angle. This equation essentially represents muscle volume (mass / density) divided by fibre length and has units of area (cm^2).

1.1.4.3 Functional Impact of Architectural Parameters

Angle of Pennation

The first of the architectural arrangements that will be reviewed is the fascicle angle of pennation (θ_p), and the consequences of this on functional measures. Pennation can facilitate a number of different packing arrangements for the muscle fibres within the space available, without the need for (or minimal) modification of fibre length (Gans and de Vree, 1987). As the angulation of a muscle fibre increases, the effective pull or proportion of fibre force directed along the aponeurosis or tendon is actually reduced. Based on parellelogram models of bipennate muscles, PCSA increases proportionally to sin θ_p, muscle effective force reduces in proportion to cos θ_p. The net result of the opposing effects of pennation angle (i.e. $\sin(\theta_p) \times \cos(\theta_p) 1/2 \sin(2\theta_p)$), is that maximal muscle force is expected to increase with increases in pennation angle to an upper limit of 45 degrees (Alexander and Vernon, 1975, Rutherford and Jones, 1992) however the loss of effective pull is also thought to be minimal when fascicle angles are moderate (i.e. $<25^o$ (Blazevich, 2006)).

There are three main ways in which the angle of pennation is expected to increase muscle force. Firstly, as mentioned above, PCSA is increased as pennation angle is increased for any given muscle volume. As a result, and again as mentioned previously, PCSA is the only measurement that is directly proportional to maximum tetanic tension in pennate muscle. The arrangement of the fibres in this instance allows a greater amount of contractile tissue to attach to any given area of tendon or aponeurosis (Rutherford and Jones, 1992).

Secondly, the pennation angle can allow sarcomeres (and each muscle fibre) to exert their force at (or close to) their optimum lengths. Evidence has shown that as muscle contracts,

fibres also rotate as well as shorten. For example, in the unipennate rat gastrocnemius, Zuurbier and Huijing (1993) noted that during an isotonic contraction, resting θ_p changed from 30^o to 60^o, whilst the angle between the muscle aponeurosis and the muscle axis rotated from about 10^o to 15^o. Additionally in humans, Fukunaga *et al.* (1997) demonstrated in the quadriceps that during low level voluntary contraction, *vastus lateralis* pennation angle changed from 14^o with the knee extended, to 21^o with the knee flexed, with a simultaneous decrease in fascicle length from 126mm to 67mm, indicating that fibre shortening and rotation occur simultaneously and are part of normal muscle contractions (Lieber and Friden, 2000). As a result, the tendon excursion is greater than the shortening distance of the individual muscle fibres. In accordance with the length-tension relationship (discussed in more detail in subsequent sections), there will be an optimum sarcomere length, at which the fibres will produce their greatest active force. Sarcomeres tend to produce greatest force at lengths where the highest forces are demanded (as opposed to resting lengths for example). Thus, reducing fibre shortening for any given tendon excursion is likely to allow the sarcomeres to remain operating closer to their optimum length. Thirdly, since the time-dependant shortening distance of a fibre decreases during a contraction, the shortening speed will also decrease, and the force will increase, as a characteristic property of the force-velocity relationship (Gans and de Vree, 1987, Blazevich, 2006).

Fibre Length

Muscle fibre length (or the more commonly reported fascicle length) generally refers to the amount of sarcomeres placed in series. Placement of sarcomeres in such a fashion has

two main impacts on muscle function; (1) a greater serial sarcomere number increases the maximal shortening velocity of the fibre, and (2) the fibre will produce active force over a larger range of lengths. Experimentally, the relationship between fibre length and shortening velocity has been demonstrated in the frog, where isolated muscle fibres allowed for easy measurement of fibre length (and thus sarcomere number) and shortening velocity, and found that maximum shortening velocity was proportional to fibre length, as was the active range of the isometric length-tension relationship (Edman et al., 1985). Similarly, Wickiewicz et al. (1984) demonstrated this relationship in humans. Using isokinetic dynamometry, 12 untrained individuals (8 males, 4 females) produced force-velocity curves for the knee extensors (KE), knee flexors (KF), plantarflexors (PF) and dorsiflexors (DF) over speeds ranging from 0 $rad.s^{-1}$ to 5.03 $rad.s^{-1}$. The curves were then compared to the predicted maximal shortening velocities derived from cadaveric specimens. The results showed that the KE and KF estimated peak velocities were twice that of the PF and DF, and when converted from angular velocities to linear velocities, the differences were even greater. When maximal shortening velocities were actually determined, most of the large differences between muscle groups were accounted for by the difference in sarcomere number arranged in series in each muscle group. Following this, longer fibres will also allow active force production over a greater range, as demonstrated in the active range of the length-tension curve in frogs (Edman et al., 1985) as the absolute excursion will be the sum of the excursion of the sarcomeres lying in series (Gans and de Vree, 1987).

1.1.4.4. Intrinsic Muscle Characteristics

1.1.4.4.i Length – Tension Relationship

The length-tension (or force-length) relationship of skeletal muscle was first identified and described by Magnus Blix at the end of the 19th century. In a series of published articles (Blix, 1894, Blix, 1891, Blix, 1893), Blix investigated the properties of the thigh adductors and gastrocnemius of isolated frog muscles, and found that isometric force increased with increases in muscle length, reached a plateau, and then force gradually reduced as the muscle length was increased further. Further work to fully delineate this relationship was carried out by Gordon et al. (1966), on isolated muscle fibres of the frog. One of the key elements of Gordon et al's (1966) work, was to make the underlying mechanistic link between the length-tension relationship and both the sliding filament (Huxley and Niedergerke, 1954, Huxley and Hanson, 1954) and cross-bridge theories of muscle contraction (Huxley and Simmons, 1971, Huxley, 1957). There are several assumptions made for the accurate derivation of length-tension properties of skeletal muscle that arise from these theories:

1. The cross-bridges are uniformly distributed along the length of the myosin filament.
2. The actin filament has uniformly distributed attachment sites for the cross-bridges.
3. The attachment/ detachment probability of a cross bridge only depends on its location relative to an actin attachment site and is independent of its prior history or its neighbouring cross bridges.

4. Each cross-bridge, on average, exerts the same amount of force as any other cross-bridge.

The original data produced by Gordon et al.(1966) of the sarcomere length-tension relationship in isolated frog muscle fibres are incorporated into a schematic diagram of the relationship between sarcomere dynamics (cross-bridge interaction) and their effect on the length-tension curve (Figure 9). Due to cross-bridge size and attachment range, cross-bridge formation can only occur in distinct areas of the sarcomeres where actin-myosin overlap occurs. Bearing this in mind, it therefore follows that maximal isometric force produced by an individual sarcomere is linearly related to the amount of actin-myosin overlap. Identification of frog striated muscle sarcomere geometric measurements makes it a fairly easy relationship to describe based on the information provided in Figure 1.5. For example, the lengths of actin and myosin filaments are ~0.95μm and 1.6μm respectively, Z-line width is ~0.1μm and the central part of the myosin filament does not permit cross-bridge formation (0.2μm). Based on this information, maximal overlap (and cross-bridge formation) should occur at 2.0μm (i.e. twice the actin filament length; 1.9μm, plus the Z-line width; 0.1μm), and as a result maximum isometric force should also be observed at this sarcomere length. Due to the lack of cross-bridge formation in the central region of the myosin filament, further extension of the sarcomere by 0.2μm does not reduce the potential for force production (the plateau region). Further extension of the sarcomere beyond lengths of 2.2μm invariably leads to a linear reduction in force, where there are gradually fewer actin-myosin interactions, where force production gradually reaches a value of 0 at ~3.6μm. At this sarcomere length (twice the actin filament

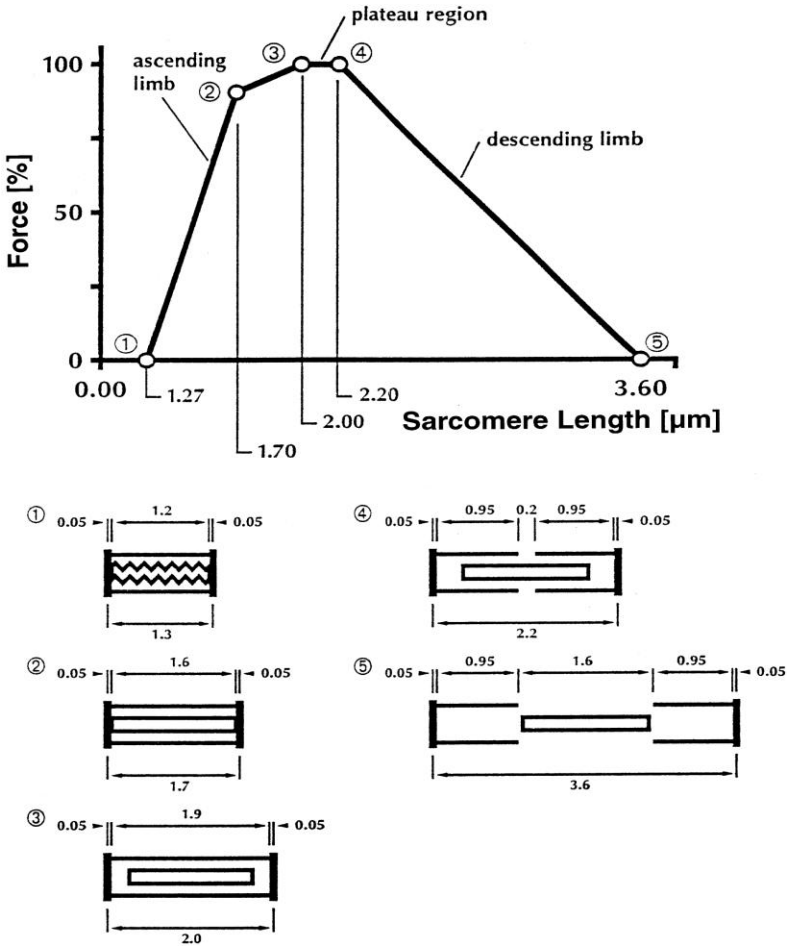


Figure 1.9. *Length-Tension relationship of frog skeletal muscle sarcomere, a derived by Gordon et al.(Gordon et al., 1966), and schematic sarcomeres corresponding to crucial points (1-5) labelled on L-T curve.*

Figure taken from (Rassier et al., 1999).

length; 1.96µm, plus the myosin filament length; 1.6µm, plus the Z-line width; 0.1µm), no further overlap and therefore cross-bridge formation can occur. This is known as the 'descending limb' of the length-tension relationship.

At sarcomere lengths <2.0µm, maximal isometric force decreases with increasing sarcomere shortening. Two distinct scenarios are hypothesized to give rise to this

observation, the first being that active force production is reduced, due to increasing lateral distance between thick and thin filaments as such reducing the probability of cross-bridge formation (Godt and Maughan, 1981). Secondly, interference of cross-bridge interaction due to double overlap of actin filaments (Gordon et al., 1966), and also at lengths <1.7μm deformation of the myosin filament may also occur (Rassier et al., 1999), where the Z-line pushes up against the ends of the myosin filaments, causing a reduction in force. Furthermore, fluid and osmotic pressures have also been suggested to contribute to opposing the active force production (Gordon et al., 1966, Sato, 1954). These sarcomere lengths are referred to as the 'ascending limb' of the relationship.

As stated throughout, the identification of the length-tension relationship was done so using isolated muscle fibres from the frog. The myofilaments used and the sarcomere forces described in these experiments offer insights into this relationship based specifically on those geometric measurements, however actin filament lengths have been shown to vary across different species. Differences in myofilaments lengths have been described in cats (Herzog et al., 1992), and in rhesus monkeys and humans (Walker and Schrodt, 1974). The result of different myofilaments lengths is the production of an alternate length-tension curve. Although the general shape of the curve is not drastically altered, the sarcomere lengths at which the plateau and descending limb of the relationship occurs, changes. For example, human actin filaments are approximately 1.27μm in length, and based on the length of the frog actin filaments mentioned earlier (~0.95μm), it is obvious that the plateau region and descending limb would occur at longer sarcomere lengths in humans compared with frog tissue (Rassier et al., 1999).

It is important also at this point to acknowledge that the length-tension relationship of skeletal muscle is a static property. For example, one measurement of force against sarcomere length gives rise to a single data point, although in general this relationship seems to be represented by a continuous line (such as in Figure 1.9 above). This gives the erroneous implication that the relationship is continuous in both time and length.

From this assumption, the notion of sarcomere instability on the descending limb of the curve has arisen, as this negative slope represents the behaviour of a softening material, which is an unstable behaviour (Rassier et al., 1999). Although this view has may not be correct. Edman and colleagues (1978) used fused tetanic contractions on single fibres from the semitendinosus of the *Rana temporaria* to produce a length-tension curve. They reported a force-enhancement following stretch that was greater than the force produced at the same sarcomere length following isometric tetani, displaying a positive sarcomere stiffness. Furthermore, at sarcomere lengths above 2.3μm the force-enhancement decreased very slowly and was still present after 4 seconds of long tetani, the force enhancement increased with increasing amplitude of stretch and increased for any given stretch amplitude with sarcomere length. This tension enhancement during and following stretch provides stability and prevents sarcomere dispersion on the descending limb (Edman et al., 1978).

Following the assumption made from the cross-bridge theory on the length-tension relationship, that isometric, steady-state force was determined by sarcomere length, several experiments have been carried out due to limitations associated with the original work of Gordon et al. (1966). The limitations associated with this work were:

1. Force was measured from a single isolated frog fibre, which would contain millions of parallel and serially arranged sarcomeres, and as such individual sarcomere forces could not be directly measured.
2. Forces that were measured did not often reach a steady state, with the associated force 'creep' denoting apparent sarcomere instability on the descending limb. Therefore force was approximated
3. Sarcomere length was approximated from a clamped mid-segment, with sarcomeres in other parts of the fibre were free to change length, and therefore some sarcomeres could have shortened at the expense of others.

More recently, Herzog et al. (2010) attempted to measure directly sarcomere force and length changes by addressing these issues. The authors of the study used isolated myofibrils from the rabbit psoas, which are organelles with serially arranged sarcomeres. The result of using myofibrils is that the forces recorded at each end of the myofibril represent the forces of all and each sarcomere, and sarcomere lengths (or half-sarcomere lengths) can also be imaged and measured. True isometric, steady state conditions were achieved and in these conditions, sarcomere length determined force, and closely mirrored the relationship outlined by Gordon et al. (1966). However, depending on the contractile history of the muscle, there is a vast range of possible isometric steady-state forces at a given sarcomere length. The results showed that, following sarcomere stretch, there was a force-enhancement observed compared to the purely isometric, steady-state condition, whilst there was a force depression following sarcomere shortening. Therefore sarcomeres of vastly different lengths can also produce the same amount force, which gives rise to sarcomere stability on the

descending limb of the relationship, allowing for a steady-state to be achieved. The conclusions of the series of experiments indicated that steady-state forces in isolated sarcomeres do not solely depend on sarcomere lengths, but the contractile history of the muscle, and that a mechanism exists that determines steady-state isometric forces that is independent of actin-myosin myofilament overlap (Herzog et al., 2010, Onambele et al., 2004).

1.1.4.4.ii Force-Velocity Relationship

The Force-Velocity (F-V) relationship was first identified by Fenn and Marsh (1935), as they described the inverse relationship between increases in muscle contraction force coupled with decreases in muscle contraction speed. However it was Hill (Hill, 1938) who extended this work and characterised the relationship as a rectangular hyperbola. A typical force-velocity curve is shown in Figure 1.10.

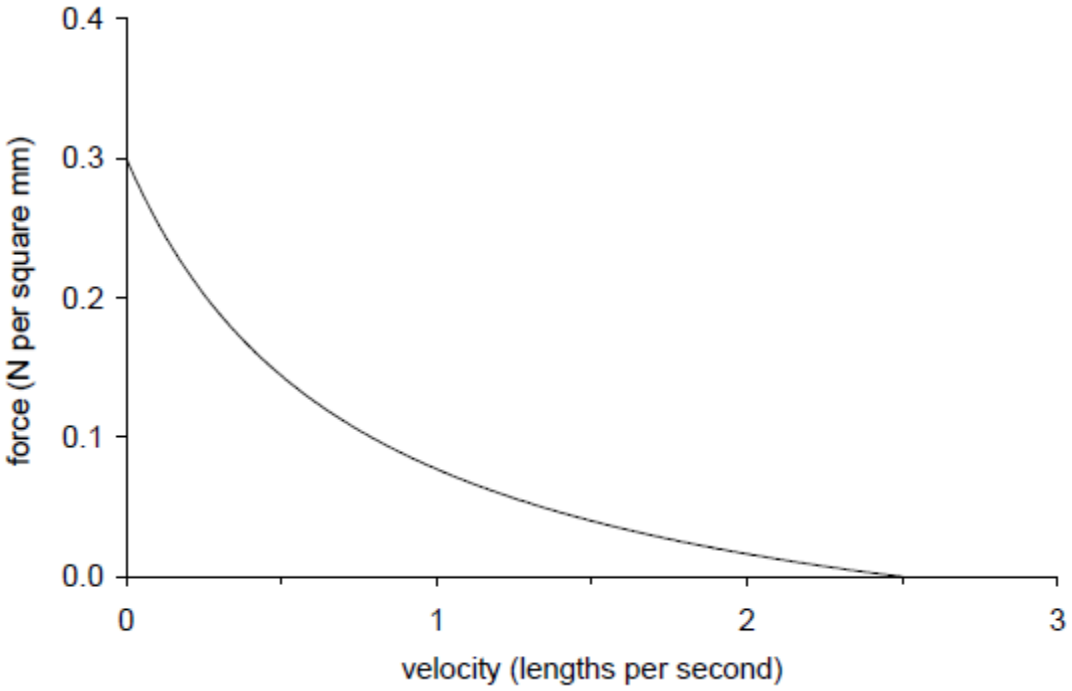


Figure 1.10. *The Force-Velocity Relationship of skeletal muscle.(Taken from Wickiewicz, 1984).*

It is apparent from the figure above, that during the production of maximal forces, muscle velocity (or speed) is equal to 0. In such conditions, the muscle is undergoing isometric contraction. Hill (1938) reported the highest velocity of shortening (Vmax) in unloaded contractions and a decrease in shortening velocity as isotonic load was increased.

The force-velocity relationship of muscle is thought to be reflective of the contractile behaviour at the sarcomere level, as the relationship maintains the same shape when measurements are made from whole muscle fibres or from short segments along the length of the same intact muscle fibre (Edman, 1988, Edman et al., 1997). However, more recently a bi-phasic force-velocity relationship has been described (Edman, 1988, Edman et al., 1997, Edman et al., 1976, Devrome and MacIntosh, 2007). This shows that the force-velocity relationship is not a simple hyperbolic function as previously thought, but actually exists as two distinct curvatures either side of a breakpoint (75-80% of the maximal isometric force in frogs (Edman, 1988, Edman et al., 1997) and ~88% in the rat (Devrome and MacIntosh, 2007)). As a result of these new findings Edman et al.(1997) expanded on the findings and models of previous authors to explain the force-velocity double curvature as a function of cross-bridge attachment states.

The model developed in this study is based on the assumptions of previous cross-bridge theory (Huxley and Simmons, 1971), where force is developed by an attached cross-bridge according to a multi-step process, in which each new step leads to tighter binding between actin and myosin. The bridge, after attachment, is assumed to undergo two steps and by each of these steps the linear cross-bridge elasticity is extended by 5nm (Huxley and Simmons, 1971, Piazzesi and Lombardi, 1995). The model contains three attached

states A_0, A_1 and A_2 and a fourth detached state A_3. Of the attached states A_0 represents the weakest binding to actin, whereas A_2 represents the strongest binding (Figure 1.11).

The force during shortening is assumed to be a function of the attachment rate constant of the cross-bridges, the distance traversed by the bridges during the working stroke and the rate constant of cross-bridge detachment. The latter rate constant determines how far the bridges may be brought into a region where they develop negative strain and resist fibre shortening (Edman et al., 1997). The detachment rate constant is therefore a major determinant (along with the size of the working stroke) of the maximum speed of shortening, V_{max}.

Briefly the model demonstrates that there is a phase early during the power stroke that cross-bridge attachment is slow; and that there is a force-producing cross-bridge state at the end of the power stroke that is only slightly occupied under isometric conditions and during shortening at low velocity but becomes significantly populated during shortening at intermediate and high velocities (A_2). The existence of a region of slow cross-bridge attachment in the beginning of the power stroke leads to a marked reduction in the number of attached cross-bridges, and therefore to a fairly large drop in force, as the speed of shortening is increased within the range 5% of maximal isometric force to 25 nm.h.s.$^{-1}$ s^{-1}. This corresponds to the flat region of the force-velocity relationship at force levels greater than 85% of maximal isometric force. The force is therefore reduced proportionately more than is the number of attached crossbridges within this range of velocities and loads. This accords with the experimental finding that there is a greater reduction of force than of stiffness as the velocity of shortening is increased from 0 to 25 nm h.s.$^{-1}$ s^{-1}.

The exact nature of the force-velocity relationship for a given muscle or muscle group will depend on a number of other factors. For example, the maximal isometric force will be dictated by the physiological cross-sectional area of the muscle. Also, the maximal shortening velocity will also be a function of fibre type (i.e. type II fibres contract more rapidly associated with greater myosin ATPase activity (Bárány, 1967, Barnard et al., 1971, Thorstensson et al., 1976)) , and also fibre or muscle length (i.e. number of sarcomeres in series (Wickiewicz et al., 1984)). The shape of the curve has

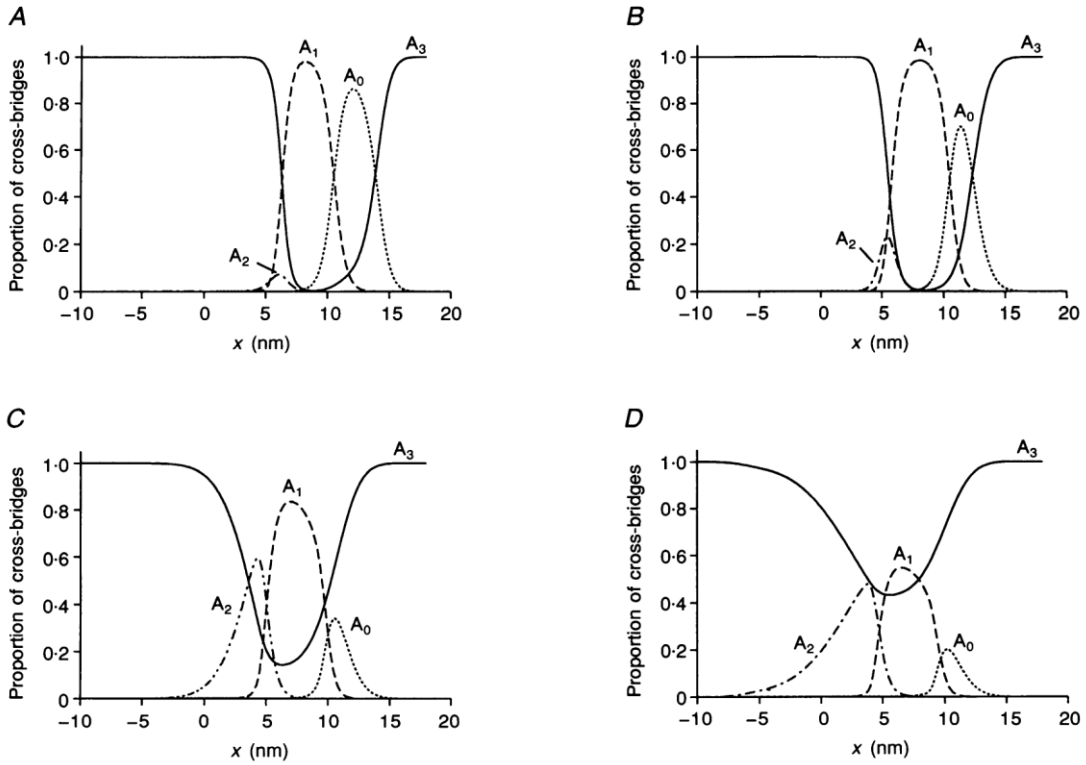


Figure 1.11. *Proportion of cross-bridges in the various states (A_0-A_3) given as a function of the distance (x) between cross-bridge and actin binding site. A) steady-state isometric tension, B) shortening at 20 nm h.s.$^{-1}$ s^{-1}, C) shortening at 200 nm h.s.$^{-1}$ s^{-1} and D) shortening at 500 nm h.s.$^{-1}$ s^{-1}.* *(Taken from Edman et al. (1997)*

also shown to be sensitive to temperature and also fibre type and myosin heavy chain isoform (Bottinelli et al., 1996).

1.1.4.5. Incorporation of muscle architecture into muscle intrinsic relationships

Throughout the description of the architectural parameters, their likely effects on the L-T and F-V relationships have been touched upon. It has been demonstrated that the effect of pennation angle increases are proportional to increases in PCSA, which in turn reflects the amount of sarcomeres activated in parallel. Thus changes to PCSA will have a major impact on force generation, which is in turn closely related with both length-tension and force-velocity relationships. Further, fibre (or fascicle or muscle length) will reflect sarcomeres placed in series, and therefore impact on the maximal rate of shortening and indeed the tension that may be developed from the L-T relationship.

Therefore, two muscles with identical intrinsic properties but different architectures will now be considered. Firstly, consider two muscles with identical fibre types and proportions, the same fibre length and pennation angles, and finally the same muscle specific tension. The difference between the muscles will be one will have twice the muscle mass i.e. tantamount to saying twice the muscle fibres and therefore twice the PCSA. The functional difference between these two muscles is outlined in Figure 1.12. The muscle has an identical shape of length-tension curve, however it is amplified upward by a factor of two i.e. it can generate twice the maximal tetanic tension. In a similar fashion, plotting these two muscles on a force-velocity curve shows that the relationship is again altered by the upward shift in maximal tension i.e. speed of

shortening is the same, although the force is greater at nearly all relative speeds of shortening with the larger PCSA. On a side note, if each of these graphs were plotted on relative scales instead of absolute scales, the two muscles would appear to have identical properties (i.e. shape and size of curves would be the same). This demonstrates how changing absolute parameters of muscle architecture can have an impact extrinsic on muscle properties (such as absolute tension) but will have no effect on intrinsic properties (such as the shape of the L-T and F-V curves).

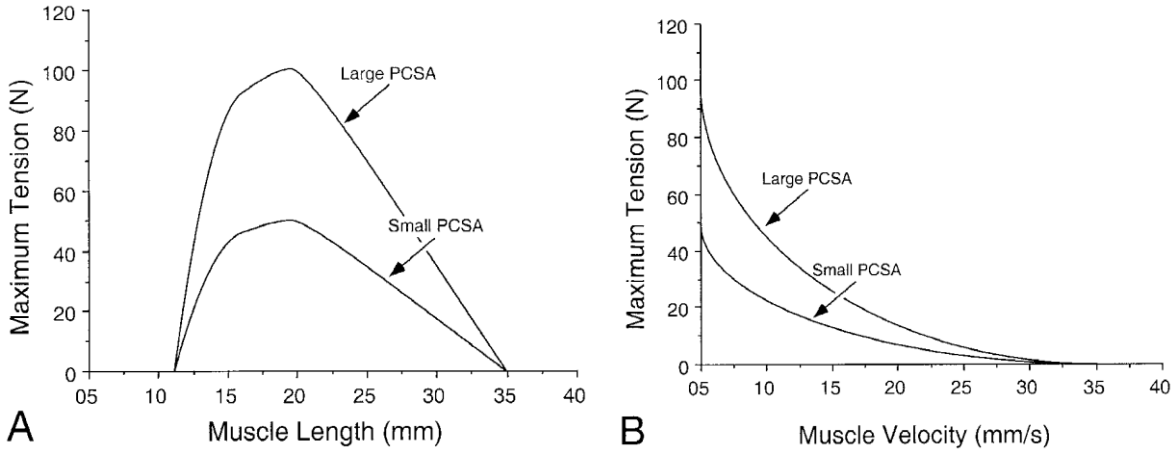


Figure 1.12. *The functional impact of one muscle with twice the PCSA of the other on (A) the Length-Tension Relationship, and (B) The Force-Velocity Relationship.*

(Taken from Lieber & Friden, 2000)

Conversely, consider now two muscles with the same properties as the two aforementioned architectural and intrinsic properties, except this time instead of one having twice the PCSA, assume that one muscle has a longer fibre length than the other and the same PCSA. The functional outcomes of such architectural discrepancies are depicted in Figure 1.13. As mentioned previously, the impact of increased fibre length is to increase the maximal shortening velocity (or enhance excursion of a muscle). It is evident from the length-tension graph that, peak tension between the two muscles is identical, but the range in which active force can be developed is larger in the muscle

with longer fibres. It is for the same reason that the longer fibres also increase the V_{max} in the force-velocity relationship. The same effect leads to the increase in active range in L-T. Once again, as with PCSA, the muscle's extrinsic properties are affected by this difference in fibre length, not their intrinsic properties. Direct experimental evidence of this comes from Bodine et al. (1982), in the study of the cat semitendinosus. The anatomical structure of this muscle presents a unique model for study because it has distinct proximal and distal heads separated by a tendinous inscription. When only the proximal semitendinosus head was activated, its V_{max} was 224 $mm.s^{-1}$, whereas when only the distal semitendinosus head was activated, its V_{max} was 424 $mm.s^{-1}$. Then, when both heads were activated simultaneously, the whole muscle V_{max} was 648$mm.s^{-1}$, or the sum of the two velocities. The values for V_{max} were proportional to the different lengths of the proximal and distal heads. These data indicate that the longer the fibres in series (equivalent of saying the greater number of sarcomeres in series), the greater the muscle contraction velocity. As expected, maximum isometric tension was essentially the same regardless of which activation pattern was used.

Following the effect of fibre length on F-V properties, as mentioned beforehand, fibre length impacts the active range (Figure 13A). It may often be misinterpreted that muscles with longer fibres are associated with joints that have larger ranges of motion. This is not the case, because as the joint rotates about its axis, the amount of fibre length change will depend on the muscle moment arm (or mechanical advantage).

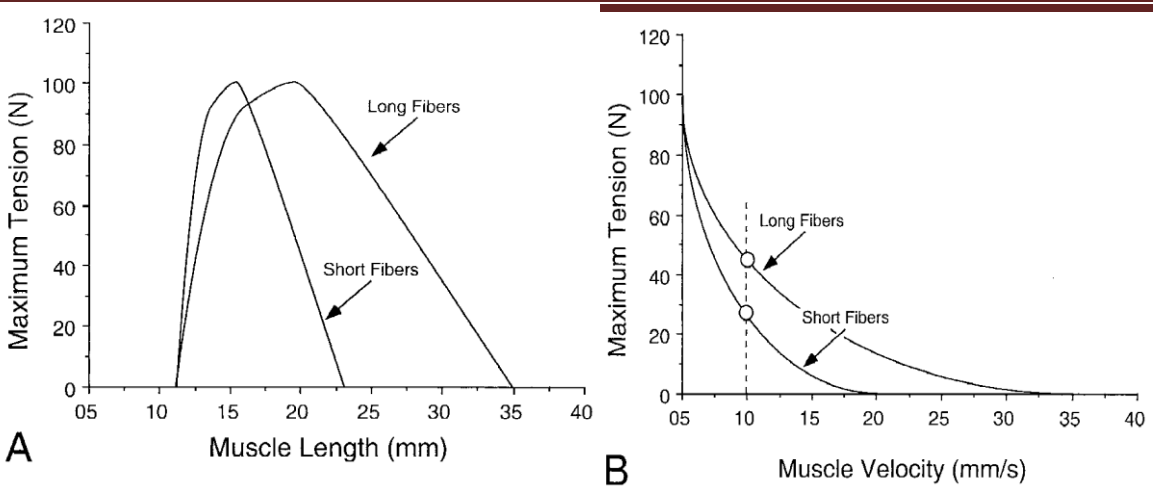


Figure 1.13. *The functional impact of one muscle with longer muscle fibres than the other on (A) the Length-Tension Relationship, and (B) The Force-Velocity Relationship.* *(Taken from Lieber & Friden, 2000)*

An important consideration when analysing the muscular force produced during muscular contraction is biomechanical influence of the moment arm. The moment arm will determine the moment (M_j) produced about a joint by muscular contraction since:

$$\overline{\boldsymbol{M}_j} = \overline{\boldsymbol{r}} \times \overline{\boldsymbol{F}} \qquad \text{Or} \qquad \mathrm{M_j} = d \cdot f$$

The first equation is written in vector form, where ***r*** is the location vector from the centre of the joint to any point on the line of action of the muscle force, ***F*** is the force vector and × is the cross product. For two-dimensional considerations, the equation may be written in its scalar form (equation 2) where M_j is the magnitude of the moment produced about the target joint, ***d*** is the perpendicular distance (moment arm) from the joint centre (axis of rotation) to the line of force application, and ***f*** is the magnitude of muscle force.

We can now consider two muscles attached to a joint with identical fibre lengths and L-T properties but one muscle (B) has a moment arm twice that of muscle A and describe the

functional differences between them. Two such muscles are outlined in Figure 1.14A, with the resultant L-T curves based on their function represented in Figure 1.14B. Muscle B can exert a peak moment twice that of muscle A, although the larger moment arm in B compared to A results in a larger change in length of muscle B compared to A for any given change in joint angle. Therefore, muscle B has only one-half the range of active force production (in terms of joint angular displacement) compared to muscle A. Therefore the design of a muscle when placed in isolation may or may not be directly related to the function of the muscle in the skeletal system (Rassier et al., 1999, Lieber and Friden, 2000).

Having looked at the various arrangements of muscle architecture in a range of animal and human models, how they impact on extrinsic muscle properties, and how these in turn relate to the very basic intrinsic muscle properties, it becomes obvious there is a clear rationale for investigation of architecture. How architecture changes or adapts following physical activity or hypoactivity should be carefully and objectively monitored, so we can manipulate training programs to bring about specific alterations to achieve our functional goals. These have implications for human performance in sport, rehabilitation and efficacy of clinical treatment (Lieber and Friden, 2000).

1.2. Responses & Plasticity of human skeletal muscle following Resistance training:

Following resistance training, a myriad of acute, co-ordinated responses take place in the physiological system, ranging from neural, endocrine, immune, muscular and

cardiovascular changes. The nature of the responses is specific to resistance exercise that has preceded them. If resistance exercise is performed over a prolonged or extended

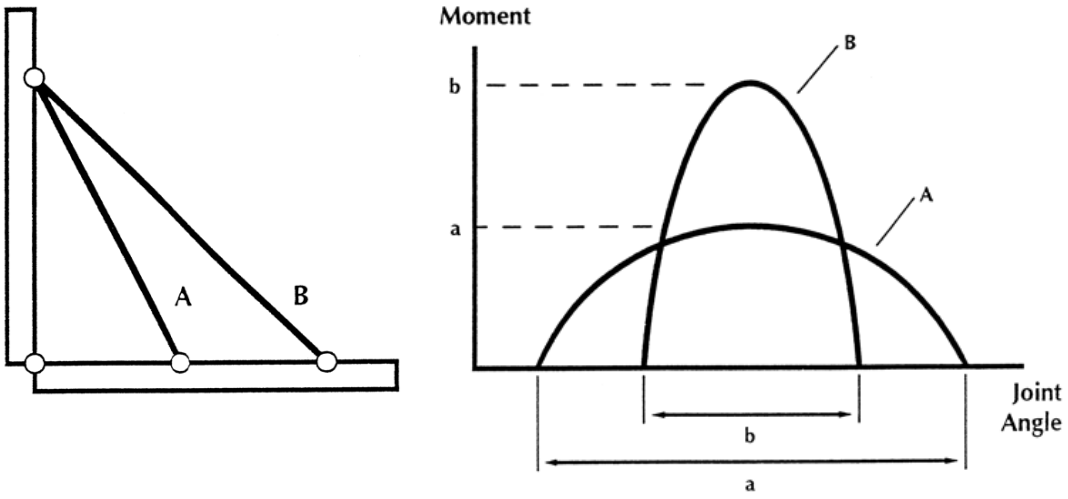


Figure 1.14. *Two muscles with different sized moment arms (A) and the corresponding moment – joint angle curves (B).* *(Taken from Rssier et al. 1999)*

period of time (i.e. weeks, months or years) then a phenomenon known as the repeated bout effect (RBE) will occur, where a system (or number of systems) will adapt to the functional needs of their external environment as a method to maintain internal homeostasis. The RBE is usually referred to as the 'chronic' effect of exercise. The first part of this section will discuss the 'acute' responses to resistance exercise, and the second will provide information on the more 'chronic' adaptations.

1.2.1 Acute Responses to resistance training (RT)

1.2.1.1 Muscle Activation

When performing RT, α-motorneurons activate muscle fibres, which in turn contract to produce force. The magnitude of force production is mainly governed by two variables; firing frequency and the number of motor units recruited. The size principle (Henneman et al., 1965) dictates that depending on the amount of weight that needs to be lifted, and therefore force produced, the smaller motor units (primarily Type I fibres) are recruited first, then the larger (primarily Type II) motor units are in turn recruited to match and overcome the external resistance. Near maximal and maximal loads will therefore recruit the whole spectrum of motor units (Spiering et al., 2008).

Crucially, this would indicate that low force activities can be matched by recruiting motor units that are resistant to fatigue, whereas the high-threshold, more easily fatigued motor units can be recruited for large force productions that are not maintained for long periods.

The level of agonist activation is usually measured by surface electromyography (SEMG), and in many cases is expressed as a level (%) of maximal voluntary contraction (or MVC). It comprises the sum of the electrical contributions made by the active motor units. The global characteristics of the surface EMG, such as its amplitude and power spectrum, depend on the membrane properties of the muscle fibres as well as on the timing of the single fibre action potentials. Thus the surface EMG reflects both peripheral and central properties of the neuromuscular system (Farina et al., 2004). However the reproducibility of sEMG is often questioned and must be carefully controlled. Issues such as relocation of electrodes, cross-talk, variable impedance of the skin and subcutaneous tissues, changes in muscle morphometry and interpretation of data make it difficult to

reliably detect longitudinal changes in sEMG (Farina et al., 2004, Türker, 1993, Folland and Williams, 2007).

In addition to the inherent reliability problems of electrode placement, another source of error in EMG measures of muscle activation is the ambiguity associated with the level of exertion during an MVC (Folland and Williams, 2007). Methods of trying to alleviate problems with less-than maximal activation include supramaximal tetanic stimulation, the interpolated twitch technique and transcranial magnetic stimulation. Even in light of these techniques, it has been reported that muscle activation may be muscle specific (Behm et al., 2002, Belanger and McComas, 1981), with quadriceps inactivation 15.5% greater than the elbow flexors (Behm et al., 2002). Furthermore, it has been demonstrated that in certain cases of muscle groups, such as that of the quadriceps femoris, activation levels are ~85-95% in an isometric MVC in healthy, untrained subjects (Nørregaard et al., 1994, Roos et al., 1999). Interesting data from Adams et al.(1993) showed that, having measured muscle activation by measuring the transverse relaxation time of water by MRI following electrical stimulation of the quadriceps, they estimated that MVC torque could be achieved by stimulating only 71% of the cross-sectional area of the muscle. This indicates that a large proportion of muscle volume mat not be activated during MVC (Enoka, 1997).

1.2.1.2. Endocrine Responses

Acute elevations in circulating blood hormones concentrations (i.e. from either increased secretion, reduced hepatic clearance, plasma volume reductions, reduced degradation rates) present a greater likelihood of interaction with cell receptors on either the target

tissue cell membrane or with nuclear/ cytoplasmic receptors located within the target tissue (Kraemer and Ratamess, 2005).

There are several hormones that are associated with the anabolic response to resistance exercise, and these include testosterone (total and free), growth hormone (GH; and its variants) and Insulin-like growth factors (IGFs). Regarding testosterone, acute elevations in total testosterone and in free testosterone in men, and in some cases, younger women also have been reported following a single bout of exercise (Ahtiainen et al., 2003b, Hickson et al., 1994, Chandler et al., 1994, Weiss et al., 1983, Häkkinen and Pakarinen, 1995, Kraemer et al., 1998, Kraemer et al., 1999a, Tremblay et al., 2004, Cumming et al., 1987). In addition, testosterone is also thought mediate its effects on response to resistance exercise through other acute influences on GH, IGF-1 and motorneurons (Giustina and Veldhuis, 1998, Brooks et al., 1998).

There have also been reports of increased GH concentrations following resistance exercise (Nindl et al., 2000, Hymer et al., 2001), although the concentrations of different GH variants appear to be influenced by within subject variation such as strength levels (Hymer et al., 2001). The acute response of IGF-1(and binding proteins) has yet to be fully elucidated, as there appears to be a delayed temporal response to IGF-1 secretion and the conclusion of the resistance exercise session. There have been studies that report small increases, and also no changes in circulating IGF-1 levels immediately following RT. Where changes have been observed, it has been suggested that peak levels of IGF-1 are reached ~16-28 hours post-stimulated GH release (Chandler et al., 1994).

The acute response to these hormones is further influenced by manipulation of the resistance protocol itself, with varying responses attributed to amount of muscle mass involved (i.e. exercise selection), volume and amount of total work done, contraction type, intensity and rest periods (Durand et al., 2003, Hansen et al., 2001, Kraemer et al., 1990, Kraemer et al., 1991, Gotshalk et al., 1997).

1.2.1.3. Mechanotransduction and Cell signalling

Following muscle activation and motor unit recruitment, loading of the muscle results in the deformation of muscle cells (or fibres), and is the critical event comprising mechanotransduction (the changing of mechanical signals into chemical signals) (Burkholder, 2007). It is obvious that the contractile elements and the adhesive complexes are the likely candidates to undergo deformation, but the sarcolemma and cytoskeleton are also deformed, and specific molecules are likely to be subjected to load-induced stretch.

Force is transmitted to the cytoskeleton and contractile unit through specialised structures, known as costameres or focal adhesions, in which adhesion and signalling molecules are abundant in number (Danowski et al., 1992, Baum et al., 2000). The primary laminin-based adhesion molecules in muscle are integrin and dystroglycan, and when bound to their extracellular matrix (ECM) targets, nucleates the condensation of the focal adhesion plaque and the polymerisation of the ECM network (Colognato et al., 1999). The adhesion plaques connect to the contractile matrix through a network of intermediate filaments, which in turn can induce a network of biochemical signalling

events, associated with integrin ligation and modulated by applied load (Iqbal and Zaidi, 2005, Carson and Wei, 2000).

Within the contractile unit, the M and Z-disks immobilise the thick and thin filaments, with the thick filament tethered to the Z-disk by titin. Titin can be loaded and elongated as the sarcomere lengthens, and there is evidence that members of the ankyrin repeat protein (ARP) family can be displaced by stretch from titin to the nucleus, where they form complexes with transcription factors (Miller et al., 2003). If cross-bridges are attached, tension will develop in the myofilaments, in the M and Z-disks, and the molecules that anchor these to the ECM, and these molecules' activity may be regulated by mechanical signals. Furthermore, the protein desmin, which links adjacent Z-disks, maintains sarcomere registry during force generation, which also suggests it is subject to loading and elongation during cellular deformation, but is not required for stretch-induced growth (Shah et al., 2004, Shah et al., 2001).

Skeletal muscle possesses multiple mechanically sensitive ion channels that can directly transducer mechanical signals to chemical signals. Although the molecular identities of many mechanically sensitive ion channels in muscle are not known, electrophysiological observations have shown that mechanical loads influence the activity of Ca^{2+} channels, Ca^{2+}-activated K^+ channels, K^+/Na^+-permeable channels, and voltage-sensitive Na^+ channels that are present in skeletal muscle membranes. Thus mechanical load-induced ion fluxes through any of these channels could feasibly affect signalling that may lead to changes in muscle growth or adaptation (Tidball, 2005).

Cell signalling stimulation from mechanical deformation can be done independently from hormones and growth factors (Hornberger et al., 2004). The main pathways involved from mechanical deformation will be presented below, and will include the protein kinase B (Akt) – mammalian target of rapamycin (mTOR) pathway, the adenosine monophosphate-activated protein kinase (AMPK) pathway, and the mitogen-activated protein kinase (MAPK) pathway. A summary of these pathways are shown in Figure 1.15.

Akt – mTOR signalling: Following muscle loading Akt phosphorylates mTOR, although mTOR can also occur independently of Akt (Hornberger et al., 2004, Bodine et al., 2001). The independent mechanism of mTOR phosphorylation occurs via phospholipase D-generated phosphatidic acid production. At rest α-actinin in the Z-band associates with and inhibits phospholipase D, however repeated mechanical deformation subsequently relieves this inhibition and promotes phosphatidic acid production and mTOR activation (Hornberger et al., 2004).

Following activation, mTOR mediates its effects through increased translational efficiency (i.e. mRNA translated per ribosome). Phosphorylation of mTOR's two primary targets, 70 kDa ribosomal protein S6 kinase (p70 S6K) and eukaryotic initiation factor (eIF) 4E binding protein 1 (4E-BP1), causes further phosphorylation of their counterparts, resulting in the increased translation of mRNAs encoding ribosomal proteins (i.e. S6) and translation initiation factors (eIF4E) (Kimball et al., 2002). The ultimate effect of mTOR signalling is an increase in protein synthesis and promotion of muscle growth.

Akt also phosphorylates glycogen synthase kinase-3β (GSK-3β), and the fork-head box O family of transcription factors (FOXO). Inhibition of GSK-3β through phosphorylation by Akt relieves the inhibition of eIF2B, which can therefore chaperone methionyl-mRNA to the 40S ribosomal unit for translation initiation. Phosphorylation of FOXO by Akt prevents FOXO from stimulating transcription of proteolytic ubiquitin ligases (Sartorelli and Fulco, 2004). Therefore this pathway can influence both protein accruement and protein degradation during and following resistance exercise.

AMPK signalling: AMPK has been described as the 'energy sensor' of the cell, due to increased activity in times of low cellular glycogen concentrations and high AMP levels (Cantó et al., 2009). If energy is needed by the cell, such as during exercise, it promotes glucose and fatty acid oxidation (i.e. energy releasing) and inhibits protein synthesis through inhibition of mTOR pathway (i.e. energy consuming (Jørgensen et al., 2006)). Following the cessation of exercise, AMPK levels are similar to baseline measures after approx 2 hours (Dreyer et al., 2006). The exact role of AMPK with *MAPK signalling*: MAPK signalling cascades are a network of four main families of parallel phosphorylation cascades; extracellular signal-regulated kinases 1 and 2 (ERK 1/2), p38 MAPK, c-Jun NH_2 –terminal kinase (JNK), and ERK 5. Martineau and Gardner (2001) found that activation and phosphorylation of JNK, ERK and p38 MAPK were done in a tension-dependent manner, with MAPK activation a better predictor of peak tension, rather than time-tension integral or rate of tension development. The authors also

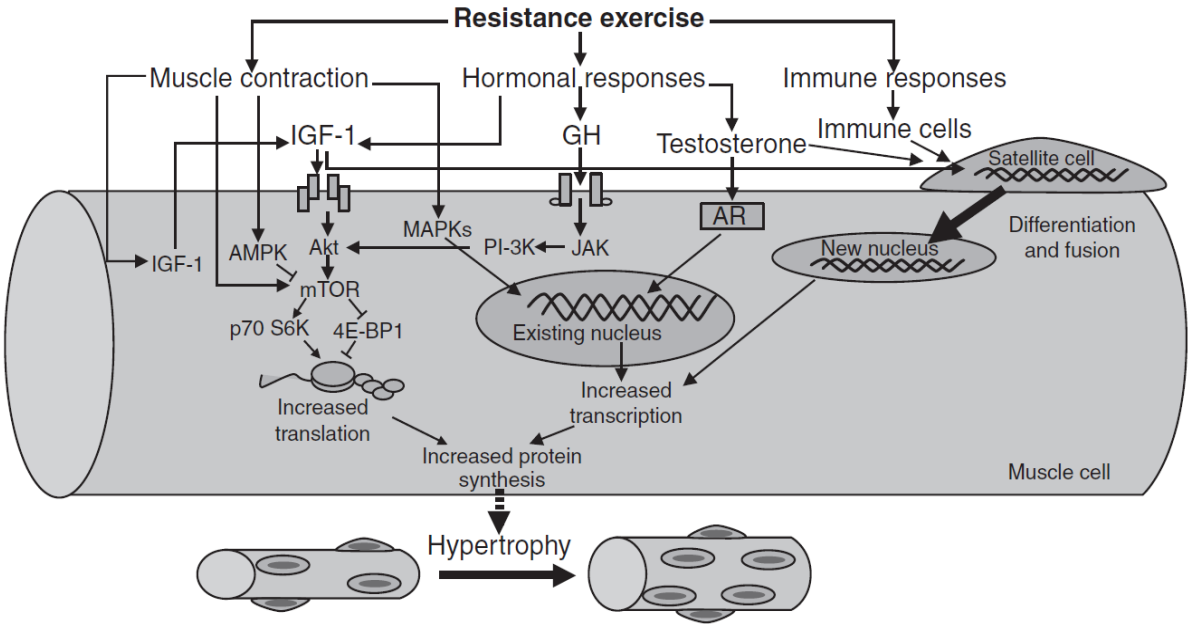


Figure 1.15. *Summary of the signalling responses to resistance exercise (see text for abbreviation definition).* *(Taken from Spiering et al. 2008)*

regards resistance exercise is yet to be fully comprehended, although it's possible role in endurance type exercise is more fully understood (Joseph et al., 2006).

suggested different roles in mechanotransduction for JNK and ERK/ p38 MAPK families, and suggested that MAPKs are a good qualitative indicator of mechanical stress applied to muscle. Activation of these pathways leads to translocation to the nucleus, where they phosphorylates a number of transcription factors (ELK-1, SRF, AP-1), thereby regulating gene expression (Garrington and Johnson, 1999). Furthermore, MAPKs can also phosphorylates histones, which alter the chromatin environment of specific genes and enhances transcription (Hawley and Zierath, 2004). However the importance of MAPKs for resistance exercise adaptations is as yet unclear (Spiering et al., 2008).

1.2.1.4 Protein Synthesis

As suggested by the ultimate events of cell signalling (i.e. increased translation and transcription), an increase in protein synthesis is a major response to an acute bout of resistance exercise. However, there is actually a net increase in protein synthesis, as degradation of protein is elevated immediately after a bout of training, but is attenuated ~24 hours later, whereas there are also significant increases in mixed muscle protein synthetic rates that peak within 3 hours post-exercise and remain significantly elevated for up to 48 hours (Phillips et al., 1997). This time-course can be different depending on training status and nutritional intake.

To investigate the relationship between translational rates, transcription rates and protein accruement, Adams et al. (2002) investigated the acute and prolonged responses to 90 days overload in the rat plantaris. The immediate 8-fold increase in translational efficiency (i.e. through phosphorylation of eIF4E and p70 S6K) returned to baseline within 15-90 days later, whereas muscle DNA continued to rise throughout the 90-day protocol. Also, total RNA increased after 3 days and continued to rise until day 7, and remained elevated at 90 days. The results suggest that initially, translational efficiency is important in the acute response to resistance training, whereas transcriptional capacity and /or translational capacity maybe more important for longer term gains in protein accruement. It should be noted however, caution should be taken when attempting to extrapolate findings in animal models to those in human models, especially regarding protein and molecular responses, as alterations of protein turnover, the fibre types

involved and the ability of static indices of protein turnover to represent dynamic changes may be misleading (Rennie et al., 2010).

1.2.2 Chronic adaptations to resistance training

1.2.2.1 Neurological Adaptations

It is well established that neural adaptations contribute to strength gains with resistance training, with particular emphasis given to the initial weeks in naïve individuals. However, some contention exists at the nature of these adaptations. The most commonly cited evidence is the disproportionate gain in muscle strength compared to muscle size following training, and also retention of strength when losses in mass have occurred following detraining or injury for example. It is usually stated that neural components account for the gains in strength after the first 8 weeks of training (Moritani and DeVries, 1979). However, more recently other morphological factors, e.g. architecture (Seynnes et al., 2007), have been proposed as accounting for some of the early rise in muscle specific tension. Others include task specificity (Nozaki et al., 2005), joint-angle specificity (Thepaut-Mathieu et al., 1988, Kitai and Sale, 1989), imagined contractions (Zijdewind et al., 2003), and the crossover (or cross-education) effect, where an untrained, contralateral limb displays an increase in strength e.g. (Komi et al., 1978). Indeed with recent work establishing an immediate increase in protein synthesis following a single bout of RT (Phillips, 2000) it is likely that the neural and muscular adaptations happen concurrently.

As mentioned previously, it is particularly difficult to decipher just how fully activated muscles are during assessment of MVC, in order to develop true maximum force. Following training, increases in agonist activation could increase through enhanced motor recruitment, or firing frequency, although this is based on the assumption that these are sub-maximal before training occurs.

Using sEMG there have been many studies carried out that have reported a significant increase in agonist sEMG recordings following a resistance training program in both males and females, and in the young and elderly (Häkkinen et al., 1996, Narici et al., 1989, Häkkinen et al., 2003, Komi et al., 1978, Moritani and DeVries, 1979, Häkkinen and Komi, 1983, Reeves et al., 2005b, Higbie et al., 1996). As mentioned previously, muscle adapts in a specific manner to the stimulus provided, and in the case of the aforementioned studies, increases in agonist muscle activation has been shown to be specific to the mode of muscular contraction employed during the resistance training period, and has been fairly well characterised. Some studies, such as that of Hakkinen and Komi (1983), found that the rate of integrated EMG paralleled and closely followed changes in torque over the course of the training period. Although other studies, such as that of Garfinkel and Cafarelli (1992) did not find a relationship between iEMG and strength changes.

1.2.2.2 Endocrine Responses

In the cases of many hormones, there appears to be little or no consensus on changes in circulating levels following extended periods of resistance training. With regards

testosterone, there have been reports of elevations in circulating levels during or following resistance exercise and especially during periods of high volume training (Marx et al., 2001, Kraemer et al., 1999b, Ahtiainen et al., 2003a, Hakkinen et al., 1988, Staron et al., 1994). However, there is an equal number of studies that report no chronic change (e.g. (Häkkinen et al., 2008)) or reductions in circulating testosterone levels with resistance training (Raastad et al., 2001).

Similarly, GH has also demonstrated no increase in resting levels following both traditional weightlifting (Kraemer et al., 1999b, Hakkinen et al., 2000, Marx et al., 2001, Mccall et al., 1999) and in elite weightlifting and/ or strength training (Hakkinen et al., 1988, Ahtiainen et al., 2003a).

IGF-I has shown similar chronic responses to both testosterone and GH with increases in both men and women of varying ages (Borst et al., 2001, Marx et al., 2001, Koziris et al., 1999), no changes (Kraemer et al., 1999b, Mccall et al., 1999) or reductions (Raastad et al., 2001, Onambélé-Pearson et al., 2010b, Onambélé-Pearson et al., 2010a) in resting levels all reported throughout the literature, again with training volume and intensity forming a potential, but tenuous link with the associated changes. An important factor to consider is that following just a few bouts of heavy resistance exercise training, an increase in androgen and IGF-I receptor expression and number is observed (Willoughby and Taylor, 2004, Bamman et al., 2001), which is significantly correlated to serum levels of circulating hormones (Willoughby and Taylor, 2004).

1.2.2.3 Changes in Muscle Size and/ or Volume

Table 1.1 shows an overview of studies that report changes in muscle size or volume following resistance training. A PubMed search using the mesh terms 'resistance exercise' and 'muscle hypertrophy' returns 563 hits. Since it is beyond the scope of the thesis to systematically review each of those, only the subsample of studies that mirrored the training protocol (resistance exercise), anatomical site of interest (quadriceps muscle group), population age and health status (young healthy) etc were included in the abbreviated summary below. In particular, the table shows a sub-sample of studies comparable to the studies in the body of work presented in this thesis in terms of: (a) duration: the vast majority are of relatively short duration, ranging from only 5 weeks to 8/9 weeks, and the rest are samples of longer duration studies 12-24 weeks. (b): gender: The predominant populations are young males (and/ or females), (c) impact: the rate of increase in size per week demonstrates the large range (0.41-1.88%) of observed adaptations. There is huge variation in the methods of analysis and also to the almost infinite variations of training program methodologies such as mode of contraction, time under tension, rest periods, order of exercises, nutritional supplementation, relative or absolute loading patterns, muscle group(s) trained, types of exercise used to name but a few. Therefore no comparisons between studies will be made directly at this point. However from these studies it is clearly evident that skeletal muscle is a plastic material and has the ability to increase in size following resistance training, and that this can vary dependent on a huge number of factors.

1.2.2.4 Changes in muscle architecture

In a similar vein to muscle morphological changes, architectural changes following resistance exercise will again be influenced by the methodology of training regime and measurements and no direct comparisons between studies will be made at this stage either, however it does provide also clear evidence of the plasticity of muscle architecture in response to resistance training. Research into changes in muscle architecture is shown in Table 1.2.

Study	***Muscle Group***	***Duration of training (weeks)***	***Parameter***	***Measurement Tool***	***Size Increase (%)***	***Rate of size Increase (% per week)***
Fiatarone et al. (1990)	Quads	8	Mid-thigh CSA	CT Scan	8	1.00
Jones & Rutherford, 1987	Quads	12	Mid-thigh CSA	CT Scan	5	0.41
Narici et al. 1996	Quads	24	Prox, mid, distal thigh CSA	CT Scan	17	0.70
Tracey et al. 1999	Quads	9	Volume	MRI	12	1.33
Kubo et al. 2006	Quads	12	Volume	MRI	12	1.00
Erskine et al. 2010	Quads	9	Volume	MRI	6	0.66
Abe et al. 2000	Quads	12	Muscle thickness	Ultrasound	8	0.66
Garfinkel & Cafarelli, 1992	Quads	8	Mid-thigh CSA	CT Scan	15	1.88
Tesch et al. 2004	Quads	5	Volume	MRI	6	1.20

Table 1.1 *Table showing changes in muscle size following various training regimes.*

Study	***Muscle/ Muscle Group***	***Duration of training***	***Parameter***	***Changes Reported***
Rutherford & Jones, 1992	VL, VI	3 months	θ_P, MTH, L_F	No change
Kawakami et al. 1995	Triceps	16 weeks	θ_P, L_F	29%, no change
Aagaard et al. 2001	VL	14 weeks	θ_P	34%
Blazevich & Giorgi 2001	Triceps	12 Weeks	θ_P,	40%
Blazevich et al. 2003	VL, VI, VM	5 weeks	θ_P, L_F	No change
Raj et al. 2012	VL, GM	16 weeks	θ_P, MTH, L_F	-3%, 5%, 5%
Suetta et al. 2008	VL	12 weeks	θ_P, MTH,	22%, 15%
Matta et al. 2011	Biceps	12 weeks	θ_P, MTH,	5%, 12%
Blazevich et al. 2007	VL, VI	10 weeks	θ_P, MTH, L_F	18%, 11%, 5%
Cromie et al. 2010	VL	10 weeks	θ_P, MTH,	No change
Seynnes et al. 2009	VL	10 weeks	L_F	No change

Table 1.2 *Table showing changes in muscle architectural parameters following various training regimes.* (θ_P = pennation angle, MTH = muscle thickness, L_F = fascicle length)

1.3. Structure, function and adaptation of human tendons:

This section will provide a brief overview of the major features of tendon structure, function. Critically also, this section will describe tendon adaptations to the stimulus of mechanical loading, and also some of the current evidence surrounding such adaptations.

1.3.1 Tendon function & behaviour

Tendons function to magnify and transmit forces generated in the muscle to the skeleton for movement, and also act as a mechanical buffer by absorbing external forces to prevent muscle damage (Kirkendall and Garrett, 1997, Sharma and Maffulli, 2006). Their parallel arrangements of fibres (see Figure 1.18) allow this to be achieved efficiently, and are designed for minimal deformation and energy loss during this process. Further, tendons possess a low resistance to shear forces, as well as the characteristics of a viscoelastic material, which display stress-relaxation and creep, influencing force transmission and energy storage/ return(Galantis and Woledge, 2003).

The behaviour of the tendon will also reflect the number and type of inter- and intra-molecular bonds. The mechanical properties of tendon are demonstrated in a stress-strain curve whilst undergoing loading of increasing magnitude (Figure 1.16). The initial 'toe region' represents the flattening of the crimp pattern of a healthy tendon (Stouffer et al., 1985), from which their usually appears a linear pattern, which reflects the intramolecular sliding of the collagen triple helices (see Figure 1.18) and the fibres gaining a parallel arrangement. If strain remains low enough, the tendon behaves in an elastic fashion,

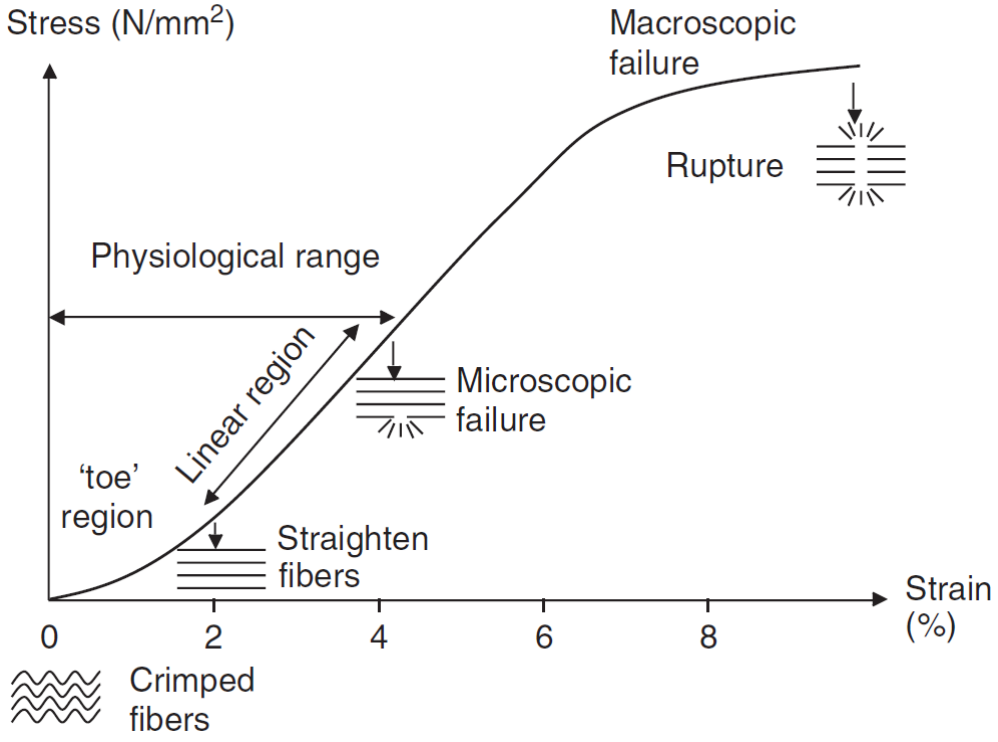


Figure 1.16. *Stress-strain curve demonstrating the mechanical properties of a typical tendon. N.B. stress & stain values vary between tendons and the above values are simply representative of a typical tendon* (taken from Wang, 2006).

returning to its original length following unloading. However, in the cases where strain exceeds the elastic limits, microscopic and eventually macroscopic damage occurs through intrafibril damage from molecular slippage (Sasaki et al., 1999). After this complete failure rapidly ensues with the tendon fibres recoiled into a tangled bud at the ruptured end. The tensile strength of the tendon will be related to the collagen content and also the thickness of the tendon (Wang, 2006).

From the stress/ strain curve it is also possible to calculate the tendon modulus (modulus = stress/ strain), which represents the properties of the actual tendon material

independently of the CSA (material properties (Maganaris and Paul, 1999)). This makes it possible to compare structures with different dimensions. A high tendon modulus indicates a relatively stiff tissue. This parameter should not be confused with tendon stiffness, which is the relationship between the change in tendon length (L) to the amount of force (F) applied (i.e. stiffness = $\Delta F / \Delta L$). However this parameter is dependent on the CSA and length of the tendon (i.e. a shorter length and greater tendon CSA will be more stiff – tendon's mechanical property (Maganaris and Paul, 1999, Heinemeier and Kjaer, 2011)).

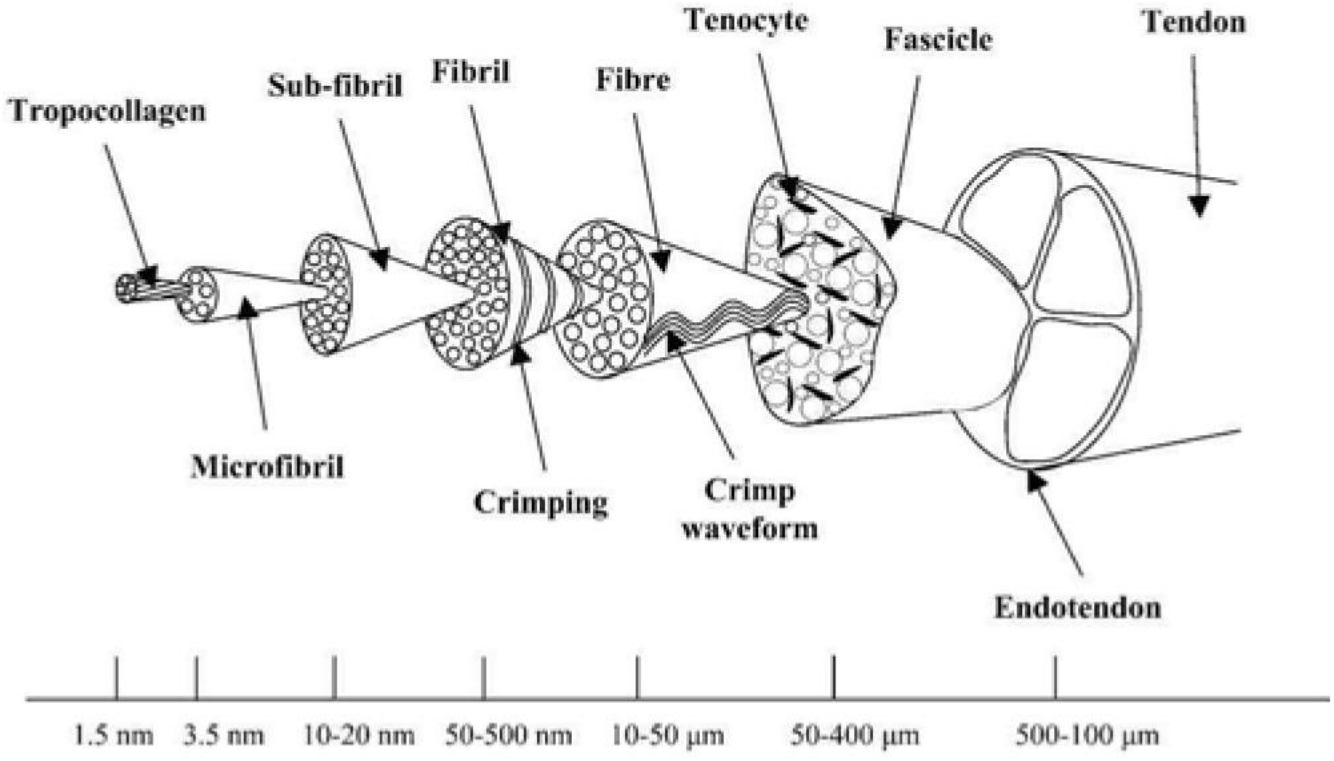


Figure 1.17. *Schematic hierarchal structure of a normal tendon.* (taken from Katelic et al.1978)

1.3.2 Tendon structure

The basic structure of a tendon is shown in Figure 1.17 with the microstructure shown in Figure 1.18. Structurally, tendon is composed of a relatively low number of cells - fibroblasts (in the form of tenoblasts and tenocytes) lying within a network of extracellular matrix (ECM). Tenoblasts are immature tendon cells. As tenoblasts age, they become elongated and transform into tenocytes, which have a lower nucleus-to-cytoplasm ratio than tenoblasts, with decreased metabolic activity. Together, tenoblasts and tenocytes account for 90- 95% of the cellular elements of tendons (Bayer et al., 2010). Tenocytes synthesize collagen and all components of the ECM, such as matrix precursors, collagen, elastin, proteoglycans and are also active in energy generation (O'Brien, 1997).

The major component of the ECM is collagen Type I assembled in Type I collagen fibrils, which are arranged mainly in the longitudinal direction of the force imposed on the tendon during muscle contraction. The collagen molecules are structurally arranged into fibrils, in an imbricate pattern with cross-links in-between. The cross-links reduce the strain at failure and increase the elastic modulus. In mature tendon tissue, fibroblasts are arranged in rows along the force-transmitting axis of the tendon, and can be connected to each other by gap junctions (Wang, 2006). An integrin-based connection of fibroblasts to the ECM exists, providing the intracellular cytoskeleton with a connection to the surrounding matrix. This is thought to be a critical factor in allowing the mechanotransduction of mechanical signals (in a similar way to muscle) that allows the tendon to respond and adapt specifically to the various magnitudes and patterns of mechanical loading (Heinemeier and Kjaer, 2011).

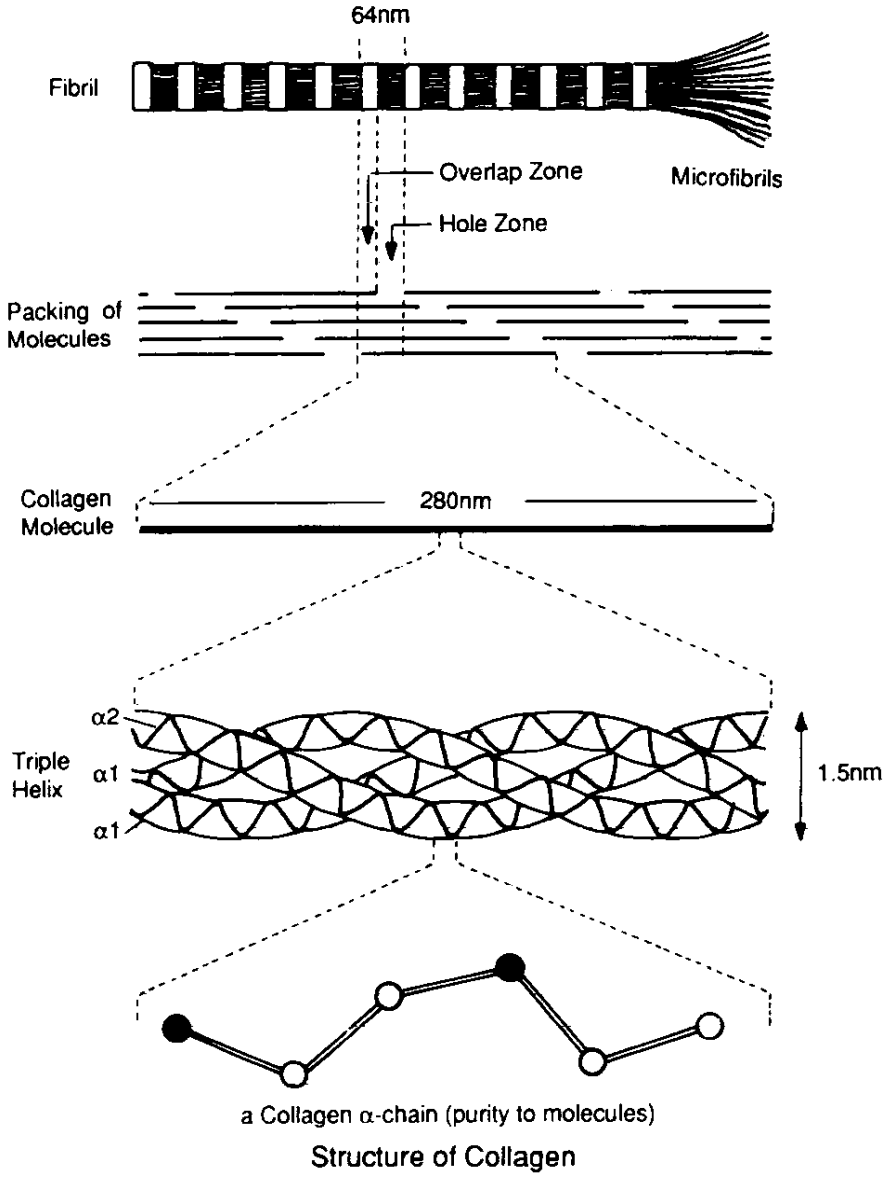


Figure 1.18. *The microstructure of collagen.* (taken from Kirkendall & Garrett, 1997)

1.3.3. *In Vivo* Tendon responses to mechanical loading

1.3.3.1. *Morphological adaptations*

The extent of morphological adaptations to increased loading (such as an increase in tendon cross-sectional area) remains contentious throughout the literature. Early reports

suggest that the free tendon does not increase significantly in size as a result of resistance training in the Achilles tendon (Kubo et al., 2002, Kubo et al., 2007) and the patella tendon (Kubo et al., 2006b, Kubo et al., 2009). In each of these cases, tendon CSA was measured by ultrasonography at the mid-point of the tendon length. Furthermore, the study of Reeves et al. (2003a) measured patella tendon CSA at 25%, 50% and 75% (i.e. proximal, mid and distal) of patella tendon length at rest using ultrasonography and found no significant changes following 14 weeks of high-intensity resistance training in older individuals. In contrast, Kongsgaard et al. (2007) measured patella tendon CSA at proximal, mid and distal points along the length by MRI before and after 12 weeks of heavy or low intensity resistance training in young men. In the heavy group, patella tendon hypertrophy occurred at proximal and distal regions, with the authors concluding that previous studies which calculated modulus from individual, mid-point CSA measurements, would have over-estimated modulus values. In, a similar vein, Seynnes et al. (2009) reported tendon hypertrophy at 20-30%, 60%, and 90-100% of patella tendon length following 9 weeks of resistance exercise. Therefore it is contentious throughout literature the extent to which tendon changes in size following resistance training, although from examination of published evidence it would suggest that multiple sites of tendon CSA measurements are required in order to accurately gauge such changes (Kongsgaard et al., 2007, Seynnes et al., 2009).

1.3.3.2. In Vivo Mechanical & Material Adaptations

It is evident from a multitude of investigations, that free tendon (and tendon aponeurosis) has the ability to adapt its mechanical properties in response to resistance training

(Maganaris et al., 2004, Seynnes et al., 2009, Kubo et al., 2009, Kubo et al., 2010, Kubo et al., 2006a, Kubo et al., 2006b, Kubo et al., 2007, Onambele-Pearson and Pearson, 2012, Reeves et al., 2003a, Arampatzis et al., 2007). However, similar to the case of comparison of studies regarding changes in muscle size, there are is a large range of changes reported in the mechanical (stiffness – Figure 1.19) adaptations following training. For example the relative increase in stiffness following resistance training in the study of Seynnes et al. (2009) was 24%, compared to for example the study of Kubo et al. (2009) which reported an 83% increase in similar populations. Once again the difficulty in directly comparing studies arises from variations in tendon measurement techniques, calculations of stiffness (Pearson and Onambélé, 2012), and intensity, volume and duration of training protocols to name but a few of the methodological discrepancies. With regards modulus, again evidence has shown tendon has the ability to increase its material properties also (e.g. (Reeves et al., 2003a)). As such there also exists the debate over the extent of changes in tendon (Young's) modulus following training, such as mentioned in the previous section.

1.3.3.3. Cellular events for adaptation following mechanical loading

Following mechanical loading, tendon restructures, and changes both composition and mechanical properties, which is achieved primarily by tendon fibroblasts altering the gene expression of ECM proteins (Kjaer et al., 2009, Kjaer, 2004). ECM proteins and polysaccharides act as scaffolds that help define tissue shape and structure, whilst also as a substrate for cell adhesion, , growth and differentiation (Silver et al., 2003).

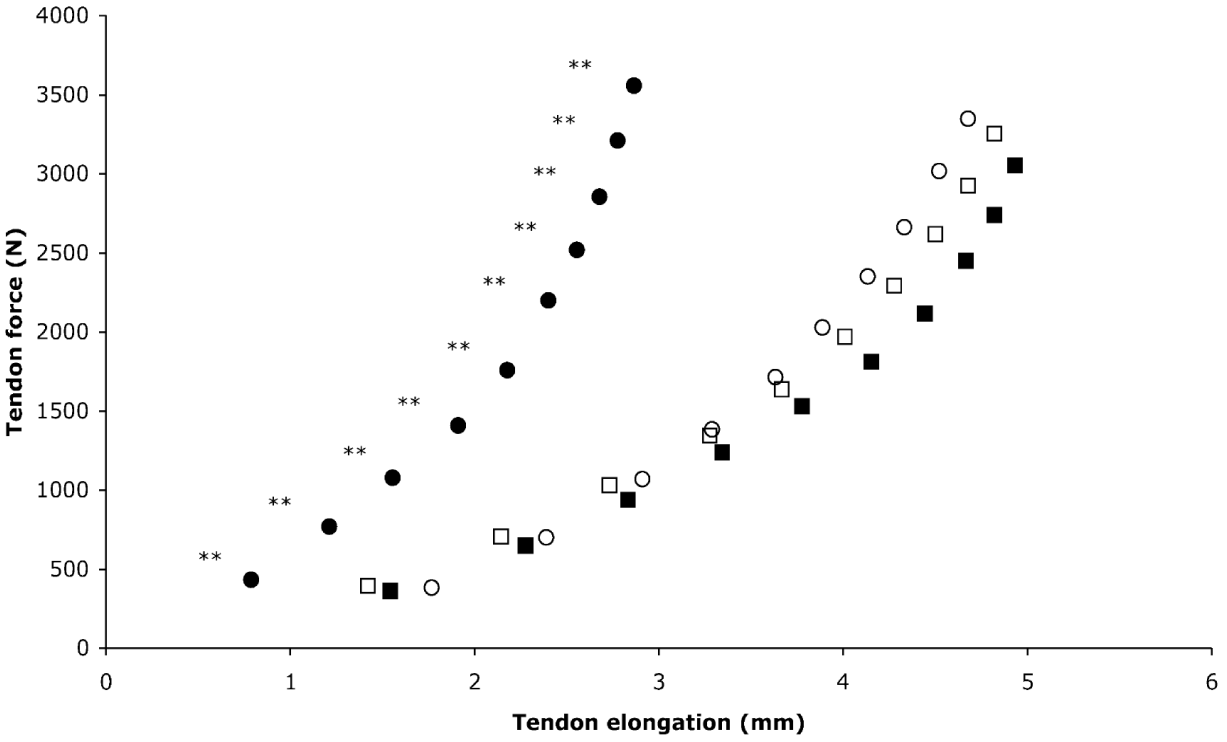


Figure 1.19. *Changes in the force-elongation relationship of the patella tendon following 14 weeks resistance training from pre-training (white circles) to post-training (filled circles). ** p<0.01.* (taken from Reeves et al. 2003)

Both uni- and bi-axial stretching of tendon fibroblasts *in vitro* have revealed increased expression of growth factors such as TGF-β1 (involved in regulation of collagen gene expression), IGF-I (promotes proliferation and migration of tendon fibroblasts subsequently increasing collagen and proteoglycan production), PDGF (stimulates production of other growth factors), VEGF (stimulates cell proliferation and capillary permeability), and bFGF (regulates cellular migration and proliferation, and also promotes angiogenesis). For a review of these see Molloy et al. (2003). In turn, these growth factors are then involved in ECM protein regulation.

Mechanically, the ECM proteins transmit mechanical loads, store and dissipate loading-induced elastic energy. As in muscle, there are pathways and interactions that mediate the transduction of mechanical signals to chemical signals that alter cellular function (Figure 1.20). In tendon this includes interaction and integration of the cytoskeleton, integrins, G-Proteins, RTKs and MAPKs, and stretch-activated ion channels (Wang, 2006). From the complex array of these biological actions, tendon appears to respond and adapt to mechanical loading by altering the mechanical/ material properties through changes in tendon fibril morphology, crimp pattern, collagen packing density and cross-link content.

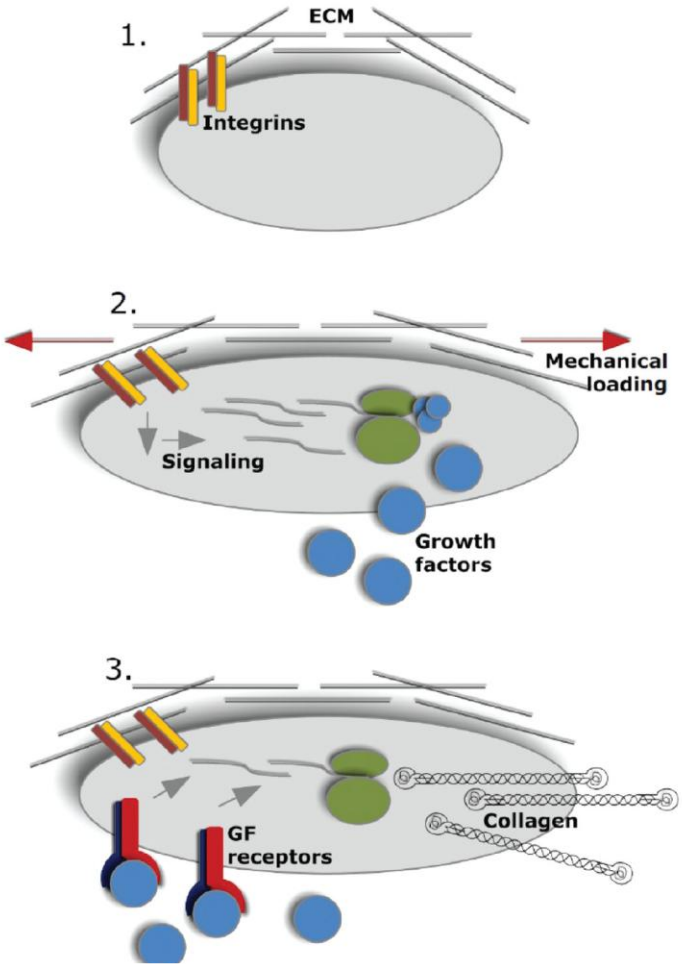


Figure 1.20. *Possible mechanism for loading induced collagen synthesis: 1) Fibroblast connected to extra cellular matrix via integrins. 2) Transcription and synthesis of growth factors induced by mechanical loading via changed intracellular signaling. 3) Autocrine/paracrine action of growth factors leading to increased collagen transcription and synthesis* (taken from Heinmeier et al. 2011)

HYPOTHESES

The main hypotheses of this body of work were that:

1. There would be a superior enhancement of muscle morphology and architecture following training with the muscle at a lengthened position, due to the greater combined and separate mechanical effects of stress and strain as stimuli for adaptation on the muscle.

2. There would be greater increases in tendon mechanical and material properties following resistance training in the LX and LL groups compared to SL group due to mechanical stress and/ or strain on the muscle-tendon complex.

3. There would be superior maintenance of muscle-tendon complex characteristics following the detaining period in the LX/ LL groups compared to SL due to greater initial adaptations attained in the preceding training program.

4. The acute physiological responses of the cardiovascular and neuromuscular systems to a bout of the three resistance exercise protocols of interest, would indicate greater stress in the order of LX>LL>SL groups.

5. The chronic changes in endocrine factors following the resistance training (and detraining) programs would reflect the observed changes to the *in vivo* muscle-tendon complex characteristics.

CHAPTER 2:

METHODOLOGY

2.1 MUSCLE GROUP:

The *quadriceps femoris* was the muscle group of choice for analysis in this study. The quadriceps femoris are made up of four constituent muscles; rectus femoris (RF), *vastus lateralis* (VL), *vastus medialis* (VM) and *vastus intermedius* (VI- see Figure 2.1). The VL, VM and VI muscles are termed the mono-articular vasti due to that they provide action across one joint in the body, thus being the knee joint. The RF is termed as a bi-articular muscle as its actions are across both the hip and knee joints.

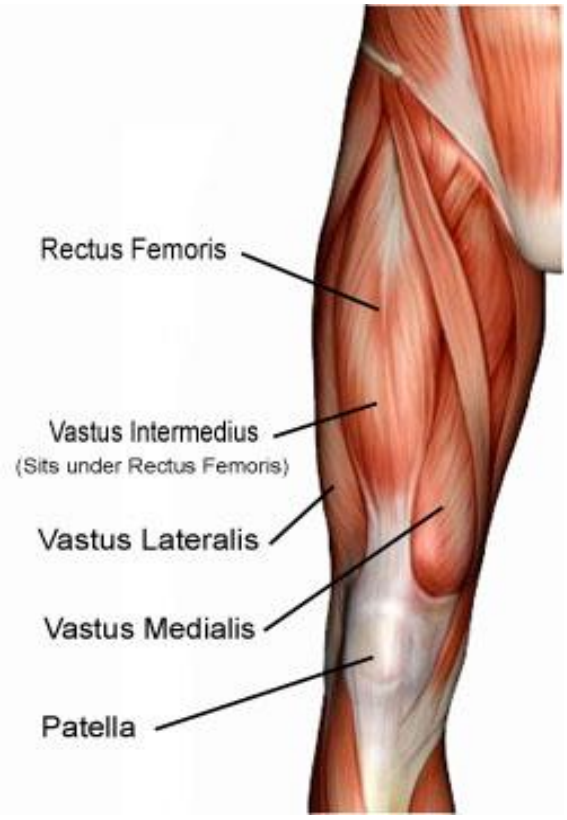


Figure 2.1. *Diagram of the quadriceps femoris muscle group showing its four individual constituent muscles.* *(Taken from floota.com)*

The quadriceps muscle group exists as large anti-gravity muscles, but for the human are also key to many other functional tasks. As the quadriceps cross the knee joint, they function mainly as

knee extensors and are used in locomotion (walking, running), jumping and squatting. The RF plays a unique role in walking and running because it crosses the hip joint, and so is also engaged in flexion of the hip joint. Due to their vital role, changes to this muscle group and ultimately their function is of great interest.

2.1.1 The Vastus Lateralis

It is relatively easy to identify changes in quadriceps function (i.e. function of the whole group) through the use of isokinetic dynamometry for instance. However, time and resource management and ability to gain accurate measurements (Blazevich et al., 2006) dictate that every individual muscle of the constituent muscles cannot be observed in any great detail, and therefore a representative muscle of the group must be considered. The *vastus lateralis* is regularly chosen to be a representative muscle of the quadriceps due to its fibre type size and distribution, enzymatic activity, EMG, morphology and function characteristics (Sinha-Hikim et al., 2002, Froese and Houston, 1985, Housh et al., 1995, Green et al., 1984). Of particular interest for this study is the fact that the VL possesses one of the largest cross sectional areas (CSA) for measurement (Lieber, 1992), and therefore will help to reduce any potential measurement error and increase reliability, especially during repeated measures designs. Due to its location on the lateral thigh, the VL is easily accessible, and images for measuring aCSA and architectural parameters using B-mode ultrasonography can be visualised to relatively high and accurate degree (Reeves et al., 2004a, Blazevich et al., 2006, Ichinose et al., 1997). Taking the above factors into consideration, the VL muscle was used for assessing the training-induced responses to resistance exercise as a representative muscle of the quadriceps femoris.

2.2. Measurement of Muscle Parameters

The following section will describe the various muscle parameters of interest, their anatomical location and details of their measurement including VL muscle cross-sectional area, VL volume, VL architecture (pennation angle and fascicle length), VL muscle width, VL activation and thigh girth.

2.2.1. Anatomical Location of Muscle Parameter Measurement

Muscle morphological and architectural parameters were measured at three distinct anatomical locations along the length of the femur (Figure 2.2): 25%, 50% and 75% of total femur length. Femur length was chosen as opposed to muscle length, because muscle length can change for various reasons (such as stretch) and may be different at various points in time (i.e. pre- and post-training), whereas bone length would not change significantly over time in physically mature adults. Three locations were chosen for analysis as there is anecdotal evidence of a heterogeneous hypertrophic response to resistance training, and heterogeneous architecture along the muscle length of the VL (Narici et al., 1996, Blazevich et al., 2006). Additionally, by taking multiple sections of anatomical CSA (aCSA) scans along the length of muscle, the muscle volume measurements can be reliably and accurately estimated (Esformes et al., 2002, Morse et al., 2007a).

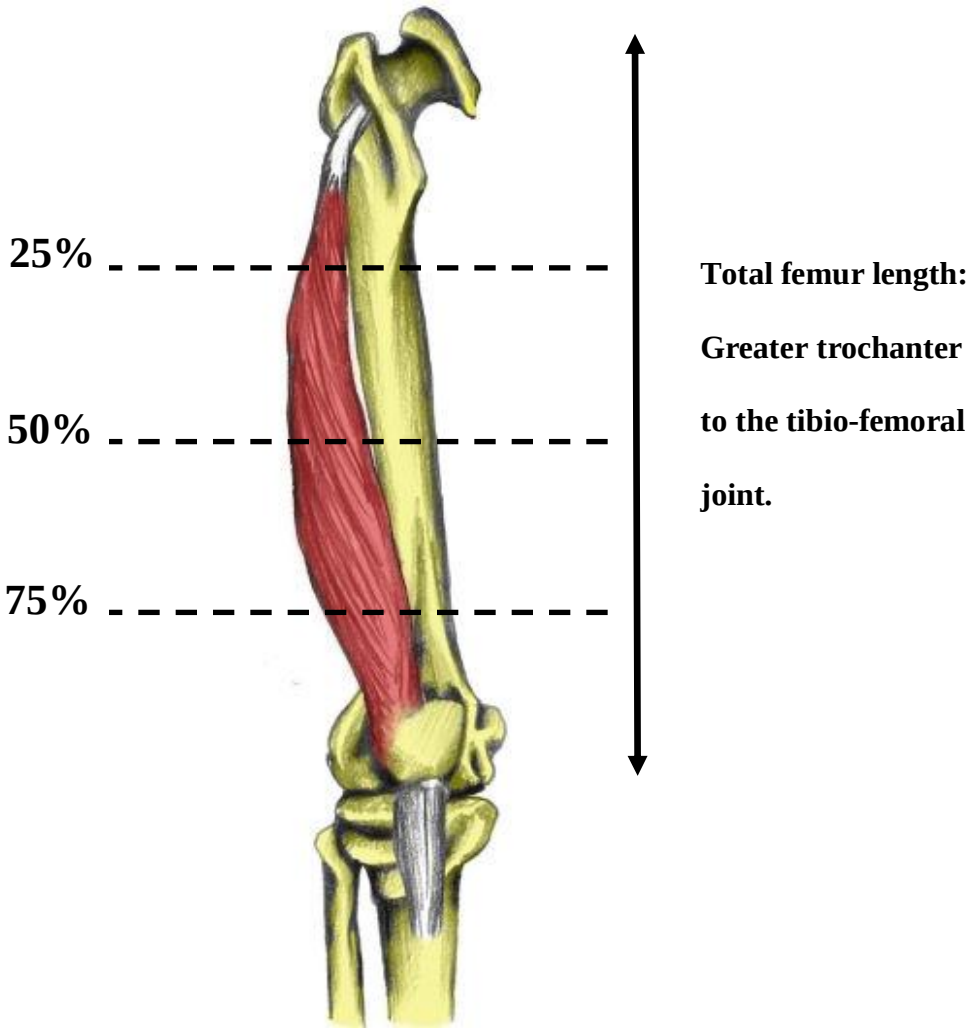


Figure 2.2. *Shows the anatomical measurement location of VL muscle parameter measurements.*
(anatomical picture taken from teachpe.com)

2.2.2 Anatomical CSA

VL muscle anatomical cross-sectional area (aCSA) was measured using real-time B-mode ultrasonography (Frequency:7.5MHz, 40-mm width linear array, B-mode ultrasound probe, AU5, Esaote Biomedica, Italy). Axial-plane images were taken on the right leg of each participant following approximately 15-20mins in the seated position, with the knee placed at 90^{o} flexion (0^{o} = full extension). Echo-absorptive tape was placed at regular intervals (~3cm) along the muscle width at each site so that when the probe was placed onto the leg; two distinct

shadows were cast on the ultrasound image. Therefore each ultrasound image provided a section of VL within the boundaries set by the two shadows and fascia surrounding the muscle. Individual images were reconstructed using the femur and superficial markers as reference points, with the total aCSA measured using Image J (Wayne Rasband, National Institute of Health, USA). The validity and reliability of this technique has previously been reported (Reeves et al., 2004a). All sonographs were taken by the same researcher throughout the study to reduce any variability/operator error within the data collection. It should be noted here that, sonographs were taken ~3-4 days post-training to avoid osmotic fluid shifts that may confound architectural or morphological measurements (Berg et al., 2008).

2.2.3. Muscle Architecture & Muscle Length

Architecture was measured at rest with each participant seated in an upright position on an isokinetic dynamometer (Cybex, Phoenix Healthcare Products, UK). Following equipment calibration, each participant was positioned with a hip angle of 80^{o} (straight back 90^{o}) and knee at 90^{o} knee flexion (straight leg 0^{o}). All muscle architectural measurements were determined using real-time ultrasonography (7.5-MHz, 40-mm linear array, B-mode ultrasound probe, AU5, Esaote Biomedica, Italy) at rest, with images captured using a digital video recorder (Tevion, UK). *Vastus Lateralis* fascicle pennation angle (θ) was measured as the angle of fascicle insertion into the deep aponeurosis (Rutherford and Jones, 1992). Images were obtained perpendicular to the dermal surface of the VL and orientated along the plane of the muscle fascicles. Images were taken at 25% (proximal), 50% (mid-diaphysis) and 75% (distal) of total femur length and 50% of muscle width at each point (where 50% muscle width is defined as the mid-point between the fascia separating the VL and Rectus Femoris, and fascia separating the

VL and Biceps Femoris muscles). Fascicle length was defined as the length of the fascicular path between the deep aponeurosis and superficial aponeurosis of the VL. The majority of fascicles extended off the acquired image, where the missing portion was estimated by linear extrapolation. This process was achieved by measuring the linear distance from the identifiable end of a fascicle to the intersection of a line drawn from the fascicle and a line drawn from the superficial aponeurosis. This method has previously been shown to produce reliable results, with a CV of 1.7% (~1.4mm) in the VL muscle (Blazevich et al., 2007). All images were analysed and measured using Image J (Wayne Rasband, National Institute of Health, USA). An example of muscle architectural measurements is shown in Figure 2.3.

VL muscle lengths were also determined using ultrasound in the mid-sagittal plane. This was determined as the length from the myotendinous junction of the VL and patellar tendon to the point where VL adjoins to the *Tensor Fascia Latae* and *Rectus femoris* muscle. These points were marked on the skin and measured with standard anthropometric measuring tape.

2.2.4. Muscle Width & Thigh Girths

The ultrasound probe was held in the transverse plane and used to locate the lateral borders of either side of the VL muscle (i.e. the two visible borders at either side of the VL as shown in Figure 2.3). Each of these junctions was marked onto the skin and the distance between them measured. In addition, at each of the aforementioned sites (25%, 50%, 75%), thigh girths were also measured using standard anthropometric techniques.

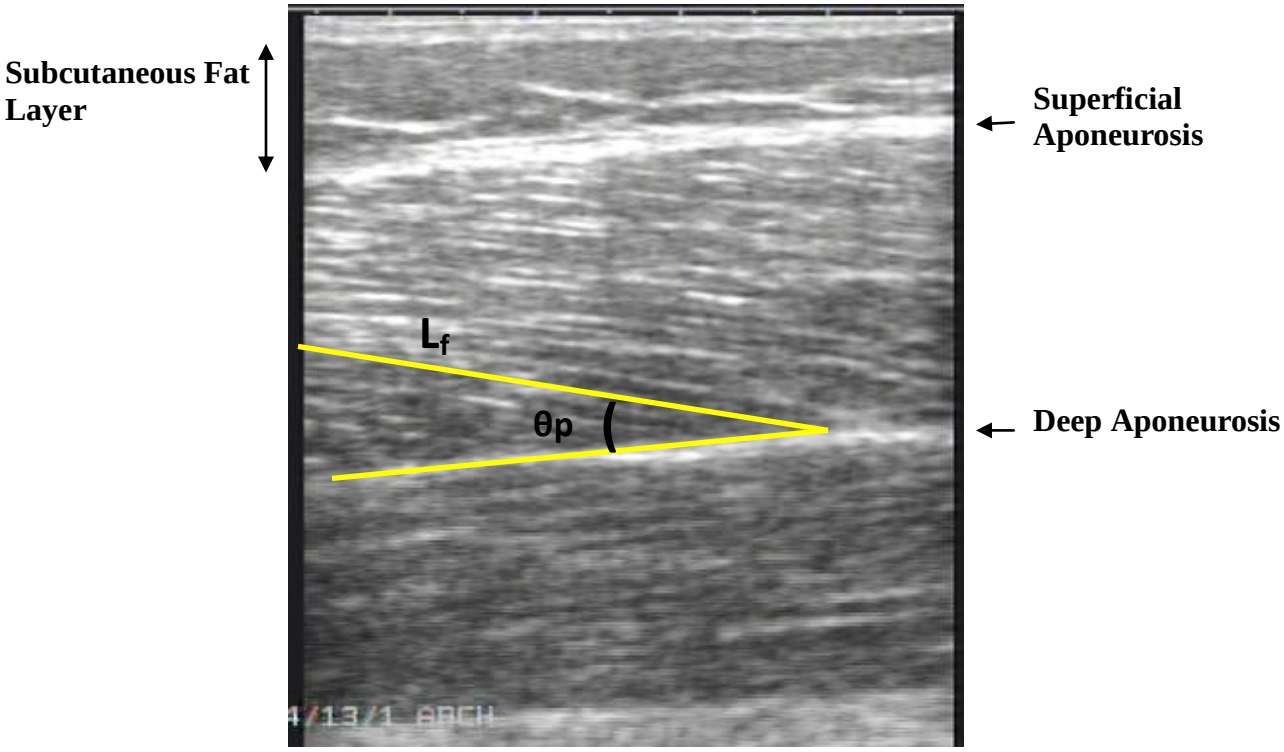


Figure 2.3. *Shows a Muscle architecture image taken at 50% femur length and muscle width. θp; pennation angle, L_f ; fascicle length (extrapolation required- taken from study participant).*

2.2.5. Subcutaneous Fat

Subcutaneous fat was estimated using the same sagittal plane images that were taken for muscle architecture. After calibration in Image J to coincide with the scale of the ultrasound image, a line from the top to the bottom of the layer of subcutaneous adipose tissue was drawn at three regular intervals on the ultrasound image. The mean lengths of these three lines were taken to estimate the average thickness of the subcutaneous fat layer in millimeters. Care was taken not to deform or compress the subcutaneous fat by allowing minimal pressure being applied to the dermal surface by the ultrasound probe.

2.2.6. Muscle Volume & pCSA

Muscle volume was estimated using the truncated cone method. This method has been shown to be a highly valid reproducible tool (intra-class correlation co-efficient 0.99) and comparable to MRI scans when estimating muscle volume (Esformes et al., 2002). The method of estimating *vastus lateralis* muscle volume is shown in Figure 2.4. The final muscle volume is the summation of muscle volumes between 25-50% femur length and 50-75% femur length in cm^3. This however only estimates approximately the muscle volume measured along 50% of femur length (i.e. the muscle volume between 25-75% femur length; 50% in total).

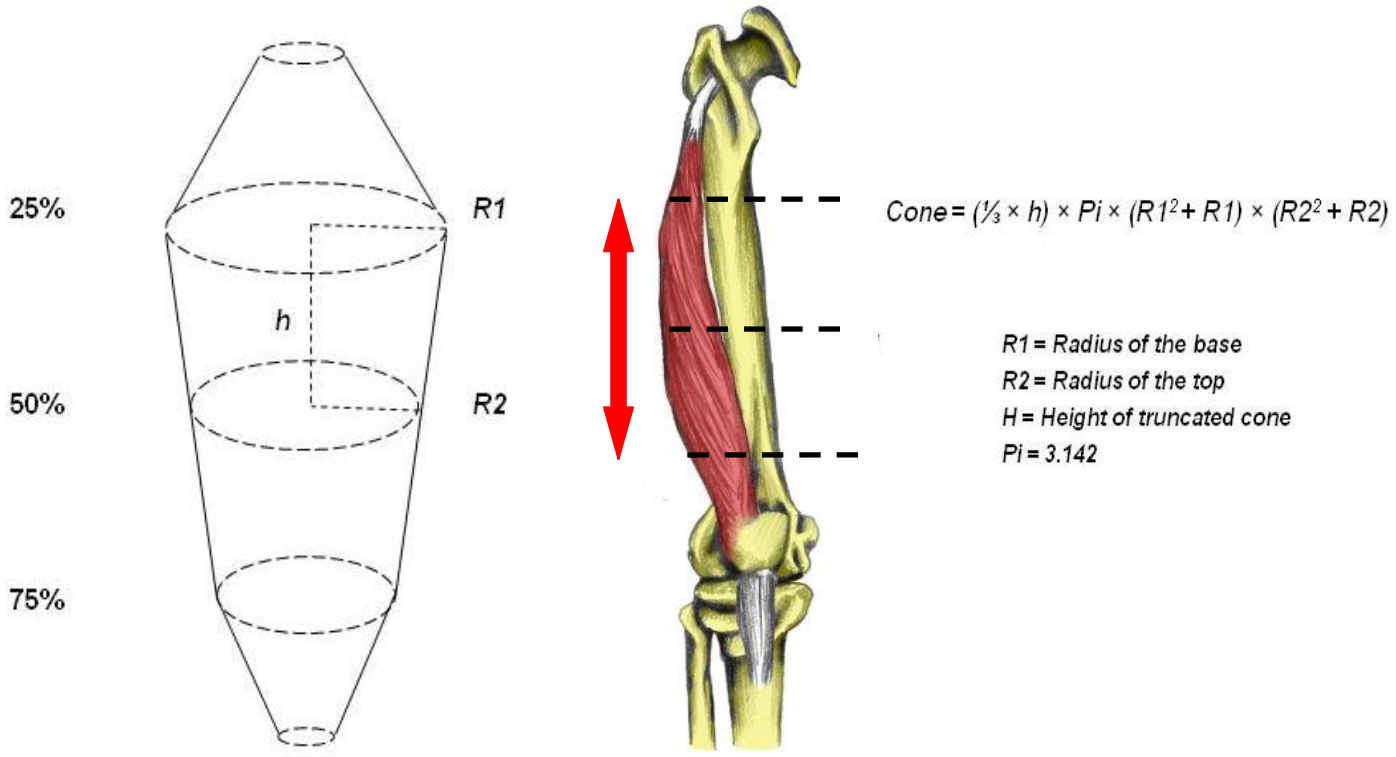


Figure 2.4. *Shows the measurement and estimation of muscle volume. Arrow indicates total muscle volume estimated.*

From these measurements pCSA was calculated using the formula:

$$\textbf{PCSA } (\text{cm}^2) = \frac{\textbf{Muscle Volume} \times \cos \theta_P}{\textbf{Fascicle Length}}$$

Note: PCSA at rest can be calculated by muscle volume divided by fascicle length when pennation angles are moderate ($<25^o$) and therefore have a negligible difference on calculation (Alexander and Vernon, 1975) as used by Reeves et al. (2004b)

2.2.7. Electromyography

Electromyographic (EMG) activity of a representative muscle in the Knee extensors (*vastus lateralis*) and knee flexors (long head of the *biceps femoris*) was measured to correct for coactivation during isometric knee extension. The skin was prepared by shaving, abrading, and cleaning with an alcohol-based solution to minimize resistance (i.e., reduce the interelectrode resistance to values below 5 kΩ). A pair of self-adhesive Ag–AgCl electrodes ~15 mm in diameter (Medicotest, Rugmarken, Denmark) were then placed in a bipolar configuration with a constant interelectrode distance of ~20 mm, at 50% of total femur length on each muscle. These electrodes were placed in the midsagittal plane of the muscle, and the reference electrodes was placed on the lateral tibial condyle and the medial femoral condyle. The raw EMG signal was pre-amplified (×2000) and bandpass-filtered between 10-500 Hz and, sampled at a rate of 2000 Hz by the same system that handled the torque data (Biopac Systems), and displayed in real time on the same graph so that all data would be synchronized (MacBook Air, Apple, California). The

reported EMG activity in this study corresponds to the root mean square (RMS) after correcting for baseline values.

The RMS EMG activity corresponding to the peak torque period was analyzed and averaged for 500ms peak torque duration, either side of instantaneous peak. As mentioned earlier, the EMG of the long head of the biceps femoris muscle was measured to ascertain the level of antagonist muscle co-contraction during the required isometric knee-extension performances. The biceps femoris torque during a knee-flexion contraction was calculated as the method described by Reeves et al.(2003a). The biceps femoris EMG activity during knee extension was divided by the biceps femoris peak flexor EMG at 90^{o} knee flexion (both described above); the maximal flexor torque is then multiplied by this value to determine co-contraction torque. The co-contraction torque values are used to correct the voluntary knee-extension torques (and hence the forces during the ramped contractions) as shown in Equation 1:

$$CT = OT + CcT \qquad \textit{[Equation 1]}$$

From the above equation CT represents corrected knee-extensor torque, OT is the observed knee-extensor torque, and CcT antagonist co-contraction torque (i.e. the calculated hamstrings torque during knee extension). The calculation of co-contraction torque is valid because the relationship between knee flexion torque and BF EMG has been shown to be linear (Reeves et al., 2004b)

2.2.8 Strength & Torque-Angle measurement

Maximal isometric knee extension torque was measured with the knee at a range of angles i.e. 30°, 50°, 60^{o}, 65°, 70°, 75° and 90° (full knee extension = 0°) on the right leg of all participants.

The order of testing by knee angle was randomised so as to minimise any systematic fatigue effect. After a series of warm up trials consisting of ten isokinetic contractions at $60°\cdot s^{-1}$ at self-perceived 50-85% maximal effort, participants were instructed to rapidly exert maximal isometric force against the dynamometer lever arm (Cybex, Phoenix Healthcare, UK). Participants were given both verbal and visual encouragement/feedback throughout their effort. Joint torque data was displayed on the screen of a MacBook Air computer (Apple Computer, Cupertino, CA, USA), which was interfaced to an A/D system (Acknowledge, Biopac Systems, Santa Barbara, CA, USA) with a sampling frequency of 200Hz. Isometric contractions were held for ~2s at the plateau with a 60s rest period between contractions. Peak torque was expressed as the average of data points over a 200ms period at the plateau phase (i.e. 100ms either side of the instantaneous peak torque). The peak torque of three extensions was used as the measure of strength in each participant.

2.2.9 Patella Tendon Moment Arm Measurement

The patella moment arm was estimated from sagittal scans of the right leg of each participant using a single Dual Energy X-Ray Absorptiometry (DEXA) scan (Hologic QDR, Vertec, Reading, UK), with the knee placed at 90^{o} of knee flexion. The patella tendon moment arm was defined as the perpendicular distance between the tibiofemoral contact point and the mid-portion of the patella (Figure 2.5). DEXA imaging has been used to estimate moment arm previously at other anatomical sites and has shown to be reliable and repeatable (Wang et al., 2009). The single energy scanning method has also been compared with MRI ((0.2-Tesla Magnetic Resonance Imaging (MRI) scanner (E-scan, Esaote Biomedica, Genoa, Italy)) images (taken in

the sagittal plane using a spin-echo TI half fourier (HF) sequence with a slice thickness of 8mm, inter-slice gap of 0.6mm and the parameters time to repetition/echo time/number of excitations (TR/TE/NEX), 420/18/1; field of view, 160·160mm; matrix, 256·256 pixels)) in our lab and does indeed provide externally valid measurements.

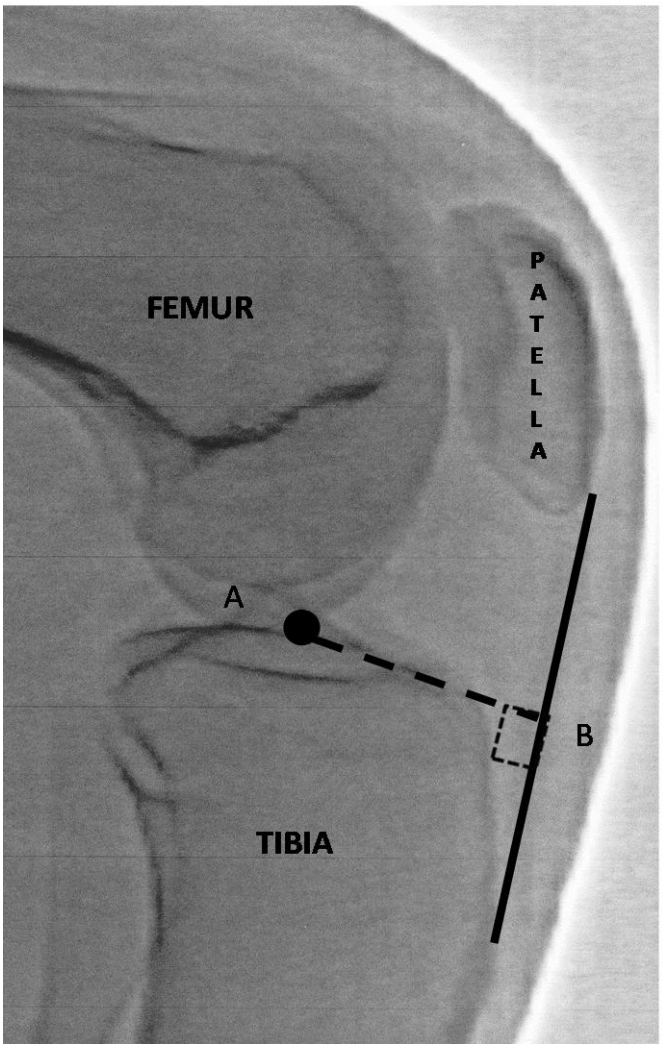


Figure 2.5: *Shows a typical sagittal plane image taken by Dual Energy X-ray Absorptiometry (DEXA) with the knee at 90^{o} flexion for measurement of patella tendon moment arm (Line A-B).*

2.2.10 Tendon properties

Tendon elongation was measured using ultrasound imaging (7.5-MHz, 40-mm linear array, B-mode ultrasound probe (AU5, Esaote Biomedica, Italy)) and set to a depth resolution of 55.3 mm. The probe was placed over the apex of the patella in the sagittal plane, with the knee fixed at 90^0 flexion. Forces were simultaneously determined by the measured external torque applied to the dynamometer with the addition of the estimated co-contraction torque from the antagonistic muscles by utilising electromyography (EMG). Prior to each measurement taken, five pre-conditioning trials were carried out to ensure reproducibility. The method for applying this *in vivo* tendon assessment technique has been published and has been shown reliable previously by Reeves et al. (2003b) and Pearson & Onambele, (2005). Briefly, an echo-absorptive marker was placed between the probe and the skin to act as a fixed reference from which relative measures of displacement could be made. Following each completed contraction (6-second graded isometric ramp with a constant low rate of force development), the distance between the original position of the tissue under the skin, relative to the new position of the tissue was recorded. Ultrasound images were recorded in real-time and captured onto a PC at 25 Hz. The ultrasound output was synchronized using a square wave signal generator (custom-made) to allow temporal alignment with the torque and EMG data. Tendon displacements were determined at intervals of 10% of the maximal force (from 10 to 100%) using Image J (National Institutes of Health, Bethesda, Maryland, USA). Three efforts were recorded and analysed, with tendon function also being measured from the effort that peak torque was recorded.

Patella moment arm at 90^o knee flexion was measured from DEXA scans (see *moment arm measurement*). Tendon forces were then calculated as: Measured Torque (corrected for antagonist co-activation) / Patella Moment Arm. The force–elongation relationship was fitted with a second-order polynomial function, forced through zero. Tendon stiffness (K in $N \cdot mm^{-1}$)

was calculated from the slope of the tangents at 10% force intervals. Tendon stiffness was also calculated at a standardised force level (2000N), which corresponded to just under the maximum baseline force value of the weakest study participant. Patellar tendon cross-sectional area (PTCSA) and resting length (PTL) were assessed with the knee joint at 90^{o} of flexion. PTCSA was determined from the mean of transverse plane ultrasound images taken at 25, 50 and 75% PTL. PTL was measured from the inferior pole of the patellar to the superior aspect of the tibial tuberosity as determined from sagittal-plane ultrasound images (see Figure 2.6).

2.2.11 Resistance Training Programme

Resistance training was performed 3 times per week by the three training groups (see below) on Tuesdays, Thursdays and Saturdays for 8 weeks, using a combination of free, machine (Technogym, UK) and body weights to complete the exercises. All exercise sessions were supervised by a member of the research team, to ensure completion and correct technique was employed throughout the study. A generalised warm-up was completed at 70-75% age-predicted maximum heart rate on a treadmill for 5mins (Gordon, 2009), after which a goniometer was attached to the central point of rotation of the knee. The training group excursions are shown in Figure 2.7. Briefly, the SL group performed all exercises from 0^{o} (full extension) to 50^{o} knee flexion, LL from 40^{o} to 90^{o} knee flexion and LX group from 0^{o} to 90 knee flexion. The LL group received the weight at 40^{o} knee flexion and unloaded on the final rep at 40^{o} knee flexion to make sure they did not perform extra work at the beginning or end of each set. All exercises involved eccentric and concentric contractions held for 2 seconds at the end

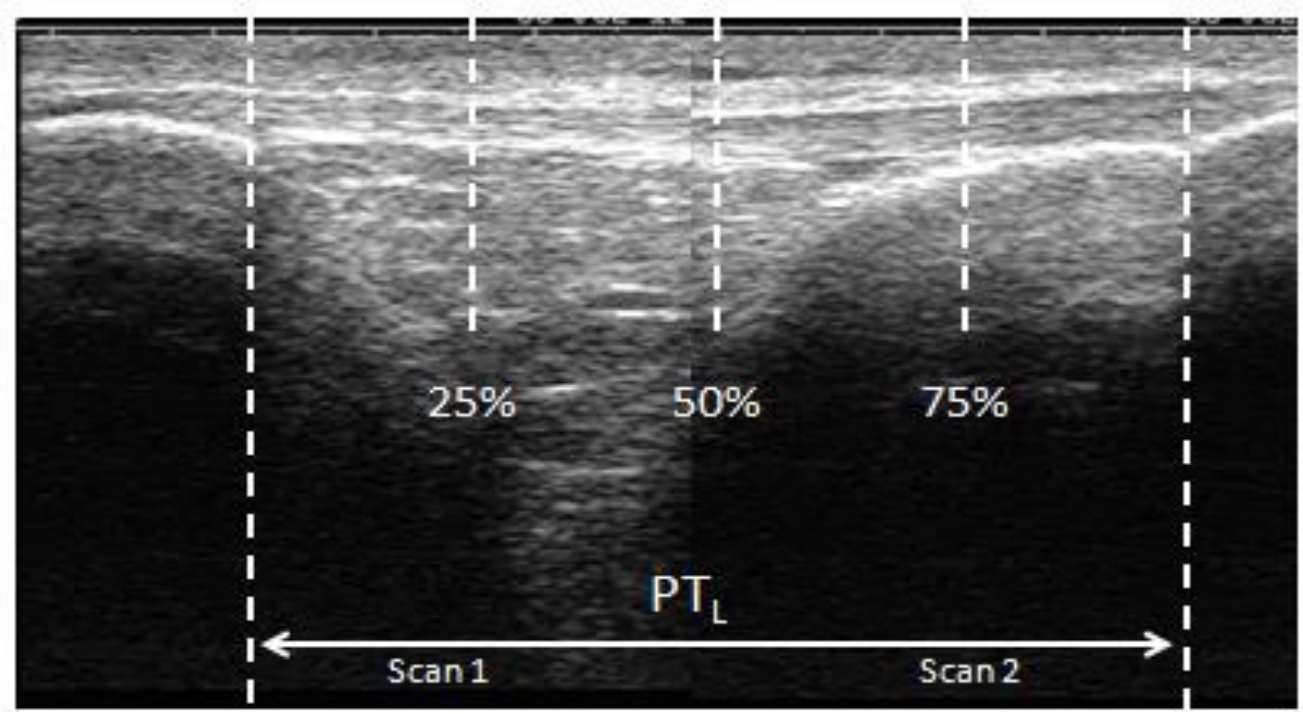


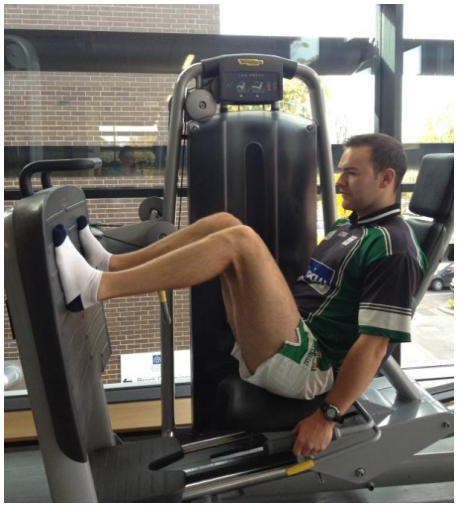
Figure 2.6: *Shows a sagittal plane ultrasound image of a study participant (composed of 2 scans) of the patella tendon denoting measurement locations of tendon cross-sectional areas (knee at 90^o flexion).*

range-of-motion joint angle, before returning to the desired starting joint angle. Joint angles were confirmed by a training partner from the goniometer scale (custom-made) and rhythmically controlled using a metronome (Wittner, Germany). Dynamic exercises included the barbell back squat, Bulgarian split squat, leg press, leg extension and dumbbell lunges (Figures 2.6A-E respectively). In addition, static loading exercise was also included (Sampson chair: Figure 2.6.F). The participants completed two familiarisation sessions at 70% of 1RM prior to commencing the resistance training programme a week later. During the training programme, exercises were performed at 80% of 1RM in SL and LX groups, whereas LL performed exercise at 55% of 1RM (see *muscle force modelling*). 1RMs were determined as the maximal amount of weight that the participant could lift covering the excursion of their designated training group

(e.g. an SL participant performed a 1RM between 0-50^{o}; LL between 40-90^{o}), these were measured every two weeks and weight adjusted accordingly. Training volume (in terms of repetitions and sets) was identical for each training group, with each training session consisting of four exercises and performing three sets of ten repetitions per exercise for the first four weeks, and four sets of eight per exercise thereafter.

A

B



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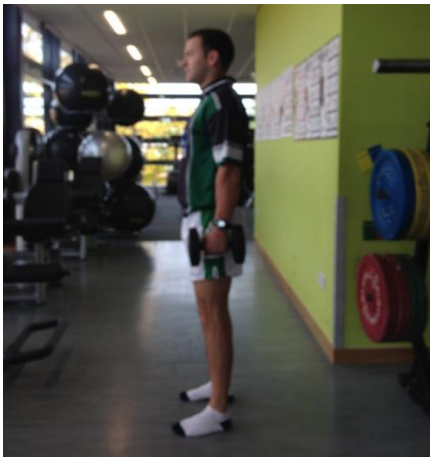
D



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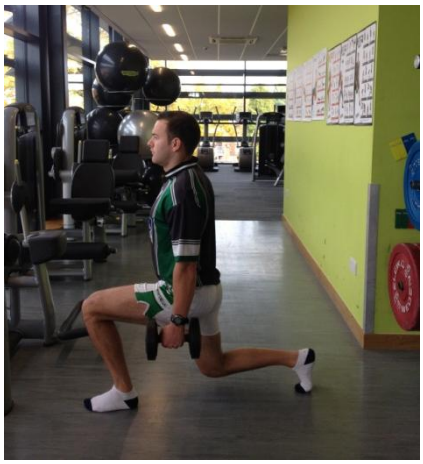

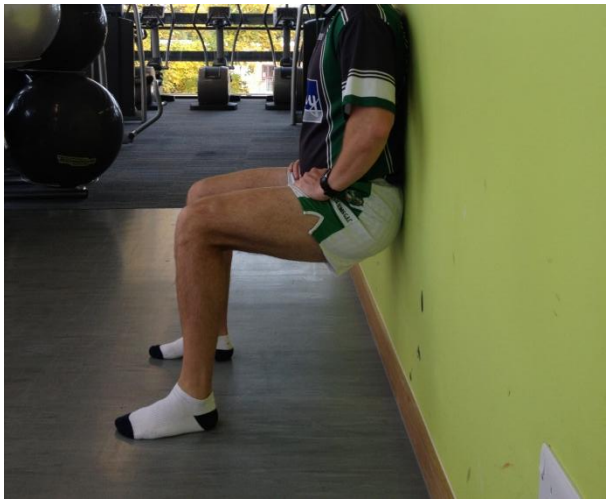

F

Figure 2.6A-F. *Shows the exercises regimes used during the resistance training program***.**

2.2.12 Muscle Force Modelling

Due to the changing moment arm length of the patella tendon at discrete knee joint angles, differences in muscle force produced between the groups had to be taken into account. Thus, quadriceps forces at the patella tendon were calculated as shown in Equations 2 & 3:

$$Quad_{Force} = (Quad_{MaxTorque} + Ham_{CoTorque}) / \text{Moment Arm}_{PT}$$

Equation [2]

where

$$Ham_{CoTorque} = (\text{Co-Con}_{EMG} \times Flex_{MaxTorque}) / (\text{Max } BF_{EMG})$$

Equation [3]

Where Co-Con$_{EMG}$ is the EMG recorded from the *biceps femoris* acting as an antagonist muscle i.e. co-contracting, and Max BF$_{EMG}$ is the maximum EMG of the *biceps femoris* when in agonist mode. Flex$_{Max}$ is maximum flexion torque and Moment Arm$_{PT}$ being the moment arm of the patellar tendon (see section 2.2.9). Based on tendon forces estimated from the 1RM data at the end range-of-motion from the SL (50^{o}) and LX (90^{o}) training groups where a short isometric hold would take place, tendon forces produced at 90^{o} were on average ~32% greater than those produced at 50^{o}.

Taking the above biomechanical facts into account, the objective was to then standardise the tendon forces between the SL and LL groups. Thus, in order to quantify the training load to apply in LL group, the mass of the external resistance (in Nm) is added to the left hand side of Equation 2, therefore through mathematical iteration the training load can be identified. Hence, it was calculated that whilst SL and LX would exercise at a high-intensity external load of 80% 1RM (for a more detailed description see resistance training programme section), LL would train at an external load of lower intensity at 55% 1RM to ensure the absolute load seen by the tendon in the SL/LL groups is equated.

In order to accurately assess internal muscle forces produced, the change in resistance moment arms of the CAM pulley machine used during leg extensions were also measured. Based on the training load for the leg extension exercise, the resistance machine load component yielded on average a 7% increase in external torque produced in the SL group compared to LX (SL; 137Nm vs. LX; 128Nm).

2.2.13 Vascular Ultrasound measures

Following five minutes of prone rest in a quiet, half-darkened room to allow for regulation of vascular tone (Venturini et al., 1993); resting measurements of blood flow velocity and diameter were obtained using an echo Doppler ultrasound machine (Technos, ESAOTE, Genova, Italy) with a 5.0- to 13.0- MHz broadband linear array transducer. To ensure probe placement was identical on subsequent sessions, the position of the probe in relation to anatomical landmarks was traced onto a sheet of acetate. Measurement of the left SFA was obtained at 75% of femur length on the posterior thigh with the subject in a prone position. As this was a within group study, limb dominance was ignored and all tests were performed on the left-hand limb which is consistent with previous literature.(Morse et al., 2007b, Morse et al., 2005) It is assumed that the studied limb reflects the adaptations of both. Measurement of the left CA was obtained with the subject in a seated position, immediately following the SFA assessment. A perpendicular measurement from the media/adventitia interface of the near arterial wall to the lumen/intima interface of the far arterial wall was taken to determine arterial diameter. The average of three measurements per frame were taken for artery diameter at the end-diastolic phase for both the SFA and CA. Blood flow velocity measurements were conducted using ~60° angle of insonation, (Naylor et al., 2005, Sugawara et al., 2004) and the average of ~15 Doppler waveforms was used to calculate mean blood flow velocity. These measurements allowed for calculation of mean blood flow (Bleeker et al., 2005a) as:

$$\textit{Mean blood flow } (ml \cdot min^{-1}) = \tfrac{1}{4} \cdot \pi \cdot (\textit{mean diameter (cm)})^2 \cdot \textit{mean velocity (cm/s)} \cdot 60$$

Mean shear rate was used as an estimate of the more invasive measure of shear stress and was calculated as:(Parker et al., 2009, Rakobowchuk et al., 2005)

Mean shear rate = 4·mean velocity/mean diameter

Resting heart rate and resistance index were taken as the average from ~ 15 Doppler waveforms, resistance index was calculated as:

Resistance index = (peak systolic velocity – end-diastolic velocity)/ peak systolic velocity

Inter- and intra-day coefficients of variation (CV) were calculated from pilot data using four subjects. For femoral artery diameter, blood flow velocity and resistance index, inter-day CV were 0.25%, 1.35% and 3.38% respectively and intra-day CV were 0.68%, 3.53% and 5.04% respectively. These values are comparable with previous research and suggests high inter- and intra-day reliability.(Dinenno et al., 2001, Bleeker et al., 2005a).

2.2.14 Exercise Protocols (acute study)

Each participant was required to perform exercise over one of three ranges-of-motion as shown in Figure 2.7, hence completing all ranges-of-motion protocols over three separate sessions. The three ranges-of-motion were; 0-50^{o} knee flexion (shorter muscle lengths, SL), 40-90^{o} knee flexion (longer muscle lengths, LL) and 0-90^{o} knee flexion (complete range-of-motion incorporating shorter and longer muscle lengths, LX).

A goniometer was attached to the knee joint centre of rotation, from which the investigator confirmed each angle was met during exercise performance. Each exercise session required participants to perform one set of five repetitions back squats at an absolute load of 20kg, 40kg and finally 60kg. Sets were interspersed by two minutes of recovery. Following a further ten minutes rest, each participant performed a further set of five back squats at 40%, 60% and 80% 1RM, interspersed by two minutes of rest. Participants would then return 2-3 days later to

complete the same trials but over another range-of-motion, so that all three sets would be completed within 7 days.

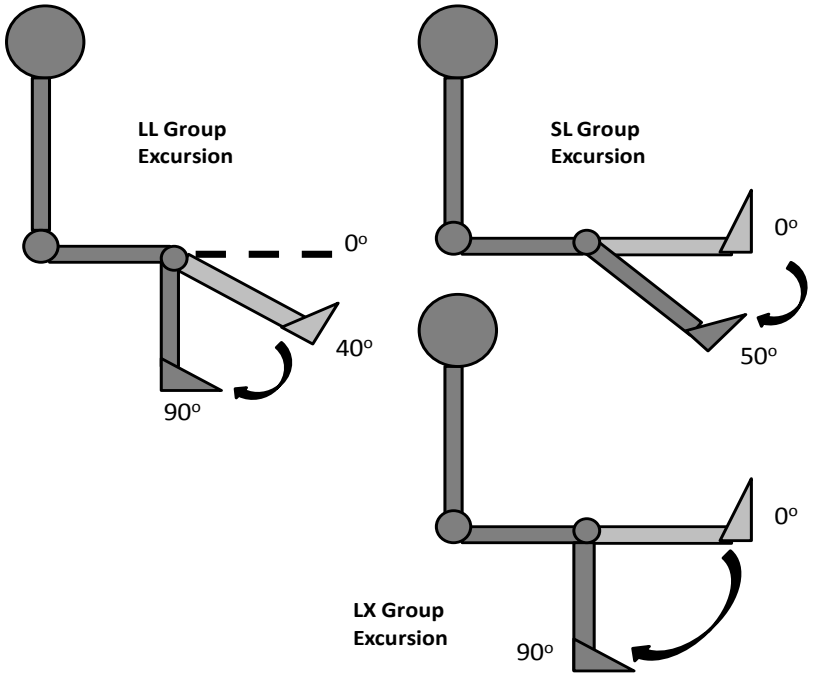

Figure 2.7. *Ranges-of-motion used in each of the training groups during the both acute exercise study and the 8-week training program.*

2.2.14 Oxygen Consumption (VO_2)

VO_2 consumption was collected using standard Douglas Bag techniques. Prior to the beginning of each set of exercise, a nose clip was placed on the nose of the participant, and the Douglas bag mouthpiece was inserted into the mouth and the valve on an empty bag subsequently opened. After the set of exercise was complete, 30 seconds were allowed to elapse before the valves were closed at the end of an expiration phase. This was to allow for any excessive post-exercise oxygen consumption during the immediate recovery period. A separate Douglas bag was used for every set of exercise completed. Each bag was then analysed using a gas analysis program (Servomex 5200 Multiuse, Crowborough, UK) and percentages of $FECO_2$ and FEO_2 were

calculated. For these calculations, the data from Gas which had been evacuated for 60 s with a flow rate of 2.1 L/min, the total gas volume which was obtained using a Harvard Dry Gas vacuum (NB. the flow rate (2.1 L) was added to the final figure to give the VE atps (L/min^{-1})). The time period in which the Douglas bag was open (secs), load (kg) and subjects' heart rate were also inserted into the gas analysis programme, alongside the VO_2 ($ml/kg^{-1}/min^{-1}$) also being recorded.

2.2.15 Heart rate & Blood Pressure

Heart rate and blood pressure were recorded at rest in the supine position before the onset of exercise using a standard heart rate monitor (Polar, UK) and electronic blood pressure monitoring device (Panasonic Diagnostec, UK). These parameters were also measured immediately post-exercise, after every set of exercise. Rate of perceived exertion (RPE) was also recorded following the conclusion of each individual set of exercise.

2.2.16 Biochemical analyses

At each of the designated testing intervals (i.e. baseline, weeks 8, 10 and 12) and following an overnight fasting period (~10 hours for all participants), participants reported to the laboratory. It should be noted here that at week 8, sampling took place 3-4 days following the last resistance training session, as to avoid the inclusion of the acute effects of a resistance training bout on IGF-I levels (Chandler, 1994). A 21-gauge 1-inch ultra-thin wall needle (Terumo Medical Corporation, New Jersey, USA) was inserted into the antecubital vein of the forearm. Using a vacutainer assembly and serum separator tubes (Monovette, Sarstedt, Numbrecht, Germany),

5mL blood samples were collected. After being kept on an ice bed for up to 2-hours, the sample was then centrifuged at 4°C for 10 min at 4,800 rpm, with the supernatant being removed and stored in eppendorfs at −20°C for later analysis.

IGF-I;

The IGF-I (R&D Systems Europe, UK. Sensitivity of 0.026 ng/mL; intra-assay variability of 4.0% CV, manufacturers' data, 4.8% CV current study) was analysed using standard enzyme-linked immuno-sorbent assay procedures. Initially, the samples were pre-treated to release IGF-I from binding proteins. This included addition of 20 µL serum to 380 µL of a pre-treatment solution A, which was vortexed and incubated for 10mins at room temperature. 50 µL of this sample was added to 200 µL of pre-treatment B, was mixed, and then assayed immediately.

50 µL of sample was added to 150 µL of the assay diluent in each well and was covered and incubated at 2-8^{o}C for 2 hours. Following this, each plate was aspirated and washed with wash buffer for four washes, before being blotted against paper towels. 200 µL of cold IGF-I conjugate was added to each well, covered, and then incubated at for one hour. The wash/ aspiration process was repeated once more; following which 200 µL of substrate solution was added to each well, before being incubation at room temperature and covered from light for 30mins. After the incubation period 50 µL of stop solution was added to each well and analysed by a microplate reader (EL808, Biotek, UK) set t 450nm, and corrected at 540nm using Gen 5 v.1.06.10.

TGF-β1

TGF-β1 (R&D Systems Europe, UK. Sensitivity 4.61pg/mL; Serum/ plasma intra-assay variability of 2.5% CV, manufacturer's data, 3.3% CV current study CV) was first activated

before the assay procedure with the reagents HCl and NaOH/ HEPES. To activate the cells, 40µL of serum was added to 20 µL HCl, mixed, and incubated at room temperature for 10mins. The samples were then neutralised by adding 20µL of NaOH/ HEPES, and mixed. 50µL of activated samples were added to 50µL of assay diluents, and tapped gently to mix. The wells were then covered and incubated at room temperature for 2 hours. Following the allotted time, each plate of wells was aspirated and washed with wash buffer for a total of 4 washes, with the plates blotted against paper towels. 100µL of TGF-β1 conjugate was added to each well and incubated for a further 2 hours at room temperature. The aspiration/ wash procedure was carried out once more. Following this, 100µL of substrate solution was added to each well, and was incubated for a further 30mins at room temperature protected from light. 100µL of stop solution was then added and mixed thoroughly. Optical density of the wells were analysed using a microplate reader () set to 450nm.

TNF-α

TNF-α (R&D Systems Europe, UK. Sensitivity 1.6pg/mL; Serum/ plasma intra-assay variability of 4.6% CV, manufacturer's data, 8.1% CV current study) levels were analysed by first adding 50µL of assay diluent to the wells, which were then mixed well and to which 200µL of sample was also then added. The wells were left to incubate at room temperature for 2 hours, and were followed by the same aspiration/ wash process as described above. 200µL of TNF-α conjugate was added to each well and then left to incubate for a further 2hrs at room temperature. Following the incubation period, the aspiration and wash process was repeated again. 200µL of substrate solution was then added to each well, and was again left to incubate for 20mins at room temperature, protected from light. At the conclusion of this incubation period 20µL of stop

solution was added to each well, with optical density being recorded within 30mins using a microplate reader set to 450nm.

2.2.17 Statistics

Data were analysed with IBM SPSS version 19.0.0. TheShapiro-Wilk and Levene tests revealed the data sets to be parametric, and they were therefore analysed using a mixed design repeated measures 4 × 4 ANOVA was used. The within factor was the phase of training (i.e. week 0, 8, 10 and 12) and the between factor was training group (i.e. SL, LL, LX and Con). Post-Hoc contrast analyses with bonferroni corrections were used to compare data to baseline (within factor) and to control group (between factor). All data are presented as mean (±S.E.M.). Statistical significance was set with alpha at ≤ 0.05. In terms of the sample size in the present study, the average statistical power of the measured muscle and tendon parameters was statistically adequate at beta = 0.89.

2.2.18 Participants

Forty two volunteers were recruited from the local university campus, and gave written informed consent to participate in the study. All procedures and experimental protocols were approved by the local Ethics Committee. Inclusion criteria were participants are to be young (18-39 years old) and healthy (assessed by completion of a pre-exercise questionnaire). Exclusion criteria included the presence of any known musculoskeletal, neurological, inflammatory or metabolic disorders or injury and taking any nutritional or any other form of ergogenic aid. Participants took part in recreational activities such as team sports, and had either never taken part in lower limb

resistance training or not within the previous 12 months. All participants habitually took part in up to 3-5 hours of non-resistance based activity per week, as assessed by a training diary/ questionnaire. Team sports included rugby union and league, soccer, hockey and netball. Training group allocation was done in a 'part-concealed randomisation' fashion in that the author was aware of the identity of the participants but the information on who was paired in the groups was not revealed to the participants. Participants were pair-matched in terms of gender, habitual physical activity levels (or sport) and physical characteristics. One of each pair was then randomly assigned to either SL, LL or LX training groups following a blind folded selection. Thirty two participants were allocated to a training group – SL (shorter muscle length; 6 males, 4 females; aged 19±2.2 years, 1.76±0.15m, 75.7±13.2Kg), LL (longer muscle length; 5 males, 6 females; 21±3.4 years, 1.75±0.14m, 74.9±14.7Kg), or LX (complete ROM; 6 males, 5 females; 19.2±2.6 years, 1.71±0.11m, 73.8±14.9Kg). Ten participants (6 males and 4 females; 23±2.4 years, 1.76±0.09m, 77.9±13.1Kg) were assigned to the non-training control group (Con), and continued their normal habitual activity throughout the study period. A One-way ANOVA comparing across all groups revealed that the population was homogeneous at baseline for all parameters of interest ($p>0.05$).

2.2.19 Main Outcome Measures

Table 2.1. *Main outcome measures of studies, method used and chapter the data appears in thesis.* $L_{F;}$ *fascicle length,* θ_P *; pennation angle, K; patella tendon stiffness, E; Young's Modulus, Tq-A; Torque-Angle relation, US; ultrasonography, Cal; calculation, Dyn; dynamometry, EMG; electromyography, Mech; mechanical, Mat; Material.*

	Muscle				**Tendon**		**MTC**	**Neuro-muscular**	**Endocrine**
	Morphology		*Architecture*		*Mech*	*Mat*			
Measure	Volume	aCSA	L_F	θ_P	K	E	Tq-A	Activation	Serum Concentration
Method	US + Cal	US	US	US	US+ Cal	US+ Cal	Dyn	EMG	Immuno-sorbent assay
Chapter	3	3	3	3	4	4	4	5	6

Table 2.2. *Secondary outcome measures of studies, method used and chapter the data appears in thesis.*

	Cardiovascular		
Measure	Heart Rate	Blood Pressure	Oxygen Consumption
Method	Heart rate monitor	Sphygmometer	Douglas Bag
Chapter	5	5	5

Pilot Study Data (Validity & Reliability):

A pilot study was conducted on a representative subsample of the study population (see table 2.3). Measurements taken included the main muscle and tendon outcome measures, or any measures to be used in the calculation of these. Repeated measures were performed on this group of 5 individuals (2 males, 3 females), with data collected on three separate occasions. The within and between day repeatability of the main measures are shown in Tables 2.4 and 2.5.

Table 2.3 *Pilot Study Participants' Physical Characteristics*

Participant No.	Age (years)	Height (m)	Mass (Kg)	Male/ Female
1	23	1.78	75.5	M
2	24	1.88	86.2	M
3	22	1.69	68.8	F
4	22	1.58	53.6	F
5	28	1.63	64.9	F
Mean (±S.D.)	24 ± 2	1.71 ± 0.11	69.8 ± 12.1	

Table 2.4. *Within and Between Day Muscle Measurements Repeatability*

	aCSA	Volume	M_L	F_L	L_F	θ_P	Fat	Strength
	Co-efficient of Variation % (range)							
Within	1.5 (0.5-4.1)	2.9 (1.7-5.6)	0.7 (0.5-1.2)	1.4 (1.1-3.1)	1.9 (1.4-3.2)	3.3 (2.4-7.7)	2.6 (1.8-5.0)	0.8 (0.4-1.6)
Between	2.6 (1.4-6.1)	3.3 (2.3-6.0)	1.1 (0.7-2.3)	2.5 (0.8-4.0)	2.1 (1.2-4.4)	3.6 (2.7-6.8)	2.9 (1.8-5.4)	1.8 (0.8-4.0)

Table 2.5. *Within and Between Day Tendon Measurements Repeatability*

	PT_L	PT_{CSA}	K	E
	Co-efficient of Variation % (range)			
Within	6.5 (5.0-8.8)	2.3 (1.6-4.2)	3.7 (2.3-5.6)	5.4 (3.7-8.0)
Between	7.0 (5.5-9.1)	3.6 (2.3-5.4)	5.5 (4.0-8.2)	6.8 (4.3-8.4)

With the mean CV% <5-10% in all of the muscle-tendon measures, the repeatability of the measures were within an acceptable range of error (Atkinson and Nevill, 1998).

CHAPTER 3:

Effects of Resistance Training & Detraining on Muscle Size, architecture & function

3.1 Introduction:

When considering the design of a resistance training program, it should incorporate a selection of exercises that will ideally reflect both functional tasks of a chosen movement, and also the ability of the exercise to bring about a desired set of adaptations to enhance function and ideally therefore, physical performance. As muscle force is proportional to muscle cross-sectional area (Maughan et al., 1983), is associated with power (Wickiewicz et al., 1984), and is a key determinant to success in many sports, muscular hypertrophy is often a key outcome following resistance training. The degree of hypertrophy arises from manipulation of the training stimulus; exercise selection/ order, mode of contraction, intensity, recovery and volume (Ratamess et al., 2009). Therefore, the key to optimising adaptations in function through resistance training, is to fully understand the mechanical stimuli that induce alterations to the muscle's characteristics/ properties.

As muscle length changes during force production to bring about movement, the moment arm of the series elastic component (i.e. the tendon) also changes. Therefore the internal tension a muscle experiences at different joint angles will change despite no alterations in external absolute load. Simultaneous to the change in moment arm, is the effect of changing muscle length on actin-myosin interactions and thus cross-bridge states (Huxley and Simmons, 1971). A changing muscle length will vary both of these cellular factors, thus impacting on the force-length relationship in the muscle (Rassier et al., 1999). The magnitude of mechanical stress is known to induce muscle hypertrophy(McDonagh and Davies, 1984), therefore increased mechanical stress at one joint angle compared to another could act as a signal for additional sarcomerogenesis at that muscle length based on the differential stress imposed through the moment arm changes.

In relation to muscle architecture, there are reports of increases in fascicle length following resistance training (Reeves et al., 2009, Seynnes et al., 2007, Alegre et al., 2006), which impacts the force-velocity and force-length relationships of muscle, and also muscle excursion. An increase in fascicle length is thought to be brought about by in-series sarcomerogenesis. Muscle length (or passive tension/ stretch) and muscle excursion have been shown to be major regulators of serial sarcomerogenesis in animal models (Williams and Goldspink, 1973, Koh and Herzog, 1998, Tabary et al., 1972, Goldspink et al., 1974) and appears to be relatively independent of both muscle activation level and tension (Gajdosik, 2001). For example, in the soleus muscle of guinea pigs, when muscle length and tension had been independently varied, results indicated that muscle length, rather than tension, appeared to be the determining factor in sarcomere number regulation (de la Tour et al., 1979). Furthermore, research has demonstrated that the observed increase in functional length is associated with increased protein synthesis (Loughna et al., 1986). However in humans, conflicting evidence from studies on the major mechanical stimuli for such adaptation exists. In young adults, muscle contraction type (eccentric vs. concentric) was investigated as a possible primary candidate for fascicle length change (Blazevich et al., 2007). The authors concluded that other factors (possibly excursion of muscle during resistance training) were the main mechanical stimuli for changes in fascicle length, rather than contraction type. In contrast, in older individuals, Reeves et al. (2009) found that eccentric contractions (through enhanced training stimulus and associated greater muscle-tendon stress and strain), were the driving force behind greater increases in fascicle length, compared to conventional weight training (i.e. a combination of relatively low concentric loads and even lower eccentric torques than those encountered in the eccentric-only program). Therefore, the primary constituent in resistance training for regulating fascicle length in humans remains ambiguous. Alterations to the fascicle angle of pennation impacts on the physiological cross-

sectional area (pCSA) and therefore force-generating capacity (Aagaard et al., 2001) of muscle. Regarding fascicle angle, there appears to be a strong relationship between increases in muscle size and increases in pennation angle (Blazevich et al., 2007, Kawakami et al., 1993, Kawakami et al., 1995). Although an increase in pennation angle is expected to allow an increase muscle force (up to an upper limit of 45^{o}), at greater pennation angles the effective contractile force exerted on the aponeurosis is reduced to a greater extent, off-setting the increase in force production from the increased number of acto-myosin crossbridges activated in parallel (Narici et al., 1992, Rutherford and Jones, 1992). Hence, it is important to monitor both fascicle angle and functional changes in strength in the muscle of interest.

This identifies 'average muscle length-specific training' as a potential modulator of the training-induced hypertrophic/ architectural response. Only one previous study to our knowledge, Kubo et al.(Kubo et al., 2006a), has investigated training at different joint angles on muscle size and function *in vivo*, but their results do not reflect what the theory would have predicted. Nine males completed a 12-week unilateral isometric training program (70% MVC × 15secs × 6 sets) on the knee extensors at either a short (50^{o} of knee flexion) or a long (100^{o}) muscle length. The authors found that whole quadriceps volume increased significantly in both short (+10 ± 1%) and long (+11 ± 2%) muscle lengths, although there was no significant difference between the groups. However, it should be noted that the isometric only protocol adopted by Kubo et al.(25) may not reflect the practices of individuals training to optimize gains in strength and hypertrophy in addition to the limited transfer of the functional aspect of an isometric exercise.

At the other end of the loading spectrum, it is also important to describe how the muscle responds to a reduction in loading. Detraining is the partial or complete loss of training-induced

adaptations, in response to an insufficient loading stimulus (Mujika and Padilla, 2000). Significant decrements in strength, EMG and mean fiber CSA have been reported in as little as two weeks of detraining(Hortobagyi et al., 1993), with similar observations in chronic detraining periods (≥4wks) alluding to either losses in mass, strength or neural activation, or combinations of these factors (Gondin et al., 2006, Kubo et al., 2010, Narici et al., 1989). Also, most studies have tended to report changes following detraining after similar time courses to the preceding resistance training i.e. between 3-6 months. If there appears to a be a greater hypertrophic response at one muscle length over another, it would also be of interest to determine whether there is a differential modulation of detraining-induced mal-adaptations following greater initial gains from resistance training at different muscle lengths. The *in vivo* changes to muscle architecture during a relatively shorter period of time (≤4 weeks – such as that found in short-term injury, illness or tapering), have not yet previously been described. Significant increases in fascicle length have been reported in as little as 10 days from the onset of resistance training (Seynnes et al., 2007), so it would be of interest to monitor the rate of mal-adaptation in a relatively small time-frame of detraining. Counter-intuitively, Blazevich et al.(2007) documented an increase in VL fascicle length during 3 months of detraining following 10 weeks of resistance training. Therefore following resistance training, the impact of detraining from the subsequent training appears to follow an unpredictable/unchartered pattern. Thus, if these greater gains are still evident following detraining, it would further highlight the value of using more optimal training mechanics within a resistance training program.

3.2 Methods:

Thirty two activity-matched participants were allocated to a training group – SL (shorter muscle length; 6 males, 4 females; aged 19±2.2 years, 1.76±0.15m, 75.7±13.2Kg), LL (longer muscle

length; 5 males, 6 females; 21±3.4 years, 1.75±0.14m, 74.9±14.7Kg), or LX (complete ROM; 6 males, 5 females;19.2±2.6 years, 1.71±0.11m, 73.8±14.9Kg). Ten participants (6 males and 4 females; 23±2.4 years, 1.76±0.09m, 77.9±13.1Kg) were assigned to the non-training control group (Con), and continued their normal habitual activity throughout the study period.

B-mode ultrasonography was used to assess muscle aCSA, muscle architecture and subcutaneous fat of the *vastus lateralis* muscle at 25%, 50% and 75% of femur length at baseline, week 8 (post-training), week 10 (detraining 1) and week 12 (detraining 2). From these measurements muscle volume and pCSA was also calculated. Other general muscle morphological measurements were also made (for details see chapter 2).

3.3 Results:

Sub-group population characteristics versus study population characteristics

Independent *t*-tests revealed that there were no significant differences between the sub-group population (used for quantification of muscle-tendon complex stretch) and the main study population for age, height, mass, VL muscle length and femur length. Therefore the sub-group was representative of the study population.

Quantification of muscle-tendon complex stretch

In order to measure the extent of lengthening (or passive stretch) at each training joint-angle compared to full extension, the VL muscle length was measured as the distance between the two myotendinous junctions of the muscle, with the results shown in Table 3.1. At 90^{o} knee flexion,

the VL was significantly ($p<0.05$) lengthened compared to full extension, but not at either 40^o or 50^o.

Table 3.1. Resting *Vastus Lateralis* length at various knee-joint angles. NB. In this population, the average femur length was 44.6±1.0 cm (*n*=6). * Significantly different to full extension (p<0.05)

Knee Joint angle (^{o} knee flexion)	**VL Muscle Length (cm)**	**Muscle Length to Femur Length Ratio**
0^o (Full extension)	32.8±1.1	0.74 : 1
40^o	34.7±0.9	0.78 : 1
50^o	35.3±0.9	0.80 : 1
90^o	37.0±1.1*	0.83 : 1

Muscle Volume; Muscle volume increased following the resistance training protocol at week 8 in all training groups ($p<0.01$, Figure 3.1). The mean relative increase in VL volume in SL was 38±8% (576±89cm^3 to 768±109cm^3) at week 8, compared to LL group's 67±10% (482±62cm^3 to 805±111cm^3) and LX 70±15% (516±97cm^3 to 783±103cm^3). At this juncture, there was a significant difference between SL and LL groups ($p<0.05$) relative to baseline. However despite even larger increases than LL, LX group's muscle volume changes did not reach statistical significance compared to SL at week 8 ($p<0.05$), with no difference between LL and LX ($p=0.952$). Significant reductions in muscle volume occurred following two weeks and four weeks post-training ($p<0.05$) in all training groups, with no difference between groups in rate of loss. There was no main effect of group at week 10 between SL and LL ($p=0.063$), however all

groups remained significantly elevated compared to baseline following the completion of detraining ($p<0.05$).

Muscle aCSA; Absolute changes to aCSA are shown in Table 3.2 and relative changes in Figure 3.2. VL aCSA increased significantly ($p<0.01$) relative to baseline following training at all sites in each training group, which was still evident at the conclusion of the detraining period in each training groups at proximal, central and distal sites (pooled data; $p<0.01$) . There was also a trend for both LX and LL to exhibit greater relative gains in aCSA compared to SL at all sites at week 8 ($p<0.06$), but only significantly so distally. Here there was a main group effect ($p<0.05$) with LX and LL exhibiting a 63±15% and 53±12% increase respectively compared to SL (18±8% increment) in VL aCSA. The superior adaptations were retained distally at both week 10 (LX; 51±12%, LL; 45±13% vs. SL; 11±10%, $p<0.05$) and week 12 (LX; 33±10%, LL; 32±9% vs. SL; 2±7%, $p<0.05$). There was no notable VL aCSA change over the 12 week period for the controls (0±2%, 4±6%, 3±4% proximal, central and distal respectively; $p>0.05$).

Following the larger than anticipated relative changes in aCSA, a sub-group sample of 3 participants from SL, LL and LX groups were re-analysed (total *n*=9) for VL aCSA at baseline and post-training (Table 3.2) . Following a personal communication with Dr. Neil Reeves, author of the validation of the analysis method (Reeves et al. 2004), the individual images used to reconstruct the VL muscle were done so using the leading edge of the subsequent image as well as the shadow reference point for contour matching. Following analysis, there was no significant difference ($p>0.05$) between the original and re-analysis aCSAs at baseline, post-training or relative changes from baseline to post-training. Therefore the results of the aCSA measurements and analysis have been further validated.

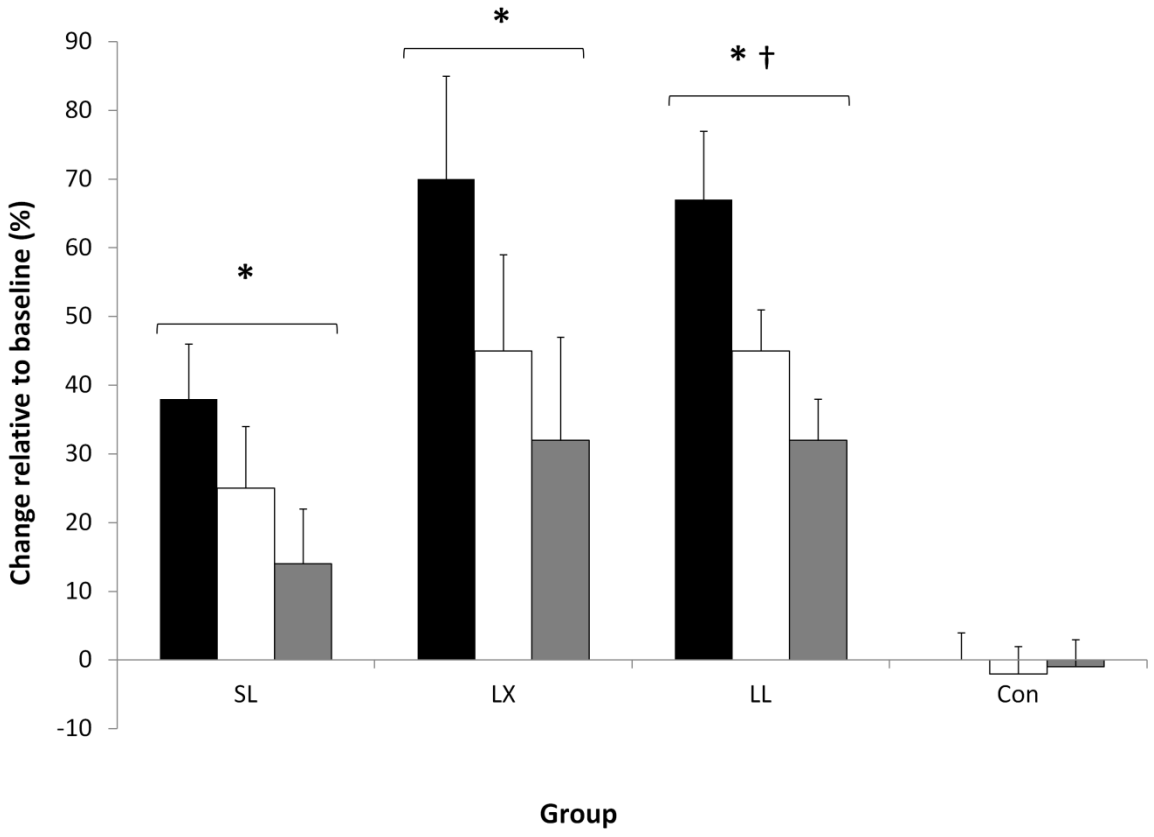

Figure 3.1. Changes to muscle volume at week 8 (black bars), week 10 (white bars) and week 12 (grey bars) in all groups. * Significant change from baseline (p<0.01). † Significant difference between SL and LL training groups at week 8 (p<0.05).

Muscle pCSA; At week 8, pCSA had increased significantly ($p<0.01$) as a result of the training regime compared to baseline in SL (56±8 cm^2 to 66±9 cm^2; Δ17%), in LL (52±9 cm^2 to 65±7 cm^2; Δ25%), and in LX (54±6 cm^2 to 71±8 cm^2; Δ31%) with no differences between groups ($p>0.05$). During detraining, pCSA remained significantly above baseline values at both week 10 and week 12 ($p<0.05$) in all training groups. The control group pCSA did not change significantly during the 12 week period ($p>0.05$).

Muscle architecture;

Fascicle Length: There were significant relative increases in VL fascicle lengths as a result of the training protocol in both groups post training and detraining compared to baseline, at all three measurement sites (see Figure 3.5, $p<0.001$). Post-training, fascicles increased in length to a greater extent at all sites in LL and at central and distal sites in LL compared to SL (LL; Δ 27±3mm, 21±3mm, 24±3mm, LX; Δ 26±4mm, 25±5mm, 20±5mm vs. Δ18±4mm; Δ9±6mm; 12±5mm; $p<0.05$). This significant main effect of group was retained through the entire detraining period at all sites in LL ($p<0.01$) and at the aforementioned sites in LX ($p<0.05$). The control group did not display any significant changes in fascicle length during the training and detraining periods (averaged over 3 sites Δ 3±4mm; $p>0.05$).

Fascicle pennation Angle: All training groups experienced significant increases in fascicle pennation angle post-training at proximal (SL; 9.9±0.4^o to 10.7±0.4^o Δ9±4%, LX; 9.9±0.4^o to 10.7±0.6^o Δ9±5%, and LL; 9.0±0.4^o to 10±0.3^o Δ14±7%, $p=0.034$), central (SL; 16.2±0.5^o to 17.2±0.4^{o}Δ7±2%, LX; 15.6±0.7^o to 16.6±0.8^o Δ8±4%, and LL; 15.3±0.4^o to 16.2±0.5^o Δ6±3%, $p=0.033$) and distal (SL; 16.5±1.2^o to 18.1±1.0^o Δ11±4%, LX; 16.2±0.7^o to 17.8±0.8^o Δ11±4%, and LL; 18.1±0.9^o to 19.2±0.8^o Δ7±3%, $p<0.05$) sites compared to baseline. Fascicle pennation angle remained elevated compared to baseline ($p<0.05$) at week 10 at the distal site only, but not at week 12 in any training groups at any measurement site. There was no difference ($p>0.05$) between SL, LX or LL groups in fascicle angle at any stage. The control group displayed no changes in fascicle angle over the 12 week period (averaged over 3 sites - 15.9±0.5^o to 15.7±0.5^o Δ 1±1%, $p>0.05$).

Table 3.2. Changes to aCSA following training and detraining in all groups. * Significant change from baseline (p<0.05). † Significant difference between training groups (p<0.05). Values are in mm^2

Site	*Baseline*	*Week 8*	*Week 10*	*Week 12*
Proximal	SL 2,989±280	SL 3,511±260*	SL 3,412±307*	SL 3,290±248*
(25% Femur L)	LX 2,916±388	LX 3,614±256*	LX 3,462±310*	LX 3,329±296*
	LL 2,790±151	LL 3,642±265*	LL 3,251±243*	LL 3,269±218*
	Con 3,201±253	Con 3,086±259	Con 3,079±240	Con 3,086±259
Central	SL 3,288±284	SL 3,999±370*	SL 3,737±334*	SL 3,559±267*
(50% Femur L)	LX 3,080±325	LX 3,875±359*	LX 3,424±349*	LX 3,233±338*
	LL 2,966±211	LL 4,029±295*	LL 3724±284*	LL 3,625±283*
	Con 3,326±354	Con 3,314±364	Con 3,294±357	Con 3,314±364
Distal	SL 1,190±157	SL 1,350±161*	SL 1,263±128*	SL 1,158±127*
(75% Femur L)	LX 1,100±197	LX 1,744±226* †	LX 1,369±200*	LX 1,219±191*
	LL 1,110±122	LL 1673±202* †	LL 1,543±164*†	LL 1,458±222*†
	Con 1,366±165	Con 1,370±185	Con 1,358±175	Con 1,370±185

Table 3.3 Re-analysis of aCSAs in each training group

Group	**Sex**	**Baseline**		**Post-Training**	
		Measure 1	**Measure 2**	**Measure 1**	**Measure 2**
SL	M	3318	3121	4487	4288
SL	M	3360	3140	3873	3588
SL	F	2320	2389	2825	2850
LL	M	3994	3780	4874	4588
LL	M	2574	2648	3510	3489
LL	F	2204	2090	2479	2286
LX	M	2887	2922	3893	3768
LX	M	3843	3616	4256	3951
LX	F	2295	2097	2532	2571
Mean ± SEM		2997±227	2867±205	3637±289	3487±269

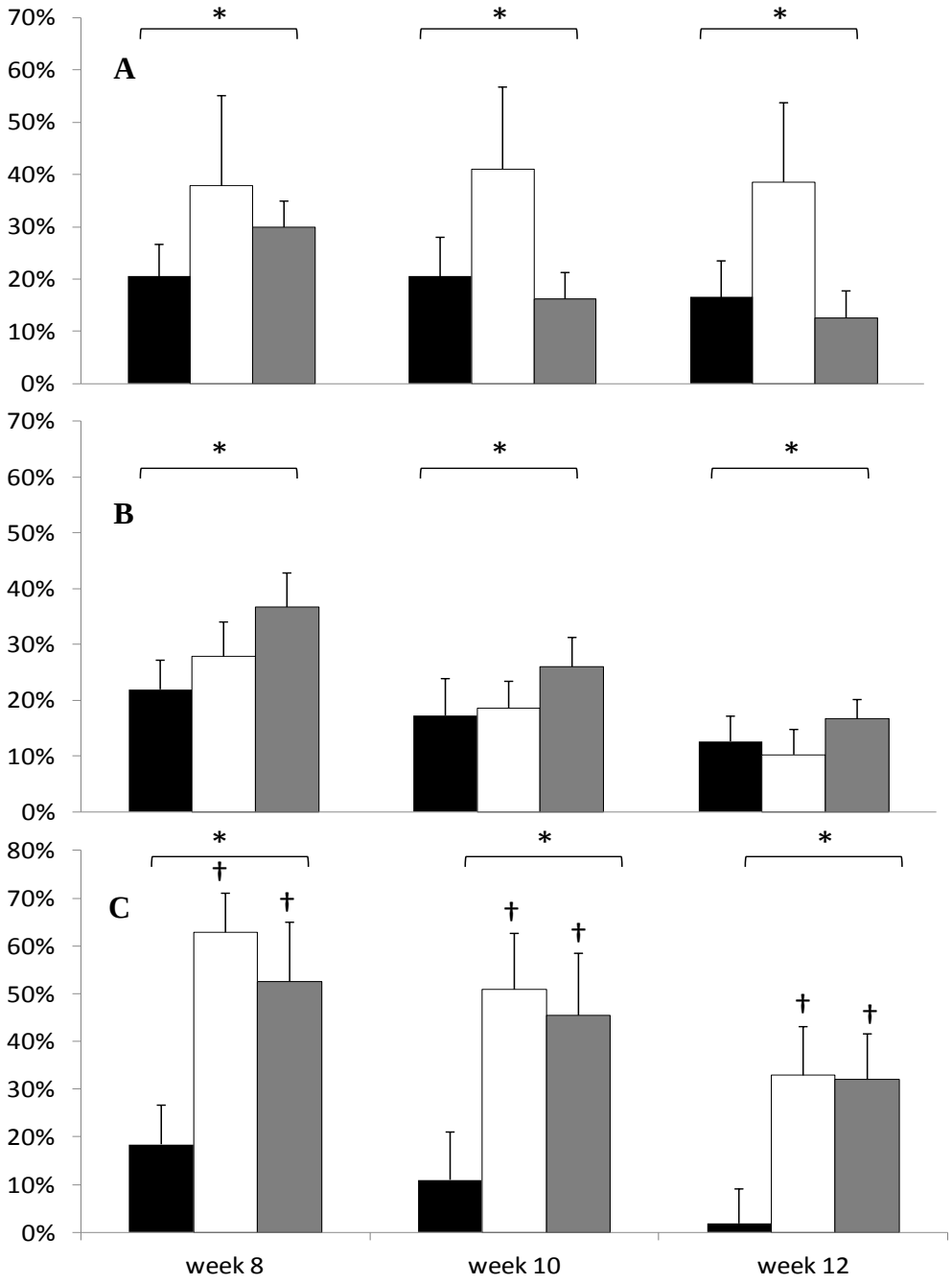

Figure 3.2: Relative changes following training and detraining to aCSA in SL group (black bars) LX group (white bars) and LL group (grey bars) at (A) proximal, (B) central and (C) distal sites of VL muscle. * Significantly different to baseline (p<0.05) † Significant group difference in aCSA (p<0.05).

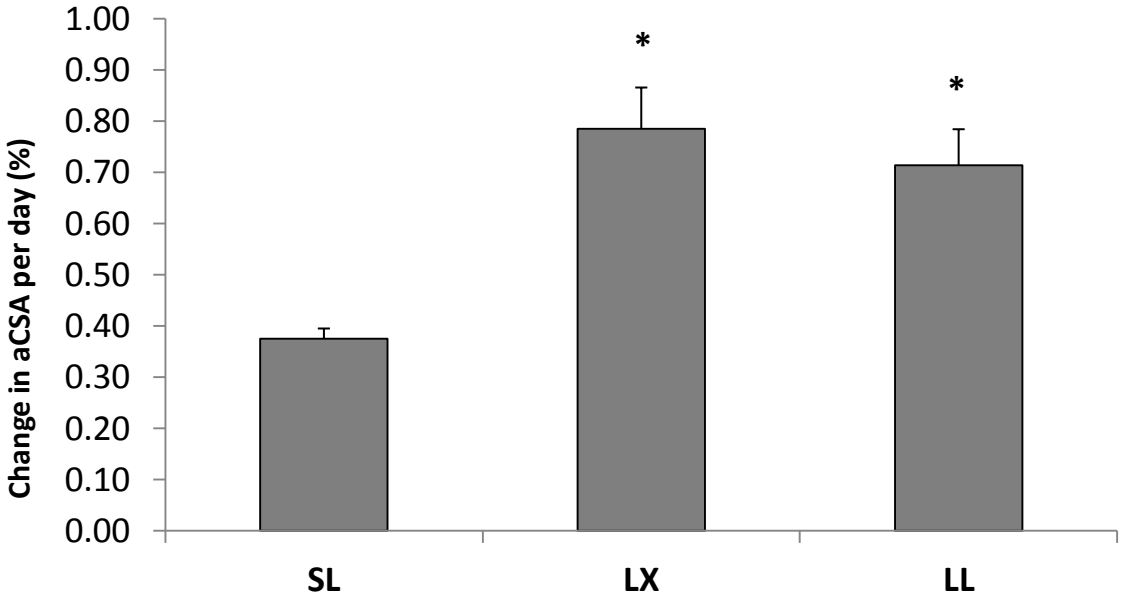

Figure 3.3 . Relative changes in aCSA per day in each training group from baseline to week 8. * Significantly different to SL group ($p<0.05$).

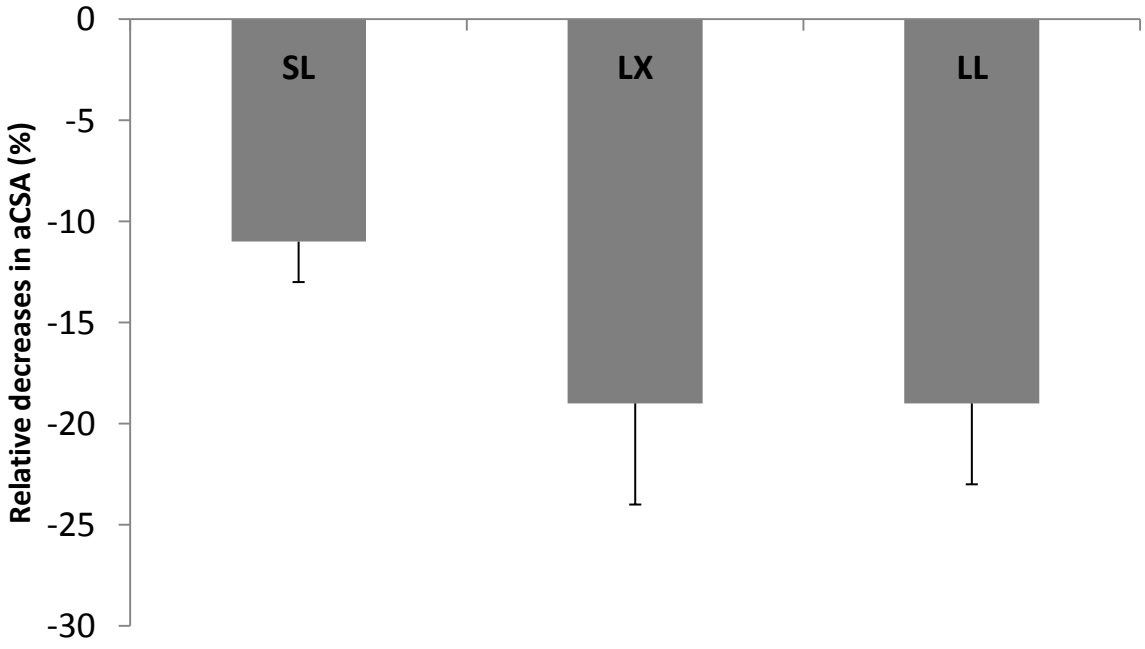

Figure 3.4. Relative changes in aCSA between week 8 and week 12 (detraining). No significant difference observed between groups

Table 3.3. Paired changes to pennation angle at different femur lengths over the training and detraining phases. *Significantly above baseline # Significantly different to other training group. Values are degrees (mean ± S.E.)

Site (% Femur L)	*Baseline*	*Week 8*	*Week 10*	*Week 12*
25PEN	SL 10.1±0.3	SL 10.3±0.3*	SL 10.0±0.7	SL 9.9±0.2
	LX 10.2±0.3	LX 11.2±0.6*	LX 11.1±0.6	LX 11.0±0.6
	LL 9.0±0.4	LL 10.0±0.3*	LL 9.7±0.2	LL 9.2±0.1
	Con 11.9±0.2	Con 12.2±0.1	Con 12.0±0.1	Con 12.2±0.1
50PEN	SL 16.5±0.5	SL 17.2±0.3*	SL 16.8±0.9	SL 16.4±0.4
	LX 15.9±0.2	LX 17.1±0.8*	LX 16.6±0.8	LX 16.0±0.6
	LL 15.3±0.3	LL 16.2±0.5*	LL 15.4±0.2	LL 14.8±0.5
	Con 16.4±0.8	Con 16.4±0.7	Con 16.3±0.7	Con 16.4±0.7
75PEN	SL 16.0±1.4	SL 17.9±1.2*	SL 16.8±0.9*	SL 16.8±0.9
	LX 15.5±0.6	LX 17.5±0.7*	LX 17.2±0.6*	LX 16.3±0.5
	LL 18.1±0.9	LL 19.2±0.8*	LL 18.9±0.7*	LL 18.1±0.5
	Con 19.4±0.7	Con 18.5±0.7	Con 18.4±0.6	Con 18.5±0.7

Muscle Length; Values obtained for absolute changes in muscle length are displayed in Table 3.4. Following training at week 8, muscle length increased significantly ($p<0.001$) in all groups relative to baseline (SL; 5±1%, LX; 6±2%, LL; 4±1 %) but there was no differences between training groups ($p=0.593$). The training groups remained significantly above baseline at week 10 ($p<0.05$), however only LX group remained above baseline values at week12 ($p<0.05$) and was significantly different to both SL and LL groups at the conclusion of the detraining phase.

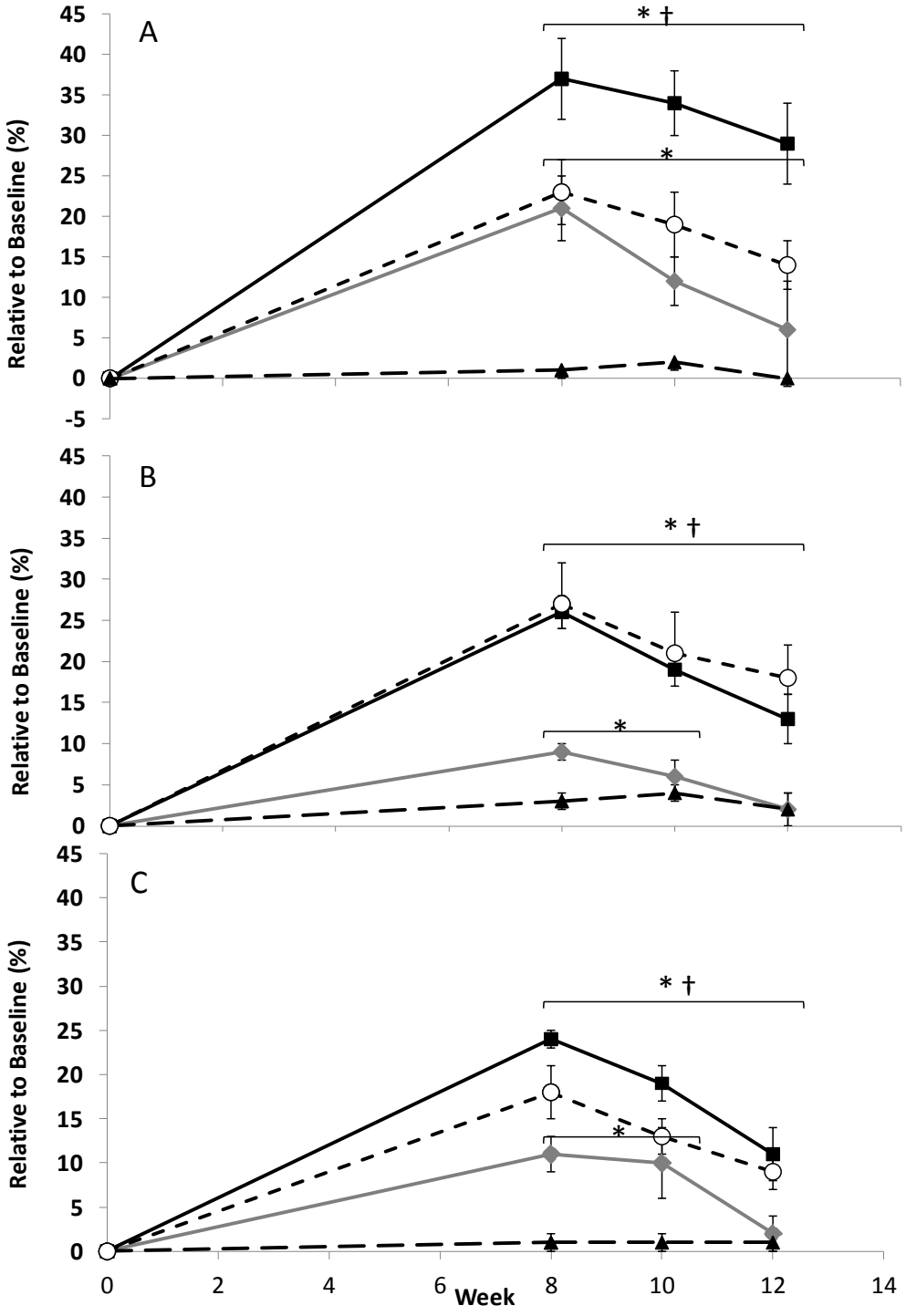

Figure 3.5: Relative changes to fascicle length following training and detraining in SL (grey diamonds), LL (black squares), LX (white circles) and controls (black triangles) groups at A) proximal, B) central and C) distal sites of the VL muscle. * Significant difference compared to baseline ($p<0.05$) † Significant difference between LL/LX and SL groups ($p<0.05$).

Table 3.4. Changes in *Vastus Lateralis* muscle length at each stage of the training and detraining protocols. * Significantly different to baseline (p<0.05) † significantly different to SL & LL groups (p<0.05)

	Muscle Length (cm)			
	Wk 0	**Wk 8**	**Wk 10**	**Wk 12**
SL	34.0±0.9	35.6±0.8*	34.6±1.0*	33.5±0.7
LX	32.3±1.1	34.4±0.8*	33.6±0.8*	33.6±1.0*†
LL	33.1±0.7	34.3±0.8*	34.7±0.7*	34.4±0.7
Con	34.8±0.8	35.1±0.9	34.7±0.8	34.6±0.9

Muscle Width; Changes in muscle widths are shown in Table 3.5. At week 8, VL muscle widths had increased significantly at all three measurement locations in all training groups compared to baseline ($p<0.001$). Following the first period of detraining at week 10, the SL group had returned to baseline values at all three measurement sites ($p>0.05$), however both LL and LX groups retained adaptations at this juncture compared to baseline ($p<0.01$). At week 12 LX group had returned to baseline levels at 25% and 50% width but still remained significantly elevated at 75% femur length compared to baseline ($p<0.05$). The LL group retained their significant gains in muscle width at all three sites for the duration of detraining ($p<0.01$). There were no significant ($p>0.05$) mean relative changes between training groups post-training or following detraining at 25% and 50% femur length (SL; 12±13%, LL; 11±7%, LX; 13±11%). However, LL and LX groups had a greater significant ($p<0.05$) relative increase in muscle width at week 10 (LL; 26±13%, LX; 21±9%) compared to SL (13±8%) at 75% femur length. This was also the case at week 12, however only LL group was significantly greater ($p<0.05$) than SL group at this measurement site.

Table 3.5. Muscle widths a baseline, post-training and detraining. * Significantly different to baseline (p<0.05) † Significantly different to SL group (p<0.05)

		Muscle Width (cm)			
		Wk 0	**Wk 8**	**Wk 10**	**Wk 12**
SL	**25**	12.7±0.7	13.9±0.6*	13.2±0.6	12.9±0.6
	50	12.8±0.9	14.2±0.8*	13.6±0.7	13.3±0.7
	75	9.6±0.7	11.0±0.6*	10.1±0.4	9.6±0.4
LX	**25**	12.2±0.9	14.2±0.7*	13.0±0.7*	12.4±0.6
	50	12.1±0.9	14.3±0.8*	13.1±0.7*	12.3±0.7
	75	8.2±0.8	10.6±0.5*	9.6±0.6*†	8.8±0.4*
LL	**25**	14.0±0.4	15.3±0.5*	15.6±0.4*	15.3±0.3*
	50	13.8±0.5	15.6±0.6*	15.8±0.5*	15.4±0.4*
	75	9.4±0.5	11.4±0.4*	11.5±0.4*†	11.1±0.4*†
Con	**25**	14.2±0.7	14.0±0.6	13.9±0.6	13.9±0.7
	50	13.7±0.9	13.9±0.8	13.7±0.8	13.8±0.8
	75	10.0±0.7	10.4±0.6	10.4±0.6	10.1±0.5

Thigh Girths; Thigh girths increased following training at week 8 in all training groups and at all sites (mean over three sites SL; 3±2%, LL; 4±3%; LX; 4±2%), however this was not significantly different to baseline values (p>0.05) with no differences between groups. Thigh girths also did not differ significantly at weeks 10 or 12 compared to baseline or between groups.

Subcutaneous Fat; Changes in subcutaneous fat are displayed in Figure 3.6. The training intervention resulted in appreciable reductions in fat in SL, LX and LL training groups between week 0 and 8 (p<0.01) at all measured sites. Proximally, the SL group reduced fat levels from 14.4±3.3mm to 14.0±3.2mm (-2±1%), compared to16.7±4.0mm to 15.4±3.4mm (-7±6%) in LX and to18.7±4.1mm to 17.6±3.3mm (-4±9%) in LL post-training; however no

Table 3.6. Thigh girths at baseline, post-training and detraining. No significant differences between baseline or groups were detected.

		Thigh Girth (cm)			
		Wk 0	**Wk 8**	**Wk 10**	**Wk 12**
SL	**25**	62±2	65±2	63±2	62±3
	50	55±1	59±2	57±5	56±3
	75	45±1	46±3	46±3	45±2
LX	**25**	62±3	66±3	65±4	65±3
	50	55±3	61±4	59±4	58±3
	75	44±3	49±3	49±4	48±3
LL	**25**	61±2	65±3	65±3	64±4
	50	53±2	57±3	56±2	56±4
	75	43±3	48±2	48±3	47±2
Con	**25**	61±1	62±2	61±2	62±3
	50	54±1	55±1	54±2	54±3
	75	44±3	45±2	45±2	45±3

group effect existed. All training groups retained fat loss through week 10 compared to baseline at this site ($p<0.05$), but not at week 12 ($p>0.05$). The control group did not fluctuate significantly from baseline values during weeks 8, 10 and 12 ($p>0.05$). There was also considerable subcutaneous fat loss at the belly of the VL following resistance training, with greater losses achieved by the LX and LL groups at week 8 ($p<0.05$) compared to SL. All training groups retained fat losses compared to baseline at week 10 ($p<0.05$), but had lost training adaptations by week 12 ($p<0.05$) with no difference between groups at either of these stages. A marked effect of the training protocol was found distally, where a main effect of both group and training existed at week 8 (training $p<0.01$, between group $p<0.05$). The group effect had diminished by week 10 and 12 despite a trend for both LX and LL retaining superior losses

(week 10; p=0.069, week 12 p=0.074), although all groups still had greater subcutaneous fat losses relative to baseline at week 10 and 12 ($p<0.05$).

Table 3.7. Results of Within-Subject Contrasts following a significant ($p<0.05$) time*group interaction in the main outcome variables

Measure	Site (% femur Length)	Degrees of Freedom	F- Ratio	P Value
Volume	-	1	5.177	.035
aCSA	75	2	4.908	.015
Fascicle Length	25, 50, 75	2, 2, 2	4.250, 8.022, 5.682	.024, .002 .008
Subcutaneous Fat	50, 75	2, 2	4.104,4.137	.040, .041

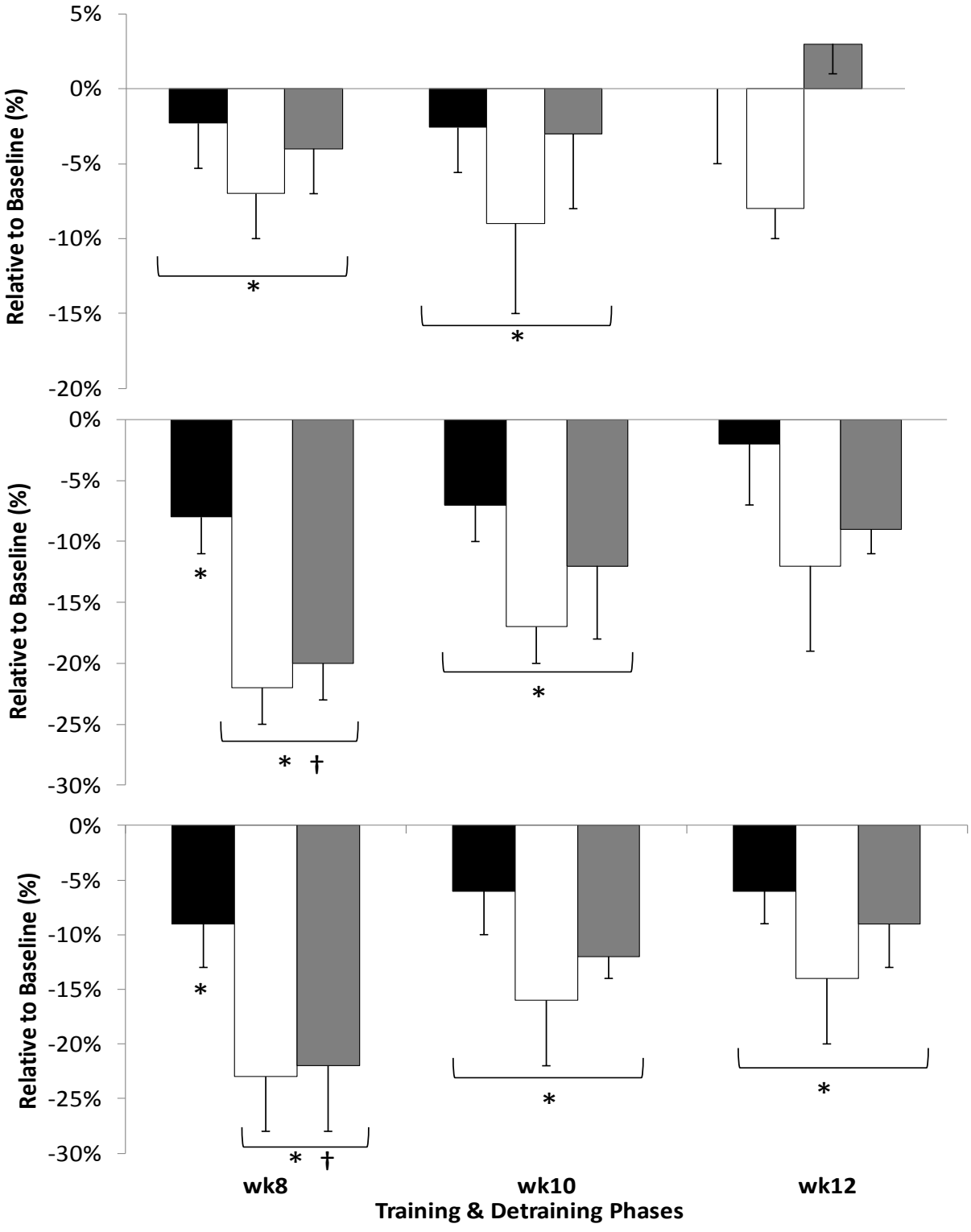

Figure 3.6. Relative changes in subcutaneous fat following training and detraining in SL (black bars), LX (white bars) and LL (grey bars) groups. * Significantly different to baseline ($p<0.05$) † Significantly different to SL group ($p<0.05$)

3.4 Discussion:

The current investigation aimed to study the *in vivo* effect of resistance exercise training and detraining over longer average muscle length (40-90^{o} knee flexion – LL) versus a shorter average muscle length (0-50^{o} knee flexion – SL) and also over a wide range of motion covering both shorter and longer muscle lengths (i.e. complete ROM 0-90^{o} – LX) on morphological, architectural and subcutaneous fat levels in the VL. It was hypothesized that the groups training over longer muscle lengths (LL & LX) would undergo a greater amount of skeletal muscle hypertrophy due to increased physiological (mostly in the form of mechanical) stress and stretch on sarcomeres compared to the group training over a shorter muscle lengths (SL). It was also hypothesized that the LL and LX groups would still have a greater muscle mass following detraining, probably due to greater initial gains. Our findings partly support these hypotheses in that whilst there were no notable changes to any of the muscle variables in the control group during a 12-week non-training period, significant adaptations were observed in SL, LX and LL training groups across nearly all of the muscle measurements. What is more, there was a significant main effect of training where VL muscle length, fascicle length, VL volume, anatomical cross-sectional area and muscle width increased, whilst mid-thigh and distal subcutaneous fat decreased to a greater extent following training and detraining at a longer muscle lengths and combination of long/ short muscle lengths compared to a shorter muscle length.

- **Changes in volume**

The relative increases in VL volume following 8 weeks of resistance training reported in this study (38±8% in SL, 70±15% in LX and 67±10% in LL) are much greater than those previously reported in a similar study (Kubo *et al.* 2006). It is perhaps surprising that such large between-

group differences in relative volume changes are not significantly different between the SL and LX groups, especially when LL was significantly different to SL despite 3% less relative increase than LX. The lack of statistical significance between these two groups is due to the large variation present within the data (as represented in the standard error).

The observed muscle volumes in the current study are larger than those from (Kubo et al., 2006a) who produced the only other study investigating changes in VL volume following training at longer vs. shorter muscle lengths (~11±7% SL and ~13±12% LL – values estimated from Figure 2 in their results section). Variation in the results would not arise from differences between measurement methods of VL volume, as both MRI and B-mode ultrasonography have been found to be valid and reliable tools for measurement of quadriceps CSA and volume (Walton et al., 1997). The differences are likely down to the difference in training protocols (isometric vs. combined isotonic and isometric) between Kubo et al (2006) and the present study. Indeed metabolic cost and work done is greater during concentric compared to isometric contractions (Wilkie, 1968). Therefore a greater work-induced hypertrophic effect of concentric training may have produced the variation in hypertrophy gain differences between the two studies, an inference supported by previous work (Goldberg et al., 1975). In a review of hormonal responses and adaptations to exercise (Kraemer et al., 1990), the authors suggest exercises involving large muscle masses are superior to more isolated exercises to elicit greater hormonal responses, which could impact on the hypertrophic responses to exercise. Therefore, the large mass exercises such as the bilateral squat in our study would elicit a greater hormonal response to that of a seated unilateral knee extension on a dynamometer as in the study of Kubo et al (2006). Also, the volume of exercise undertaken in the current study per week was much greater. The SL, LX and LL groups all performed 3-4 sets of 8-12 reps (depending on the stage of the training protocol)

per exercise with 4 different knee extensor dominant exercises (55% or 80% 1RM) performed per training session, 3 days per week. This volume is much greater than the 6 sets of 15 seconds at 70%MVC, 4 days per week in the study of Kubo et al. (2006). This is important to note since training volume has been proposed to be very important for eliciting a hypertrophic response in the early phases of resistance training in untrained subjects (Staron et al., 1994).

There were limitations of the estimated VL volume in the current study that may also impact on direct comparisons with other studies. Currently, VL muscle volume was estimated via the truncated cone method (see chapter 2), where 3 aCSAs were taken at 25, 50 and 75% femur length. By estimating muscle volume within these set boundaries, a certain percentage of muscle volume will not have been included in the calculation (i.e. some of the muscle mass more proximal than 25% femur length and more distal than 75% femur length). Morse et al. (2007) used magnetic resonance imaging (MRI) techniques, which are the 'gold standard' to measure the volume of the quadriceps and their individual constituents in a population similar to the one of the current study. The authors reported a mean (± S.D.) VL muscle volume of 702±108cm^3 in a group of 18 young recreationally active men. The mean (± S.D.) estimated VL volume at baseline of the current group (i.e. males from SL, LX and LL pooled n=16) was 741±139cm^3, and therefore indicates that the baseline measure of muscle volume appears to be reasonably accurate and representative of a similar homogenous population. In addition to this, a previous study that used the truncated cone method (Esformes et al., 2002) for estimating the muscle volume of the tibilais anterior, did so by taking 11 axial-plane scans of the muscle. In the current study, only 3 scans were taken as previously mentioned at 25, 50 and 75% femur length. Morse et al. (2007) demonstrated that just a single aCSA axial scan of the quadriceps at either 40, 50 or 60% of femur length correlates significantly and highly (R^2 = 0.84, 0.93 and 0.90 respectively)

with the observed quadriceps volume as measured by MRI. In fact, a single scan at 50% or 60% femur length is associated with standard error of the estimate (SEE) of only 13% and 10% respectively. As such, taking only 3 axial scans along the femur length should have estimated volume with sufficient accuracy.

- **Changes in aCSA**

The magnitude of hypertrophy at 75% of femur length was greater for LL and LX following training. Relative increases were 63±15% and 53±12% for LX and LL respectively with SL increasing 18±8%, in contrast at 50% of femur length relative increases were 22±5%, 28±6% and 37±6 % (SL, LX and LL respectively) for the corresponding training length; this would provide evidence region-specific hypertrophy. This has been observed previously following knee extensor resistance training, with greater relative hypertrophy in the distal region of the muscle (Carey Smith and Rutherford, 1995, Narici et al., 1989, Seynnes et al., 2007, Narici et al., 1996, Housh et al., 1992, Häkkinen et al., 2001). With both force generation and stretch being effective stimuli for muscle growth (Goldspink et al., 1991), the discrepancies in CSA between the groups may be due to regional differences in the total stimulus transmitted along the length of the muscle, with experimental evidence demonstrating variations in proximo-distal transmission of muscle forces (Yucesoy et al., 2003). Evidence exists that there is relatively high serial and parallel distribution of muscle fibre strain during transmission of myofascial force (Huijing and Jaspers, 2005). Thus the LL and LX groups could have experienced greater strain at a more distal portion of the VL, which was also transmitted laterally, resulting in a greater stimulus and therefore enhanced hypertrophy at this site.

Previous research on resistance training induced whole quadriceps aCSA showed changes of 18.8±7.2%, 13.0±7.2% and 19.3±6.7% at distal, central and proximal sites respectively (Narici et al., 1989). It is nonetheless difficult to compare the studies directly however, not only owing to the fact the current study measured aCSA of the VL as opposed to all four quadriceps muscles, but also the earlier report (Narici et al., 1989) showed a significant difference in hypertrophic response between the four components of the quadriceps muscle group, so without knowing the responses of the remaining three muscles it is difficult to compare whole quads CSA vs. VL CSA. Despite the differences between these studies, the magnitude of aCSA increment between that of Narici et al. (1989) and SL group are very similar (~20% at each measurement site), and additionally SL was the only group that displayed homogeneity in relative increases along the length of the VL. In contrast, the LL group tended to respond with greater relative increases at proximal and distal lengths of the VL, whereas the LX group tended to have improved relative increases centrally and distally. Therefore each training regimen appears to have favoured regional hypertrophy at discrete lengths along the VL depending on the type of exercise undertaken (i.e. range of muscle lengths and magnitude of loading), which again most likely reflects the variation in distribution of myofascial forces in each training condition. Furthermore, at 75% femur length, the relative increase in aCSA in LL and LX groups are quite large (~70%) compared to other increases. This may have been due to the much lower (~25-40%) absolute muscle mass at this location, therefore if the participants did not routinely exercise and stress this part of the VL previously, it would indeed predispose this part of the muscle to the greater relative increases in muscle mass following longer muscle length training.

- **Changes in fascicle length**

A major finding of the current study was the greater increase in fascicle length at all sites in the LL group, and at 50% and 75% femur length in LX group, compared to SL group. *In vivo* increases in fascicle length are associated with addition of sarcomeres in series (assuming a fixed sarcomere length) and appear to be strongly influenced by muscle length or stretch.

Observations from animal models have demonstrated the importance of stretch in modulation of sarcomere number (Tabary et al., 1972, Williams and Goldspink, 1973, Goldspink et al., 1974, de la Tour et al., 1979), and is associated with an increase in protein synthesis (Loughna et al., 1986). Furthermore, in humans, a study by Boakes et al.(2007) where surgery placed the thigh muscles under constant stretch to address a leg-length discrepancy, resulted in 4cm femoral lengthening. Fascicle and sarcomere length was measured in VL post-operatively and after twelve months. Results showed *in vivo* fascicle length increased, and sarcomere length decreased, with sarcomerogenesis from approximately 25,000 to 58,650 as a result of adaptation to stretch.

Table 3.1 shows that by placing the leg at 90^{o} of knee flexion (i.e. the joint-angle encountered by both LL and LX groups during training), that the VL muscle was stretched to a greater degree than any other training knee joint angle. By performing load bearing exercise with the muscle-tendon complex in a relatively lengthened position, an enhanced mechanical stimulus is experienced (i.e. greater stretch), thereby augmenting fascicle length increase by an increase in the number of in-series sarcomeres to allow each sarcomere to work at optimal length in line with the length-tension relationship of this unit. It should be pointed out here that, although fascicle length was measured at 90^{o} of knee flexion and the SL group did not encounter this joint-angle during training specifically, they would still have experienced this joint angle during their normal daily routine including sit-to-stand transitions. Therefore when measuring fascicle

length in the laboratory, the in-series and parallel elastic components should not be sufficiently 'stiffer' in this group to justify the large between group changes we describe here.

Excursion range has been suggested to be important for regulating sarcomere number in the rabbit (Koh and Herzog, 1998), although our results suggest that an excursion with the muscle in a predominantly lengthened position, that is the major regulator of fascicle length *in vivo*. This is due to the fact that both SL and LL groups performed the same degree of excursion of 50^{o}, yet had different levels of adaptation. Additionally, in favour of the excursion offset hypothesis, is that muscle forces at the tendon were also matched during resistance training in the SL and LL groups. Therefore no additional mechanical stimulus for fascicle length change (i.e. greater force) was present in the LL group. The fact that LL group adaptations were in general superior to LX group's, despite having exercised at 55% 1RM compared to 80% 1RM, supports previous observations from literature that increased muscle activity or activation is not necessary for the adaptation of serial sarcomere number (de la Tour et al., 1979, Williams and Goldspink, 1978).

- **Pennation Angle**

Further architectural adaptations included a significant increase of the VL pennation angle ($P\theta$) in all training groups. A functional consequence of an increase in $P\theta$ is that more contractile material can be packed in parallel for a given anatomical cross-section (Kawakami et al., 1993, Rutherford and Jones, 1992). $P\theta$ has been shown to increase following resistance exercise (Blazevich et al., 2007, Aagaard et al., 2001, Kawakami et al., 1995, Seynnes et al., 2007, Reeves et al., 2009) and is usually closely associated with an increase in physiological and anatomical CSA in the quadriceps (Rutherford and Jones, 1992, Aagaard et al., 2001). The lack

of significant differences between groups in $P\theta$ is reflected also by the lack of between-group differences observed in pCSA. Despite not reaching between group significance, the average increase in $P\theta$ across the three sites exhibited a trend towards being greater for LX (11±5%) and LL groups (9±3%) than SL (7±4%).

- **Subcutaneous Fat**

A further benefit experienced by the LX and LL groups was a greater reduction in subcutaneous fat at 50% and 75% of femur length at week 8. In the athletic world it is often considered beneficial to reduce levels of subcutaneous fat. For example in running events (or events where the body is not supported), excess body weight has been shown to significantly decrease relative VO_2 max and performance during a running test as a direct consequence of an increased energy cost of running at submaximal speeds (Cureton et al., 1978). Additionally in sports, such as the high jump, where a given body mass is accelerated against gravity, a more lean muscle mass would be advantageous for performance and energy consumption. This is not to mention the effect of body composition on cardiovascular risk factors and mortality for the average person (Lee et al., 1999). It would be tempting to suggest that the possible mechanism for an increased fat loss in the LL and LX groups may be linked to the greater internal physiological stress on the muscle. Studies have shown greater activation and oxygen consumption of the VL muscle at longer muscle lengths compared to shorter muscle lengths, even when loading is normalized to maximal torque capacity (Kooistra et al., 2006, Kooistra et al., 2008, de Ruiter et al., 2005). Furthermore, strenuous resistive exercise may elevate post-exercise metabolic rate for a prolonged period, and may enhance post-exercise lipid oxidation (Melby et al., 1993). However, more recently Singhal et al.(2009) found that there was no significant difference in post-prandial

lipemia in groups undertaking either moderate or high-intensity resistance exercise. An alternative hypothesis for the relatively greater fat loss in longer muscle length training could be through an increase in AMPK activity (Dreyer et al., 2006), which in turn has also been shown to mediate effects of IL-6 stimulated increases in glucose disposal and free fatty acid oxidation. Further, AMPKα2 activity has been shown to be intensity dependent (Chen et al., 2003), therefore training at longer muscle lengths could affect upstream factors of adiposity, However a direct link with training at longer muscle lengths remains to be reported.

- **Other muscle measures**

It can be useful at times to have a more gross measure of muscle changes to act as a possible surrogate for a more time-consuming assessment, or indeed to gain insight to how a muscle group responds as opposed to an individual muscle within a group. From the more slightly crude estimates of muscle size increments such as muscle width and thigh girths, it would appear that only muscle width (measured in a medial-to-lateral fashion across the VL) was representative of the changes in muscle morphometry observed from more accurate sources. For example, at 75% femur length, muscle widths increased significantly in all groups following training, however there were significant differences between groups during detraining at this location with muscle widths being greater in both LL and LX groups compared to SL group, which was very similar to trends identified in aCSA at this measurement site. An increase in muscle width would likely reflect parallel addition of sarcomeres which is also why it would probably mimic changes in aCSA quite closely, and therefore could possibly be used as a rapidly measured surrogate for aCSA. Total thigh girths did not display a relationship with other measures of muscle size, as there were no significant changes to thigh girths in any group, despite small increases. This could

be for a number of factors that such a crude measure does not take into account. Firstly, the total thigh girth would include the hypertrophic response of not only the knee extensors (i.e. quadriceps), but also the antagonist Knee flexors (i.e. hamstrings muscle group). Major functional exercises such as the two squat variations and the lunge exercise used in the training regime also require considerable stabilisation and work from this muscle group (Escamilla, 2001), therefore with no accurate morphological data on the hamstrings group, one does not know their contribution to changes in total thigh girths. Furthermore, following resistance training, the individual constituent quadriceps muscles have been shown to have a heterogeneous hypertrophic response (Narici et al., 1996), and therefore following length-specific training, without measuring the other knee extensor muscles' individual contribution to total thigh girth, estimations using thigh girths may provide inaccurate information regarding the response of the muscle group as a whole. And finally, following significant changes in subcutaneous fat after prolonged training, using gross measures such as thigh girths would not distinguish between an increase in lean muscle tissue or just an general increase in both muscle size with no change in adipose layer thickness.

- **Effects of detraining**

Another aim of the present study was to determine if there was a differential response to detraining between the training groups. The detraining period resulted in significant losses at weeks 10 and 12 ($p=0.001$,) in the vast majority of measured parameters in all training groups. Although generally there were no significant difference between groups, LL and LX groups consistently exhibited a trend ($p=0.067$) towards greater absolute and relative decrements in muscle dimensional parameters over the four-week detraining period. This is in agreement with a previous study (Tokmakidis et al., 2009), which found following 12 weeks of resistance

training, a group of older adults performing high intensity (HI) training increased total thigh CSA and strength to a greater ($p<0.05$) extent than a moderate intensity (MI) training program. Following a subsequent 12 weeks of detraining, total relative thigh CSA and strength in the HI group diminished significantly more than MI group. Despite these reductions, HI group strength and CSA remained significantly greater than in MI group due to greater initial adaptations. In the present study in terms of average VL aCSA across the 3 sites, there was a decrease from 44±13% to 25±11% above baseline between week 8 and 12 in the LX group, 40±5% to 21±5% in LL, compared to SL group's decrease from 21±8% at week 8 to 10±7% at week 12. Therefore the relative changes in aCSA to LL and LX groups following four weeks detraining (+21±5 and +25±11% respectively) are still similar or superior to those made by SL immediately post-training (21±8%). This suggests that although greater initial gains may be lost at a greater rate, training using a relatively wider range of motion and longer muscle length may still confer an advantage for the longer term. Secondly it is also evident that both LL and LX groups, who had similar average increases at week 8, actually experienced identical relative decreases between week 8 and 12 in aCSA (both -19% compared to week 8 – Figure 3.6) demonstrating that groups with similar relative increases will display at a similar rate of detraining-induced loss of hypertrophic gains.

It is difficult to present a definitive rationale for the reason for greater gains to be lost more rapidly, such as in the LL and LX groups. We would propose here however, that it may be due to an inability to stimulate sufficient protein synthesis to support a larger muscle mass. One would not expect to observe a difference in daily protein synthetic rate between groups with no change in activity levels and dietary habits (Kimball et al., 2002). Therefore basal protein synthetic rates would support a greater relative percentage of a smaller muscle mass than a larger mass. Also,

since resistance exercise causes perturbations to the intramuscular environment, and there are subsequent adaptations, a new homeostatic point is reached, where only a greater stimulus than the original will stimulate further adaptations (Kraemer et al., 1996). The LL/ LX groups may have set a higher threshold to maintain adaptations, whereas training activities influence on the internal muscle environment would have been lower in the SL group owing to the presence in the latter, of a still relatively low homeostatic threshold. Therefore since the phosphorylation of extracellular signal-regulated kinase (ERK1/2) and the 38-kDa stress-activated protein kinase (p38) both appear to be intensity dependent (Rennie et al., 2004), a smaller muscle mass may find a certain daily task relatively more intense than a larger mass because of differences in force-generating capabilities, and therefore disturb homeostasis more and could be a contributing factor to the difference in cellular response and adaptation.

It was also interesting to note that there did not appear to be any difference in relative losses in muscle volume from week 8 through detraining between SL and LL/ LX groups as was evident in aCSA (Figure 3.6). This suggests as a whole, relative muscle volume losses may be similar, the rate of actual losses may be different along the length of the muscle as reflected by aCSAs at different locations.

Study limitations

It should however be noted that with the differences observed between SL and LX, whilst the ROM has been presented as different, the manner in which the two training groups were required to hold the contractions (over 2secs) means that the greatest difference in the loads experienced by the quadriceps muscle was due to the internal joint architecture. In other words, the difference

between the two training protocols was not so much owing to the ROM (thought this differed at the start of the movement) but to the length of the muscle when the muscle group was experiencing the 'isometric hold' phase of the exercise. The authors do also recognize that in covering a greater ROM, the muscle is loaded for a longer duration in LX than SL (i.e. 0.25-0.50secs). However, previous studies (e.g. (Adams et al., 2004)) have concluded that measures of work production during resistance training do not directly scale with the adaptation responses seen in skeletal muscle. Furthermore, it is difficult to give substance to a mechanism whereby such a small difference in duration of loading (i.e. load-time product) would lead to serial sarcomerogenesis and give rise to such striking differences in fascicle length.

Conclusion

Previous evidence in young humans (Blazevich et al., 2007) demonstrated that eccentric training was not the major determinant for fascicle length adaptations. However the current study has shown that greater increases in fascicle length of the VL were present following longer muscle length training and a complete ROM, and this was true even when the overall degree of excursion and muscle forces at the tendon were made comparable. In addition, current evidence suggests that prolonged dynamic resistance training at predominantly longer compared to shorter muscle lengths, resulted in more marked improvements in muscle volume, regional muscle hypertrophy, and muscle width . Furthermore, with adaptations retained more successfully over 4 weeks of detraining with the muscle lengthened, such training could also be used to offset deleterious effects of detraining or hypoactivity.

CHAPTER 4:

Effects of Resistance Training & Detraining on Muscle-Tendon Complex Mechanical & Material Properties

4.1 INTRODUCTION:

During resistance exercise, muscles and their in-series tendon (the muscle-tendon complex or MTC) work together in a concerted effort to bring movement about a joint (Ker et al., 1988). Research using resistance exercise over the past few decades has focused mainly on adaptation of the force generating muscle, with less focus on the adaptation of the force transmitting tendon, probably due to the traditional view of the relatively inert nature of the tendon (Neuberger et al., 1951). However, in contrast, more recent evidence from an *in vivo* study of the patellar tendon, shows that it is not an inert material and that tendon properties can change significantly even over the course of a day (Pearson and Onambele, 2005). Additionally during acute periods of activity, the tendon has also shown to be metabolically active with significant increases in blood volume (Kubo et al., 2009), oxygen consumption (Kubo et al., 2008) and glucose uptake (Bojsen-Møller et al., 2006, Kalliokoski et al., 2005). Miller et al. (2005) showed that after 6 and 24 hours following 60 mins of resisted one-legged kicking on an ergometer, tendon collagen, muscle collagen and myofibrillar protein fractional synthesis rates had increased by 1.7-fold, 3.5-fold and 2.8-fold respectively in the patellar tendon and quadriceps. This once again highlights the synchronization of adaptation in response to mechanical loading throughout the muscle-tendon complex.

Following prolonged periods of resistance training, it has been demonstrated that tendon (e.g. patella and Achilles) has the ability to adapt both morphologically (Seynnes et al., 2009, Kongsgaard et al., 2007, Arampatzis et al., 2007) and in terms of intrinsic material properties (i.e. increased stiffness and/ or Young's modulus (Reeves et al., 2003a, Maganaris et al., 2004,

Kongsgaard et al., 2007, Seynnes et al., 2009, Kubo et al., 2006a, Arampatzis et al., 2007)). Muscle has been shown to adapt to high mechanical loads and stretch (McDonagh and Davies, 1984, Tabary et al., 1972). Like its in-series muscular counterpart, tendon appears to respond and adapt at varying magnitudes to the stimulus provided by mechanical loading and stretch (Arampatzis et al., 2007, Kongsgaard et al., 2007, Kubo et al., 2006a, Yang et al., 2004). For example, Arampatzis et al. (2007) demonstrated that when training under low cyclic magnitude tendon strain (2.97%) compared to high magnitude tendon strain (4.72%) with equal volume and loading frequency, tendon-aponeurosis stiffness and Achilles tendon modulus increased significantly only following training under high magnitude tendon strain. In parallel, Kubo et al. (2006a) trained the quadriceps and patella tendon MTC isometrically for 12 weeks at either a longer (LT) or shorter (ST) muscle length. Stiffness of the tendon-aponeurosis increased significantly (47%) following training in LT whereas ST did not (5%). Furthermore, the internal forces estimated from their data suggested that, based on the differences of the patella moment arm, although both groups trained at external torques of 70% MVC, the LT group in fact experienced internal forces of 2090N compared to 908N in ST (i.e. ~3.2 times greater). The above evidence suggests that the mechanics of the resistance exercise protocol has profound effects on the changes to tendon mechanical and material properties.

During dynamic resistance training, joint angle will change as bone rotates about its joint axis. A change in joint angle will also alter the length of the muscle-tendon unit. For example, the muscle-tendon unit length of the three monoarticular *vasti* (*medialis*, *lateralis* and *intermedius*) of the quadriceps has been shown to increase by ~15% during movement from full extension (0^{o}) to 100^{o} of knee flexion in cadaveric specimens (Visser et al., 1990). This pattern has also been demonstrated *in vivo*, on both the gastrocnemius and tibialis anterior (Herbert et al., 2002). The

authors found that passive movement of the ankle joint from full plantarflexion to dorsiflexion induced considerable changes in muscle-tendon unit lengths, with 72% and 45% of the total change in MTC length being accounted for by the tendon of the gastrocnemius and tibialis anterior respectively. This demonstrates that a change in joint angle also alters the passive tension and therefore strain experienced by the in-series tendon.

As mentioned above, force and strain through the tendon appears to be a major mechanical stimuli following long-term exposure to resistance exercise. Indeed it appears that the application of force coupled with greater strain appear to be effective stimuli for muscle hypertrophy, through training at longer muscle lengths (chapter 3). However, to the author's knowledge, no study exists that systematically investigates both separate and combined effects of load and/ or strain components of resistance training on the *in vivo* adaptations of the MTC. The data could provide valuable insight into how the mechanical and material properties of tendon are altered in response to these stimuli, and can be used in the application of resistance training for performance or for injury prevention/ recovery (Sharma and Maffulli, 2006).

At the other end of tendon loading spectrum, is chronic unloading such as seen in disuse models where tendon has been shown to alter its mechanical properties in an almost inverse manner to those following training. De Boer et al.(2007) showed that after 14 and 23 days of unilateral lower limb suspension, patella tendon stiffness decreased by ~10% and ~30% respectively, concluding that a time-course exists in tendon adaptation to unloading. Alterations to the mechanical properties of tendon following bed-rest are in agreement with these findings (Reeves et al., 2005a, Kubo et al., 2004a). However following a period of 3 months of isometric knee extensor training, Kubo et al. (2010) reported a fairly linear decrease in tendon stiffness measured a one, two and three months post-training, and that stiffness had returned to baseline

values following 2 months of detraining. Obviously a difference exists between unloading and detraining, with detraining allowing habitual loading of the MTC through normal activity. Periods of detraining (such as through illness, injury and tapering) would often fall within a shorter time period of ≤4 weeks (i.e. less than the 1-3 months time-frame described by Kubo et al. (2010)), and not necessarily result in bedrest/ immobilisation and therefore also allow for habitual loading. However, no previous study has investigated if there is a distinct variation of tendon adaptation during the early and latter stages of a relatively shorter detraining period.

The aim of the current investigation was therefore to describe the adaptation of the muscle-tendon complex to 8 weeks of combined dynamic and static resistance exercise, with the MTC following three different excursions ranges. To vary magnitude of stretch, the first two excursions (0-50^{o} or 40-90^{o} knee flexion) were to place the MTC in a relatively shortened position (MTCS; 0-50^{o}) or lengthened position (MTCL; 40-90^{o}) with matching tendon forces (and work) during exercise, and the third excursion range (which is normally encountered in a training program) was a combination of the entire first two ranges (MCTX; 0-90^{o} knee flexion) but with higher tendon forces. The second aim of the current study was to establish a time-course and quantitative/ qualitative changes in MTC properties following a subsequent shorter period of detraining. The hypothesis being that the tendon would undergo a chronic stiffening response, the magnitude of which would present a step-wise increment (i.e. control<MTCS<MTCL<MTCX) reflecting the combination of both force and stretch magnitude placed on the MTC in each group.

4.2 Methods:

Patella tendon (PT) mechanical and material properties (force-elongation, stiffness, stress-strain, young's modulus) were measured using B-mode ultrasonography during isometric knee

extensions. Additionally patella tendon CSA was measured at25%, 50% and 75% PT length, as was PT length and co-contraction of the antagonist hamstrings muscles. Knee extensor strength was measured over a range of joint-angles ranging from 30-90^{o} knee flexion (0^{o} = full extension). Please refer back to chapter 2 for full details.

4.3 Results:

Strength & Torque Angle:

There was a significant main effect of training on all three training groups (p=0.013). The strength and torque angle data are presented in Figure 4.1, with each group displaying varying magnitudes of torque increment at each angle. MTCS achieved a significant ($p<0.05$) relative increase in torque at four angles (mean Δ6±4%; 50^{o}, 60^{o}, 65^{o}, 70^{o}), with MTCL recording a significant relative increase in torque at all angles (mean Δ21±8%; 30^{o}, 50^{o}, 65^{o}, 90^{o} $P<0.05$; 70^{o}, 75^{o} $p<0.01$), as did MTCX (mean Δ16±5%; 50^{o}, 65^{o}, 75^{o} $p<0.05$; 30^{o} 70^{o}, 90^{o} $p<0.01$) except 60^{o}. There was no change in the angle of peak torque production from pre-training to post-training in MTCL and MTCX groups (70^{o}), however interestingly, angle of peak torque production shifted to the left from 75^{o} pre-training to 70^{o} post-training in MTCS. There was no significant change in strength at any angle, or angle of peak torque in the control group ($p>0.05$).

Tendon Dimensions & Antagonist Co-contraction:

Changes in tendon dimensions and co-contraction of the biceps femoris are shown in Table 4.2. There was no significant change in tendon length or CSA at 25%, 50% or 75% of PT length following training and detraining in any training or control group ($p>0.05$). Additionally, no change in maximal antagonist activation or co-contraction during 100% knee flexion MVC were detected at any stage of the study ($p>0.05$).

Table 4.1. Muscle-Tendon Complex Lengths at various knee joint angles. Values are mean ± S.D. * Significantly different from full extension (*P*<0.05). * Significantly different from full extension (p<0.001). PTCSA at 25%, 50% and 75% PT Length.**

Knee Joint angle (^{o} knee flexion)	MTC Length (cm)	PTCSA (mm^2)	
0^o (Full extension)	36.5±2.9	25	89±23
		50	94±14
		75	90±17
40^o	38.9±2.4*	25	84±9
		50	93±10
		75	101±9
50^o	39.7±2.4*	25	86±10
		50	79±12
		75	98±5
90^o	42.0±2.7***	25	83±11
		50	87±16
		75	86±11

Patella Tendon Properties: Figure 4.3 and Table 4.2 shows the relationship between tendon force and elongation, and changes to tendon stiffness (K) at each measurement period. In MTCS, there was a significant effect of training (p<0.001) at week 8 on tendon elongation at force levels of 40-100%MVC, while MTCL and MTCX displayed a main training effect (p<0.001) at force levels of 30-100%MVC at week 8. Also at week 8, there was a group effect with MTCL and MTCX having significantly less tendon elongation at force levels of 70% (p=0.017), 80% (p=0.007) and 90% (p=0.005) MVC, with a trend for a

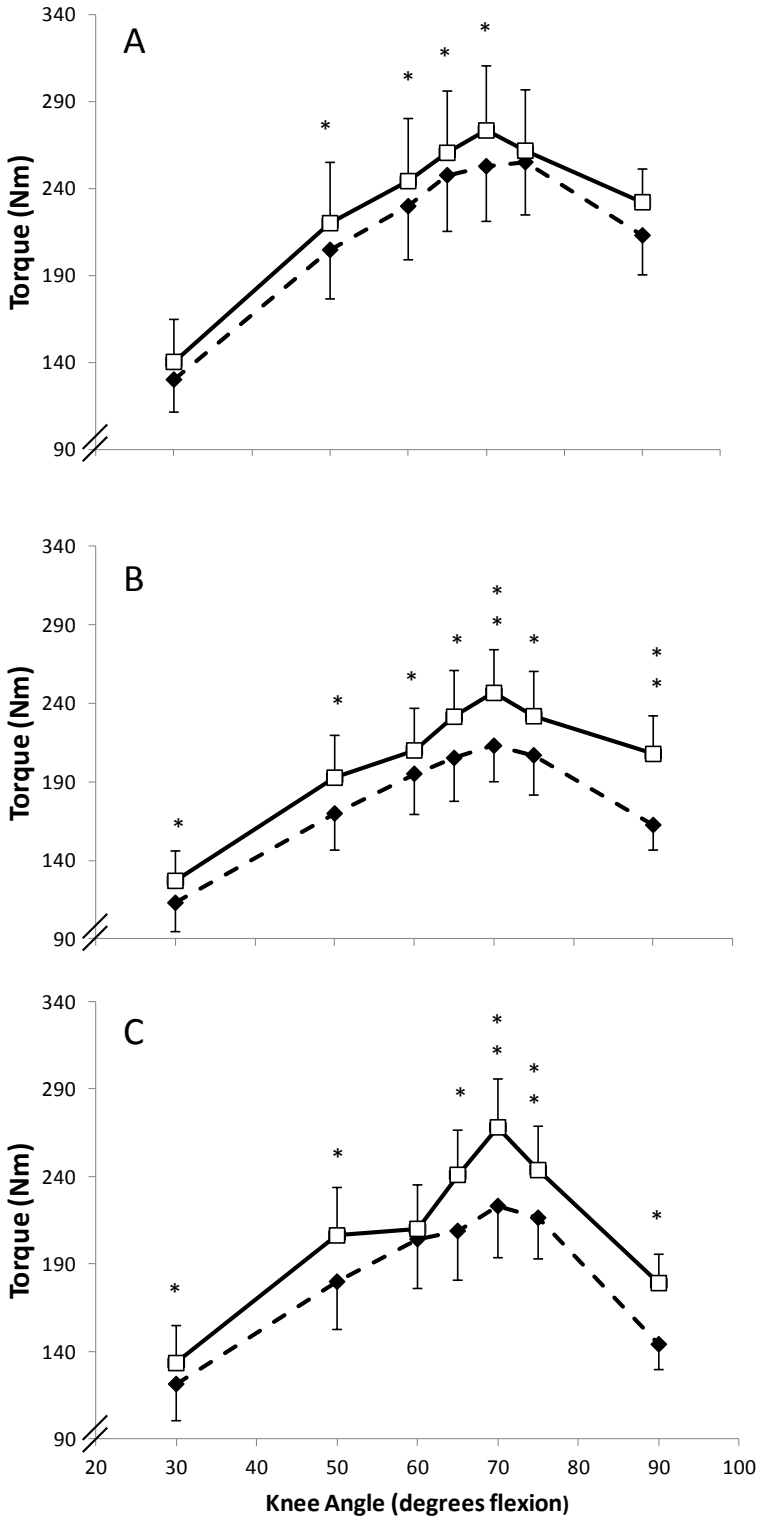

Figure 4.1. Changes in torque levels at various joint angles at baseline (dashed line) and at week 8 (solid line) in A) MTCS, B) MTCX, and C) MTCL. * Significantly different relative to baseline ($p<0.05$). ** Significantly different relative to baseline ($p<0.01$).

group effect at 100%MVC ($p=0.07$). Following detraining, elongation values had returned to baseline at week 12 in MTCS, however it remained significantly above baseline ($p<0.001$) at week 12 in both MTCL and MTCX groups.

Average Patella tendon stiffness across the entire range of normalised forces increased post-training in all training groups ($p<0.001$) compared to baseline, with relative increases in K significantly greater in both MTCL (43±11%) and MTCX (50±18%) groups compared to MTCS (35±6%; $p<0.05$). Interestingly following two weeks of detraining, MTCL group K increased further by 11% (i.e. to 54±16%), whereas K decreased in the other training groups (-7±4% MTCX; - 12±6% MTCS), however there was no difference between MTCL and MTCX ($p>0.05$). Group differences persisted throughout the detraining period with MTCL and MTCX groups remaining above baseline ($p<0.05$) following detraining at weeks 10 and 12, whereas MTCS had returned to baseline values by week 12.

Table 4.2. Tendon dimensions & properties, and antagonist activation. * Significantly different to Baseline (p<0.05) ** Significantly different to other training groups (p<0.05)

Variable		Baseline (Wk0)				Post-Training (Wk8)				Detraining 1 (Wk10)				Detraining 2 (Wk12)			
		MTCS	MTCL	MTCX	CON	MTCS	MTCL	MTCX	CON	MTCS	MTCL	MTCX	CON	MTCS	MTCL	MTCX	CON
PT Length (mm)		52.3 ±4.7	53.1 ±5.3	50.5 ±5.1	53.5 ±5.2	52.0 ±4.4	53.9 ±5.4	50.8 ±4.9	52.9 ±4.4	52.1 ±4.5	54.1 ±5.4	51.3 ±5.1	53.3 ±5.0	52.0 ±4.4	53.8 ±5.0	50.9 ±4.7	53.1 ±4.9
PTCSA (mm^2)	25%	67±11	69±14	71±11	74±12	69±12	76±18	74±15	72±12	67±14	74±18	75±12	74±11	68±8	73±16	70±14	73±14
	50%	70±9	78±13	69±13	73±11	74±15	83±13	75±16	76±14	74±10	82±13	73±11	75±13	71±10	82±12	73±15	75±13
	75%	74±18	69±15	73±15	78±14	77±15	71±25	78±17	79±15	75±17	77±13	77±16	79±14	73±16	77±13	75±15	78±14
Max Flexion RMS-EMG (V)		0.55 ±0.30	0.50 ±0.23	0.69 ±0.42	0.58 ±0.44	0.48 ±0.24	0.47 ±0.23	0.57 ±0.18	0.57 ±0.30	0.47 ±0.37	0.35 ±0.25	0.52 ±0.30	0.52 ±0.28	0.48 ±0.31	0.43 ±0.23	0.67 ±0.33	0.55 ±0.31
Co-Contraction RMS-EMG (mV)		0.046 ±0.024	0.058 ±0.050	0.044 ±0.015	0.051 ±0.021	0.050 ±0.037	0.045 ±0.040	0.046 ±0.030	0.046 ±0.030	0.047 ±0.028	0.043 ±0.026	0.054 ±0.027	0.054 ±0.027	0.045 ±0.032	0.046 ±0.035	0.054 ±0.032	0.050 ±0.032
PT Stiffness		916	837	765	1008	1221	1124	1167	1041	1115	1206	1070	1027	1024	1043	989	1025
(Nmm^{-1})		±441	±379	±242	±265	±594*	±471*	±353*	±244	±530*	±503*	±346*	±251	±439	±428*	±344*	±248
Δ Relative		-	-	-	-	35	43	50	3	23	54	43	2	14	35	32	2
Stiffness (%)						±6*	±11**	±18**	±2	±12*	±16**	±17**	±3	±8	±14**	±11**	±2
Δ Relative		-	-	-	-	38	43	51	3	17	51	31	3	14	33	38	3
Stiffness 2kN (%)						±19*	±14*	±30*	±3	±18*	±19**	±17**	±3	±14	±16**	±18**	±2
Young's Modulus (GPa)		0.83	0.74	0.78	0.99	1.10	0.99	1.15	1.03	1.02	1.03	1.11	1.04	0.91	0.91	1.02	1.03
		±0.09	±0.09	±0.10	±0.11	±0.12	±0.11	±0.11	±0.12	±0.10*	±0.11	±0.12	±0.10	±0.05	±0.13	±0.14	±0.11
Δ Relative		-	-	-	-	36	33	48	4	23	40	42	5	13	24	31	4
Young's Modulus (%)						±7*	±5*	±6**	±4	±7*	±5**	±12**	±4	±2*	±13**	±13**	±4

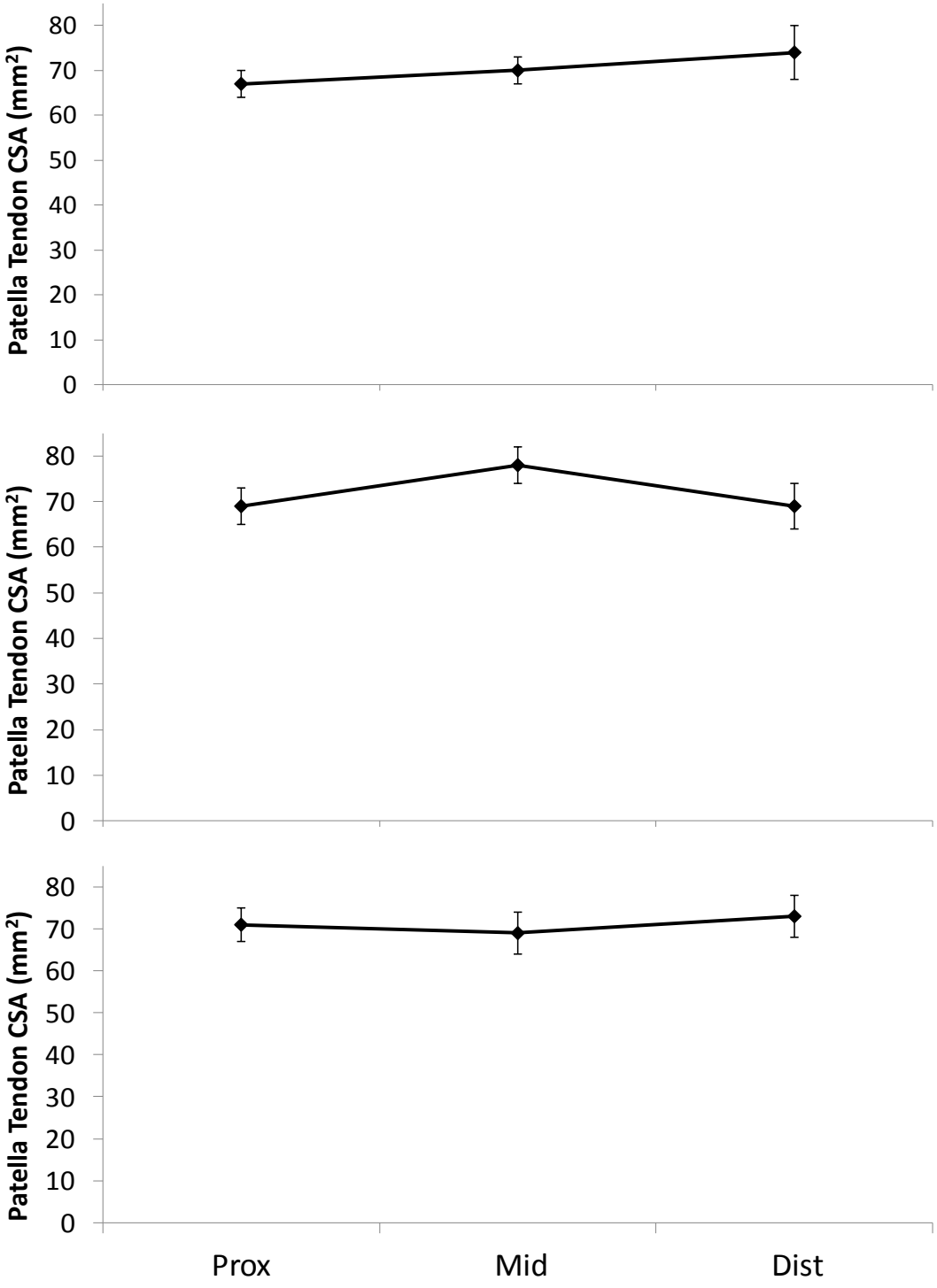

Figure 4.2. Patella Tendon CSA at proximal, mid and distal sites in SL (A), LX (B) and LL (C) groups. Note. There were no between site effects or between group effects detected.

Relative changes in patella tendon stiffness at a standardised tendon force (2000N), followed a similar trend to those at all force levels (Table 4.2). At week 8, there was a main effect of training observed ($p<0.001$), however there was no significant effect of group evident ($p>0.05$).Main effects of training phase ($p<0.001$) and group ($p<0.05$) were shown at week 10 with MTCS (17±18%) once again displaying reduced adaptations compared to both MTCL (51±19%) and MTCX (31±17%; $p<0.05$). Following the conclusion of the detraining period at week 12, relative changes in stiffness values at 2000N in MTCL (33±16%) and MTCX (38±18%) remained significantly enhanced compared to baseline ($p<0.05$), although MTCS tendon stiffness at that force level did not (14±14%; $p>0.05$). Neither stiffness at 2kN, nor the force-elongation relationship did not significantly change ($p>0.05$) at any stage of the 12-week period in the control group.

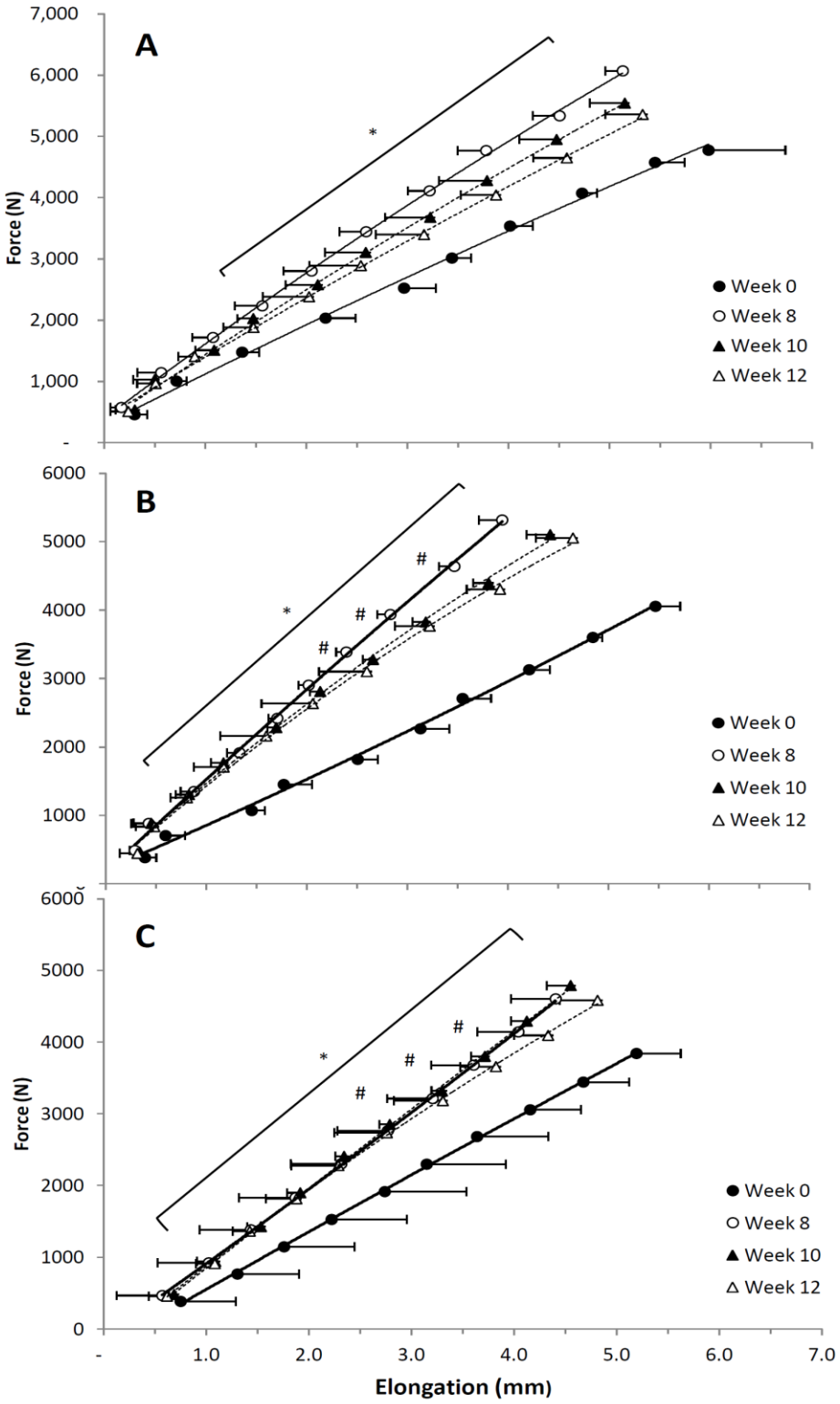

Figure 4.3. Changes in patella tendon force-elongation relationship from 10-100% MVC at baseline, week 8, week 10 and week 12 in A) MTCS, B) MTCX, and C) MTCL training groups. * Significantly different to baseline . # Significantly different to MTCS ($p<0.05$).

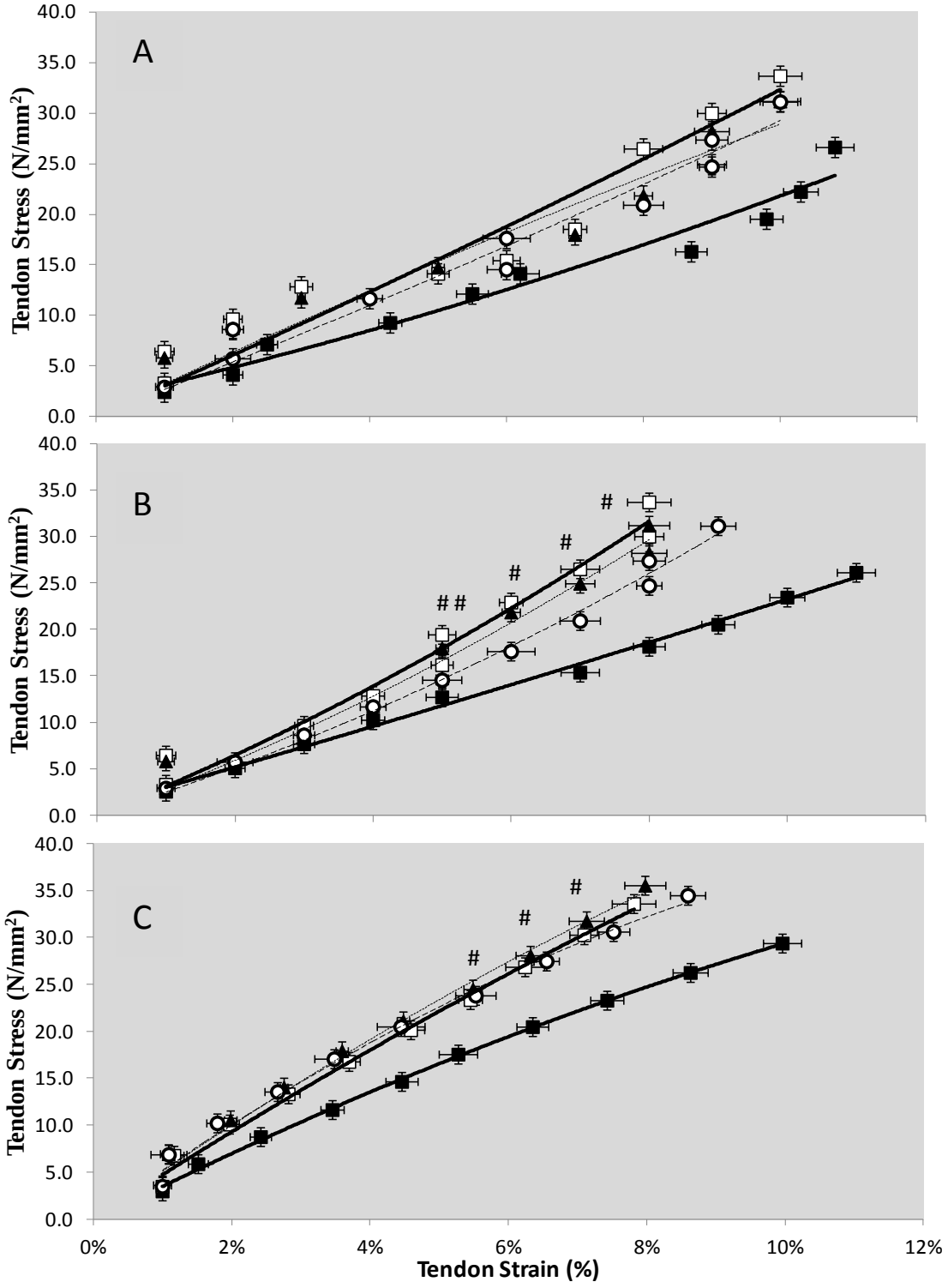

Figure 4.4. Stress- strain relationship in the patella tendon at baseline (black squares), week 8 (white squares), week 10 (black triangles) and week 12 (white circles) in MTCS (A), MTCX (B) and MTCL (C) # Significantly different to MTCS at week8 ($p<0.05$) ## Significantly different to MTCS at week 10 ($p<0.05$).

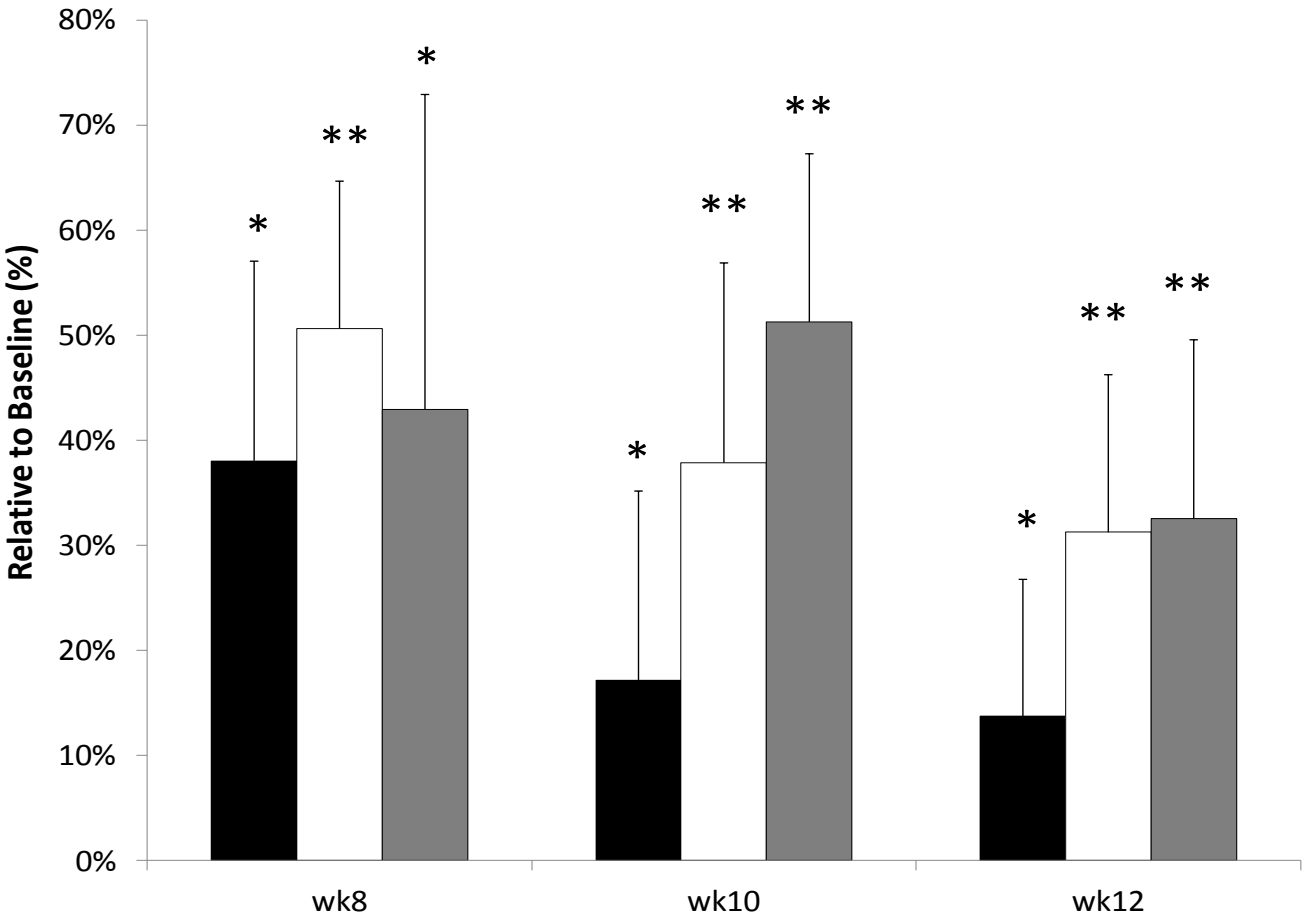

Figure 4.5. Relative changes in stiffness at standardised force (2kN) following training and detraining in SL (black bars), LX (white bars) and LL (grey bars). * Significantly different to baseline (p<0.01) ** Significantly different to baseline and SL group (p<0.05)

Table 4.7. Results of Within-Subject Contrasts following a significant (p<0.05) time*group interaction in the main outcome variables

Measure	Degrees of Freedom	F- Ratio	P Value
Stiffness	2	4.598	.049
Modulus	2	32.084	.001
Strength	2	5.178	.012

4.4 DISCUSSION:

The current study aimed to describe the changes to the muscle-tendon complex (MTC) following 8 weeks of resistance training with the MTC in a relatively shortened position (MTCS), lengthened position (MTCL) or moving from a shortened position into a lengthened position (i.e. a range-of-motion normally encountered in resistance training; MTCX). In line with our first hypothesis, the MTCL group showed increased patellar tendon stiffness following training compared to MTCS, but surprisingly MTCL and MTCX groups displayed no difference in relative training-induced increments in tendon stiffness (p>0.05). As a result we must reject our second hypothesis that on the basis of greater degree of motion and stress during loading, that MTCX would show the greatest adaptation.

- **Changes in PT mechanical & material properties**

Following resistance training, there are concurrent adaptations to both the force transmitting connective tissue i.e. tendon, as well as the contractile unit (Kongsgaard et al., 2007, Seynnes et al., 2009). Following prolonged periods of resistance training, it has been demonstrated that tendon (e.g. patella and Achilles) has the ability to adapt both morphologically (Seynnes et al., 2009, Kongsgaard et al., 2007, Arampatzis et al., 2007) and in terms of intrinsic material properties (i.e. increased stiffness and/ or Young's modulus (Reeves et al., 2003a, Maganaris et al., 2004, Kongsgaard et al., 2007, Seynnes et al., 2009, Kubo et al., 2006a, Arampatzis et al., 2007)).

As demonstrated in chapter 3 of this thesis, and previous research, resistance training mechanics (i.e. forces transmitted through the tendon) appear to be major mechanical stimuli for enhancing muscle-tendon adaptations (Kongsgaard et al., 2007, Arampatzis et al., 2007, Kubo et al., 2006a). However, to the authors' knowledge, no study has previously attempted to systematically differentiate between the impacts of load versus strain *per se* on the chronic adaptations to resistance training on the tendon properties. In the present study, to bring about the effects of stretch, the MTC during training was

positioned over two different range-of-motions. Evidence from cadaveric specimens (Visser et al., 1990) and from *in vivo* research (Herbert et al., 2002) has shown that the muscle-tendon complex is lengthened to varying levels depending on the joint-angle achieved. The lengths of the MTC during the different joint-angles used in the study participants were also measured and found that the MTC was lengthened significantly greater at 90^{o} of knee flexion more so than at 50^{o} of knee flexion, compared to full extension (Table 4.1).It can therefore be concluded that, if MTCS covered a range-of-motion of 0-50° and MTCL a range-of-motion of 40-90°, the latter would be experiencing greater MTC stretch.

Indeed, tendon stiffness was significantly greater in the current study in the MTCL group (43±11%) compared to MCTS (35±6%), (which also translates to significantly less elongation of the tendon at 30%, 70%, 80% and 90% of MVC in MTCL following training). In ligaments, cyclic stretch has been shown to increase expression of both Type I and Type III collagen, and also the expression of Transforming Growth Factor (TGF)-β1, which plays an important role in connective tissue remodelling (Kim et al., 2002). Also, Yang et al. (2004) investigated the effects of cyclical uniaxial stretching on tendon fibroblast numbers and collagen production. Fibroblasts were either stretched by 4%, 8% or not stretched (control) with both stretch frequency and duration kept constant. The results showed that tendon fibroblast proliferation only increase significantly following stretching at 8%, with Type I collagen level and TGFβ-1 mRNA levels both increasing in a stretch-magnitude-dependent manner. From this evidence, plus that presented in Table 4.1 and cadaveric data , it can be proposed that in addition to the forces produced by the knee extensors, the stretch experienced by the MTC over 8 weeks of training in MTCL has led to the accumulation of cellular responses resulting in the changes observed between the two groups in tendon properties. It is important to remember here that the muscular work performed between MTCS and MTCL was also matched (both groups covered an excursion of 50^{o}), so that the loading histories of the tendon would be very similar, and therefore not result in group differences (Seynnes et al., 2009).

In addition to the difference between MTCS and MTCL training-induced changes, there were no significant differences between stiffness and elongation measurements in the MTCL compared with the

MTCX groups (43±11% vs. 50±18%). This was somewhat surprising, as the MTCX group would have had a more extensive loading history (in terms of muscular work done; 90^{o} excursion) and also experienced greater peak tendon forces due to exercising at 80% 1RM compared to MTCL who only exercised at 55% 1RM. The fact that the combined effects of higher contraction duration, wider contraction range-of-motion and higher absolute load resulted in a lesser stiffening of the tendon compared with that where the MTC was under constant lengthening (and comparatively lower load) would support the idea that the relative contribution of stretch to tendon adaptation may indeed be more substantial than previously considered.

The shapes of the tendon curves displayed in the current study are slightly more linear in fashion, with a less distinct 'toe' region in the force-elongation relationship (see Figure 1.16 – chapter 1). The shape of the curve in the 'toe' region reflects changes at the fibre level, with the crimping pattern of the tendon fibres becoming straightened under relatively low force or strain, and is evident in both males and females. However as each of the training groups were composed of both males and females, differences between absolute levels of force and absolute tendon elongation meant that when the individual curves in each group were combined to produce a mean curve, the toe regions were less distinctive (i.e. curvilinear). Onambele and Pearson (2012) showed that in older females, greater increases in tendon stiffness following resistance training at ~10-40% MVC (i.e. lower force levels), whereas the older males increased tendon stiffness to a greater degree at levels above ~40% MVC. Therefore at baseline and following training, the current linear shape of curves may have been produced due a chronic stiffening adaptation to habitual activity (at baseline) or the resistance training program, in females at lower force levels compared to the males at the higher force levels.

Furthermore, the curves in the current study are second-degree polynomial curves forced through zero, and therefore will not show the same shape as a point-by-point mean curve fit.

- **Changes to PT dimensions**

There were no changes in patella tendon morphology following training or detraining in any of the training groups or control groups, which is in agreement with another study measuring PTCSA proximally, centrally and distally by ultrasound (Reeves et al., 2003a). Other previous studies have reported region-specific increases in patella tendon cross-sectional area following prolonged resistance training (Kongsgaard et al., 2007, Seynnes et al., 2009). Kongsgaard et al. (2007) measured tendon CSA at proximal, mid and distal areas along the length of the patellar tendon by MRI, with the knee placed at 90^{o} flexion which is similar to the current study. Differences may have arisen due to a number of reasons. Firstly, there may have been very minute discrepancies at the exact intervals of where the measurement sites occurred. They measured PTCSA proximally just distal the patellar insertion, just proximal to the tibia insertion, and mid-way between those two points. In the current study PTCSA was measured at 25%, 50% and 75% of total tendon length (apex of patella to tibial tuberosity). On inspection the regions appear to be in a very similar area along the patella tendon length, however the research of Seynnes et al. (2009) using MRI which shows that along the length of the tendon , there were only five of ten discrete areas in which there was evidence of region-specific hypertrophy measured. This highlights the intricate nature of the measurement, and the degree to which small inter-study differences in methods could lead to contradicting findings. Indeed, Seynnes et al. (2009) report significant region specific hypertrophy very close to the mid-region of the tendon. Furthermore, other tendon regions found to have undergone hypertrophy (in those studies that report such an effect) also appear extremely close to the areas measured in the present study.

Another confounding factor when comparing the studies is the sex of the participants involved. The current study used an approximately equal number of both males and females, whereas the two aforementioned studies reporting hypertrophy used males only. Considering that for example Miller et al.

(2005) showed that following exercise, women do not have the same elevated levels of protein synthesis rate of tendon collagen. Furthermore it has also been reported that patella tendon CSA is significantly greater in well trained male runners compared to untrained counterparts, but there is no difference between well trained and untrained females (Magnusson et al., 2007), it therefore is possible that a region-specific hypertrophy, if present, may have been in part concealed owing to a gender effect (see *Appendix 1* for a gender comparison).

- **Effects of detraining**

Detraining resulted in a reduction in tendon stiffness in MTCS (-12%) and MTCX (-7%) groups compared to week 8, following 2 weeks of detraining, with absolute values remaining superior to baseline measures. Interestingly, the MTCL group relative stiffness increased further by 11% compared to week 8 following two weeks detraining. This is reflected in figure 4.3, where at week 10, elongation of the tendon is virtually identical to week 8 although the force through the tendon is actually slightly higher, therefore reflecting a stiffer tendon. Following a further two weeks of detraining (week 12), MTCL stiffness had reduced by 19% which was the greatest relative loss during the detraining out of any other training groups, whereas MTCS and MTCX displayed identical relative reductions in stiffness of 11% in the last two weeks. By week 12, the magnitude of increases in tendon stiffness relative to baseline was very similar between MTCL (35±14%) and MTCX (32±11%) groups, which again highlight the effectiveness of performing under the lengthened condition.

Our current data regarding the effect of an acute period of detraining on tendon properties is in support of the findings of de Boer et al.(2007) who showed that during 23 days of unilateral lower-limb suspension in younger men, stiffness and Young's modulus decreased significantly after only 14 days, and accelerated during the next 9 days, hence describing a temporal pattern to losses in tendon mechanical properties. However this temporal response only appeared in the MTCL and MTCX groups, who

displayed much greater relative decreases following cessation of training. From days 14-28 (i.e. week 10 to week 12) both of these groups experienced larger changes in tendon stiffness than the initial 14 days of detraining (i.e. week 8 to week 10). MTCX showed an initial -7% reduction in stiffness, followed by a greater -11% reduction in the second period of detraining, whereas MTCL, as mentioned previously, actually increased stiffness +11% in the initial period of detraining, experienced a large -19% reduction in the following 14 days. Kubo et al. (2010) reported a linear decrease in tendon properties (as seen in MTCS) following 3 months of training and proceeded by 3 further months of subsequent detraining (tendon properties measured every month). The current findings are in agreement with this study in that tendon properties are still enhanced compared to baseline following one month of detraining, however because tendon properties were only assessed following one month detraining Kubo et al. (2010), it is still possible that a temporal pattern may have eventually formed into a linear pattern by that stage. It is suggested from the current results that tendon properties may be modulated depending on the degree of the initial adaptations to resistance training i.e. greater increases are lost more rapidly.

- **Changes to torque-angle relationship and strength**

Torque levels increased significantly at all angles (except 60^{o}) in MTCL but only at four of the seven angles measured in MTCS. Previous data from studies involving isometric training has indicated that strength training adaptations are joint-angle specific (Thepaut-Mathieu et al., 1988, Kitai and Sale, 1989, Kubo et al., 2006a), and that training at a more flexed knee angle (100^{o}) length resulted in significant changes in MVC over a greater range of angles (40^{o}-110^{o} of knee flexion) compared to a more extended knee angle i.e. 50^{o} (40^{o}-80°). What is also interesting from the data in Figure 4.1 is that the MTCL group increased strength significantly at 30^{o} of knee flexion, despite not encountering this specific joint angle during the training excursion (i.e. 40-90^{o}). This is in contrast to MTCS who did not increase torque levels at this angle despite actually dynamically passing through the joint angle during the training excursion (i.e.0-50^{o}). Furthermore, MTCX group had also significantly increased MVC at 30^{o} following training, again only passing dynamically through the joint angle. This study reaffirms the consensus from isometric

training studies, that there is a less marked effect of joint-angle specificity when training occurs with the muscle (or MTC) in a relatively lengthened position compared to a relatively shortened position (Kubo et al., 2006a, Thepaut-Mathieu et al., 1988). Another relationship that was evident following training was the change in the torque-angle relationship in MTCS, but not in MTCL or MTCX. Whilst, the mechanisms for such observations remain unclear, we propose below, an argument for the likely modulators of the observed changes. Concentric training has also been purported to alter the operating range of muscle (e.g. (Lynn et al., 1998)), with a left-shift on the length-tension relationship of muscle. Conversely, an increase in tendon stiffness would decrease sarcomere shortening, shifting the length-tension relationship to the right. The VL muscle has been shown to operate over the ascending (<70°), plateau region (70°) and descending limbs of the length –tension relationship (Ichinose et al., 1997). In the MTCL and MTCX, peak torque occurred at 70^{o} (plateau region) before training, therefore an increase in K would have theoretically resulted in a shift toward the descending limb ($>70^{o}$). However, in the same group of subjects, in chapter 3 significant increases in fascicle length were observed (possibly indicating serial sarcomerogenesis in MTCL and MTCX). This may explain why there was no change in angle of peak torque following training, as the two adaptations to training may have mitigated each other allowing for the muscle to remain operating over the same portion of the length-tension relationship (Reeves et al., 2004c). In MTCS, the optimal angle of torque production was 75^{o} pre-training and 70^{o} post-training. Due to training at a shorter muscle length for an extended period, the muscle may have adapted in such a way as to produce higher forces (and keep individual sarcomeres operating around the plateau region) closer to the angle at which the muscle has been routinely operating, despite increases in tendon stiffness which would result in a shift towards longer muscle lengths. The current data implies that when designing resistance training programs for sports performance, it is important to consider resistance training kinematics to bring about specific adaptations that could influence the torque-angle relationship.

Conclusion

Training with the MTC in a lengthened position appears to enhance the improvements in the mechanical properties of the muscle-tendon unit to a greater degree than training in a shortened position. Interestingly, the adaptations to lengthened-position resistance loading are in fact comparable to those where the entire range-of-motion (and in fact higher loads) is used, and the effects are associated with adaptations to both tensile properties of the in-series tendon and the torque-angle relationship. It is proposed that such adaptations result from a difference in the magnitude of mechanical stretch owing to the relative difference in length of the complex.

CHAPTER 5:

Effects of acute & prolonged Resistance Training & Detraining on Muscle Electromyographical & cardiovascular responses

5.1 Introduction:

Frequent adaptations that are sought from resistance exercise regimes include an increase in muscle cross-sectional area (CSA) and strength (Maughan et al., 1983), alterations to muscle architecture (spatial arrangement of muscle fibres within a muscle (Kawakami et al., 1995)), and greater maximal activation of the musculature (Moritani and DeVries, 1979).

Muscle activation has been widely assessed using surface electromyography (sEMG), and in many cases is expressed as a relative level (%) of maximal voluntary contraction (or MVC). It comprises the sum of the electrical contributions made by the active motor units in proximity to the measurement site. The global characteristics of the surface EMG, such as its amplitude and power spectrum, depend on the membrane properties of the muscle fibres as well as on the timing of the single fibre action potentials. Thus the surface EMG reflects both peripheral and central properties of the neuromuscular system (Farina et al., 2004). For many muscles, optimal firing rate, which is that elicited by a maximal voluntary effort, is sufficient to generate a fused tetanus in individual motor units. In predominantly fast-twitch muscles (e.g. biceps brachii), this firing rate is ~30Hz whereas in predominantly slow-twitch muscles (e.g. soleus), this firing rate is ~10Hz (Bellemare et al., 1983). This electromyographic signature is warranted in order for the muscle to express its maximal force generating capabilities, and there have been many studies carried out that have reported a significant increase in agonist sEMG recordings following a resistance training program in both males and females, and in the young as well as the elderly (Häkkinen et al., 1996, Narici et al., 1989, Häkkinen et al., 2003, Komi et al., 1978, Moritani and DeVries, 1979, Häkkinen and Komi, 1983, Reeves et al., 2005b, Higbie et al., 1996). As

mentioned previously, muscle adapts in a specific manner to the stimulus provided, and in the case of the aforementioned studies, increases in agonist muscle activation has been shown to be specific to the mode of muscular contraction employed during the resistance training period, and has been fairly well characterised. It is however unclear whether chronic changes in the magnitude of the EMG signal occur with training.

However, one aspect of resistance training that is scarcely reported in the literature is the acute (and/ or chronic) responses to resistance training programs where the length of the muscle when it is loaded is being manipulated. Acutely, it has been demonstrated that there are significantly different responses to exercise at different joint-angles (and thus different muscle lengths). De Ruiter et al. (2005) showed that following isometric MVC exercise at 30^{o}, 60^{o}, and 90^{o} of knee flexion, maximal activation of the knee extensors was significantly greater at 90^{o} than the other two angles, despite having identical torque production as 30^{o} (90^{o}; 199±22Nm, 30^{o}; 199±29Nm) and significantly lower torque production than 60^{o} (298±41Nm). A subsequent study (Kooistra et al., 2006) found that maximal muscle oxygen consumption was reached significantly later, and was on average ~60% less at 30^{o} compared to 60^{o} and 90^{o} knee flexion. Furthermore, Hisaeda et al.(2001) found that when performing isometric contractions at 50% MVC to failure at either 50^{o} or 90^{o} of knee flexion, endurance time was significantly shorter at 90^{o} than 50^{o}. This effect was present both when the exercise was performed with the local circulation occluded and not occluded, thereby highlighting local events as being key to the performance of the musculature at discrete knee angles (or muscle lengths). In addition to this, the slope of the iEMG-time regression was significantly greater in the 90^{o} condition compared to 50^{o}. It is proposed that one of the reasons for an increase in oxygen consumption at longer muscle lengths (or more flexed

joint angles) is that to produce the same external torque, the internal mechanical stress must be higher at more flexed angles (90^o) compared to extended angles (30^o or 50^o) because the moment arm of the in-series elastic component (i.e. the distance between the tendon and the joint centre of rotation, a factor which impacts on the forces required at the muscle) is shorter (Krevolin et al., 2004) at more flexed angles. The above studies provide compelling evidence of the link between the muscle length during a bout of resistance exercise and the acute impact on muscle activation levels, energetic provision and fatigability, and torque production. It has therefore been important to determine the nature of the acute effects of length-specific training because it is the accumulation of the acute responses that ultimately are reflected in the chronic muscle adaptations (known as the repeated bout effect; RBE).

As demonstrated in chapter 3 and 4 of this thesis, training over a longer muscle lengths and at a limited range over longer muscle lengths results in greater gains in muscle size, strength and tendon mechanical properties. Previous investigations have also identified the link between muscle length (or joint-angle) and gains in strength and/ or levels of muscle activation following a more extended period of resistance training (Lindh, 1979, Gardner, 1963, Thepaut-Mathieu et al., 1988, Kitai and Sale, 1989, Kubo et al., 2006a). Briefly, these studies have shown that significantly greater increases in isometric strength are attained when tested at the training length or position, and that these changes in strength are accompanied by significantly greater activation of the muscle in the training position. Furthermore, several studies have outlined that at shorter muscle lengths, the phenomenon of length-specific adaptations are more marked compared to those at longer muscle lengths (Kubo et al., 2006a, Thepaut-Mathieu et al., 1988). For example, performing resistance training at a shorter muscle length results in increases in strength at, and

close to the training muscle length, whereas training at longer muscle lengths results in strength increases at, and around a larger range of muscle lengths. However, all of the above information is provided via controlled isometric (static) contractions, when indeed resistance training programs for most individuals are predominantly of a dynamic nature, and therefore warrants further research to extend the knowledge in this area. Therefore the aims of the body of work presented here were:

1. To describe the acute differences in activation (RMS-EMG relative to max RMS-EMG during MVC) of the *Vastus Lateralis* (VL) muscle whilst performing dynamic resistance exercise over relatively short muscle lengths (SL) compared to long muscle lengths; here comparisons were carried out a) where the external 'perceived' workload is matched (LX), and b) when the internal workload is systematically matched between the two training modalities (LL).

2. To describe the changes in oxygen consumption and cardiovascular responses during these exercise protocols.

3. To describe the more chronic changes in VL activation and *biceps femoris* co-contraction following 8 weeks of resistance exercise and 4 weeks of detraining

5.2 Methods:

Acute; VL muscle electromyographical activity was assessed by surface electromyography. Following preparation of the skin, electrodes were attached to the *vastus lateralis* (knee extensor) and *biceps femoris* (BF - knee flexor) muscles. Oxygen consumption was measured via the Douglas bag method with cardiovascular responses assessed by heart rate analysis and blood pressure monitoring. Participants performed multiple sets of standard barbell back squats over shorter and longer muscle lengths whilst undergoing absolute loading of 20Kg, 40Kg and 60Kg and also relative loading of 40%, 60% and 80% of 1RM. For more details see chapter 2.

Prolonged responses; VL and BF activation and co-contraction were assessed at baseline, week 8 (pos-training), week 10 (detraining 1) and week 12 (detraining 2)

Results:

5.3.1 Acute training Responses - *Muscle Activation***;** As expected *vastus lateralis* activation increased linearly with absolute external load, with activation being significantly greater ($p<0.05$) when lifting 40Kg compared to 20Kg, and also when lifting 60Kg compared to 40Kg ($p<0.05$) and 20Kg ($p<0.001$). When comparing activation between ranges-of-motion as a percentage of MVC, activation of the muscle was significantly ($p<0.05$) less at SL (59±6%, 63±7%) compared to LL (73±7%, 77±5) and LX (70±7%, 75±6%) at 40Kg and 60Kg loads respectively (Figure 5.1A). During relative loading, performing exercise at 60% 1RM did not

increase activation compared to 40% 1RM ($p>0.05$) at any ROM, though activation was increased at 80% 1RM compared to 60% ($p<0.05$), and 40% ($p<0.001$). There were no significant differences in activation at 40% and 60% 1RM between the three ranges-of-motion ($p>0.05$), whilst at 80% 1RM, VL activation was significantly greater during exercise in LL and LX compared to SL (Figure 5.1B; $p<0.05$). It is notable that these effects were similar for all two 'long muscle' training protocols so that there were no significant differences between the longer muscle length ROM and the complete ROM under any loading conditions ($p>0.05$).

Oxygen Consumption (VO_2); There were no significant changes in VO_2 between any of the absolute loading conditions or between any ROM ($p>0.05$). Furthermore, in the relative loading conditions, mean VO_2 was significantly greater at 80% 1RM compared to 40% 1RM (6.4±0.9 $ml \cdot kg^{-1} \cdot min^{-1}$ vs. 9.93±1.3 $ml \cdot kg^{-1} \cdot min^{-1}$, $p<0.05$). VO_2 was greater at 40% and 60% 1RM in LL and LX than SL, however there were no significant differences between these ROMs. At 80% 1RM there was a significantly greater VO_2 (Figure 5.2) in the LL ROM compared to SL ($p<0.05$), however there were no significant differences between LL and LX, or SL and LX at this loading intensity ($p>0.05$).

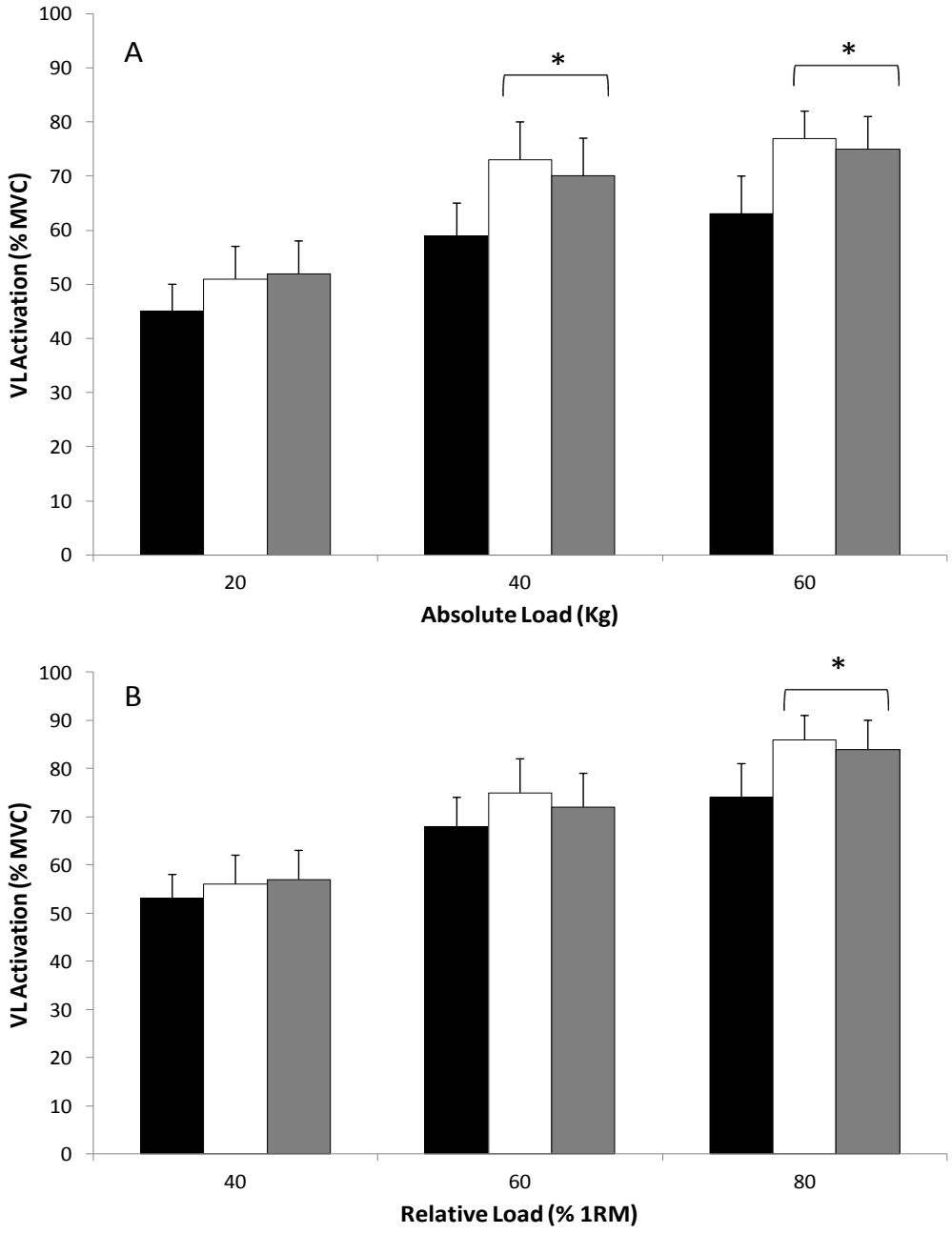

Figure 5.1. Vastus Lateralis muscle activation in SL (black bars), LL (white bars) and LX (grey bars) following varying magnitudes of absolute and relative loading. * Significantly different to SL ($p<0.05$).

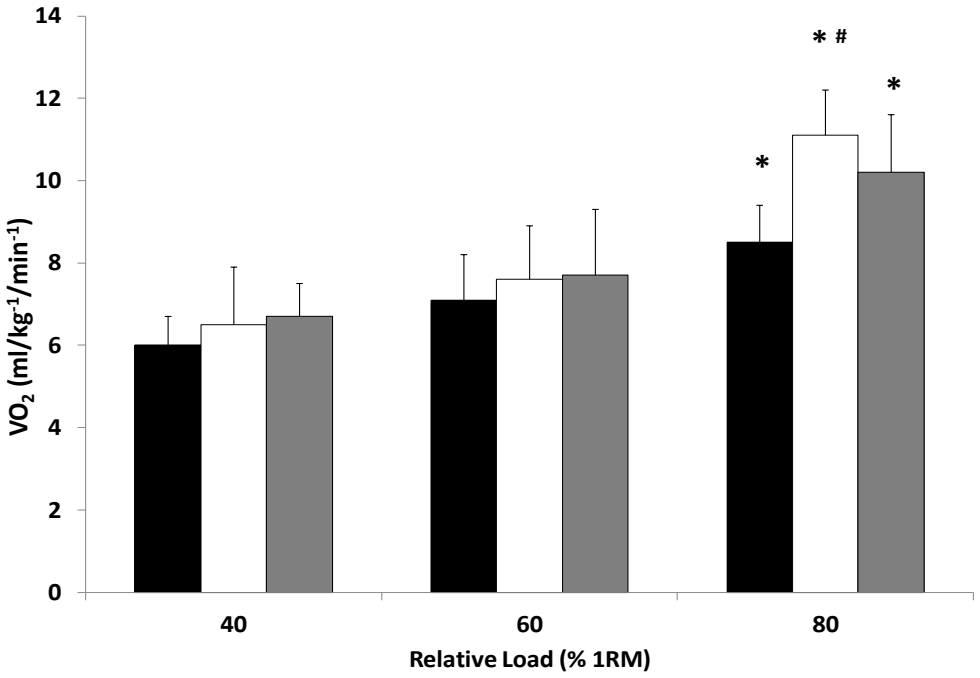

Figure 5.2. Oxygen consumption (VO_2) during relative loading in SL (black bars), LL (white bars) and LX (grey bars). * Significantly different to 40% 1RM. # Significantly different to SL.

Heart rate & Blood Pressure; There was a significantly greater ($p<0.05$) mean heart rate difference between LL (139±10 beats per minute) and LX (136±11bpm) compared to SL (118±12bpm) in both absolute and relative loading conditions, with no difference between LL and LX ($p>0.05$). Mean systolic blood pressure yielded no significant differences ($p>0.05$) between the three ROMs under relative loading conditions, however LX (148±8 mmHg) mean systolic blood pressure was significantly greater than both SL (138±6 mmHg) and LL (135±8 mmHg) following loading under absolute loads ($p<0.05$). There were no significant differences in diastolic pressure between the ROMs ($p>0.05$). For a brief look at vascular adaptations to prolonged training in SL and LX groups please refer to *Appendix 2.*

5.3.2 Prolonged Resistance Training Responses

Agonist (VL) Muscle Activation; Figures 5.3 shows absolute (i.e. raw RMS-EMG signal) and relative (i.e. RMS-EMG normalised for values at baseline - Figure 5.4) changes in muscle activation at baseline and post-training. At week 8, absolute maximal agonist activation did not increase significantly in a chronic response to the training protocols, with no significant difference between training groups at any knee angle. There was also no significant changes observed in the control group ($p>0.05$).

However, on further investigation, it was found that in fact, post-training there was a significant relative increase in maximal activation at 50^o (23±15%, $p<0.05$), 70^o (26±15%, $p<0.01$) and 90^o (16±13%, $p<0.05$) in the LX group and at 70^o (24±9%, $p<0.01$) and 90^o (25±9%, $p<0.01$) in LL group. In the SL group there was no significant change at 50^o, although there were significant ($p<0.05$) reductions in VL activation at both 70^o (-15±6%) and 90^o (-13±5%).

Following detraining, muscle activation at 70^o decreased at week 10, and levelled off for the remainder of the detraining period (week 12) in both LL and LX groups with no significant changes compared to week 8. In the SL group, activation reduced at both weeks 8, 10 and 12 compared to baseline, however despite larger decrements in this group, there was no significant differences between all three training groups (Figure 5.5, $p>0.05$). The control group displayed no significant ($p>0.05$) relative changes in muscle activation at any joint angles during the training and detraining periods (Figure 5.4).

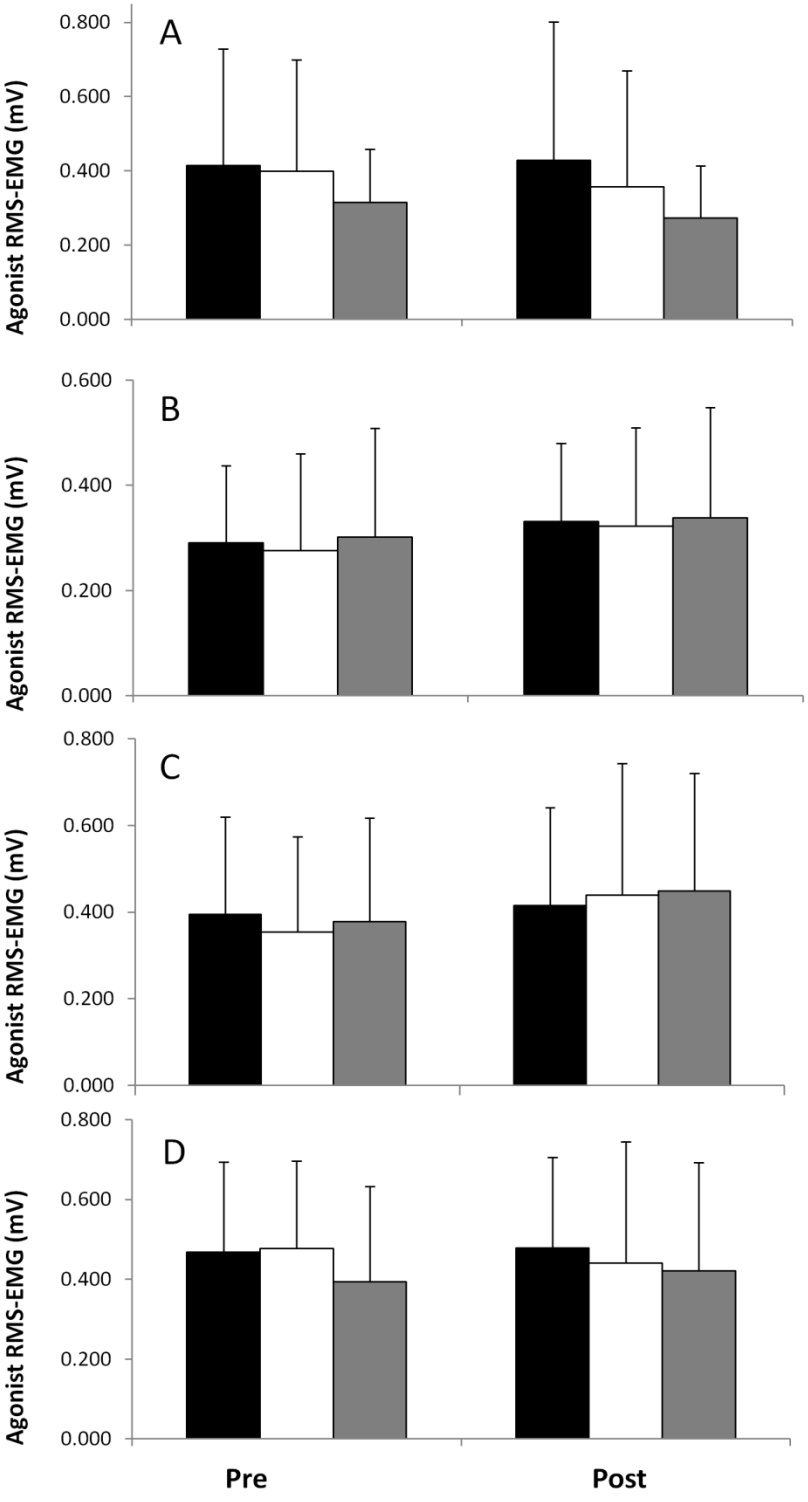

Figure 5.3. Absolute Changes in VL MVC at baseline (pre) and week 8 (post) training at 50^{o} (black bars), 70^{o} (white bars) and 90^{o} (grey bars) knee flexion in A) SL, B) LX, C) LL and D) Control groups.

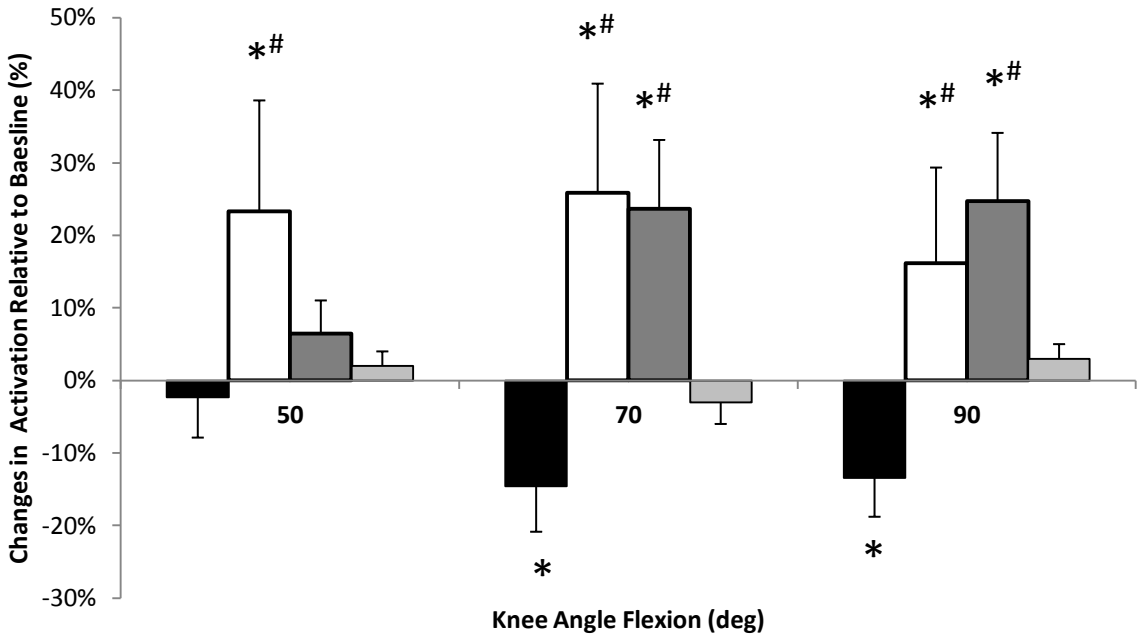

Figure 5.4. Relative changes in VL MVC at week 8 at three knee joint-angles in SL (black bars), LX (white bars), LL (dark grey bars) and controls (light grey). * Significantly different to baseline. # Significantly different to SL group.

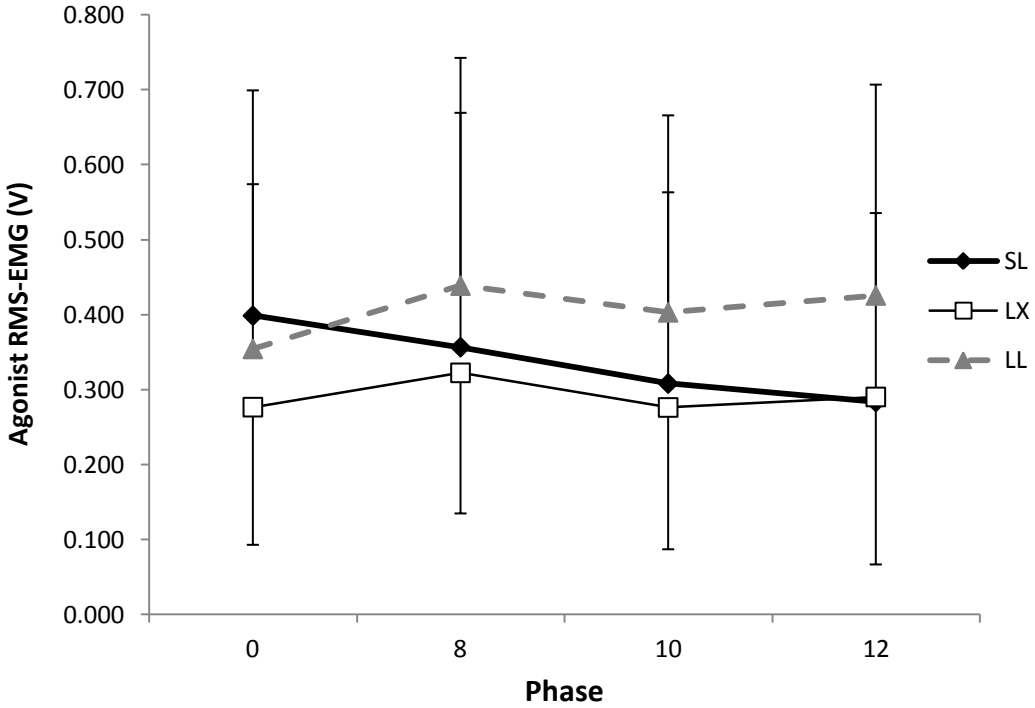

Figure 5.5. Absolute changes in VL MVC throughout training and detraining periods at 70^{o} knee flexion. No significant were detected between phases or training groups ($p>0.05$).

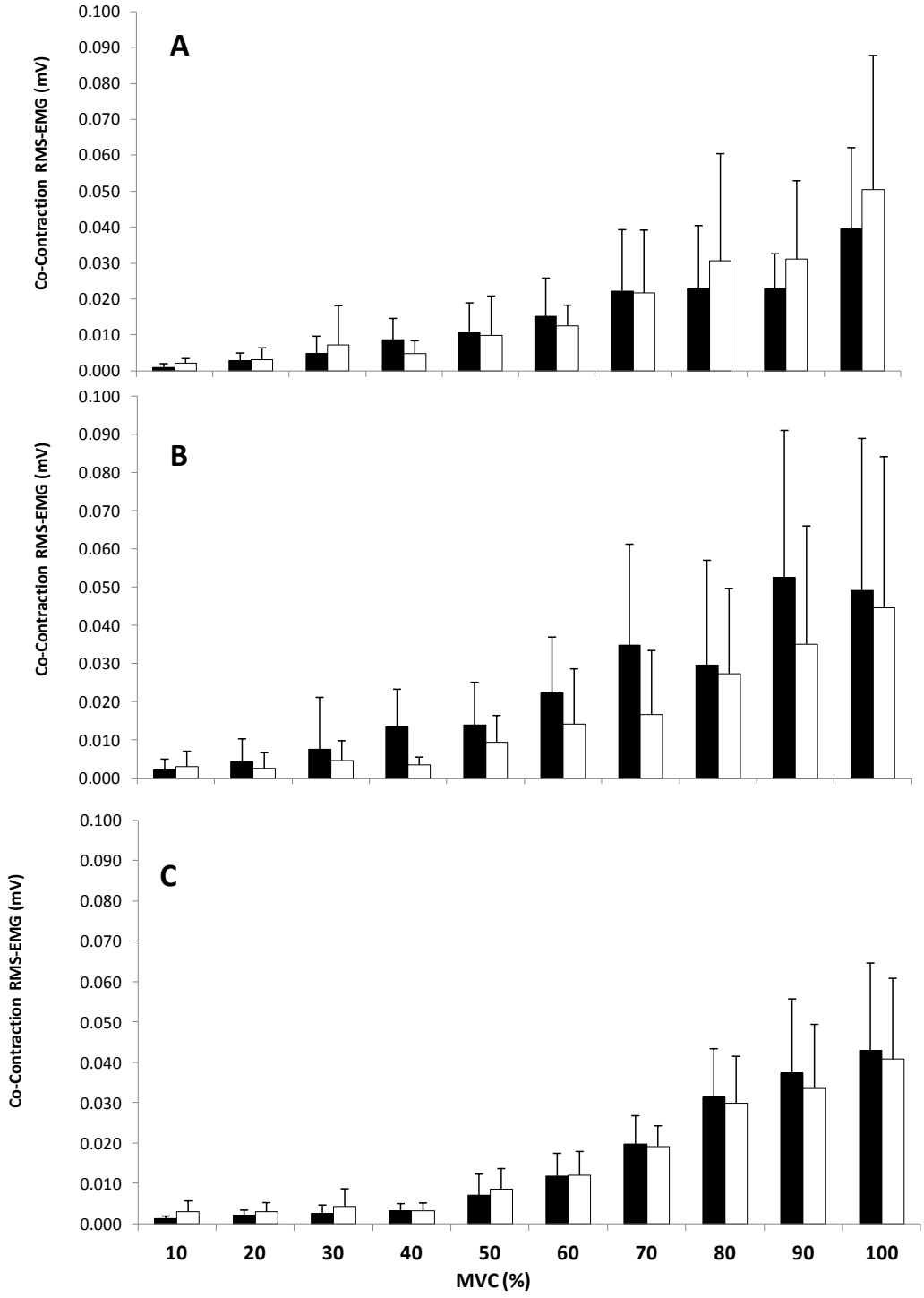

Figure 5.6. Biceps Femoris co-contraction at baseline (black bars) and post-training (white bars) at various force levels in SL (A), LX (B) and LL (C) groups.

Co-contraction – There was a significant difference in antagonist co-activation between force levels ($p<0.01$), with force levels ≥70% MVC greater than those a 10% MVC. Following the resistance training programme there were no significant changes in antagonist co-activation levels at any force level when compared to baseline in training groups (Figure 5.6) or control group ($p>0.05$).

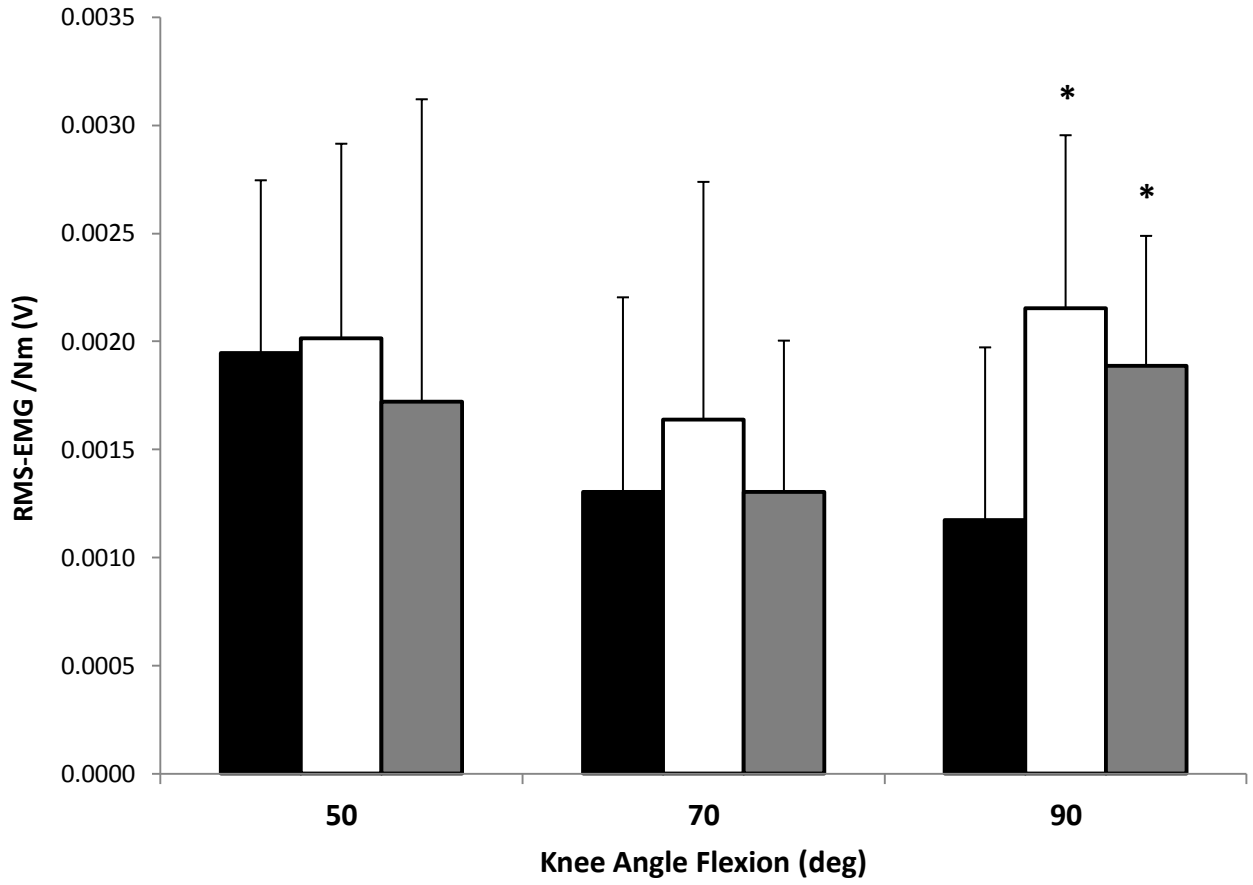

Figure 5.7. Vastus Lateralis muscle efficiency (i.e. activation per unit of torque) at week 8 at three joint-angles in SL (black bars), LL (white bars) and LX (grey bars). * Significantly different to SL ($p<0.05$). N.B. there were no between group differences at baseline.

Discussion:

Resistance training presents a medium through which muscular function can be enhanced. In order to devise an appropriate and effective resistance training program tailored with functional and structural enhancement objectives, it is necessary to understand the responses to both an acute bout of exercise, and the adaptations to exercise over a prolonged period of training. An important aspect for muscular performance is the degree to which the muscle can be activated. Previous work using isometric contractions of the knee extensors, has demonstrated that the magnitude of maximal muscle activation is dependent on the joint-angle (and thus muscle-length) used during exercise, even when external torque produced is maintained at a similar level at the different joint angles (de Ruiter et al., 2005). This earlier study showed that activation of the quadriceps is significantly greater at 90^{o} knee flexion compared to both 30^{o} and 60^{o}, despite isometric MVC torque being significantly less than 60^{o} and identical to 30^{o}. In the current study, unlike the isometric training of previous research, our participants exercised dynamically over a range-of-motion that was predominantly over shorter muscle lengths ($0\text{-}50^{o}$), longer muscle lengths ($40\text{-}90^{o}$) or over both short and long muscle lengths ($0\text{-}90^{o}$) using absolute and relative loading patterns.

During absolute loading, weight lifted increased in a graded manner, and was reflected by significantly increased muscle activation between each absolute load in the training groups. This result was a more easily predicted outcome and reflects one of the fundamental properties of the neuromuscular system, i.e. the size principle (Henneman et al., 1965), where a greater number of

motor units are recruited in order to meet the increasing demands of force production. When exercising over longer muscle lengths (LL) and the complete ROM (LX), muscle activation was significantly greater during absolute and relative loading compared to shorter muscle lengths (SL). So why would a muscle exhibit greater activation whilst moving the same external weight but at different muscle lengths?

By moving through a range of muscle lengths or joint-angles, the moment arm of the in-series elastic component (i.e. the tendon) also changes. As the amount of force needed to lift an external load (F) is $F = f \times d$, where f is the internal force produced by the muscle and d is the length of the moment arm, when d is greater f will be smaller and vice versa, and therefore when the external force produced is the same but the moment arm (d) is smaller, the contribution from internal muscle force production increases. An example of this experienced in daily living is the increased difficulty in rising from a low seat position compared to a higher seated position (however this is also reflective of the length-tension relationship). It has been demonstrated previously that when the joint-angle in the knee extensors is at 90^o flexion (such as the end of LL and LX group ROM), the moment arm is considerably shorter (Krevolin et al., 2004) than when at 50^o (the end of SL ROM). Therefore when exercising at 90^o, internally the muscle must produce a greater amount of contractile force to overcome the external weight than that required at 50^o knee angle. Again due to the overloading principle of training response, a larger number of motor units will have needed to be recruited to match the force demands at the longer muscle lengths, reflected by the increase in RMS-EMG activity of the VL muscle.

In support of this hypothesis, Kubo et al. (2006a) trained the knee extensors isometrically at either 50^o or 100^o of knee flexion. Based on their MVC and EMG recordings, they estimated that

the internal force on the quadriceps muscles was 2.3 times greater at longer muscle lengths (i.e. 100^{o}) than at shorter lengths.

A further variable that must be considered is the influence of changing muscle lengths on the force-length relationship of muscle (for review see (Rassier et al., 1999)). In short, when one alters the length of a muscle, the basic contractile units of individual muscle fibres, known as sarcomeres, also change length. The ability of sarcomeres (and thus muscle) to exert force is determined mainly by actin and myosin filaments interaction and cross-bridge formation. As sarcomere (or muscle) lengths increase, cross-bridges number and force is increased up to an optimal length. Beyond this length (i.e. with further lengthening), decreases cross-bridges formation and force are seen (NB. The caveat here lies with contractile speed, and preceding type and degree of muscle contraction (Onambele et al., 2004)). If longer muscle lengths are less optimum muscle lengths for force production and cross-bridge formation than shorter muscle lengths, then greater motor unit recruitment will be necessary to overcome the external resistance. Therefore the two factors likely for greater activation in LL and LX compared to SL may be due to the greater internal mechanical stress on the muscle because of a shorter moment arm, and/ or the length of the muscle reducing cross-bridge formation and force production per sarcomere, all other factors (contraction type, speed and history) being equal.

In the current study, oxygen consumption (VO_2) was shown to be significantly greater at 80%1RM compared to 40% 1RM, and also significantly greater in both the LL and LX ROMs compared to SL ROM at 80% 1RM loading. VO_2 is used in exercise physiology as an indicator of energy expenditure. Oxygen is the terminal electron acceptor in mitochondrial oxidative phosphorylation and determines whether pyruvate enters the Krebs cycle during the resynthesis

of ATP for use in the cross bridge cycle. An increase in VO_2 represents a greater demand for the resynthesis of ATP and therefore a greater calorific expenditure through an increase in the energy substrates (glucose, and indirectly fat and protein) used in ATP resynthesis. Therefore, an increase in VO_2 during training represents an increase in intensity and therefore calorific expenditure. This has implications for developing training guidelines regarding ROM. As it was previously demonstrated in chapter 3, training over longer muscle length results in greater reductions in subcutaneous fat than over shorter length, and therefore calorific expenditure may also be used as a training goal at longer muscle lengths. It has been demonstrated previously in humans that oxygen consumption increases with work intensity during constant isometric loading of the knee extensors (de Ruiter et al., 2005, Kooistra et al., 2006). This is reflected by the increased VO_2 at 80% 1RM compared to 40% 1RM, where although performing the same ROMs, participants were exerting greater force, requiring more energy to supply muscular contraction. The relative VO_2 levels are much lower than normally encountered during aerobic exercise for example, due to the shorter duration of exercise bouts and greater contribution to energy supply from anaerobic sources such as ATP-PCr system and glycolysis.

Of more interest in the present study was the fact that both LL and LX ROMs had significantly greater VO_2 compared to SL at 80% 1RM. Previous research using near-infrared spectroscopy has demonstrated that during isometric exercise of the knee extensors, VL muscle VO_2 is significantly increased at longer muscle lengths (60^o and 90^o) compared to shorter muscle lengths (30^o). This was even despite the fact that MVC torque relative to the maximum torque capacity (MTC) tended to be greater (~85% of MTC) at 30^o compared to both 60^o and 90^o (~75% MTC). A subsequent study by Kooistra et al. (2006) demonstrated that knee extensor muscle activation

and VO_2 were significantly less, and time to VO_{2max} significantly longer at the same relative torque levels at 30^o compared to 60^o and 90^o. An additional indication of the increased stress at longer muscle lengths was the observation that at 80% 1RM, although covering almost half the ROM of LX, LL group showed a trend (though not statistically significant) to consuming greater volumes of oxygen. This suggests that the energetic cost of constantly working at longer muscle lengths is at least just as, if not more demanding than, alternating between longer and shorter muscle lengths even when over a relatively large ROM. With significantly higher heart rates in both LL and LX groups (and also greater blood pressure in LX) compared to SL during exercise, the results also suggest that the cardiovascular system was also under greater stress at longer muscle lengths. Taken into consideration with both the aforementioned differences between the LL and LX groups compared to SL with regards muscle activation and oxygen consumption, it appears that performing exercise over predominantly longer muscle lengths (or incorporating longer muscle lengths into a full ROM) present a more potent stress to both neuromuscular and cardiovascular systems than performing exercise over mainly shorter muscle lengths. So what factors are present that would require greater oxygen consumption at longer muscle lengths?

First of all, as mentioned previously, there are a number of processes that occur in order for a muscle to contract and produce force. One such process has been termed excitation-contraction coupling, where an action potential induces the release of calcium ions (Ca^{2+}) from the muscle membrane (sarcoplasmic reticulum) and these ions interact with the thin filaments of a sarcomere, allowing muscle contraction to occur. Ca^{2+} ions are then transported back into the sarcoplasmic reticulum for storage, allowing the muscle to relax. These processes are ATP-dependent (i.e. energy consuming) and as energy is consumed during activation, the amount of

energy is measured as heat (Homsher et al., 1972). Therefore if greater activation of the muscle is occurring at longer muscle lengths, the possibility exists that the energy cost of this activation is also greater, and that this mechanism requires greater oxygen consumption to supply the energy. In addition, potentiation is force enhancement following muscle contraction, and is dependent on the contractile history. Place et al.(2005) showed that following fatiguing contractions in the quadriceps muscles at either shorter (35^{o}) or longer (75^{o}) muscle lengths, peak twitch potentiation and doublet force were significantly greater at shorter muscle lengths, which may also allow for a reduction in energy cost as activation may be reduced. Secondly, we have already discussed the likelihood that due to the internal architecture of muscles and tendons, that the length of the moment arm will dictate that greater muscle force will have to be produced at longer muscle lengths compared to shorter muscle lengths. Production of the additional force through recruitment of more motor units would mean that more of the contractile machinery would be used and be consuming energy, as muscular contraction from cross-bridge cycling also requires ATP (Huxley, 1957, Huxley and Simmons, 1971). Therefore the additional oxygen consumption observed at longer muscle lengths may be the result of both the energetic requirements of muscle activation and the increased energetic requirements of force production. This hypothesis is consistent with the fact that endurance performance is significantly reduced with time to fatigue at longer muscle lengths compared to shorter muscle length , regardless of of the intensities of loading and circulatory conditions (Hisaeda et al., 2001, Place et al., 2005, Ng et al., 1994). Consistent with an increased oxygen demand, would be an increase in heart rate which was observed between the groups.

When exercise is performed on a regular basis, the above acute responses to a bout of exercise will eventually result in long-term adaptations, which will allow the body to complete the same exercise bout as before but with relatively less disturbance to homeostasis. During the resistance training program, the three groups performed exercise over the same range-of-motion as during the acute bouts (i.e. SL, LL and LX), with the only differences being the degree of loading. SL and LX exercised at 80% 1RM, whereas LL exercised at 55% 1RM, where this was to allow the length of muscle excursion (50^{o}) and the internal muscle forces to be as similar as possible during resistance training between SL and LL.

Following 8 weeks of resistance training, absolute changes in muscle activation did not increase significantly at any of the angles tested (50^{o}; shorter lengths, 70^{o} more optimal lengths, 90^{o} longer lengths) during an isometric MVC. There have been conflicting reports throughout the literature concerning the possible increase in agonist activation following resistance training, as there have been studies published that have reported significant changes (Häkkinen et al., 1996, Narici et al., 1989, Häkkinen et al., 2003, Komi et al., 1978, Moritani and DeVries, 1979, Häkkinen and Komi, 1983, Reeves et al., 2005b, Higbie et al., 1996), whereas some have not (Garfinkel and Cafarelli, 1992, Narici et al., 1996, Weir et al., 1995, Aagaard et al., 2002). However, comparing longitudinal changes in agonist EMG both within and between studies can prove difficult due to methodological differences (Folland and Williams, 2007). In one length-specific resistance training study, Thepaut-Mathieu et al. (1988) reported an increase in iEMG-force relationships at the specific joint angles used during training. These findings were also supported by Kubo et al. (2006a) who found that iEMG of the quadriceps (rectus femoris, vastus lateralis and vastus medialis) increased significantly in groups that trained at either shorter or

longer muscle lengths, with no differences between the groups at any of the joint-angles tested. In the current study there were also no significant differences in maximal activation levels between groups and muscle lengths. However, one of the main findings from the current study was the significant relative increases in activation at all muscle lengths in LX, at longer muscle lengths in LL, and significant decreases in activation at longer muscle lengths in SL. This is further evidence of the muscle length (or joint-angle) specificity phenomenon following resistance training. Whereas a previous study (Kubo et al., 2006a) found that following 12 weeks of isometric resistance training at shorter muscle lengths, relative quadriceps iEMG increased at all measured knee angles (40-110^{o}), whereas the current results show a decrease in activation at longer muscle lengths following training at shorter muscle lengths. Interestingly from the study of Kubo et al. (2006a) was the fact that although iEMG increased within the range of ~25-45% over all testing angles (40-110^{o}) following training at shorter muscle lengths, MVC only significantly increased between 40-80^{o} in this group. Previous work has shown that MVC torque did not change significantly at longer muscle lengths following a period of resistance training at shorter muscle lengths (see chapter 4), and results from the current investigation show that this could be in part be mediated by a reduction in maximal activation at these lengths. Further evidence of muscle-length specificity was the fact that only LX group, who covered an entire ROM, actually demonstrated a significant relative increase in activation at each angle tested, and also that LL only showed significant relative increases in activation at longer muscle lengths (lengths where the majority of training would have taken place). In order to allow us to describe the impact of changes in activation on strength changes, we have shown that there was significantly greater muscle efficiency (EMG per unit of torque) at longer muscle lengths (i.e. in LL and LX) compared to SL, following the 8-week training program. Changes in torque

generating capacity are not accounted for solely, or at times at all by increased muscle activation. Changes in muscle architecture, morphology and/ or muscle specific tension are just a few of the many other factors that can impact a muscle's ability to produce force following resistance training as well as neural adaptations (for review see (Folland and Williams, 2007)). However in this case, there appears to be a relationship between the increased activation of the VL muscle and the changes in torque production following resistance training in LL and LX at longer muscle lengths.

Levels of antagonist co-contraction did not change in any of the training groups following training at any force level (i.e. 10-100% MVC). In the current study, maximal quadriceps activation and co-contraction were measured at 90^{o} knee flexion. Kubo et al. (2004b) measured levels of antagonist co-contraction during maximal knee extensor efforts between 40-110^{o} knee flexion using electrical muscle stimulation. The authors found that co-activation was significantly greater at 90-110^{o} compared to other joint angles, which would suggest that in the current study we measured antagonist co-activation levels at a joint angle that evokes their greatest activity during maximal extension efforts. The results suggest that training at longer muscle lengths does not confer any beneficial training adaptations regarding antagonist co-activation at knee-flexed positions than training at shorter muscle lengths.

Conclusion

Following acute resistance exercise, agonist (VL) electromyographical activity was significantly greater when performing the exercise at longer muscle lengths than at shorter muscle lengths. Furthermore, there was also an increase in oxygen consumption and heart rate at longer muscle

lengths compared to shorter. This acute evidence suggests that the muscle is working more intensely to produce mechanical work at longer muscle lengths through both the neuromuscular system, and also though the cardiovascular and energy systems. Following prolonged resistance training, activation of the VL was altered depending on the trained muscle length and also the joint-angle MVC activation was assessed. This new dynamic resistance training evidence supports previous research into the joint-angle activation specificity associated with isometric length (or joint-angle) specific resistance training.

CHAPTER 6:

Effects of Prolonged Resistance Training & Detraining on Endocrine Profiles

6.1 Introduction:

Following delivery of the exercise stimulus, systemic and local responses (endocrine, immune, muscle, neural, cardiovascular) drive a cascade of downstream signalling and responses that cumulatively result in functional adaptation (Spiering et al., 2008). Skeletal muscle and tendon development and regeneration are sensitive to the extracellular milieu. There are many hormones associated with an exercise-induced response to mechanical loading of muscle and tendon such as testosterone, human and insulin-like growth factors, cortisol, TGF-β superfamily and fibroblast growth factor to name but a few. However, much research has focused on particular growth factors, such as insulin-like growth factor -1 (IGF-I) and transforming growth factor beta (TGF-β1), that appear to exert potent levels of control over gene expression (Kollias and McDermott, 2008).

Regulation of the muscle-tendon unit (MTU) is important, in terms of increasing/ maintaining size and function, to a variety of populations from athletes, to elderly, those suffering from illness/ disease (myopathies, sepsis, cancer, HIV/ AIDS), following injury and astronauts. From the skeletal muscle mass perspective, IGF-I has been shown to mediate hypertrophy in animal models by stimulating satellite cell proliferation, and also myoblast proliferation and differentiation (Edwall et al., 1989, Barton-Davis et al., 1999). Furthermore, a study by Lee et al. (2004), showed that rats who performed resistance training and who were administered with viral IGF-I, had significantly greater increases in muscle mass and peak tension compared to rats administered with IGF-I alone or took part in resistance training alone. Additionally from the same study, during detraining, muscle mass in the IGF-I plus resistance trained rats maintained muscle mass to a significantly greater degree than those who were not injected with IGF-I. The

hypertrophic response to IGF-I has been shown to be mediated through the PI3/Akt/mTOR cascade, and precedes myotube hypertrophy (Rommel et al., 2001), whilst the retention of mass may be due to the prevention of the expression of atrophy-induced ubiquitin ligases (Stitt et al., 2004). IGF-1 mRNA has also been shown to increase to a much greater extent in response to stretch combined with electrical stimulation, compared to stimulation or stretch alone in adult skeletal muscle (Goldspink et al., 1995). Furthermore IGF-I has also been shown to induce Type I collagen synthesis *in vitro* in rabbit tendon (Abrahamsson and Lohmander, 1996), whilst it has also been shown that the presence of IGF-I protein in rat tendon can be induced by mechanical loading *in vivo* (Butt and Bishop, 1997).

TGF-β1 is a member of the TGF-β superfamily of ligands which includes TGF-βs, activins, inhibins and bone morphogenic proteins (Danielpour and Song, 2006). TGF-β1 isoform has been found to have a plethora of effects such as regulation of cell growth, proliferation, differentiation, adhesion, migration, and apoptosis, with a unifying feature of its cell signalling is that it is cell context specific i.e. signalling outcome is dependent on the intracellular content (Kollias and McDermott, 2008). For example, from two *in vitro* studies, in skeletal muscle tissue, TGF-β1 has been shown to depress proliferation and inhibit differentiation (Allen and Boxhorn, 2005), whereas in patella tendon fibroblasts, TGF-β1 has been shown to increase proliferation of fibroblast number and at least in part mediate increases in collagen Type I production following cyclical stretch in serum-free conditions (Yang et al., 2004). Furthermore from Yang et al. (2004), TGF-β1 expression increased in a stretch-magnitude-dependent manner, with stretching of 4% and 8% resulting in greater levels of TGF-β1 mRNA compared to controls (i.e. no stretch). However, the role of TGF-β1 *in vivo* following exercise has not been fully well established.

In rodent models, TGF-β1 mRNA increased 27% following strain injury (induced from stretching) in the gastrocnemius muscle (Smith et al., 2007). Additionally, TGF-β1 mRNA increased significantly in muscle (gastrocnemius) and tendon (Achilles) following 4 days of resistance training with eccentric, isometric or concentric contraction types (Heinemeier et al., 2007).

In humans, Heinemeier et al. (2011) took biopsies from the patella tendon and *vastus lateralis* following one hour of kicking exercise at 67% workload max. TGF-β1 mRNA levels did not increase in tendon but TGF-β1 and -β2 along with TGF-β receptor II increased significantly in muscle. This is in agreement with another study that reported a significant increase in TGF-β1 mRNA in young and older men following downhill interval running in the *vastus lateralis* (Hamada et al., 2005). In contrast, previous work from Heinemeier et al. (2003) found significant increases in both systemic (plasma) and local (dialysate samples from peritendon tissue) of TGF-β1 levels immediately and 3 hours post treadmill running respectively (60 minutes, 3% incline, 1km/hr). There is also evidence to suggest that chronic training can influence circulating levels of TGF-β1. In Type II diabetes patients who took part in 8 weeks of combined resistance training and aerobic training, TGF-β1 serum levels increased by 50.4% compared to baseline (Touvra et al., 2011).

Tumor necrosis Factor alpha (or TNF-α) is a pro-inflammatory cytokine that is thought to play both a direct and an indirect role in reductions in skeletal muscle mass, as acute administration of TNF-α causes increased muscle proteolysis in rats (Goodman, 1991). It is also implicated in muscle wasting in inflammatory diseases such as cancer, AIDS, congestive heart failure, chronic obstructive pulmonary disease and models of Duchenne muscular dystrophy (Reid and Li, 2001). This is hypothesized to be due to activation of the proteolytic nuclear factor kappa-light-chain-

enhancer of activated B cells (NF-kB) cell signalling pathway (Leong and Karsan, 2000). TNF-α has been shown to decrease (Phillips et al., 2012, Ogawa et al., 2010), not change (Libardi et al., 2011, Touvra et al., 2011), or increase (Lucotti et al., 2011, Lee et al., 2011) following a period of resistance training. Vast differences in measurement timings and other methodological procedures exist and make it extremely difficult to provide a consensus on the response of TNF-α to prolonged resistance training (De Salles et al., 2010). However no studies have been carried out on a young population, and also most of the studies that report on the effect of resistance exercise on TNF-α levels have been done on those with health disorders or dysfunction e.g. obesity, Type II diabetes etc.

From the literature, it appears that no *in vivo* study in humans has systematically compared the effects of relatively higher vs. lower muscle stretch (where the degree of internal muscle loading is normalised in the two stretch conditions – SL and LL groups) on circulating IGF-I, TGF-β1 and TNF-α levels following resistance training and detraining and the evidence of presence of possible interactions.

6.2 Methods:

At each of the designated testing intervals (i.e. baseline, weeks 8, 10 and 12) and following an overnight fasting period (~10 hours for all participants), participants reported to the laboratory. Venous blood samples were obtained using a 21-gauge 1-inch ultra-thin wall needle (Terumo Medical Corporation, New Jersey, USA) inserted into the antecubital vein of the forearm. Using a vacutainer assembly and serum separator tubes (Monovette, Sarstedt, Numbrecht, Germany), 5 mL blood samples were collected. After being kept on an ice bed for up to 2-hours, the sample

was then centrifuged at 4°C for 10 min at 4,800 rpm, with the supernatant being removed and stored in eppendorfs at −20° Celsius for later analysis of IGF-I, TGF-β1 and TNF-α levels.

Two training groups performed resistance training for a total of 8weeks with one group performing training over shorter (SL), and one group over longer (LL) muscle lengths, under comparable loading conditions. Following training, each group refrained from any physical training (detraining) for four weeks. A control group who retained habitual activity levels were also monitored during the entire 12-week period (for more details see Chapter 2). The LX group was not analysed for growth factors and cytokines due to insufficient biochemical analyses materials.

6.3 Results:

Insulin-Like Growth Factor - 1(IGF-I):

Changes in IGF-I are shown in Figure 6.1 and 6.4. IGF-I levels increased significantly as a result of training in LL group but not SL group at week 8 (SL, 407 ± 25 ng/mL – 429 ± 30 ng/mL, p=0.438; LL, 375 ± 18 ng/mL – 489 ± 29 ng/mL, p=0.033), with a significant between group effect (31±6% vs. 7±6%; p<0.01). Results of within-subject contrasts were $F(1, 18) = 8.795$, p=0.008. LL group maintained greater IGF-I levels compared to baseline (p<0.01) and SL group (p<0.05) at week 10, but had returned to baseline levels by week 12 with no main group effect evident at the conclusion of detraining (p>0.05). The control group showed no significant change in the circulating levels of this hormone at any stage of the study period (p>0.05, Figure 6.1).

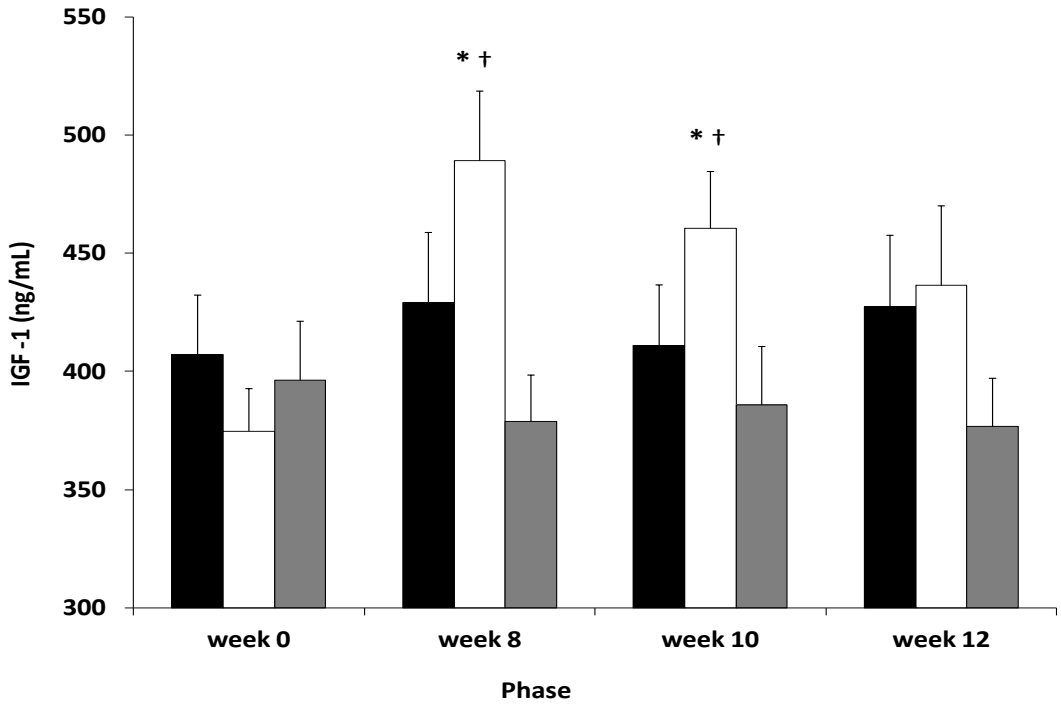

Figure 6.1. Circulating IGF-I levels at each stage of the research protocol in SL (black bars), LL (white bars) and Con (grey bars). * † Significantly different to baseline and to SL group.

Transforming Growth Factor – Beta 1 (TGF-β1):

After 8 weeks of resistance training, circulating levels of TGF-β1 did not change significantly in SL (4,976±1279 pg/mL to 4,274±1071 pg/mL) or LL (4,572±807 pg/mL to 4,755±752 pg/mL) training groups ($p>0.05$), with no differences observed between groups ($p>0.05$). Following four weeks of detraining TGF-β1 levels remained similar to those reported at baseline and at week 8 in both training groups (SL; 3,726±519 pg/mL, LL; 4,439±815 pg/mL). Controls were not analysed for changes in TGF-β1 due to insufficient serum.

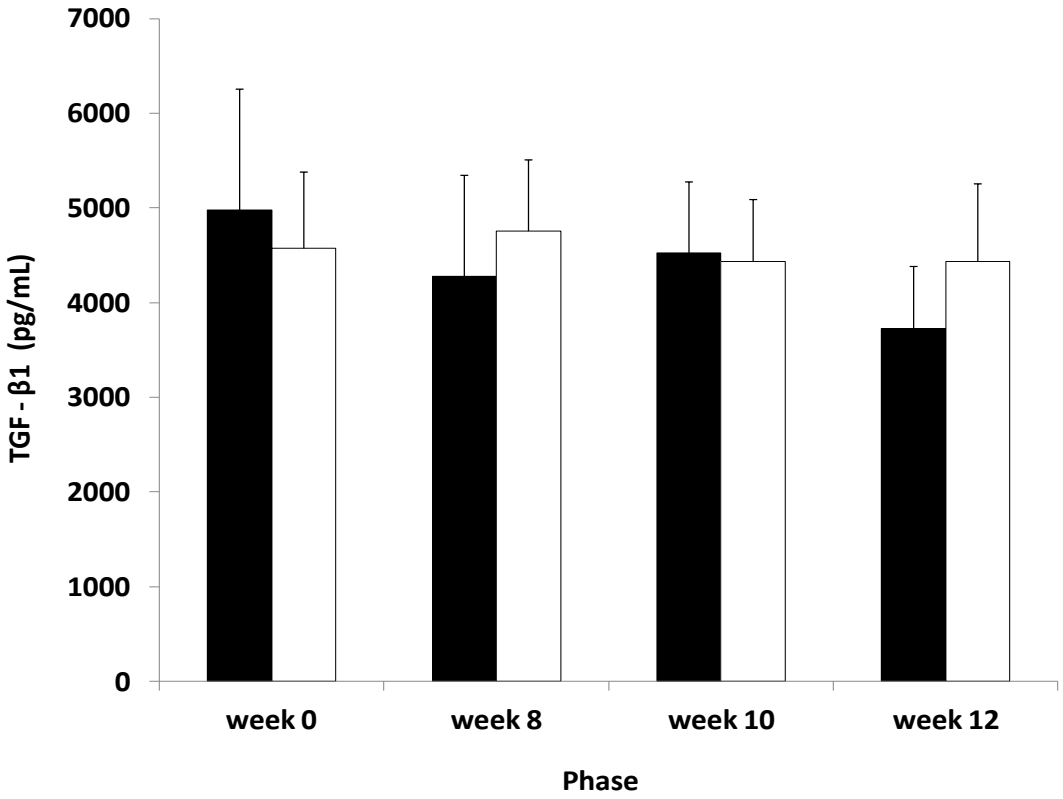

Figure 6.2. Circulating TGF-β1 levels at each stage of the research protocol in SL (black bars), LL (white bars) training groups.

Tumor Necrosis Factor – alpha (TNF-α):

The circulating levels of the TNF-α cytokine did not change significantly at week 8 in either training group ($p>0.05$) with no difference between groups ($p>0.05$), despite a tendency for levels to increase in SL (86±11 pg/mL to 91±12 pg/mL) and decrease in LL (93±9 pg/mL to 84±7 pg/mL, Figure 6.3). Following four weeks of detraining cytokine levels were shown to increase and were identical in SL and LL groups with 93±10 pg/mL and 93±9 pg/mL in SL and LL respectively, but there was no difference in changes to TNF-α levels relative to week 8 in

either group (p>0.05, Figure 6.5) . There were no changes in TNF-α levels in the controls during this period (p>0.05).

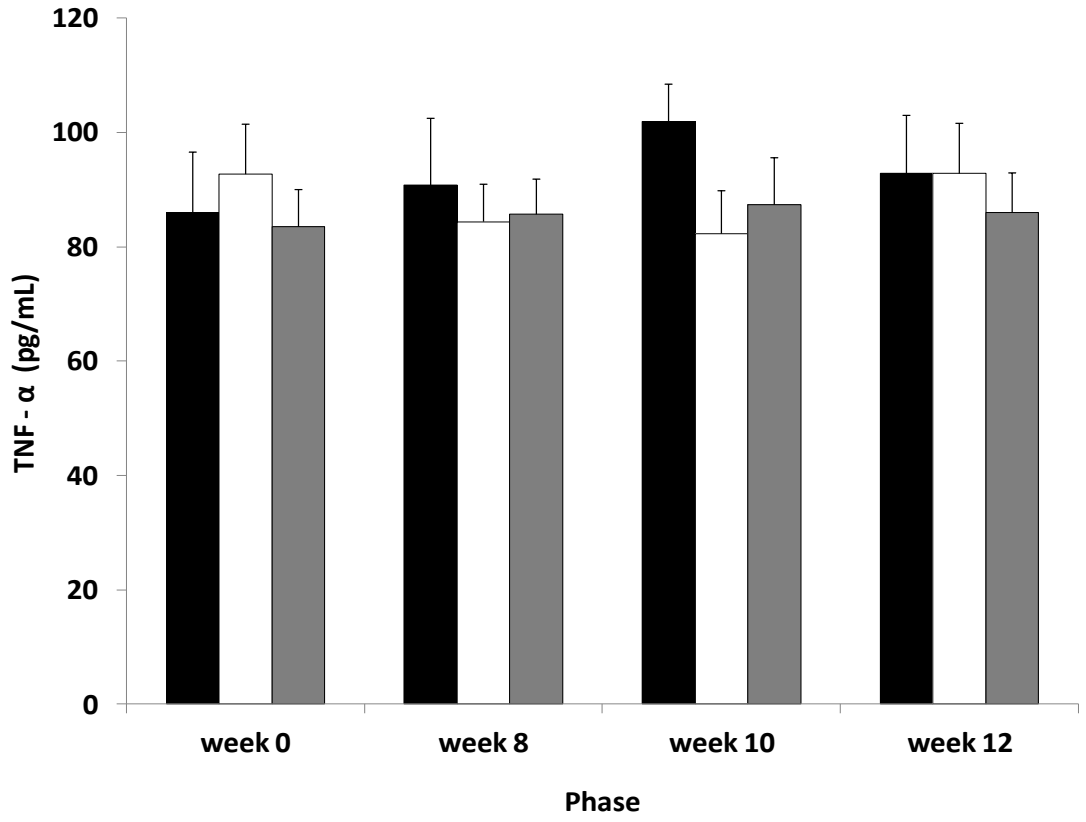

Figure 6.3. Circulating TNF-α levels at each stage of the research protocol in SL (black bars), LL (white bars) and Con (grey bars).

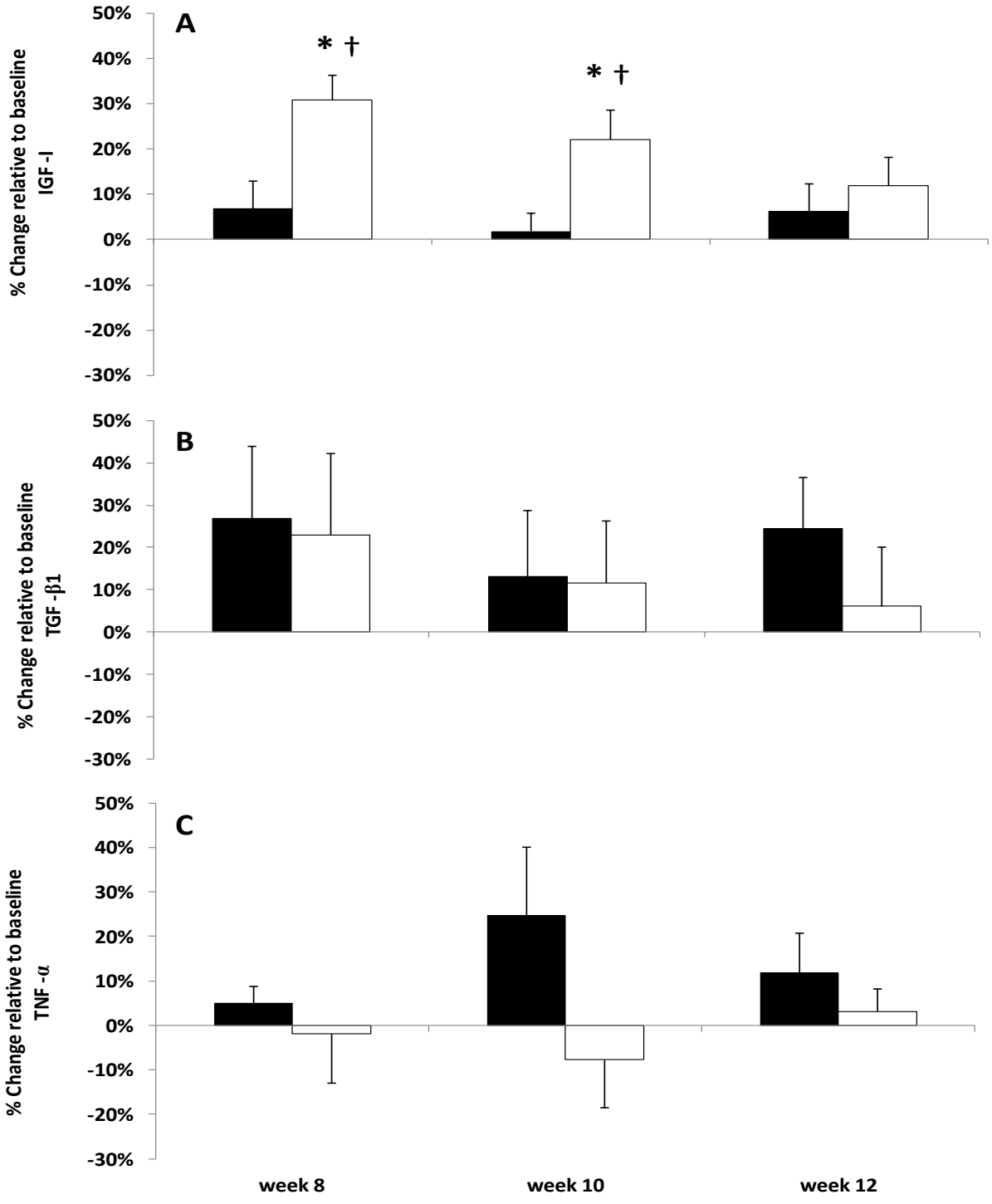

Figure 6.4. Relative changes in growth factors & cytokines in SL (black bars), LL (white bars). * † Significantly different to baseline and to SL group ($p<0.05$).

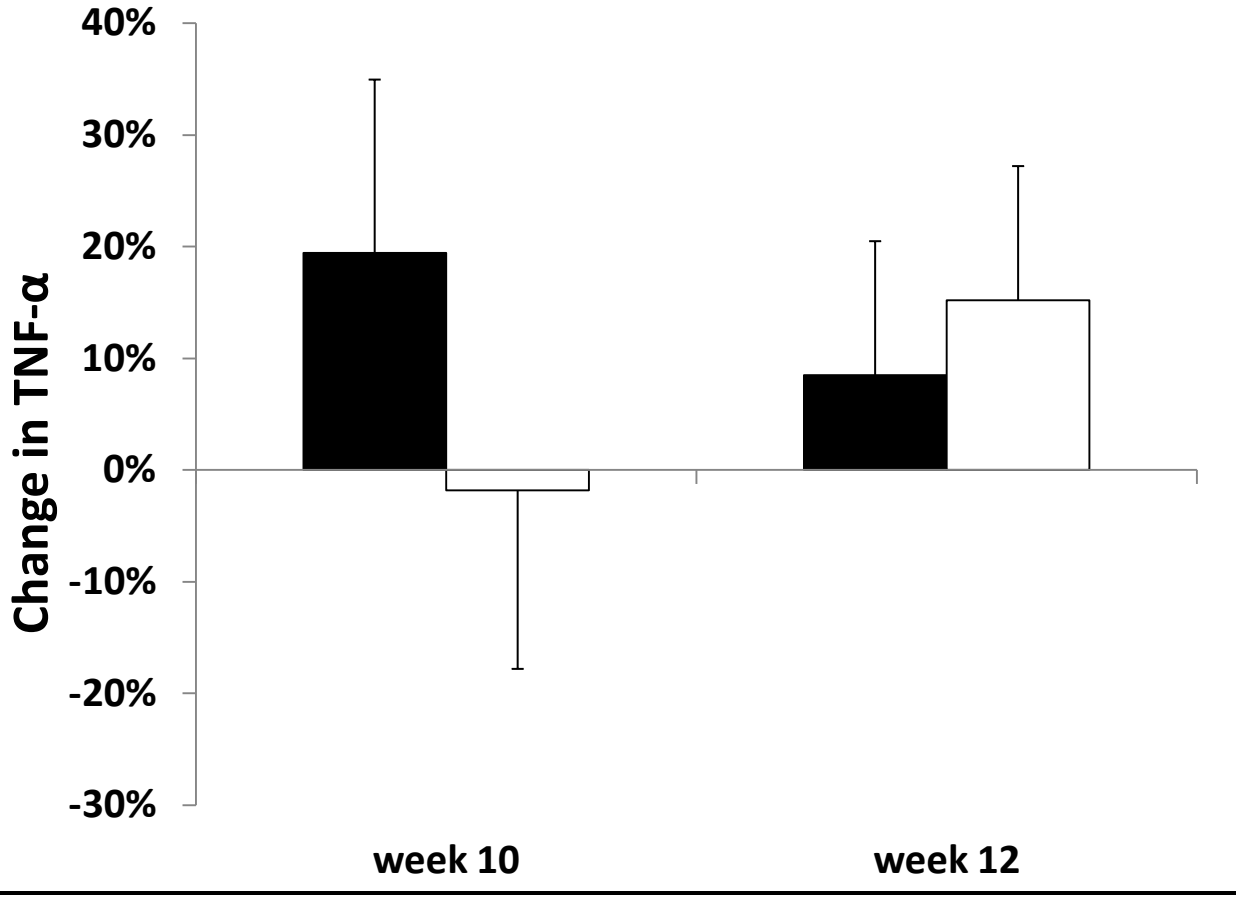

Figure 6.5. Changes in TNF-α relative to week 8 during detraining in SL (black bars) and LL (white bars) groups. No significant differences were detected (p=0.924).

6.4 Discussion:

Following eight weeks of resistance training and four weeks of detraining, the changes in circulating growth factors and cytokines that are hypothesised to play a role in the modulation of training-induced adaptations at the cellular level, showed different trends that in part reflected the *in vivo* events at the level of the whole muscle-tendon complex level (Figure 6.4).

- **6.1 Changes in circulating IGF-I levels**

Increases in IGF-I and IGF-I mRNA following acute resistance training in humans have been reported previously (Borst et al., 2001, Greig et al., 2006), with extensive *in vitro* research showing that IGF-I is an important regulator of muscle mass *in vivo*, and that experimental manipulation of IGF-I levels can cause a significant increase in muscle mass and protein synthesis (Barton et al., 2002, Barton-Davis et al., 1999, Lee et al., 2004, Coleman et al., 1995, McKoy et al., 2004, Musarò et al., 2001, Yang et al., 1996, Goldspink et al., 1995). One of the major findings was that IGF-I levels in the current study were significantly greater in LL post training compared to SL (31% vs. 7% increase, $p<0.05$). This finding is consistent from *in vivo* data from adult skeletal muscle that has shown, although electrical stimulation failed to increase levels of growth or protein synthesis, static stretch of muscle increased IGF-I mRNA 12-fold with a concomitant increase in fractional (138%) and total (191%) protein synthesis rates. Furthermore, stretch combined with electrical stimulation increased IGF-I mRNA concentration 40-fold , with fractional and total protein synthesis rates increasing by 345% and 450% respectively in this condition (Goldspink et al., 1995). The above evidence suggests that by training at longer muscle lengths (i.e. LL group), muscle will experience a greater mechanical strain and the fact that IGF-I is induced by mechanical signals (Yang et al., 2002), it may have been the case that the LL group induced a more potent mechanical signal. In response, there is probably a co-ordinated activation of several signalling cascades, which may be mediated, at least in part, by the activity of IGF-I through the PI3/Akt/mTOR cascade (Rommel et al., 2001). This evidence may explain the larger hypertrophic response of the LL group compared to SL that was outlined in chapter 3.

- **6.2 Does IGF-I play another role during detraining?**

It has been previously shown that IGF-1 has been implicated in the suppression of protein degradation by preventing the expression of the FOXO class of transcription factors and the mRNA increases of MAFbx and MuRF1 seen during muscle atrophy (Stitt et al., 2004, Sacheck et al., 2004). The LL group continued to have significantly greater IGF-I levels than SL at both week 8 and week 10. Although not strictly speaking a 'chronic increase' in IGF-I levels, the LL group's responses certainly lasted longer than the transient increase in IGF-I observed with acute bouts of resistance exercise. Therefore, there may have been a protective role played by IGF-I allowing greater retention of muscle mass in LL. In support of this notion is the observation that the LL group IGF-I levels at week 12 had returned to baseline levels, and that this period also coincided with the relatively larger magnitude of aCSA decrement in LL group at the conclusion of the detraining phase (see chapter 3).

- **6.3 Systemic changes in TGF-β1 and possible *in vivo* roles following resistance training on the muscle-tendon complex**

Circulating TGF-β1 levels did not change significantly in either SL or LL training groups compared to baseline, but on average tended to decrease over the course of training and detraining, from 4,774 pg/mL at week 0 to 4,082 pg/mL at week 12 (mean SL + LL). With regards to the adaptation of the muscle-tendon complex, TGF-β1 plays a pleiotropic role, as it has been shown to depress proliferation and inhibit differentiation in adult skeletal muscle cells (Allen and Boxhorn, 2005), whereas in patella tendon fibroblasts, TGF-β1 has been shown to

increase proliferation of fibroblast number and at least in part mediate increases in collagen Type I (Yang et al., 2004). Mice deficient in fibrillin-1 have increased TGF-β1 signalling activity that causes failure of skeletal muscle regeneration (Cohn et al., 2007), with inhibition of TGF-β1 signalling resulting in a more improved skeletal muscle profile in many genetically invoked myopathies (Kollias and McDermott, 2008). Furthermore, increases in myostatin promoter activity have been shown to increase following TGF-β treatment, which is a further indication of the negative regulation of muscle mass by this family of cytokines (Allen and Unterman, 2007).

From the evidence presented in chapter 3, it would appear that due to the significant increase in aCSA observed in both SL and LL training groups along the length of the VL, that any impact of TGF-β1 was minimal in disrupting the hypertrophic response of skeletal muscle in a healthy, young, mixed-gender population. Furthermore, circulating TGF-β1 levels did not significantly change following training in either group, whereas IGF-I levels increased significantly in LL group. Although there is evidence to suggest that there is cross-talk between IGF-I and TGF-β1 signalling pathways, and that IGF-I can block TGF-β1-induced apoptosis in human hepatoma and prostate cancer cell lines through the PI3 kinase/Akt pathway (Chen et al., 1998), there is no evidence that delineates their interaction and effects on adult skeletal muscle cell signalling, and also signalling following exercise. It is difficult to decipher from the current evidence if there was any interaction between these two growth factors, and if there was such interactions, it would appear that IGF-I had a more pronounced effect on skeletal muscle adaptation.

In contrast to a possible conflict in cellular signalling outcomes in skeletal muscle, in tendon, these growth factors would appear to produce a similar effect, by increasing collagen

Type I expression (Abrahamsson and Lohmander, 1996, Yang et al., 2004). The fact that TGF-β1 levels did not change compared to baseline and between groups, whilst evidence from chapter 4 shows that there was no increases in tendon CSA in any training group along the length of the patella tendon following training, suggests that if TGF-β1 does indeed play a significant role in mechanical loading-induced collagen synthesis in tendon, a lack of increase in circulating TGF-β1 may have contributed to the observed lack of change in tendon CSA.

It should be noted at this point, the difficulty in trying to extrapolate findings of circulating growth factors to their function and role in adaptation, compared to the impact and role of local growth factors performing autocrine/ paracrine roles in cellular adaptation. Indeed, the correlation between the effects of the two sources may not be entirely reflective of one another (Velloso, 2009).

- **6.4 Does resistance training and a period of detraining alter TNF- α?**

The role of TNF-α in the decline of skeletal muscle mass through increased levels of protein loss *in vivo* has been demonstrated previously in rats (Goodman, 1991). Furthermore, cell culture studies have shown that TNF-α can stimulate a time- and concentration dependent decrease in total muscle protein content and losses in muscle specific proteins such as MHC, which are not accompanied by a change in protein synthesis rates (Li et al., 1998, Li and Reid, 2000). The results from the current study show that following 8 weeks of resistance training at shorter and longer muscle lengths, that there was no significant difference between groups or compared to baseline in circulating TNF-α levels. This is in agreement with several other studies following prolonged resistance training regimens (Libardi et al., 2011, Touvra et al., 2011), although these

studies used different populations (i.e. middle-aged men and older Type II diabetes sufferers respectively) there was no change in TNF-α levels throughout the training regimen. As mentioned previously, diverse ranges of populations in studies makes direct comparisons difficult, as no study has been carried out in a young population as of yet.

With regards the two current training groups, mechanical stress (through stretch) has been shown to activate the NF-kB pathway in mdx mice muscle fibres, resulting in increased expression of IL-1β and TNF-α (Kumar and Boriek, 2003). The mechanical stress (also through stretch) would have been higher in the LL group, although it is only speculative to suggest that mechanical stress activation of such a catabolic pathway may or may not be magnitude dependent. Therefore without acute data, one cannot say whether TNF-α levels would have been transiently increased to a greater degree in one group or another, although more chronically there were no differences.

One study in elderly women found that the reduction in circulating TNF-α levels were significantly correlated with increases in muscle thickness following 12 weeks of resistance training (Ogawa et al., 2010). In the current study, both training groups experienced significant gains in VL hypertrophy with no changes in TNF-α levels and increases in IGF-I levels (chapter 3). The exposure of human myoblasts to TNF-α reduced or completely stopped IGF-I induced protein synthesis rates in a dose dependent manner (Frost et al., 1997). In addition, Frost et al. (1997) also demonstrated that administration of TNF-α inhibited protein synthesis rates for upto 48 hours after treatment and also inhibited protein synthesis in myoblasts that had differentiated into myotubes. Furthermore, administration of TNF-α reduced circulating levels of liver and skeletal muscle IGF-I and IGF-IBP-1 in fasted rats (Fan et al., 1995). The effect of exercise intensity on *in vivo* IGF-BP3 and TNF-α levels were shown to increase and decrease respectively

following low-intensity training, whereas in the high-intensity group neither changed significantly in an elderly population (Onambélé-Pearson et al., 2010c). Once again, due to the lack of change in TNF-α levels, increases in IGF-I levels and the muscular hypertrophy observed, it would appear that TNF-α did not influence or modulate a possible IGF-I mediated hypertrophic response to resistance training.

It is also particularly interesting to note, that there was no significant difference in TNF-α compared to week 8 at week 12, following 4 weeks of detraining (Figure 6.5). One would possibly expect during periods of detraining, where muscle mass decrements are evident, that this may at least in part be mediated by TNF-α levels. However, in a disuse atrophy model in healthy young rats, 7 days of unloading resulted in activation of the proteolytic NF-kB pathway that was not induced by TNF-α, as cytokine levels actually moderately decreased even though muscle mass was reduced by 30% (Hunter et al., 2002).

CONCLUSION

In conclusion, there were significant increases in IGF-I levels in the LL group following training at week 8, that were still evident at week 10 compared to SL. Such elevations in this growth factor may have contributed to the increases in muscle mass observed, and also retention of the muscle mass during detraining. On the other hand, it appears that circulating cytokines TGF-β1 and TNF-α may not have had an inhibitory effect on either IGF-I or the ability to induce skeletal muscle hypertrophy (discussed further in chapter 7). Non-significant changes in TGF-β1may also have been a contributing factor in the lack of changes noted in tendon CSA.

CHAPTER 7:

Discussion, Conclusions & Future Directions

- *7.1 Thesis findings regarding muscle morphology, architectural and strength adaptations*

It was hypothesised that following resistance training that the morphological, architectural and functional adaptations of the vastus lateralis and quadriceps muscle group would be improved to a greater extent by training at longer muscle lengths, or over a complete range-of-motion compared to shorter muscle lengths. The hypothesis has been verified from the results presented in Chapters 3 and 4.

From Chapters 3, 4 and 5, it is evident that in terms of *vastus lateralis* muscle electromyographical activity, volume and size (regional aCSA), fascicle length and quadriceps strength, that training at a longer muscle length increased these parameters to a significantly greater extent. The link between muscle volume (Fukunaga et al., 2001, Aagaard et al., 2001) or aCSA (Maughan et al., 1983, Jones and Rutherford, 1987) and strength has been demonstrated previously, which displays a positive relationship between muscle size and strength (Figure 7.1A). This relationship was verified further in the current study, where VL muscle volume correlated significantly ($r = 0.698$, $p<0.01$) with torque levels (Figure 7.1B).

Also, in this thesis, greater volume (and aCSA) in longer muscle length training groups following resistance training were accompanied by a significantly greater increase in peak torque following training compared to shorter muscle length training. However, despite no significant changes between groups in pennation angle ($P\theta$), both LL and LX tended to have greater increments in $P\theta$ compared to SL.

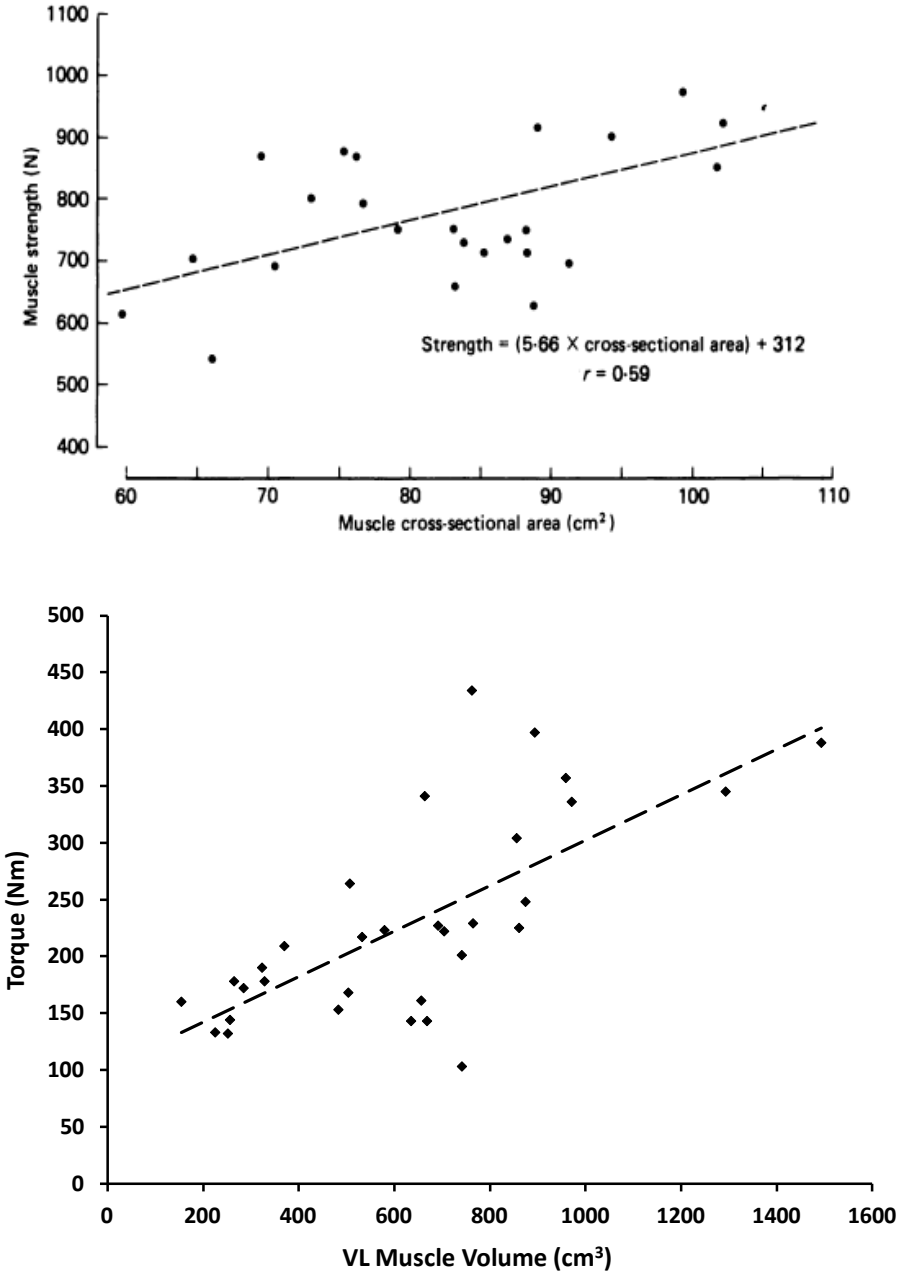

Figure 7.1(A). Relationship between muscle cross-sectional area and strength. (Taken from Maughan, Watson & Weir, 1983). (B) Relationship between measured muscle volume and torque from the current study – r = 0.698, p<0.01).

Thus, one may speculate that this could also have been a contributing factor to LL and LX group's greater strength following training compared to SL, as previous work has demonstrated that an increase in $P\theta$ is highly correlated with changes in maximal contractile strength in human quadriceps (Aagaard et al., 2001). When this relationship was investigated in the current study, unexpectedly there was a significant negative correlation between changes in pennation and muscle strength ($r = 0.409$, $p<0.05$, Table 7.1). As previously mentioned in Chapter 1, based on parellelogram models of bipennate muscles, pCSA increases proportionally to sin $P\theta$, and muscle effective force reduces in proportion to cos $P\theta$. The net result of the opposing effects of pennation angle (i.e. $\sin(P\theta) \times \cos(P\theta) \propto 1/2\sin(2P\theta)$), is that maximal muscle force is expected to increase with increases in pennation angle to an upper limit of 45 degrees (Alexander and Vernon, 1975, Rutherford and Jones, 1992). However, the loss of effective pull is also thought to be minimal when pennation angles are moderate (i.e. $<25^{o}$ (Blazevich, 2006)). The pennation angles in the present study were moderate (ranging from $8^{o} – 23^{o}$), so it is difficult to identify the exact mechanism by which such a relationship exists in the current study.

Apart from the increased mechanical stimuli experienced through greater stretch (as shown in Table 3.1) in the longer muscle length groups compared to shorter, a significantly greater amount of circulating IGF-I (Figure 6.1) may have also created a more favourable cellular environment for protein synthesis, and thus increasing muscle size. Indeed, there was a significant positive correlation between changes in muscle aCSA and IGF-I at the post-training week 8 phase ($r = 0.399$, $p<0.05$, Table 7.1).

Also architecturally, with regards the significantly greater increases in fascicle length in the longer muscle length training groups, especially in LL, compared to SL, the current work has shown for the first time , that muscle length over training excursion is the main mechanical

stimulus for fascicle length changes *in vivo* in a young population. This is most evident from the fact that both SL and LL groups performed the same volume of training, identical ranges-of-motion (in terms of absolute excursion) with matched forces at the tendon, yet LL fascicle lengths during loading were significantly greater. This additional protein also may have been a contributing factor to the differences observed in volume, by the significant ($p<0.01$) correlation between changes in volume and fascicle length below. Table 7.1 shows the correlations between absolute and changes in muscle parameters, which may provide an insight to the related responses to resistance training. As hypothesized in Chapter 3, the additional protein accruement in fascicle length may have been translated externally into greater muscle volume, as there does appear to be a significant correlation between these relative changes.

Table 7.1. Correlations between various muscle parameters. Significance set at alpha $p \leq 0.05$. aCSA (anatomical cross-sectional area), Vol (VL muscle volume), Pen (pennation angle), FL (fascicle length), Str (strength), IGF-I (insulin-like growth factor I).

Parameters	**Absolute Changes**		**Relative Changes**		**Significant**
	Pearson Correlation	**P Value**	**Pearson Correlation**	**P Value**	
aCSA vs. FL	0.622	<0.01	0.089	0.628	✓
Vol vs. FL	0.173	0.342	0.695	<0.01	✓
aCSA vs. IGF-I	0.237	0.302	0.399	<0.05	✓
Pen vs. Str	0.234	0.227	-0.409	<0.05	✓
FL vs. Str	0.357	0.098	0.427	<0.05	✓
Vol vs. IGF-I	0.218	0.342	0.286	0.222	×

- *7.2 Future work regarding muscle morphology, architectural and strength adaptations*

As mentioned previously there were significantly greater increases in fascicle length following training at longer muscle lengths, which appears to heavily influence serial sarcomere adaptations during resistance training. However, excursion is not the only purported stimuli for fascicle length change. There is evidence that performing exercise at high velocity or in an explosive manner can also result in significant changes to fascicle length (Alegre et al., 2006), with fascicle length of *vastus lateralis* in 100m sprinters being significantly greater than both non-trained controls and endurance runners (Abe et al., 2000) and significantly correlated to sprint time performance (Kumagai et al., 2000). Therefore, future work may investigate the relationship between performing explosive or high-velocity resistance/ plyometric training at longer and shorter muscle lengths to see if there is an additive effect of both muscle length and speed of contraction during training. This should also be coupled in relation to power performance measures to determine whether an increase in fascicle length translates to a change in the functional performance of the muscle group.

In addition, following an increase in circulating IGF-I levels in LL group following training, future studies should investigate the acute responses of IGF-I intracellular signalling and protein synthesis rates, following training at longer and shorter muscle lengths, thus to establish the relative contribution of both mechanical stretch in addition to contraction and endocrine signalling to the hypertrophic response.

- *7.3 Thesis findings on changes to tendon properties*

Both mechanical and material properties of the patellar tendon were enhanced significantly as a result of training, with both longer muscle-tendon complex (MTCL/ X) length groups being more markedly improved than the shorter (MTCS) group following training. What was particularly surprising was the fact that the training adaptations observed were comparable between MTCL and MTCX despite the much lower loading but more constant stretch in MTCL. The acute response of patella tendon fibroblasts has been shown to be graded in a stretch-magnitude-dependent manner, and is associated with a greater expression of Collagen Type I and TGFβ-1 (Yang et al., 2004). However, currently there were no changes to patella tendon dimensions following training, although the modulus increased suggesting possible increases in the cross-linking proteins (Kjaer, 2004). Indeed, TGFβ-1 is purported to be a regulator of collagen turnover in tendon, with evidence from Chapter 6 demonstrating no increase in TGFβ-1 levels. However, there was no significant correlation ($r = 0.297$, $p>0.05$) between changes in TGFβ-1 and tendon CSA. There was also no significant correlation identified between absolute TGFβ-1 and tendon CSA ($r = 0.112$, $p>0.05$). Table 7.2 shows the correlations between some of the muscle-tendon parameters. In most cases, it appears that absolute changes in pre-post training are very closely related, however the relative changes do not appear to be as closely linked. The finding of a significant relationship between relative changes in muscle size (aCSA) and patella tendon stiffness in the current study is in agreement with the work of Seynnes et al. (2009), who also identified this relationship, but using physiological CSA. This relationship makes sense from a mechanical perspective as a larger muscle that can generate greater force, should in theory have these forces transmitted by a stiffer tendon. Thus, being able to withstand them and transmit them effectively to the bone.

Table 7.2. Correlations between various muscle-tendon complex parameters. Significance set at alpha $p \leq 0.05$. aCSA (anatomical cross-sectional area), K (patella tendon stiffness), E (young's modulus), Vol (VL muscle volume), FL (fascicle length), tCSA (tendon CSA).

Parameters	Absolute Changes		Relative Changes		Significant
	Pearson Correlation	P Value	Pearson Correlation	P Value	
aCSA vs. K	0.617	<0.01	0.717	<0.05	✓
Vol vs. K	0.576	<0.01	0.356	0.09	✓
K vs. E	0.837	<0.01	0.659	<0.05	✓
E vs. tCSA	0.510	<0.01	0.224	0.731	✓
FL vs. K	0.430	<0.05	0.375	0.07	✓

- *7.4 Future work*

Similarly to muscle, one may want to elucidate the acute signalling responses between the three training regimes. Obtaining samples of TGFβ-1 directly from the source tendon under investigation may give a more accurate indicator of its role in tendon adaptation *in vivo* following the performance of these varying types of training methods, and also indicate the signalling cascade that mediates these changes. Indeed the circulating concentrations of a ligand may only be evident locally and yet not at the systemic level (hence our inability to detect changes in sera levels of TGFβ-1 for instance). This direct local measurement may also demonstrate not only the difference between MTCS and MTCL/ X groups, but also provide an insight to how the MTCL group's adaptations were comparable in magnitude MTCX, despite the aforementioned differences between the protocols.

The IGF-I and TGFβ-1 signalling pathways have been shown to interact (Chen et al., 1998), with both growth factors having differing roles depending on the target tissue (i.e. muscle or tendon). No study so far has looked at the acute interaction between these growth factors and the resultant signalling outcomes in muscle or tendon. For example, TGFβ-1 is a negative regulator of myoblast differentiation and proliferation, whereas one of the roles of IGF-I is supposed to be a positive regulator of these processes. Therefore, following the resistance exercise protocols it would be interesting to determine what governs which of these endocrine effects will dominate the outcome phenotype (in terms of training adaptations).

- *7.5 What does the information tell us about the general effects of muscle (-tendon) length specific training on adaptation, and how can we use it?*

The data presented in each individual chapter provides specific detail on adaptation to resistance training in relation to a number of dependent variables. However, if one wishes to provide a more standardised measure of the overall impact of each training protocol, a composite Z-score analysis tool can be used. A Z-score analysis allows bringing together several different measurements, regardless of the original dimension scale, into one single dimensionless measurement. The resulting Z-scores can then be unit weighted and combined in to a single outcome value called the composite z-score. The calculation of the z-scores and composite z-scores applied in the current set of data was as follows:

$$\text{Z-score} = (\text{Observation} - \text{mean}) / \text{standard deviation}$$

$$\text{Composite z-score} = \Sigma(\text{Z-scores})$$

Where the mean and standard deviation, are computed across all time points.

Main outcome variables chosen were representative of the changes in; muscle response (VL muscle volume), tendon response (patella young's modulus), function (knee extension peak torque) and endocrine system (circulating IGF-I levels). Note: modulus was chosen as opposed to tendon stiffness as it is a normalised measure, peak torque was chosen instead of peak force as it is a more functional measure of a muscle acting around a joint, and muscle volume was chosen as this reflects changes in muscle length as well as cross-section. Following multiple correlation analyses, it was found that changes in muscle volume did not significantly correlate with the other variables ($r = 0.303$, $p>0.05$) and therefore was replaced by mean aCSA across the three measurement sites as the muscle response variable, which did correlate significantly with the other variables. Indeed in composite Z-score analysis, those factors that are correlated with each other are considered to be measuring the same effect and are thus considered good candidates for inclusion in the composite.

Following the Z-score analysis (see Figure 7.2), there was a significant effect of each training protocol ($p<0.001$) on the Z-scores. Interestingly a group effect was also evident with SL group being significantly different to LL and LX groups ($p=0.028$) at week 8, which was also observed at week 10 ($p=0.047$), but not at week 12 ($p=0.383$). This result reinforces the evidence from each Chapter that suggests that the two training protocols that include training to some degree over longer muscle(-tendon unit) lengths confers an overall advantage over shorter. These composite Z-scores also confirm that undertaking these training regimes would be more

beneficial to the individual both in terms of immediate adaptations and short-term retention of them.

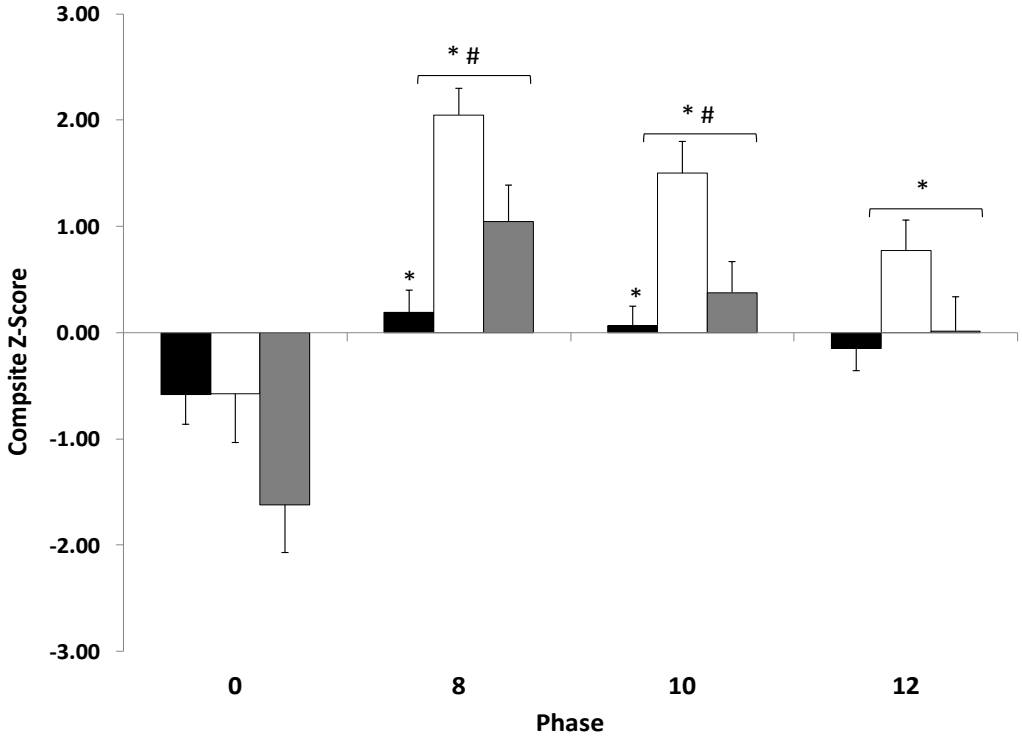

Figure 7.2. Composite Z-score analysis comparing the overall effectiveness of the training program in SL (black bars), LL (white bars) and LX (grey bars). * Significantly different to week 0 ($p<0.05$) # Significantly different to SL group ($p<0.05$).

A further way of exploring the data within this thesis is by attempting to use the data gathered to produce a mathematical model of the observed changes. Thus, in order to predict changes in muscle volume, a simple entry multiple linear regression was performed, with changes in fascicle length, aCSA, IGF-I, modulus, peak torque and stiffness as the predictors. However this created an unstable model. A simple linear regression was then run with the only significant predictor, muscle aCSA, and the model produced the following equation ($r = 0.944$, $r^2 = 0.892$, $p<0.001$):

$$\Delta \text{ Muscle Volume} = 1.592 \times \Delta \text{ aCSA} + 0.035 \pm 1.2\%$$

The regression equation derived shows a very positive and significant correlation with mean changes in aCSA across 25%, 50% and 75% femur length and changes in muscle volume. It is therefore possible to use the above equation in order to accurately assess changes in muscle volume following training from the mean change in three axial aCSA scans at the three aforementioned measurement sites if one does not have the facilities to measure muscle volume. One may have expected a high correlation between these two variables as aCSA was used to calculate muscle volume, and therefore the regression serves to highlight the validity of the aCSA measurement.

In conclusion, training at various muscle (-tendon unit) lengths induces specific and different physiological responses to resistance training. Training at longer lengths but with a comparable internal load is much more effective for physical and functional conditioning than training at shorter lengths. Covering a full rage-of-motion incorporating both shorter and longer muscle (-tendon unit) lengths with a higher internal load produces similar adaptations to that as training at exclusively longer lengths. This can directly impact the end-user in several ways.

1. Athletes, elderly, or people suffering from diseases associated with muscle mass loss who are seeking to increase muscle mass should incorporate training at longer muscle lengths or full ROM into their training. Training at longer muscle lengths compared

to full ROM may reduce the requirement for magnitude of loading and therefore reduce residual fatigue and possibly muscle damage/ risk of injury following training.

2. Additionally, any individual recovering from injury or surgery can improve tendon mechanical properties using lower loads with longer length training compared to full ROM, again reducing the incidence or likelihood of injury.
3. In relation to time, the retention of training-induced adaptations is greater over a short-term period of detraining using longer muscle length training and therefore may be of importance to the injured/ ill or tapering athlete and also clinically for those recovering from injury or surgery.

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Appendices

APPENDIX 1

Was there a gender effect concealed regarding patella tendon cross-sectional area (CSA) following resistance training?

Evidence was outlined in Chapter 4 that there were no significant increases in patella tendon CSA as a result of training, although the author also posited it is possible that there is a sex-difference in how males and females respond to exercise in terms of collagen turnover (Miller et al., 2005) and adaptation of cross-sectional area (Magnusson et al., 2007). As a result of using a mixed gender training population, it is therefore important to investigate the possibility that a gender-effect may have existed and therefore would have implications on the outcome of the study. Changes in PT CSA along the length of the tendon were then compared between genders.

Group	**Baseline (week 0)**			**Post-training (week 8)**			**Change (%)**		
PT_L (%)	*25*	*50*	*75*	*25*	*50*	*75*	*25*	*50*	*75*
Males	78±12	79±12	81±14	87±17	81±12	87±17	5±5	4±6	9±15
Females	66±13	68±10	65±8	68±15	81±12	74±13	2±3	3±4	5±12

Table 8.1. Changes in patella tendon CSA from baseline to week 8 in males and females. There were no significant differences detected in the relative change in CSA at any measurement site between groups or compared to baseline ($p>0.05$). Values are in mm^2 (mean and S.D.)

Conclusion; The author concludes from the results above that it appears that there was no gender effect concealed by the results in Chapter 4, and therefore no PT hypertrophy was observed in this study following resistance training.

APPENDIX 2

Vascular adaptations to resistance exercise training over shorter muscle lengths and a complete range-of-motion

Introduction: Alterations to the vascular system has implications for exercise performance, with specific reference to oxygen delivery and muscle perfusion, as these impact (amongst other factors) on rate of maximal oxygen uptake or $VO_{2\ max}$ (di Prampero and Ferretti, 1990). The importance of VO_2 has been discussed in chapter 5, section 4 already. Changes in physical activity are known to lead to acute alterations in vascular properties. An increase in physical activity, such as with endurance training is known to lead to an increase in lumen diameter (Dinenno et al., 2001, Huonker et al., 2003) and arterial CSA.(Miyachi et al., 2001) Conversely, reductions in physical activity, such as from cessation of training and bed rest, results in decreased lumen diameter and arterial CSA.(Bleeker et al., 2005c, Sugawara et al., 2004). It has been also demonstrated previouslt that resistance exercise can also have beneficial effects on vascular function, even when compared with aerobic exercise, although the adaptations may be mediated by different mechanisms (Collier et al., 2008).

Furthermore, there are lines of evidence to suggest that the molecular mechanism of exercise-induced upregulation of vascular endothelial nitric oxide synthase (eNOS), which is important for vascular function, is closely related to the frequency and magnitude of physical forces in the

vasculature, and in particular fluid shear stress (Kojda and Hambrecht, 2005). It is postulated that an increased physiological intensity, such as during a high-intensity resistance training bout, may increase vascular eNOS expression by increasing the shear stress-dependent activity of c-Src in endotheleial cells (Davis et al., 2003). Additionally, oxidative stress has been shown to also impact on eNOS, as data obtained in endothelial cells have shown that hydrogen peroxide can increase the expression and activity of eNOS by phosphorylation of Ca^{2+}/calmodulin-dependent protein kinase II/janus kinase 2 (Drummond et al., 2000, Cai et al., 2001). In a review of exercise-induced oxidative stress, Cooper et al. (2002) implies that exercise intensity is one example of when oxidative stress can be increased further.

From chapter 5, oxygen consumption, activation (which also requires oxygen), heart rate and blood pressure all tended to be higher, if not significantly so, in the LX group compared to SL group. Therefore the impact of 8 weeks of dynamic resistance exercise at 80% 1RM in SL (0-50^{o} knee flexion) and LX (0-90^{o} knee flexion) on vascular adaptations were investigated, with changes during detraining also measured. It was hypothesized that the LX group would experience a more intense vascular stimulus for change compared to SL.

Methods: Vascular measurements were obtained at rest using an echo Doppler ultrasound machine at baseline (week 0), post-training (week 8), detraining 1 (week 10) and detraining 2 (week 12) including blood flow velocity, arterial diameter and shear rate (as a surrogate measure of shear rate) of the superficial femoral artery (SFA) and carotid artery (CA). For more details on measurement see Chapter 2, section 2.2.13.

Results:

Blood flow velocity; Mean resting blood flow of the training groups (pooled data) for the SFA and CA increased significantly between 0 wk and 8 wk (by 180.7 ± 12.2 ml·min^{-1}, p<0.001and 221.0 ± 26.5 ml·min^{-1}, p<0.001 respectively) with no differences observed between groups (see Figure 8.1 - SL; 151±6% vs. LX; 144±4%, p>0.05). Significant reductions were observed during detraining at week 10 and 12 in SFA (p<0.001) with blood flow returning below baseline levels in both groups, with no difference between groups (p>0.05). However, CA blood flow remained significantly above baseline levels in both training groups following detraining, once again there was no difference between the groups (SL; 22±1% vs. LL; 21±1%, p>0.05). There were no significant changes to the control group (p>0.05) at any stage.

SFA & CA arterial diameters; The diameter of the SFA and CA for the training groups (data pooled) changed significantly post-training (p<0.001 and p<0.01 respectively). The SFA diameter increased to an identical extent in both SL and LX training groups (both 26±1%) and therefore no difference between groups (p>0.05) at week 8, which was also observed at following detraining at weeks 10 and 12 (p>0.05) despite significant reductions in diameter during detraining in both groups (p<0.001). This was also the case in CA diameter, where there was no difference between groups following training or detraining (p>0.05) despite an increase at week 8 (p<0.01) and reductions at week 10 and 12 (p<0.05). There were no significant changes to the control group (p>0.05) at any stage.

Shear rate; For changes in shear stress see Figure 8.1. At week 8, SFA shear stress significantly (p<0.001) in both training groups (SL; 105±11% and LL; 102±5%) with no difference (p>0.05) between groups. Both groups remained significantly (p<0.01) above baseline at both week 10 and week 12 (SL; 33±12% and LL 36±10%) despite significant (p<0.05) reductions during detraining compared to week 8. There was significant difference between groups at any stage

(p>0.05). With regards CA shear stress, at week 8, both SL and LX experienced an identical significant (p<0.05) increase compared to baseline (38±4%). However by week 10 following a significant reduction in shear stress (p<0.001), both training groups had returned to below baseline levels. Unexpectedly, following a further two weeks of detraining, shear stress had increased significantly above baseline levels again (p<0.05) in both training groups. There were no significant changes to the control group (p>0.05) at any stage.

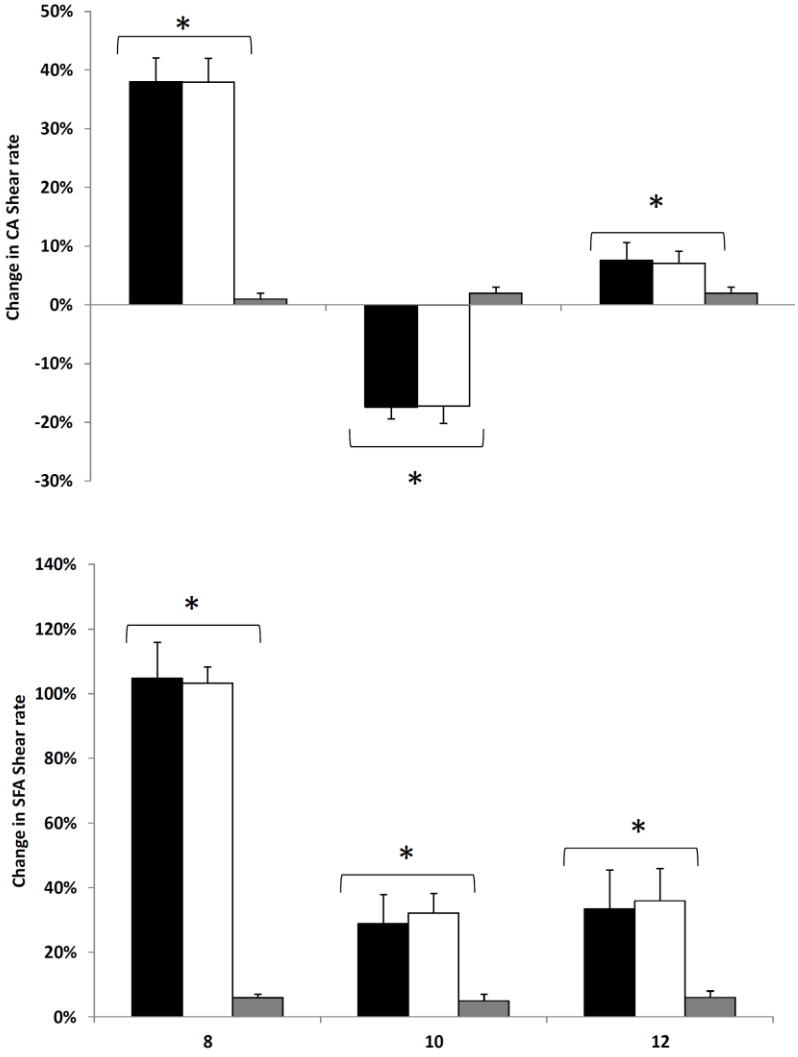

Figure 8.1. Changes in CA (top graph) and SFA (bottom graph) shear rates following training and detraining in SL (black bars), LX (white bars) and control (grey bars). * Significantly different to baseline and control group (p<0.01).

Discussion: Following 8 weeks of resistance exercise at two different ranges-of-motion, the vascular system made significant adaptations to its function in both training groups. Further, there were no significant differences between the training groups in terms of relative changes in vascular function, and therefore the hypothesis that LX group may improve vascular function in a superior fashion is rejected.

The stimuli for local and systemic increases in arterial diameter and blood flow, as observed in the present study may be attributed to changes in endothelial dilatory factors or systemic rises in pressure. It is argued that in response to exercise training or physical inactivity, functional vascular adaptations occur initially but are soon superseded by structural adaptations to the vessel walls (Laughlin, 1995), with arterial remodeling thought to be dependent on both shear stress and nitric oxide (NO) (Tuttle et al., 2001). During acute exercise training endothelial NO synthase (eNOS) expression and subsequent NO production is increased to moderate the elevated shear stress on the endothelial surface (Prior et al., 2004). With prolonged exercise training the enhanced NO levels are believed to initiate structural vessel remodeling to adjust shear levels back to a 'new normal' at which level they are maintained (Prior et al., 2004).

Shear stress on the endothelial cells is caused by fluctuations in blood flow associated with muscle hypertrophy (Rakobowchuk et al., 2005). These authors also previously proposed that the increased blood flow associated with hypertrophy could be explained by enhanced capillary and/or arteriolar proliferation (Rakobowchuk et al., 2005). Evidence from chapters 3 and 5 show that the LX group experienced greater muscle hypertrophy (chapter 3) and a greater acute cardiovascular response to the training regime (chapter 5) than SL. However it appears that although there may appear to a possible link between exercise intensity (Cooper et al., 2002), blood flow dynamics associated with hypertrophy, blood pressure and flow on arterial walls

(Kojda and Hambrecht, 2005) that could plausibly lead to superior vascular adaptation, the association and combination of these factors was not observed in this study. Therefore with regards the purported benefits of vascular adaptation to enhance amongst other things, $VO_{2\ max}$, neither training regime appears to be superior to the other to bring about such changes.

APPENDIX 3

PUBLICATIONS:

1. McMahon G, Morse CI, Burden A, Winwood K and Onambélé-Pearson GL, 2013. How deep should you squat to maximise a holistic training response? Electromyographic, energetic, cardiovascular, hypertrophic and mechanical evidence. *In* Electromyography - ISBN 980-953-307-826-2
2. McMahon G, Morse CI, Burden A, Winwood K and Onambélé GL, 2013. Impact of range-of-motion during ecologically valid resistance training protocols, on muscle size, subcutaneous fat and strength. Journal of Strength & Conditioning Research. *[Epub ahead of print]*
3. McMahon G, Morse CI, Burden A, Winwood K and Onambélé GL, 2013. Muscular adaptations and IGF-I responses to resistance training are stretch-mediated. Muscle & Nerve DOI. 10.1002/mus.23884

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1. McMahon G, Morse CI, Burden AM, Winwood K and Onambélé GL, 2011. Training at an elongated muscle length maximises muscle size and strength. Proceedings 16th Annual Congress of the European College of Sports Science. Liverpool, UK; July. POSTER P-056
2. McMahon G, Morse CI, Burden AM, Winwood K and Onambélé GL, 2011. Human *in vivo* tendon adaptations to resistance training & detraining at different muscle lengths. Proceedings of the Physiological Society. Oxford, UK, July. ORAL C108
3. McMahon G, Morse CI, Burden AM, Winwood K and Onambélé GL, 2012. Effect of differential muscle-tendon unit length during dynamic resistance training on muscle function, architecture, morphology and detraining. The Physiological society (ID: 1343596). The Biomedical Basis of Elite Performance. March, London, UK. ORAL- C6
4. McMahon G, Morse CI, Burden AM, Winwood K and Onambélé GL, 2012. Effect of differential muscle-tendon unit length during dynamic resistance training on muscle function, architecture, morphology and detraining. The Physiological society (ID: 1343596). The Biomedical Basis of Elite Performance. March, London, UK. POSTER- PC6

HOW MUCH SHOULD YOU BEND TO MAXIMISE A HOLISTIC TRAINING RESPONSE? Electromyographic, energetic, cardiovascular, hypertrophic and mechanical evidence.

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INTRODUCTION: Skeletal muscle possesses the ability to change its characteristics and properties in response to its external environment (i.e. adaptation). The exact nature of such adaptations is manipulated by the stimulus provided to the muscle. Resistance exercise is an example of one such stimulus, and it is used in a variety of settings, such as athletic performance, general health and fitness, injury prevention and rehabilitation. It is also now commonplace for resistance exercise to be used to offset the debilitating effects of illness, disease and sarcopenia (sarcopenia is the term used to describe the age-related loss in muscle mass or more generally in muscle 'quality'). The objectives of the resistance exercise protocol therefore, will vary due to the unique nature of each setting, and therefore should be optimised in order to bring about a specific and desirable set of adaptations. Frequent adaptations that are sought from resistance exercise regimes include an increase in muscle cross-sectional area (CSA) and strength (Maughan et al., 1983), alterations to muscle architecture (spatial arrangement of muscle fibres within a muscle (Kawakami et al., 1995)), and greater maximal activation of the musculature (Moritani and DeVries, 1979).

Muscle activation has been widely assessed using surface electromyography (SEMG), and in many cases is expressed as a level (%) of maximal voluntary contraction (or MVC). It comprises the sum of the electrical contributions made by the active motor units. The global characteristics of the surface EMG, such as its amplitude and power spectrum, depend on the membrane properties of the muscle fibres as well as on the timing of the single fibre action potentials. Thus the surface EMG reflects both peripheral and central properties of the neuromuscular system (Farina et al., 2004). Maximal activation of muscle is warranted in order for the muscle to express its maximal force generating capabilities, and there have been many studies carried out that have reported a significant increase in agonist SEMG recordings following a resistance training program in both males and females, and in the young and elderly (Häkkinen et al., 1996, Narici et al., 1989, Häkkinen et al., 2003, Komi et al., 1978, Moritani and DeVries, 1979, Häkkinen and Komi, 1983, Reeves et al., 2005b, Higbie et al., 1996). As mentioned previously, muscle adapts in a specific manner to the stimulus provided, and in the case of the aforementioned studies, increases in agonist muscle activation has been shown to be specific to

the mode of muscular contraction employed during the resistance training period, and has been fairly well characterised. However, one aspect of resistance training that is poorly reported in the literature is the acute (and/ or chronic) responses to muscle length-specific resistance training programs. Acutely, it has been demonstrated that there are significantly different responses to exercise at different joint-angles (and thus different muscle lengths). De Ruiter et al. (de Ruiter et al., 2005) showed that following isometric MVC exercise at 30^{o}, 60^{o}, and 90^{o} of knee flexion, maximal activation of the knee extensors was significantly greater at 90^{o} than the other two angles, despite having identical torque production as 30^{o} (90^{o}; 199±22Nm, 30^{o}; 199±29Nm) and significantly lower torque production than 60^{o} (298±41Nm). A subsequent study(Kooistra et al., 2006) found that maximal muscle oxygen consumption was reached significantly later, and was on average ~60% less at 30^{o} compared to 60^{o} and 90^{o} knee flexion. Furthermore, Hisaeda et al.(Hisaeda et al., 2001) found that when performing isometric contractions at 50% MVC to failure at either 50^{o} or 90^{o} of knee flexion, endurance time was significantly shorter at 90^{o} than 50^{o}, both when performed with the local circulation occluded and not occluded. Also the slope of the iEMG-time regression was significantly greater in the 90^{o} condition compared to 50^{o}. One of the reasons for an increase in oxygen consumption at longer muscle lengths (or more flexed joint angles) is that to produce the same external torque, the internal mechanical stress must be higher at more flexed angles (90^{o}) compared to extended angles (30^{o} or 50^{o}) because the moment arm of the in-series elastic component (the tendon) is shorter (Krevolin et al., 2004) at more flexed angles. These studies provide compelling evidence of the link between the muscle length during a bout of resistance exercise and the acute impact on muscle activation levels, energetic provision and fatigability, and torque production. It is important to determine the nature of the acute effects of length-specific training because it is the accumulation of the acute responses that ultimately are reflected in the chronic muscle adaptations (known as the repeated bout effect; RBE). Several investigations have also previously identified the link between muscle length (or joint-angle) and gains in strength and/ or levels of muscle activation following a more extended period of resistance training (Lindh, 1979, Gardner, 1963, Thepaut-Mathieu et al., 1988, Kitai and Sale, 1989, Kubo et al., 2006a). Briefly, these studies have shown that significantly greater increases in isometric strength are attained when tested at the training length or position, and that these changes in strength are accompanied by significantly greater activation of the muscle in the training position. Furthermore, several studies have outlined that at shorter muscle lengths, the phenomenon of length-specific adaptations are more marked compared to those at longer muscle lengths (Kubo et al., 2006a, Thepaut-Mathieu et al., 1988). For example, performing resistance training at a shorter muscle length results in increases in strength at, and close to the training muscle length, whereas training at longer muscle lengths results in strength increases at, and around a larger range of muscle lengths. However, all of the above information is provided via controlled isometric (static) contractions, when indeed resistance training programs for most individuals are predominantly of a dynamic nature, and therefore warrants further research to extend the knowledge in this area. Therefore the aims of the current were:

1. To describe the acute differences in activation of the Vastus Lateralis (VL) muscle whilst performing dynamic resistance exercise over relatively short muscle lengths compared to long muscle lengths; here comparisons will be carried out both between different absolute and relative loading. 2. To describe the changes in oxygen consumption and cardiovascular responses during the same exercise parameters as above. 3. To identify a link between the acute responses to loading at shorter and longer muscle lengths and the more chronic adaptations on VL muscle activation and size and following 8 weeks of length-specific resistance training and 4 weeks detraining.

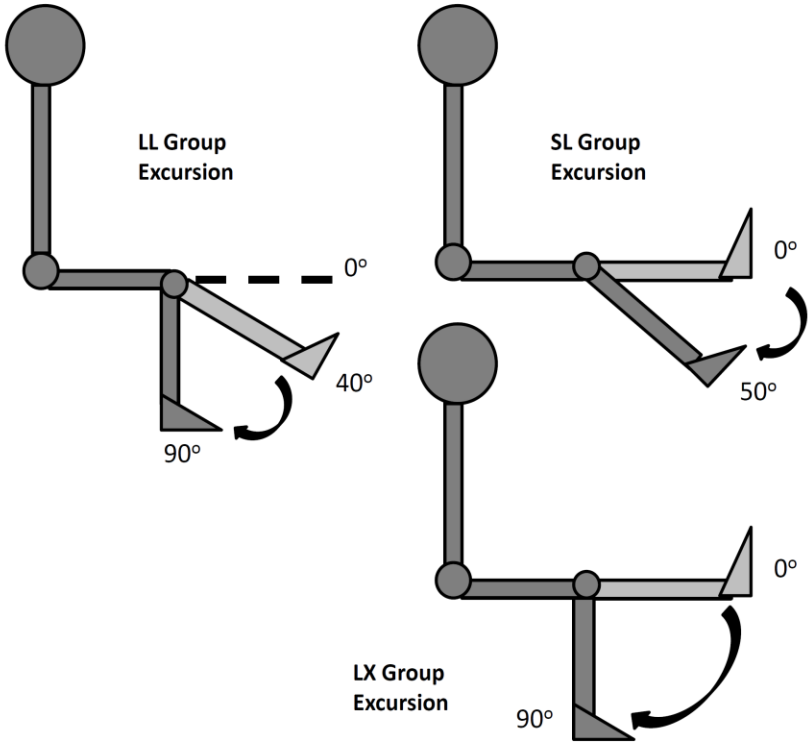

Figure 1. Diagram showing the various knee-joint ranges-of-motion used in the acute and training studies

METHODS: *Acute Study* – Ten males (23±3 years, 1.79±0.06m, 73.4±8.4Kg) gave written informed consent to take part in the study. All procedures and experimental protocols were approved by the Ethics Committee at Manchester Metropolitan University. Exclusion criteria for participation in the study were the presence of any known musculoskeletal, neurological, inflammatory or metabolic disorders or injury. Participants were physically active, involved in recreational activities such as team sports, and had either never taken part in lower limb resistance training or not within the previous 12 months. Participants attended the laboratory on a total of five occasions. The first visit included demonstration of the appropriate squat technique for a standard barbell back squat, and familiarisation of the exercise protocol and testing equipment. The following week participants returned on four occasions, firstly to record their one repetition maximum over each range-of-motion, which was defined as the maximum amount of external weight (Kg) that could be lifted in a controlled manner through the entire range-of-motion, and their MVC on an isokinetic dynamometer (Cybex, Phoenix Healthcare Products, UK) at 50^{o} and 90^{o} of knee flexion. The time-line of the sessions was as follows: Day 1; 1RM & MVC, Day 2; Rest, Day 3; Protocol 1, Day 4; Rest, Day 5; Protocol 2, Day 6; Rest, Day 7; Protocol 3. During each of the resistance exercise protocol days, the participants were randomly allocated to perform the resistance exercise session of one of the three designated ranges-of-motion. During each of the resistance exercise sessions, all acute variables (EMG, VO_2, heart rate, blood pressure) were measured. *Exercise Protocols*;

Each exercise protocol involved performing exercise over one of three ranges-of-motion (Figure 1). The three ranges-of-motion were; 0-50^{o} knee flexion (shorter muscle lengths, SL), 40-90^{o} knee flexion (longer muscle lengths, LL) and 0-90^{o} knee flexion (complete range-of-motion incorporating shorter and longer muscle lengths, LX). A goniometer was attached to the knee joint centre of rotation, from which the investigator confirmed each angle was met during exercise performance. Each exercise session required participants to perform one set of five repetitions back squats at an absolute load of 20Kg, 40Kg and finally 60Kg. Sets were interspersed by two minutes of recovery. Following a further ten minutes rest, each participant performed a further set of five back squats at 40%, 60% and 80% 1RM, interspersed by two minutes of rest. *Electromyography;* A pair of self-adhesive Ag-AgCl electrodes 15 mm in diameter (Neuroline 720, Ambu, Denmark), were placed on clean, shaved, and previously abraded skin, in a bipolar configuration with an inter-electrode distance of 20 mm. The electrodes were placed at 50% of femur length and 50% of muscle width of the *Vastus Lateralis* muscle (VL). The reference electrode (Blue sensor L, Ambu, Denmark) was placed on the lateral tibial condyle. The raw EMG signal was preamplified (MP100, Biopac Systems Inc., USA), amplified (MP100, Biopac Systems Inc., USA), bandpass filtered between 10-500 Hz (Biopac Systems, USA), and sampled at 2000 Hz. All EMG and torque signals were displayed in real time in AcqKnowledge software (Biopac systems Inc., USA) via a PC (iMac, Apple Inc., USA). The root mean square (RMS) EMG activity

was averaged for a 500ms period which coincided with the plateau of peak torque of all analysed muscle contractions. *Oxygen Consumption (VO_2)*; Gases for VO_2 consumption were collected using standard Douglas Bag techniques. Prior to the beginning of each set of exercise, a nose clip was placed on the nose of the participant, and the Douglas bag mouthpiece was inserted into the mouth and the valve subsequently opened. After the set of exercise was complete, 30 seconds were allowed to elapse before the valves were closed. This was to allow for any excess post-exercise oxygen consumption during the immediate recovery period. A separate Douglas bag was used for every set of exercise completed. Each bag was analysed using a gas analysis program (Servomex 5200 Mulitiuse, xxx) and was used to calculate the $FECO_2$ and FEO_2 percentages. Gas was evacuated for 60 s with a flow rate of 2.1 L/min. A Harvard Dry Gas vacuum was used to extract the remaining gas, the flow rate (2.1 L) was added to the final figure to give the VE atps (L/min^{-1}). The time period in which the Douglas bag was open (secs), load (kg) and subjects' heart rate were then added to the programme, and finally the VO_2 ($ml/kg^{-1}/min^{-1}$) was recorded. *Heart rate & Blood Pressure*; Heart rate and blood pressure were recorded at rest in the supine position before the onset of exercise using a standard heart rate monitor (Polar, UK) and electronic blood pressure monitoring device (Panasonic Diagnostec, UK). These parameters were also measured immediately post-exercise, after every set of exercise. Rate of perceived exertion (RPE) was also recorded following the conclusion of each individual set of exercise.

Resistance Exercise Program Study – Thirty two activity-matched participants were allocated to a training group – SL (shorter muscle length 0-50^0; 6 males, 4 females; aged 19±2.2 years, 1.76±0.15m, 75.7±13.2Kg), LL (longer muscle length; 5 males, 6 females 40-90^0; 21±3.4 years, 1.75±0.14m, 74.9±14.7Kg) or LX (6 males, 5 females0-90^0;19.2±2.6 years, 1.71±0.11m, 73.8±14.9Kg). Ten participants (6 males and 4 females; 23±2.4 years, 1.76±0.09m, 77.9±13.1Kg) were assigned to the non-training control group (Con), and continued their normal habitual activity throughout the study period. A One-way ANOVA revealed that the population was homogeneous at baseline for all parameters of interest (P>0.05). All groups were assessed at baseline (week 0), post-training (week 8), after two weeks of detraining (week 10) and following a further two weeks of detraining (week 12). *Electromyography;* Preparation of EMG site, measurement and assessment of EMG was described in the previous section. In addition to these measurements, EMG of the biceps femoris was also recorded during graded maximal contractions in order to assess antagonist co-activation. *Resistance Training Program (RT)*; RT was carried out 3 days per week for 8 weeks and ceased during the 4 week detraining period. RT included performing 3-4 sets of 8-12 reps (depending on stage of training program) of exercises designed to overload the knee extensor muscle group. Exercises included the barbell back squat, leg extension, leg press, Bulgarian split squat, and forward lunge. 1RMs were assessed and recorded every two weeks to adjust the exercise loads. *Muscle size measurements*; VL muscle widths were measured using B-mode ultrasonography (AU5, Esaote Biomedica, Italy) at 25%, 50% and 75% of femur length. The ultrasound probe was held in the transverse plane and used to locate the borders of either side of the VL muscle. Each of these junctures was marked on the skin and the distance between them measured. In addition, at each of the aforementioned sites, thigh girths were also measured using standard anthropometric techniques. All data is presented as mean ± standard deviation (S.D.)

RESULTS:

Acute Responses *- Muscle Activation***;** Vastus lateralis activation increased as a result of absolute load, with activation being significantly greater (P<0.05) when lifting 40Kg compared to 20Kg, and also when lifting 60Kg compared to 40Kg (P<0.05) and 20Kg (P<0.001). When comparing activation between ranges-of-motion as a percentage of MVC, activation of the muscle was significantly (P<0.05) less at SL (59±6%, 63±7%) compared to LL (73±7%, 77±5) and LX (70±7%, 75±6%) at 40Kg and 60Kg loads respectively (Figure 2A). During relative loading, performing exercise at 60% 1RM did not increase activation compared to 40% 1RM (P>0.05), however activation was increased at 80% 1RM compared to 60% (P<0.05), and 40% (P<0.001). There were no significant differences in activation at 40% and 60% 1RM between the three ranges-of-motion (P>0.05), however at 80% 1RM, VL activation was significantly greater during exercise in LL and LX compared to SL (Figure 2B; P<0.05). However there were no significant differences between the longer muscle length ROM and the complete ROM under any loading conditions (P>0.05).

Oxygen Consumption (VO_2); There was no significant changes in VO_2 between any of the absolute loading conditions or between any ROM (P>0.05). Further, in the relative loading conditions, mean VO2 was significantly greater at 80% 1RM compared to 40% 1RM (6.4±0.9 $ml/kg^{-1}/min^{-1}$ vs. 9.93±1.3 $ml/kg^{-1}/min^{-1}$, P<0.05). VO_2 was greater at 40% and 60% 1RM in LL and LX than SL, however there were no significant differences between these ROMs. At 80% 1RM there was a significantly greater VO_2 (Figure 3) in the LL ROM compared to SL (P<0.05), however there were no significant differences between LL and LX, or SL and LX at this loading intensity (P>0.05).

Heart rate & Blood Pressure; There was a significantly greater (P<0.05) mean heart rate observed between LL (139±10 beats per minute) and LX (136±11bpm) compared to SL (118±12bpm) in both absolute and relative loading conditions, with no difference between LL and LX (P>0.05). Mean systolic blood pressure

yielded no significant differences (P>0.05) between the three ROMs under relative loading conditions, however LX (148±8 mmHg) mean systolic blood pressure was significantly greater than both SL (138±6 mmHg) and LL (135±8 mmHg) following loading under absolute loads (P<0.05).

Prolonged Resistance Training Responses – *Agonist (VL) Muscle Activation*; Figures 4 and 5 shows absolute and relative changes in activation at baseline and post-training. At week 8, absolute maximal agonist activation did not increase significantly as a result of the training protocol in any of the training groups, with no significant difference between training groups at any knee angle (P>0.05, Figure 4). However, post-training there was a significant relative increase in activation at 50^o (23±15%, P<0.05), 70^o (26±15%, P<0.01) and 90^o (16±13%, P<0.05) in the LX group and also at 70^o (24±9%, P<0.01) and 90^o (25±9%, P<0.01) in LL group. In the SL group there was no significant change at 50^o, although there were significant (P<0.05) reductions in VL activation at both 70^o (-15±6%) and 90^o (-13±5%). Following detraining, muscle activation at 70^o decreased at week 10, and levelled off for the remainder of the detraining period (week 12) in both LL and LX groups with no significant changes compared to week 8. In the SL group, activation reduced at both weeks 8, 10 and 12 compared to baseline, however despite larger decrements in this group, there was no significant differences between all three training groups (Figure 6, P>0.05). Muscle widths and thigh girths. Changes in muscle widths are shown in Table 1. At week 8, VL muscle widths had increased significantly at all three measurement locations in all training groups compared to baseline (P<0.001). Following the first period of detraining at week 10, the SL group had returned to baseline values at all three measurement sites

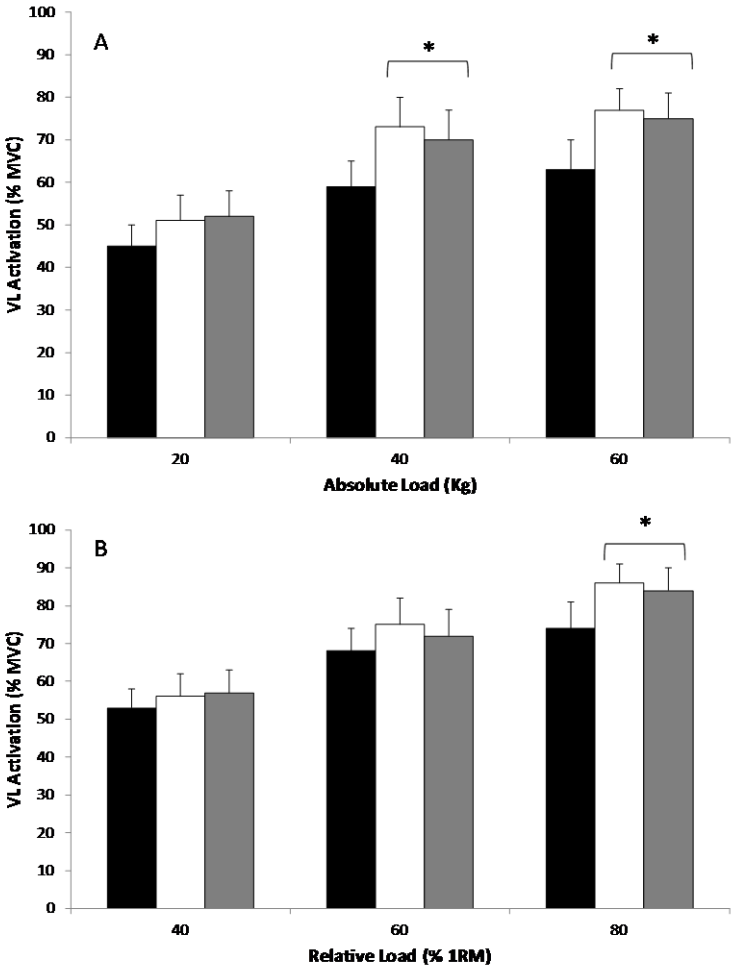

Figure 2. Vastus Lateralis muscle activation in SL (black bars), LL (white bars) and LX (grey bars) following varying magnitudes of absolute and relative loading. * Significantly different to SL

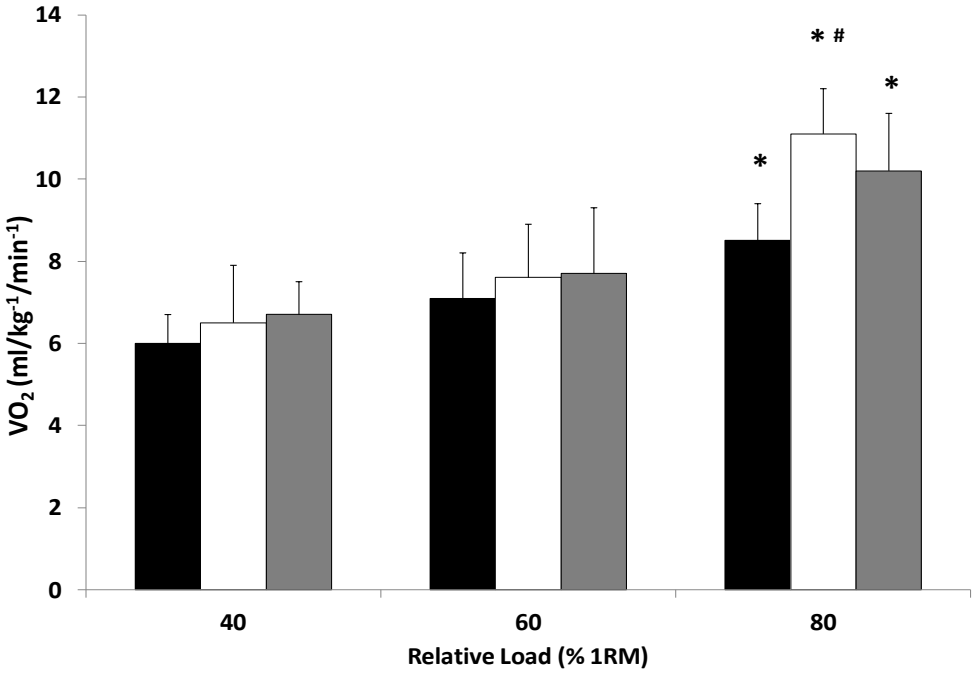

Figure 3. Oxygen consumption (VO_2) during relative loading in SL (black bars), LL (white bars) and LX (grey bars). * Significantly different to 40% 1RM. # Significantly different to SL.

(P>0.05), however both LL and LX groups retained adaptations at this juncture compared to baseline (P<0.01). At week 12 LX group had returned to baseline levels at 25% and 50% width but still remained significantly elevated at 75% femur length compared to baseline (P<0.05). The LL group retained their significant gains in muscle width at all three sites for the entire duration of detraining (P<0.01). There were no significant (P>0.05) mean relative changes between training groups post-training or following detraining at 25% and 50% femur length (SL; 12±13%, LL; 11±7%, LX; 13±11%). However, LL and LX groups had a greater significant (P<0.05) relative increase in muscle width at week 10 (LL; 26±13%, LX; 21±9%) compared to SL (13±8%) at 75% femur length. This was also the case at week 12, however only LL group was significantly greater (P<0.05) than SL group at this measurement site. Thigh girths increased following training at week 8 in all training groups and at all sites (mean over three sites SL; 3±2%, LL; 4±3%; LX; 4±2%), however this was not significantly different to baseline values (P>0.05) with no differences between groups. Thigh girths also did not differ significantly at weeks 10 or 12 compared to baseline or between groups.

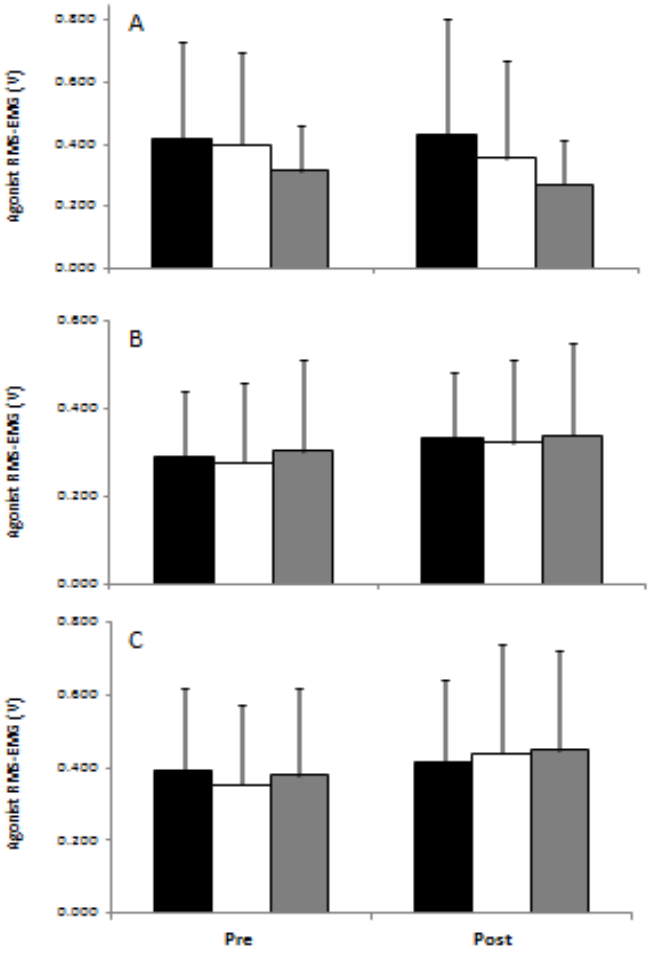

Figure 4. Absolute Changes in VL activation at baseline (pre) and week 8 (post) training at 50^{o} (black bars), 70^{o} (white bars) and 90^{o} (grey bars) knee flexion in A) SL, B) LX and C) LL groups.

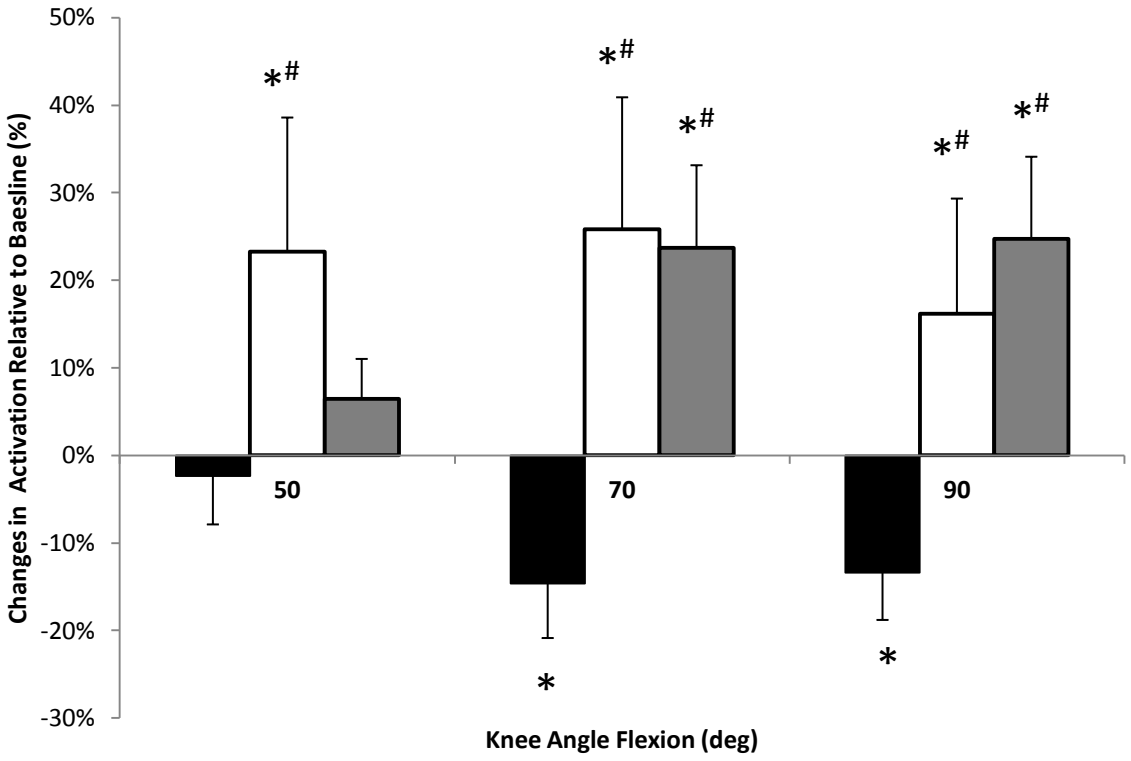

Figure 5. Relative changes in VL activation at week 8 at three knee joint-angles in SL (black bars), LX (white bars) and LL (grey bars). * Significantly different to baseline. # Significantly different to SL group.

Table 1. Changes in Vastus Lateralis muscle width at each measurement site throughout training and detraining. * Significantly different to baseline (P<0.05) ** Significantly different to baseline (P<0.01)

Group	% Femur Length	Baseline (cm)	Week 8 (cm)	Week 10 (cm)	Week 12 (cm)
SL	25	12.7±2.3	13.9±2.0*	13.2±1.8	12.9±1.6
	50	12.8±2.7	14.2±2.5**	13.6±1.9	13.3±1.9
	75	9.6±2.2	11.0±1.9*	10.1±1.2	9.6±1.1
LL	25	14.0±1.3	15.3±1.6**	15.6±1.1**	15.3±0.9**
	50	13.8±1.6	15.6±2.0**	15.8±1.5**	15.4±1.3**
	75	9.4±1.8	11.4±1.4**	11.5±1.2**	11.1±1.3**
LX	25	12.2±2.7	14.2±1.9**	13.0±2.0**	12.4±1.8
	50	12.1±2.6	14.3±2.3**	13.1±2.0**	12.3±2.0
	75	8.2±2.2	10.6±1.5**	9.6±1.6**	8.8±1.2*

Strength measures; Throughout the training program, 1RM of the knee extensors were monitored on the knee extension machine. 1RM did not increase significantly compared to baseline until week 4 of the training program (data pooled, P<0.05) with each training group making similar increments in weight (SL; 11±4%, LL; 9±5%, LX 12±5%). There were further significant increases at week 6 (mean of groups 16±6%, P<0.01) and week 8 (mean of groups 22±8%, P<0.001), with no significant difference in the rate of relative increase in 1RM between groups (P>0.05). When muscle activation was normalised against torque at week 8 (Figure 7), both LL and LX groups showed significantly greater muscle activation than SL (P<0.01) at 90^{o} of knee flexion, however there were no differences in muscle activation per unit torque at 50^{o} or 70^{o} following training (P>0.05).

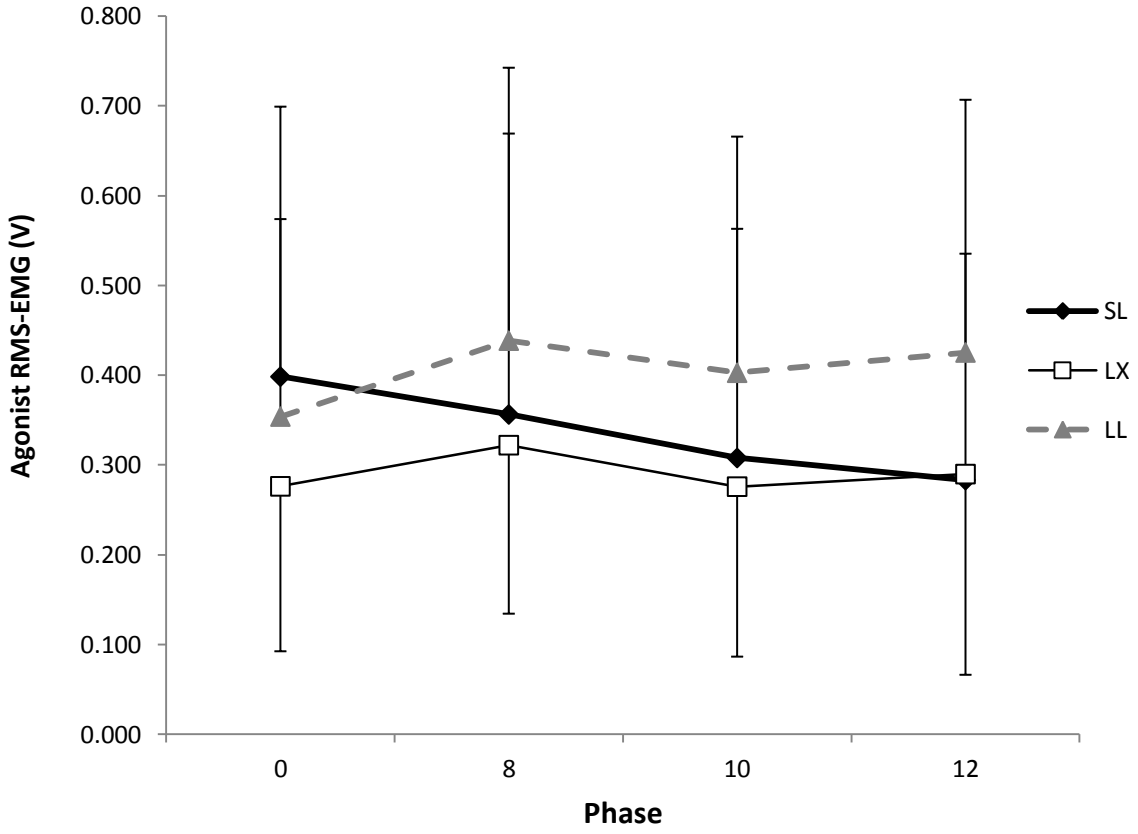

Figure 6. Absolute changes in VL activation throughout training and detraining periods at 70^{o} knee flexion. No significant were detected between phases or training groups (P>0.05).

Discussion: Resistance training presents a medium in which we can enhance our muscular function. In order to devise an appropriate and effective resistance training program tailored for specific objectives, it is necessary to understand the responses to both an acute bout of exercise, and also the adaptations to exercise over a prolonged period of training. An important aspect for muscular performance is the degree to which the muscle can be activated. Previous work using isometric contractions of the knee extensors, has demonstrated that the magnitude of maximal muscle activation is dependent on the joint-angle (and thus muscle-length) used during exercise, even when external torque produced is the same(de Ruiter et al., 2005). This study showed that activation of the quadriceps was significantly greater at 90^{o} knee flexion compared to both 30^{o} and 60^{o}, despite isometric MVC torque being significantly less than 60^{o} and identical to 30^{o}. In the current study, our participants exercised dynamically over a range-of-motion that was predominantly over shorter muscle lengths (0-50^{o}), longer muscle lengths (40-90^{o}) or over both (0-90^{o}) during exercise using absolute and relative loading patterns. During absolute loading, weight lifted increased in a graded manner, and was reflected by significantly increased muscle activation between each absolute load in the training groups. This result was a more easily predicted outcome and reflects one of the fundamental properties of the neuromuscular system, i.e. the size principle (Henneman et al., 1965), where a greater number of motor units are recruited in order meet the increasing demands of force production. When exercising over longer muscle lengths (LL) and the complete ROM (LX), muscle activation was significantly greater during absolute and relative loading compared to

shorter muscle lengths (SL). So why would muscle be more activated by moving the same external weight but at different muscle lengths? By moving through a range of muscle lengths or joint-angles, the moment arm of the in-series elastic component (i.e. the tendon) also changes. As the amount of force needed to lift an external load (F) is $F = f \times d$, where *f* is the internal force produced by the muscle and *d* is the length of the moment arm, when *d* is greater *f* will be smaller and vice versa, and therefore when the external force produced is the same but the moment arm (*d*) is smaller, the contribution from internal muscle force production increases. An example of this experienced in everyday life is the increased difficulty in rising from a lower seated position compared to a higher seated position. It has been demonstrated previously that when the joint-angle in the knee extensors is at 90^o flexion (such as the end of LL and LX group ROM), that the moment arm is considerably shorter (Krevolin et al., 2004) than when at 50^o (the end of SL ROM). Therefore when exercising at 90^o, internally the muscle must produce a greater amount of contractile force to overcome the external weight than at 50^o. Again due to the size principle, a larger number of motor units will have needed to be recruited to match the force demands at the longer muscle lengths, reflected by the increase in RMS-EMG activity of the VL muscle. In support of this hypothesis, Kubo et al.(Kubo et al., 2006a) trained the knee extensors isometrically at either 50^o or 100^o of knee flexion. Based on their MVC and EMG recordings, they estimated that the internal force on the quadriceps muscles was 2.3 times greater at longer muscle lengths (i.e. 100^o) than at shorter lengths. A further variable that must be considered is the influence of changing muscle lengths on the force-length relationship of muscle (for review see (Rassier et al., 1999)). In short, when one alters the length of a muscle, the basic contractile units of individual muscle fibres, known as sarcomeres, also change length. The ability of sarcomeres (and thus muscle) to exert force is determined mainly by actin and myosin filaments interaction and cross-bridge formation. As sarcomere (or muscle) lengths increase, cross-bridges number and force is increased until a certain optimum length is reached, upon which further lengthening decreases cross-bridges formation and force (however strictly not in all conditions). If longer muscle lengths are less optimum muscle lengths for force production and cross-bridge formation than shorter muscle lengths, then greater motor unit recruitment will be necessary to overcome the external resistance. Therefore the two factors likely for greater activation in LL and LX compared to SL may be due to the greater internal mechanical stress on the muscle because of a shorter moment arm, and/ or the length of the muscle reducing cross-bridge formation and force production per sarcomere.

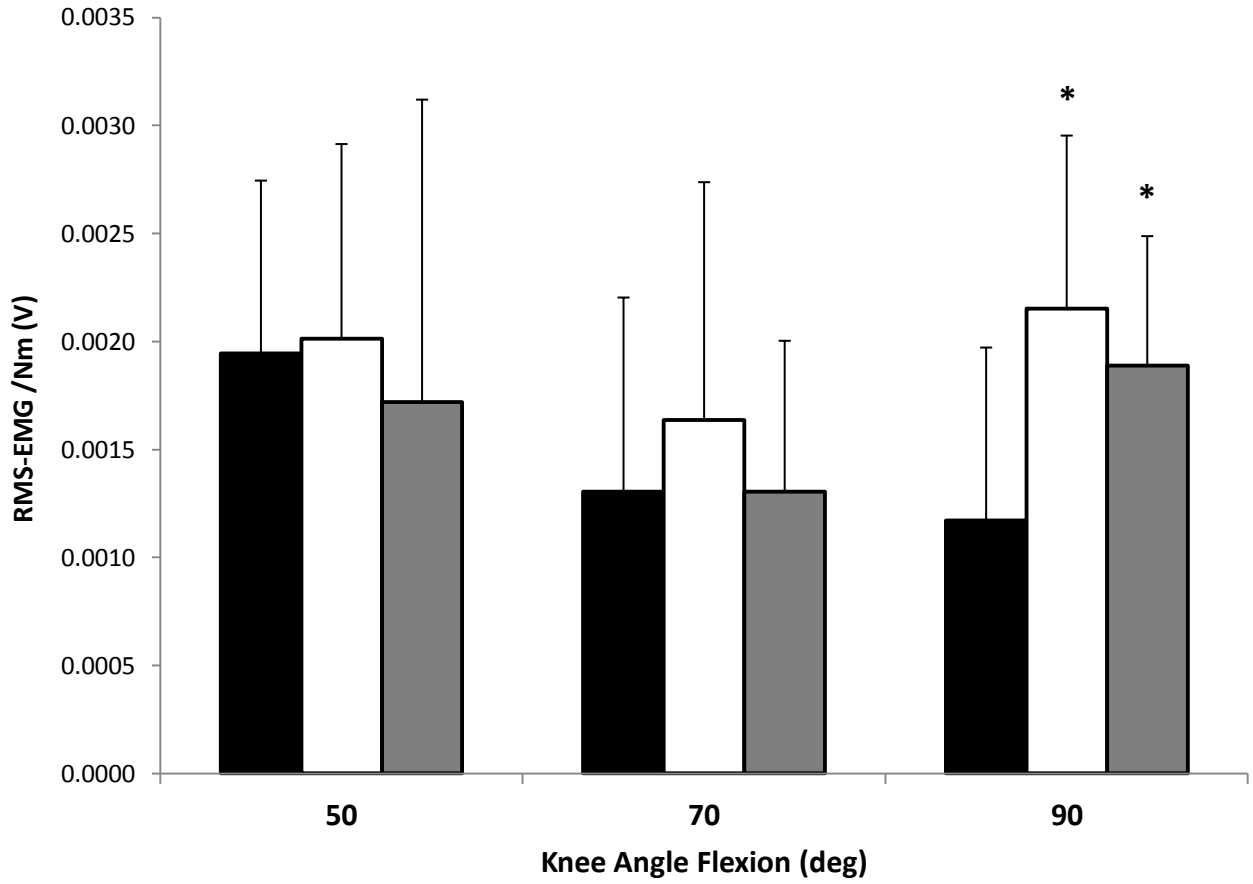

Figure 7. Vastus Lateralis muscle activation per unit of torque at week 8 at three joint-angles in SL (black bars), LL (white bars) and LX (grey bars). * Significantly different to SL (P<0.05). N.B. there were no between group differences at baseline.

In the current study, oxygen consumption (VO_2) was shown to be significantly greater at 80%1RM compared to 40% 1RM, and also significantly greater in both the LL and LX ROMs compared to SL ROM at 80% 1RM loading. VO_2 is used in exercise physiology to provide several pieces of valuable information during exercise, such as an indicator of energy expenditure. Oxygen is 'consumed' by the working muscle during oxidative phosphorylation in order to produce, maintain and/ or replenish the energy used during the many different processes involved with muscular contraction. Therefore if a particular form or type of exercise requires the use of greater volumes of oxygen, then it indicates that the system is working harder in order to do so. It has been demonstrated previously in humans that oxygen consumption increases with work intensity during constant isometric loading of the knee extensors (de Ruiter et al., 2005, Kooistra et al., 2006), and this is reflected by the increased VO_2 at 80% 1RM compared to 40% 1RM, where although performing the same ROMs, participants were exerting greater force, requiring more energy to supply muscular contraction. The relative VO_2 levels are much lower than normally encountered during aerobic exercise for example, due to the shorter duration of exercise bouts and greater contribution to energy supply from anaerobic sources such as ATP-PCr system and glycolysis. Of more interest was the fact that both LL and LX ROMs had significantly greater VO_2 compared to SL at 80% 1RM. Previous research using near-infrared spectroscopy has demonstrated that during isometric exercise of the knee extensors, VL muscle VO_2 is significantly increased at longer muscle lengths (60^o and 90^o) compared to shorter muscle lengths (30^o). This was even despite the fact that MVC torque relative to the maximum torque capacity (MTC) tended to be greater (~85% of MTC) at 30^o compared to both 60^o and 90^o (~75% MTC). A subsequent study by Kooistra et al. (Kooistra et al., 2006) demonstrated that knee extensor muscle activation and VO_2 were significantly less, and time to VO_{2max} significantly longer at the same relative torque levels at 30^o compared to 60^o and 90^o. Another indication of the increased stress at longer muscle lengths was the observation that at 80% 1RM, although covering almost half the ROM of LX, LL group consumed greater volumes of oxygen, although this was not statistically different. This suggests that the energetic cost of constantly working at longer muscle lengths is

at least just as, if not more so, demanding than alternating between longer and shorter muscle lengths even when over a greater ROM. With significantly higher heart rates in both LL and LX groups (and also greater blood pressure in LX) compared to SL during exercise, the results also suggest that the cardiovascular system was also under greater stress at longer muscle lengths. Taken into consideration with both the aforementioned differences between the LL and LX groups compared to SL with regards muscle activation and oxygen consumption, it appears that performing exercise over predominantly longer muscle lengths (or incorporating longer muscle lengths into a full ROM) present a more potent stress to both neuromuscular and cardiovascular systems than performing exercise over mainly shorter muscle lengths. So what factors are present that would require greater oxygen consumption at longer muscle lengths? First of all, as mentioned previously, there are a number of processes that occur in order for a muscle to contract and produce force. One such process has been termed excitation-contraction coupling, where an action potential induces the release of calcium ions (Ca^{2+}) from the muscle membrane (sarcoplasmic reticulum) and these ions interact with the thin filaments of a sarcomere, allowing muscle contraction to occur. Ca^{2+} ions are then transported back into the sarcoplasmic reticulum for storage, allowing the muscle to relax. These processes are ATP-dependent (i.e. energy consuming) and as energy is consumed during activation, the amount of energy is measured as activation heat(Homsher et al., 1972). Therefore if greater activation of the muscle is occurring at longer muscle lengths, the possibility exists that the energy cost of this activation is also greater, and that this mechanism requires greater oxygen consumption to supply the energy. In addition, potentiation is force enhancement following muscle contraction, and is dependent on the contractile history. Place et al.(Place et al., 2005) showed that in following fatiguing contractions in the quadriceps muscles at either shorter (35^{o}) or longer (75^{o}) muscle lengths, peak twitch potentiation and doublet force were significantly greater at shorter muscle lengths, which may also allow for a reduction in energy cost as activation may be reduced. Secondly, we have already discussed the likelihood that due to the internal architecture of muscles and tendons, that the length of the moment arm will dictate that greater muscle force will have to be produced at longer muscle lengths compared to shorter muscle lengths. Production of the additional force through recruitment of more motor units would mean that more of the contractile machinery would used and be consuming energy, as muscular contraction from cross-bridge cycling also requires ATP(Huxley, 1957, Huxley and Simmons, 1971). Therefore the additional oxygen consumption observed at longer muscle lengths may be the result of both the energetic requirements of muscle activation and the increased energetic requirements of force production. This hypothesis is consistent with the fact that endurance performance is reduced with time to fatigue significantly shorter at longer muscle lengths compared to shorter muscle under various intensities of loading and circulatory conditions (Hisaeda et al., 2001, Place et al., 2005, Ng et al., 1994). Consistent with an increased oxygen demand, would be an increase in heart rate which was observed between the groups.

When exercise is performed on a regular basis, the above acute responses to a bout of exercise will eventually result in more long-term adaptations (i.e. repeated bout effect), which will allow the body to complete the same exercise bout as before but with relatively less disturbance to homeostasis. During the resistance training program, the three groups performed exercise over the same range-of-motion as during the acute bouts (i.e. SL, LL and LX), with the only differences being the degree of loading. SL and LX exercised at 80% 1RM, whereas LL exercised at 55% 1RM, where this was to allow the length of muscle excursion (50^{o}) and the internal muscle forces to be as similar as possible during resistance training between SL and LL. Following 8 weeks of resistance training, absolute changes in muscle activation did not increase significantly at any of the angles tested (50^{o}; shorter lengths, 70^{o} more optimal lengths, 90^{o} longer lengths) during an isometric MVC. There have been conflicting reports throughout the literature concerning the possible increase in agonist activation following resistance training, as there have been studies published that have reported significant changes (Häkkinen et al., 1996, Narici et al., 1989, Häkkinen et al., 2003, Komi et al., 1978, Moritani and DeVries, 1979, Häkkinen and Komi, 1983, Reeves et al., 2005b, Higbie et al., 1996), whereas some have not (Garfinkel and Cafarelli, 1992, Narici et al., 1996, Weir et al., 1995, Aagaard et al., 2002). However, comparing longitudinal changes in agonist EMG both within and between scientific studies can prove difficult due to methodological differences (Folland and Williams, 2007). In one length-specific resistance training study, Thepaut-Mathieu et al. (Thepaut-Mathieu et al., 1988) reported an increase in iEMG-force relationships at the specific joint angles used during training. These findings were also supported by Kubo et al. (Kubo et al., 2006a) who found that iEMG of the quadriceps (rectus femoris, vastus lateralis and vastus medialis) increased significantly in groups that trained at either shorter or longer muscle lengths, with no differences between the groups at any of the joint-angles tested. In the current study there were also no significant differences in maximal activation levels between groups and muscle lengths. However, one of the main findings from the current study was the significant relative increases in activation at all muscle lengths in LX, at longer muscle lengths in LL, and significant decreases in activation at longer muscle lengths in SL. This is further evidence of the muscle length (or joint-angle) specificity phenomenon following resistance training. Whereas a previous study (Kubo et al., 2006a) found that relative quadriceps iEMG increased at all measured knee

angles (40-110^{o}) following 12 weeks of isometric resistance training at shorter muscle lengths, our results show a decrease in activation at longer muscle lengths occurred following training at shorter muscle lengths. Interestingly from the study of Kubo et al. (Kubo et al., 2006a) was the fact that although iEMG increased within the range of ~25-45% over all testing angles (40-110^{o}) following training at shorter muscle lengths, MVC only significantly increased between 40-80^{o} in this group. Previous work from our laboratory has shown that MVC torque did not change significantly at longer muscle lengths following a period of resistance training at shorter muscle lengths (McMahon et al., 2012), and results from the current investigation show that this could be in part be mediated by a reduction in maximal activation at these lengths. Further evidence of muscle-length specificity was the fact that only LX group, who covered an entire ROM, actually demonstrated a significant relative increase in activation at each angle tested, and also that LL only showed significant relative increases in activation at longer muscle lengths (lengths where the vast majority of training would have taken place). In order to allow us to describe the impact of changes in activation on strength changes, we have shown that there was significantly greater activation of musculature per unit of torque at longer muscle lengths in LL and LX at week 8 compared to SL. Changes in torque generating capacity are not accounted for solely, or at times at all by increased muscle activation. Changes in muscle architecture, morphology and/ or muscle specific tension are just a few of the many other factors that can impact a muscle's ability to produce force following resistance training as well as neural adaptations (for review see (Folland and Williams, 2007)). However in this case, there appears to be a relationship between the increased activation of the VL muscle and the changes in torque production following resistance training in LL and LX at longer muscle lengths.

As indicated above, one of the other factors influencing changes in torque or force production following resistance exercise is muscle morphology, such as size. There is a strong positive relationship between the size of a muscle and the force it is able to exert (Maughan et al., 1983). In the current study, all of the three training groups increased the size of the VL muscle at proximal (25%), central (50%) and distal (75% of femur length) measurement sites at week 8. However in the SL group, the muscle size increment was more significant centrally rather than at proximal and distal sites of the VL, whereas both LL and LX had fairly equal distribution of size increment along the length of the muscle. Firstly, this information tells us that the resistance training program was effective in increasing muscle size, which is a well established characteristic of resistance training. Secondly, the results also suggest that the ROM involved during resistance training (i.e. the muscle lengths used) may produce region specific variations in muscle growth. Our laboratory has provided more conclusive evidence that muscle size increments at distal regions are enhanced to a greater degree immediately following resistance training at longer muscle lengths (McMahon et al., 2012), however in the current study this was only apparent following two weeks of detraining, although these were still present following a total of four weeks detraining. The region specific variation in muscle size has been previously documented throughout literature (e.g. (Narici et al., 1996)), and is probably due to the unique way in which forces are transmitted along the length of a muscle when exercised at different lengths. Forces in muscles are transmitted both serially and in parallel (Huijing and Jaspers, 2005), and when train at longer muscle lengths, there may be a more pronounced parallel transmission of force at distal regions of the muscle, providing a stimulus for growth in this location. In terms of muscle growth, force production and muscle stretch are potent stimulators of muscle protein synthesis, with a combination of both having an additive effect (Goldspink, 1999). *In vitro* experiments have shown that when muscle cells are stretched to longer lengths, there is a marked increase in protein synthesis and growth factor mRNA (Goldspink et al., 1995). The LL and LX groups when performing exercise at longer muscle lengths would have experienced a larger effect of muscle stretch compared to SL, and would have also been simultaneously producing force. In addition, because LX group worked at an intensity of 80% 1RM, peak force generation would also been greater in this group. This is supported by the mean relative increase in VL muscle width being greatest in this group at all measurement sites, although due to the variation between subjects, this was not statistically significant. Even despite the greater force generation in LX compared to LL, the LL group will have remained at longer muscle lengths throughout each training session, and therefore muscle stretch would probably have persisted compared to LX group who worked between shorter and longer muscle lengths. This may have been one of the reasons why the groups were so similar in terms of muscle width increases. At week 10, VL muscle widths were significantly greater in LL and LX at all measurement sites compared to baseline, whereas the SL group had returned to baseline values. Following a further two weeks of detraining, LX group muscle widths only remained significantly greater than baseline values at 75% femur length, whereas LL group retained post-training increments in muscle width at all measurement sites for the entirety of the detraining period. Therefore not only does training at longer muscle lengths possibly confer more beneficial adaptations following training, but it also appears to allow retention of these adaptations for a longer period of time. This is a positive finding from the current study, in that following any periods on illness, injury or tapering that occur to the individual, longer-term retention of the benefits of the preceding resistance exercise will minimise the impact of such events.

Conclusion: Performing resistance training over predominantly longer muscle lengths compared to shorter muscle lengths produces diverse acute responses, and also differential magnitudes of adaptation following an extended period of training. Following evidence from literature regarding isometric exercise and acute (de Ruiter et al., 2005, Kooistra et al., 2006) and chronic (Kubo et al., 2006a) muscle length restrictions, the current study showed that dynamic exercise at longer muscle lengths also results in greater activation and oxygen consumption. The nature of the acute responses suggests that the muscle is more physiologically stressed at longer muscle lengths. Following a prolonged period of resistance training, muscle showed relatively greater muscle activation, and following detraining had retained greater increases in muscle size. It is likely that the more beneficial size increments were the result of greater physiological stress (as supported by the acute responses) as a result of changes in the moment arm and muscle stretch. These findings have implications for athletic, elderly, or post-operative populations to name but a few.

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Impact of range-of-motion during ecologically valid resistance training protocols, on muscle size, subcutaneous fat and strength

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Abstract

Muscle strength adaptations to resistance training (RT) are joint-angle specific. However the effects of RT over different ranges of motion on concomitant changes to muscle size, architecture and strength remain largely unreported. Therefore the aim of this study was to identify differences to training at a longer (LR) compared with a shorter (SR) range of motion, as well as the time-course of any changes during detraining. Participants in both LR (aged 19 ± 2.6 years; *n*=8) and SR (aged 19 ± 3.4 years; *n*=8) groups undertook 8 weeks of RT and 4 weeks detraining. Muscle size, architecture, subcutaneous fat and strength were measured at weeks 0, 8, 10 and 12. A control group (aged 23 ± 2.4 years; *n*=10) was also monitored during this period. There was a significant effect of training group (p>0.05) on strength (on average 4±2% vs. 18±2%), anatomical cross-sectional area at 75% of femur length (59±15% vs. 16±10%), fascicle length (23±5% vs. 10±2%) and mid-thigh subcutaneous fat (22±8% vs. 5±2%), with LR exhibiting greater adaptations than SR. Detraining resulted in significant (p>0.05) deteriorations in all muscle parameters measured in both groups, with the SR group experiencing a more rapid relative loss of post-exercise increases in strength than LR (p>0.05). This is the first study to show that greater morphological and architectural RT adaptations in LR (owing to higher mechanical stress) result in a more significant increase in strength compared to SR. Strength retention following detraining showed a similar advantage in the LR protocol. The practical implications for this body of work follow that LR should be observed in resistance training where strength increment is the objective.

Key words: Detraining; Hypertrophy; Muscle Architecture; Range-of-Motion; Stress; Strength Training.

INTRODUCTION:

To the strength and conditioning practitioner, the design of a resistance training program will incorporate a selection of exercises that will ideally reflect both functional tasks of a chosen sport, and also the ability of the exercise to bring about a desired set of adaptations to enhance function and ideally therefore, physical performance. As muscle force is proportional to muscle cross-sectional area(Maughan et al., 1983), is intimately linked with power, and is a key determinant to success in many sports, muscular hypertrophy is often a key outcome following resistance training. The degree of hypertrophy arises from manipulation of the training stimulus; exercise selection/ order, mode of contraction, intensity, recovery and volume(Ratamess et al., 2009). Whilst studies on several of these variables are numerous, effects of training kinematics on training mechanics remain largely unreported. Taking the squat exercise (which is an exercise often used to overload the knee extensor muscle group during a hypertrophic training phase) as an example, studies have investigated the relationship between range-of-motion (ROM) of the squat and its effects on thigh muscle activation and joint moments(Wretenberg et al., 1993), tibiofemoral shear and compressive forces (e.g. (Nissell, 1986)), and on peak velocity and force(Drinkwater et al., 2012). One potential important aspect of training kinematics (i.e. ROM) that practitioners may not have previously taken into consideration is the ROM's effects on

muscle mechanics during resistance training, and the subsequent adaptations as a result.

As muscle length changes during force production to bring about movement, the moment arm of the series elastic component (i.e. the tendon) also changes. Therefore the internal tension a muscle experiences at different joint angles will change despite no alterations in external absolute load. Simultaneous to the change in moment arm, is the effect of changing muscle length on actin-myosin interactions and thus cross-bridge states (Huxley and Simmons, 1971). A changing muscle length will vary both of these cellular factors, thus impacting on the force-length relationship in the muscle (Rassier et al., 1999). The magnitude of mechanical stress is known to induce muscle hypertrophy(McDonagh and Davies, 1984), therefore increased mechanical stress at one joint angle compared to another could act as a signal for additional sarcomerogenesis at that muscle length based on the differential stress imposed through the moment arm changes. To further augment sarcomerogenesis at different muscle lengths, are the effects stretch has on muscle. Muscles undergoing different amounts of stretch/ shortening have been shown to adapt to their new functional lengths by the addition or removal of sarcomeres in series(Goldspink, 1983, Tabary et al., 1972, Williams and Goldspink, 1973), with an increase in functional length associated with increased protein synthesis(Goldspink, 1999).
This identifies 'average muscle length-specific training' as a potential modulator of the training-induced hypertrophic response. Only one previous study to our knowledge, Kubo et al.(Kubo et al., 2006a), has investigated training at different joint angles on muscle size and function *in vivo*, but their results do not reflect what the theory would have predicted. Nine males completed a 12-week unilateral isometric training program (70% MVC × 15secs × 6 sets) on the knee extensors at either a short (50^o of knee flexion) or a long (100^o) muscle length. The authors found that whole quadriceps volume increased significantly in both short (+10 ± 1%) and long (+11 ± 2%) muscle lengths, although there was no significant difference between the groups. However, it should be noted that the isometric only protocol adopted by Kubo et al.(25) may not reflect the practices of individuals training to optimize gains in strength and hypertrophy in addition to the limited transfer of the functional aspect of an isometric exercise.

Architectural adaptations have also been shown to occur with resistance training. Alterations to the fascicle angle of pennation impacts on the physiological cross-sectional area (pCSA) and therefore force-generating capacity(Aagaard et al., 2001) of muscle, whereas changes to fascicle length are associated with alterations to the force-velocity relationship(Wickiewicz et al., 1983), which therefore impacts potential power output. Regarding fascicle angle, there appears to be a strong relationship between increases in muscle size and increases in pennation
angle(Blazevich et al., 2007, Kawakami et al., 1993, Kawakami et al., 1995). Although an increase in pennation angle is expected to allow an increase muscle force (up to an upper limit of 45^o), at greater pennation angles the effective contractile force exerted on the aponeurosis is reduced to a greater extent, off-setting the increase in force production from the increased number of acto-myosin crossbridges activated in parallel(Narici et al., 1992, Rutherford and Jones, 1992). Hence, it is important to monitor both fascicle angle and functional changes in strength in the muscle of interest. To systematically determine what changes are evident in the muscle in terms of hypertrophic and architectural changes is therefore key to optimizing training protocols with the associated training adaptations.
It is also important to describe how the muscle responds to a reduction in loading. Detraining is the partial or complete loss of training-induced adaptations, in response to an insufficient loading stimulus(Mujika and Padilla, 2000). Significant decrements in strength, EMG and mean fiber CSA have been reported in as little as two weeks of detraining(Hortobagyi et al., 1993), with similar observations in chronic detraining periods (≥4wks) alluding to either losses in mass, strength or neural activation, or combinations of these factors(Gondin et

al., 2006, Kubo et al., 2010, Narici et al., 1989). Also, most studies have tended to report changes following detraining after similar time courses to the preceding resistance training i.e. between 3-6 months. If there appears to a be a greater hypertrophic response at one muscle length over another, it would also be of interest to determine whether there is a differential modulation of detraining-induced mal-adaptations following greater initial gains from resistance training at different muscle lengths. Thus, if these greater gains are still evident following detraining, it would further highlight the value of using more optimal training mechanics within a resistance training program.

The purpose of this current study was to therefore describe the changes to Vastus Lateralis (VL) aCSA, architecture, subcutaneous fat content and strength following 8 weeks of dynamic resistance exercise at 50^{o} compared with 90^{o} of knee flexion, using an ecologically valid training regime. In brief, the specified angle is the position at which the training load is held isometrically for two seconds. With 50^{o} this involves a shorter ROM (SR) in the dynamic phase of the exercise and thus a shorter 'average muscle length'; whereas with 90^{o} this involves a longer ROM (LR) in the dynamic phase of the exercise and thus a longer 'average muscle length'. A second objective was to describe the effects of the detraining time-course over 4 weeks on the aforementioned variables. It was hypothesized that the group training at longer muscle length (90^{o} joint angle) would undergo a greater amount of skeletal muscle hypertrophy due to increased physiological stress and stretch on sarcomeres compared to the group training at 50^{o}. It was also expected that the LR group would continue to have a large muscle mass following detraining, probably due to greater initial gains. Strength related parameters were expected to follow the same pattern as those associated with hypertrophy.

METHODS:

Experimental Approach to the Problem

We sought to compare changes in muscle size, architecture, function and subcutaneous fat following 8 weeks of resistance training and 4 weeks detraining covering two distinctly different ranges of motion. Participants performed isoinertial resistance training at either a shorter average muscle length (ROM $0\text{-}50^{o}$ knee flexion) or a longer average muscle length (ROM $0\text{-}90^{o}$ knee flexion) three times per week at 80% 1RM during the training period and performed habitual activity during detraining. Dependant variables were measured at baseline, post-training, after two weeks of detraining and again after a further two weeks of detraining.

Subjects

Twenty six volunteers (14 males and 12 females) gave written informed consent to participate in this study with all procedures and experimental protocols approved by the local Ethics Committee within the Department of Exercise and Sport Science. Exclusion criteria included the presence of any known musculoskeletal, neurological, inflammatory/metabolic disorders or injury. Participants took part in recreational activities such as team sports, and had either never taken part in lower limb resistance training or over the last 12 months. Sixteen activity-matched males and females were randomly assigned to either the short ROM (SR; n=8 - 4 males, 4 females) training group or to the long ROM (LR; n=8 – 4 males, 4 females) training group. Ten participants (6 males and 4 females) were assigned to the non-training control group (Con) to monitor for random variation in the muscle parameters investigated in this population over the training and detraining periods. The physical characteristics of the study population are outlined in Table 1. A One-way ANOVA revealed that the population was homogeneous at baseline for all parameters of interest and in physical characteristics (p>0.05). As body mass and external load provide the total training stimulus in a number of the leg exercises adopted in the training program (2 out of the 6), it should be noted that the participant groups showed no difference in body mass at baseline, week 8, 10 or 12. Furthermore it was felt that training at loads relative to 1RM provided greater external validity to the practices of individuals undertaking resistance training.

< INSERT TABLE 1 NEAR HERE >

Procedures

Resistance Training Program

Resistance training was performed 3 times per week (twice supervised and one home-based session) by

both the SR and LR training groups for 8 weeks, using a combination of free, machine (Pulse, UK) and body weights. A generalized warm-up was completed at 70-75% age-predicted maximum heart rate on a treadmill for 5 minutes, after which a goniometer was attached to the centre of rotation of the knee. As the subject performed each exercise (outlined in Table 2), the goniometer rotated from 0^o (full extension) and a training partner confirmed from the scale when the participant had reached 50^o or 90^o of knee flexion during the eccentric phase and therefore could hold the load steady over 2 seconds, before beginning the concentric phase of movement. Movement speed was dictated by a 1 second metronome. All exercises involved eccentric and concentric loading, except for the Sampson chair which was isometric loading. The subjects completed 2 familiarization sessions at 70% of 1RM prior to commencing the resistance training program. Exercises were performed at 80% of 1RM as determined at the training angle e.g. SR group 1RM measured as greatest weight lifted performing 50^o knee flexion. 1RMs were measured every two weeks and training loads adjusted accordingly. Manipulation of the exercise variables (exercise selection and order, repetitions, sets, recovery and intensity shown in Table 2) were all chosen based on empirical evidence presented in ACSM's Progression Models for Resistance Training for Healthy Adults for increasing muscle hypertrophy(Ratamess et al., 2009).

< INSERT TABLE 2 NEAR HERE >

Muscle architecture & Subcutaneous Fat

Architecture was measured at rest with each participant seated in an upright position on an isokinetic dynamometer (Cybex, Phoenix Healthcare Products, UK). Following calibration, each participant was positioned with a hip angle of 80^o (straight back 90^o) and knee at 90^o knee flexion (straight leg 0^o). All muscle architectural measurements were determined using real-time ultrasonography (AU5, Esaote Biomedica, Italy) at rest, with images captured at 25Hz using a digital video recorder (Tevion, UK). Vastus Lateralis fascicle pennation angle (θ) was measured as the angle of fascicle insertion into the deep aponeurosis(Rutherford and Jones, 1992). Images were obtained perpendicular to the dermal surface of the VL and orientated along the mid-sagittal plane of the muscle. Images were taken at 25%, 50% and 75% of total femur length (as described below) and 50% of muscle width at each point (where 50% muscle width is defined as the mid-point between the fascia separating the VL and Rectus Femoris, and fascia separating the VL and Biceps Femoris muscles). Fascicle length was defined as the length of the fascicular path between the deep aponeurosis and superficial aponeurosis of the VL. The majority of fascicles extended off the acquired image, where the missing portion was estimated by linear extrapolation. This was achieved by measuring the linear distance from the identifiable end of a fascicle to the intersection of a line drawn from the fascicle and a line drawn from the superficial aponeurosis. This method has been shown to produce reliable results previously(Blazevich et al., 2007). All images were analyzed and measured using Image J (Wayne Rasband, National Institute of Health, USA).

Subcutaneous fat was estimated using the same images as taken for muscle architecture. After calibration in Image J to coincide with the scale of the ultrasound image, a line from the top to the bottom of the layer of fat visualized was drawn at three regular intervals on the ultrasound image. The average lengths of these three lines were taken to estimate the average thickness of the subcutaneous fat layer in millimeters. Care was taken not to deform or compress the subcutaneous fat with minimal pressure applied to the dermal surface with the ultrasound probe.

Muscle Force Modeling

Due to the changing moment arm length of the patella tendon at discrete knee joint angles, differences in muscle force produced between the groups had to be accounted for. Thus quadriceps forces at the patella tendon were calculated as follows:

$$Quad_{Force} = (Quad_{MaxTorque} + Ham_{CoTorque}) / Moment\ Arm_{PT} \qquad \textit{equation [1]}$$

where

$$Ham_{CoTorque} = (Co\text{-}Con_{EMG} \times Flex_{MaxTorque}) / (Max\ BF_{EMG}) \qquad \textit{equation [2]}$$

Where $Co\text{-}Con_{EMG}$ is co-contraction of the antagonist muscle (biceps femoris), and Max BF_{EMG} is the maximum antagonist EMG. $Flex_{Max}$ is maximum flexion torque and Moment Arm_{PT} being the moment arm of the patellar tendon (values obtained from DEXA scans).

Muscle aCSA

VL muscle anatomical cross-sectional area (aCSA) was measured using real-time ultrasonography at rest. ACSA was measured at three sites – 25%, 50% and 75% of total femur length. Femur length was defined as the line passing from the greater trochanter to the central palpable point of the space between the femur and tibia heads when knee was flexed at 90^{o}. Echo-absorptive tape was placed at regular intervals (~3cm) along the muscle width at each site so that when the probe was placed on the leg, two distinct shadows were cast on the ultrasound image. Therefore each ultrasound image provided a section of VL within the boundaries set by the two shadows and fascia surrounding the muscle. Each of these sections were analyzed for total area using Image J to provide a total aCSA at that particular site. This method has been validated previously (Reeves et al., 2004a).

Strength measurement

Maximal isometric knee extension torque was measured with the knee at a range of angles i.e. 30°, 50°, 60^{o}, 65°, 70°, 75° and 90° (full knee extension = 0°) on the right leg of all participants. The order of testing by knee angle was randomized so as minimize any systematic fatigue effect. After a series of warm up trials consisting of ten isokinetic contractions at $60°{\cdot}s^{-1}$ at 50-85% maximal effort, participants were instructed to rapidly exert maximal isometric force against the dynamometer (Cybex NORM, NY) lever arm. Participants were given both verbal and visual encouragement/feedback throughout their effort. Joint torque data were displayed on the screen of a Mac Book Air computer (Apple Computer, Cupertino, CA, USA), which was interfaced with an A/D system (Acknowledge, Biopac Systems, Santa Barbara, CA, USA) with a sampling frequency of 200 Hz. Isometric contractions were held for ~2 s at the plateau with a 60 s rest period between contractions. Peak torque was expressed as the average of data points over a 200 ms period at the plateau phase (i.e. 100 ms either side of the instantaneous peak torque). The peak torque of three extensions was used as the measure of strength in each participant.

Statistical Analyses

Data were parametric and were therefore analyzed using a mixed-design repeated measures ANOVA. The within factor was the phase of training (i.e. week 0, 8, 10 and 12) and the between factor was training group (i.e. SR, LR or Con). Post-Hoc contrast analyses with bonferroni corrections were used to compare data to baseline ("within" factor) and to control group ("between" factor). All data are presented as mean ± standard error of the mean (SEM). Statistical significance was set with alpha at $\leq$ 0.05. In terms of the sample size in the present study, the average statistical power of the measured muscle parameters (CSA, pennation angle, fascicle length and strength) was statistically adequate at beta = 0.86.

RESULTS:

Repeatability of the measurements:

A small pilot study was conducted at the onset of the study on a similar population (i.e. age and physical characteristics). Repeated measures of VL muscle anatomical CSA, architecture and strength on a group of 5 individuals (2 males, 3 females) were collected on three separate occasions. Within-day coefficients of variation (CV in %) of 1.5%, 1.9%, 1.3%, 2.6%. and 0.8%, and between day CVs of 2.6%, 2.1%, 1.6%, 2.9% and 1.8% were yielded for aCSA, fascicle length, fascicle pennation angle subcutaneous fat and strength respectively. Therefore the repeatability of the measurements was within an acceptable range of error(Atkinson and Nevill, 1998).

Total training load

In order to allow internal force comparisons, external training loads were monitored in each group. Total average loads (mean ± S.D.) lifted for externally loaded (i.e. not just using body mass) exercises

completed were: 1) Squat; SR 99±10kg, LR 80±8Kg. 2) Leg Press; SR 60±19Kg, LR 48±17Kg. 3) Leg extension; SR 51±17Kg, LR 46±15Kg. In order to accurately assess internal muscle forces produced, the change in resistance moment arms of the CAM pulley machine used during leg extensions were also measured. Based on the training load for the leg extension exercise stated above for each group, the resistance machine load component yielded on average a 7% increase in external torque produced in the SR group compared to LR (SR; 137Nm vs. LR; 128Nm).

The results in the subsequent sections describe paired physiological changes relative to baseline.

Muscle aCSA at 25%, 50%, 75% Femur Length: The results at all femur lengths are presented in Table 3. VL CSA increased significantly (p<0.05) relative to baseline following training at all sites in both training groups. The significant training effect remained during the whole detraining period in both training groups at both 50% and 75%, but was not evident at 25% of femur length after week 10. There was a trend for LR to exhibit greater relative gains in aCSA compared to SR at all sites, which was significant at week 8 at 75% of femur length. It was found that there was not only a main training effect (p<0.05) but a main group effect after week 8 (p<0.05) with LR exhibiting a 59±15% compared to SR showing 16±10% increment in VL aCSA. Surprisingly, following the first two weeks of detraining the group effect was no longer evident (p=0.07) although both training groups were still significantly above baseline at weeks 10 and 12. There was no notable change over the 12 week period for the controls.

< *INSERT TABLE 3 ABOUT HERE* >

Muscle Architecture:
Fascicle Pennation Angle – 25%, 50%, 75% Femur Length: Table 3 shows changes in fascicle pennation angle at each site for all three groups. At 25% there was a main effect of training (p<0.05) for each group, with no significant effect of group. Pennation angles recorded at baseline increased by 2±5% and 9±6% at week 8 for SR and LR groups respectively. However by week 10 and 12 the effect of training was negated with values returning toward baseline (p>0.05). This pattern was repeated at 50% of femur length with the main effect of training (5±3% SR, 9±3% LR) reverted following 2 weeks without the training stimulus. This was again the case for pennation angles at 75%, however the significant increase due to resistance training was observed until week 10 but had receded towards baseline following the four weeks of detraining at week 12.

Fascicle Length – 25%, 50%, 75% Femur Length: There was a significant main effect of resistance training on fascicle length at all three sites (Figure 3, p<0.05), which remained significantly elevated above baseline values after the detraining period, with both training groups significantly increasing (p<0.05) fascicle length at all sites at week 8, 10 and 12 compared to controls. Whilst there was no group effect at 25%, a significant group effect (p<0.05) did occur at both 50% and 75% of femur length. LR fascicle lengths increased 23±5%, 19±4%, 16±4% at weeks 8, 10 and 12 from baseline in contrast to SR group's less pronounced increments of10±2%, 6±2% and 2±2% during the same time period. All values were significantly enhanced compared to baseline in both groups except for SR at week 12. At 75% there was a similar significant (p<0.05) group effect with relative increases of 19%±3, 13±3% and 10±2% at weeks 8, 10 and 12 for LR from baseline compared to 11±2%, 5±4% and 2±2% for SR during the same period.

< *INSERT FIGURE 1 ABOUT HERE* >

Subcutaneous Fat 25%, 50%, 75% Femur Length: The training intervention resulted in appreciable reductions in fat in both SR and LR training groups between week 0 and 8 (p<0.05) at all measured sites. At 25%, the SR group reduced fat levels from 16.2±3.9mm to 15.6±3.8mm (-4±1%), compared to LR (18.1±4.0mm to 15.4±3.3mm (-14±3%)) post-training; however no group effect existed. After training ceased, both groups remained significantly lower than baseline at week 10 (-3±2% SL and -9±3% LR), but by week 12 there was no main effect of training/ detraining on either group (p>0.05), although there was a strong trend for a group effect at this time point (p=0.057). The control group did not fluctuate significantly from baseline values during

weeks 8, 10 and 12. There was also considerable subcutaneous fat loss at 50% following resistance training, with greater losses achieved by the LR group. Both lost 6.8±1.2mm after training, however LR produced a loss of 22±8% compared to SL with a 5±2% loss at this phase of the protocol. The main effect of group remained during weeks 10 and 12, as SR regressed toward baseline by week 12, whereas LR still possessed significant losses at this phase (-10±6%). There was a similar trend seen at 75% where a main effect of both group and training existed at week 8 (p<0.05). This difference was not present at week 10 or 12 although both training groups had significantly less fat (p<0.05) compared to baseline during the detraining period (7±3% SL and 9±1% LR).

Muscle Strength: There was a main effect of training on the MVCs of both training groups (p>0.05) at each of the training end ranges-of-motion, with SR recording an increase of 5±10% at 50^o and 30±5% for LR at 90^o. There were no differences (p>0.05) between absolute MVC in SR and LR at baseline or following training. In agreement with previous observations following isometric joint-angle specific training, there was evidence of angular specificity of training in both groups with SR significantly (p>0.05) increasing MVCs at 50^o, 60^o, 65^o and 70^o (i.e. those closest to the training angle - see table 4), whereas LR increased MVCs over the entire angular range i.e.30^o-90^o. The angle of peak torque altered from 75^o to 70^o at week 8 in SR, where it remained for the duration of detraining in week 10 and 12. In LR, the angle of peak torque was originally 70^o and did not change following training and detraining. By week 10, both groups displayed an average 6±2% strength reduction (relative to the post-training strength values), with SR not significantly above baseline (0±2%) in contrast to LR remaining significantly above both baseline (p<0.05) and SR ($p=0.027$) at weeks 10 and 12. The control group's strength did not significantly alter during the 12 week training and detraining period (p>0.05).

< INSERT TABLE 4 ABOUT HERE >

DISCUSSION:

The current investigation aimed to study the *in vivo* effect of resistance exercise training and detraining over a larger ROM and hence longer average muscle length (0-90^o knee flexion – LR) versus a smaller ROM and hence shorter average muscle length (0-50^o knee flexion – SR) on morphological, architectural and functional changes in the VL. It was hypothesized that the group training over a wider range of joint angles (LR) would undergo a greater amount of skeletal muscle hypertrophy due to increased physiological stress and stretch on sarcomeres compared to the group training over a shorter range of joint angles (SR). It was also hypothesized that the LR group would still have a greater muscle mass following detraining, probably due to greater initial gains. Our findings partly support these hypotheses in that whilst there were no notable changes to any of the muscle parameters in the control group during a 12-week non-training period, significant adaptations were observed in both SR and LR training groups across all of the muscle measurements. What is more, there was a significant main effect of training where strength, VL fascicle length, VL anatomical cross-sectional area increased, whilst mid-thigh subcutaneous fat decreased to a greater extent following training at a longer muscle length compared to a shorter muscle length. Further, as per our expectation of greater physical demands of one training set up over the other, the stresses experienced by the knee extensors, whilst comparable in terms of ecologically valid training program setting and in terms of absolute loads lifted were 10-25% greater in SR, in fact translated to ~32% greater internal stresses in the group training over the longer ROM (LR). This has a major impact on how both coach and athlete should view the impact of ROM on muscular adaptations. It is often tempting for an athlete to reduce joint range of motion in order to accommodate a larger external load in the belief that lifting heavier will confer an advantage in adaptation. However from the evidence presented, without an appreciation of internal muscle mechanics, this assumption would be erroneous.

The relative increases in VL size following 8 weeks of resistance training reported in this study (21±8% in SR and 44±13% in LR – averaged across the 3 sites)

are much greater than those previously reported in a similar study(Kubo et al., 2006a). It should be however noted that this study(Kubo et al., 2006a) is the only other known study to our knowledge reporting changes specifically in VL size following training at shorter vs. longer muscle length (~11±7% ST and ~13±12% LT – values estimated from Figure 2 in their results section). The discrepancy between the two sets of results would not only arise from differences between measurements of VL size (volume vs. aCSA), but also to the difference in training protocols (isometric vs. combined isoinertial and isometric) between Kubo et al.(Kubo et al., 2006a) and the present study. Indeed metabolic cost and work done is greater during dynamic (i.e. concentric and/or eccentric) compared to isometric contractions(Wilkie, 1968). Therefore a greater work-induced hypertrophic effect of the combined training may have produced the variation in hypertrophy gain differences between the two studies(Goldberg et al., 1975). Previous research on resistance training induced whole quadriceps aCSA showed changes of 18.8±7.2%, 13.0±7.2% and 19.3±6.7% at distal, central and proximal sites respectively (Narici et al., 1989). It is nonetheless difficult to compare the studies directly however, not only owing to the fact the current study measured aCSA of the VL as opposed to all four quadriceps muscles, but also the earlier report (Narici et al., 1989) showed a significant difference in hypertrophic response between the components of the quadriceps muscle group. In a review of hormonal responses and adaptations to exercise(Kraemer et al., 1990), the authors suggest exercises involving large muscle masses are superior to more isolated exercises to elicit greater hormonal responses. Therefore, the large mass exercises such as the bilateral squat in our study would elicit a greater hormonal response to that of a seated unilateral knee extension on a dynamometer as in the study of Kubo et al (Kubo et al., 2006a).

Kubo et al.(Kubo et al., 2006a) estimated that internal VL force during isometric MVC at 100^{o} of knee flexion was 2.3 times greater than that at 50^{o}, and mechanical stress magnitude is known to induce muscle hypertrophy(McDonagh and Davies, 1984). Using the 1RM training loads, patellar tendon moment arm and aCSA, it was found that the mean force per unit area of muscle was 5.1N/mm^2 in the SL group compared to 6.8N/mm^2 in the LR group. The results of the current study showed a non-significant trend for the LR group to exhibit a greater VL aCSA following resistance training compared to SR at each site. Importantly, in support of these beneficial morphological adaptations in the LR group, this group had a significantly greater increase in strength than SR group following training at all knee angles measured, which has previously been demonstrated following isometric training(Kubo et al., 2006a).

The magnitude of hypertrophy at 75% of femur length was greater for LR following training. Relative increases were 59±15% for LR and 16±10% for SR, displaying evidence for region-specific hypertrophy. This has been observed previously following knee extensor resistance training(Narici et al., 1989, Seynnes et al., 2007, Narici et al., 1996). With both force generation and stretch being effective stimuli for muscle growth(Goldspink et al., 1991), the discrepancies in CSA between the groups may be due to regional differences in the total stimulus transmitted along the length of the muscle. Evidence exists that there is relatively high serial and parallel distribution of muscle fiber strain during transmission of myofascial force(Huijing and Jaspers, 2005). Thus the LR group could have experienced greater strain at a more distal portion of the VL, which was also transmitted laterally, resulting in a greater stimulus and therefore enhanced hypertrophy at this site. As muscle force is proportional to CSA, this provides a basis for enhancing the force output of the muscle, which is reflected in our strength results.

A major finding of the current study was the greater increase in fascicle length at all sites in the LR group compared to SR group, although only significantly so at 50% and 75% of total femur length. *In vivo* increases in fascicle length are associated with addition of sarcomeres in series (assuming a fixed sarcomere length) and appear to be strongly - influenced by muscle length or stretch(Goldspink, 1983, Tabary et al., 1972, Williams and Goldspink, 1973). A study by Boakes et al.(Boakes et al., 2007) where surgery placed the thigh muscles under

constant stretch to address a leg-length discrepancy, resulted in 4cm femoral lengthening. Fascicle and sarcomere length was measured in VL post-operatively and after twelve months. Results showed *in vivo* fascicle length increased, and sarcomere length decreased, with sarcomerogenesis from approximately 25,000 to 58,650 as a result of adaptation to stretch. In the current study, our protocol increased the muscle excursion range of the quadriceps to a greater extent in the LR group compared to SR. The results from this investigation support pervious animal research evidence(Koh and Herzog, 1998) that 'average muscle length' (or excursion range) is a possible primary stimulus for increases in fascicle length in adult skeletal muscle. As mentioned previously, changes in fascicle length produce alterations to the force-velocity relationship in muscle(Wickiewicz et al., 1983) and could therefore impact on an athlete's potential for power production.

Further architectural adaptations included a significant increase of pennation angle ($P\theta$) in both training groups (see Table 3). A functional consequence of an increase in $P\theta$ is that more contractile material can be packed in parallel for a given anatomical cross-section(Kawakami et al., 1993, Rutherford and Jones, 1992). $P\theta$ has been shown to increase following resistance exercise(Blazevich et al., 2007, Aagaard et al., 2001, Kawakami et al., 1995, Seynnes et al., 2007, Reeves et al., 2009) and is usually closely associated with an increase in anatomical CSA in the quadriceps(Rutherford and Jones, 1992). Despite not reaching between group significance, the average increase in $P\theta$ across the three sites was greater for LR than SR (11±5% vs. 7±4% respectively). Thus this could also have been a factor in contributing to LR group's greater strength following training.

A further benefit experienced by the LR group was a greater reduction in subcutaneous fat at 50% and 75% of femur length. In the athletic world it is often considered beneficial to reduce levels of subcutaneous fat. For example in running events (or events where the body is not supported), excess body weight has been shown to significantly decrease relative VO_2 max and performance during a running test as a direct consequence of an increased energy cost of running at submaximal speeds (Cureton et al., 1978). Additionally in sports where body mass is accelerated against gravity, a more lean muscle mass would be advantageous for performance and energetic consumption. This is not to mention the effect of body composition on cardiovascular risk factors and mortality for the average person (Lee et al., 1999). It would be tempting to suggest that the possible mechanism for an increased fat loss in the LR group may be linked to the greater internal physiological stress on the muscle, since strenuous resistive exercise may elevate post-exercise metabolic rate for a prolonged period, and may enhance post-exercise lipid oxidation(Melby et al., 1993). However, more recently Singhal et al.(Singhal et al., 2009) found that there was no significant difference in post-prandial lipemia in groups undertaking either moderate or high-intensity resistance exercise. An alternative explanation therefore for the physiological processes involved is linked with the fact that acute resistance exercise has been shown to increase AMPK activity (Dreyer et al., 2006), which in turn has also been shown to mediate effects of IL-6 stimulated increases in glucose disposal and free fatty acid oxidation. Further, AMPKα2 activity has been shown to be intensity dependent (Chen et al., 2003), therefore training at longer muscle lengths could affect upstream factors of adiposity.

A second aim of the study was to determine if there was a differential response to detraining between the training groups. Both training groups showed that detraining period resulted in significant losses at weeks 10 and 12 ($p=0.001$, $p\varepsilon^2=0.34$) in all measured parameters. Although generally there were no significant difference between groups, LR group consistently exhibited a trend ($p=0.07$, $p\varepsilon^2=0.32$) towards greater absolute and relative decrements in muscle dimensional parameters over the four-week detraining period. This is in agreement with a previous study(Tokmakidis et al., 2009), who found following 12 weeks of resistance training, a group of older adults performing high intensity (HI) training increased total thigh CSA and strength to a greater ($P<0.05$) extent than a moderate intensity (MI) training. Following a subsequent 12 weeks of detraining, total relative thigh CSA and strength in the HI group diminished significantly more than MI group. Despite these reductions, HI group strength

and CSA remained significantly greater than in MI group due to greater initial adaptations. In the present study in terms of average VL aCSA across the 3 sites, there was a decrease from 44±13% to 25±11% above baseline between week 8 and 12 in the LR group – whereas SR decreased from 21±8% at week 8 to 10±7% at week 12. Therefore the relative changes in aCSA to LR group following four weeks detraining (+25±11%) are still superior to those made by SR immediately post-training (21±8%). This suggests that although greater initial gains may be lost at a greater rate, training using a relatively wider range of motion may still confer an advantage for the longer term. This is evident in the strength data where LR group was significantly stronger until the conclusion of the detraining period, whereas SR was not significantly stronger compared with the pre-training data, at week 10. It is difficult to say why greater gains are lost more rapidly, such as in LR group, but it may be due to an inability to stimulate sufficient protein synthesis to support a larger muscle mass. One would not expect to observe a difference in daily protein synthetic rate between groups with no change in activity levels and dietary habits (Kimball et al., 2002). Therefore basal protein synthetic rates would support a greater relative percentage of a smaller muscle mass than a larger mass. Also, since resistance exercise causes perturbations to the intramuscular environment, and there are subsequent adaptations, a new homeostatic point is reached, where only a greater stimulus than the original will stimulate further adaptations(Kraemer et al., 1996). The LR group may have set a higher threshold to maintain adaptations, where daily activity did not disturb the internal muscle environment as much as in the SR group with a lower threshold. Indeed to reiterate, whilst the face value load was similar in the two groups (80%1RM in both cases) the internal loads experienced are in fact greater under a relatively wide ROM, and therefore since the phosphorylation of extracellular signal-regulated kinase (ERK1/2) and the 38-kDa stress-activated protein kinase (p38) both appear to be intensity dependent(Rennie et al., 2004), could be a contributing factor to the difference in cellular response and adaptation.

It should however be noted that in the present study, whilst the ROM has been presented as different, the manner in which the two training groups were required to hold the contractions (over 2secs) means that the greatest difference in the loads experienced by the quadriceps muscle was due to the internal joint architecture. In other words, the difference between the two training protocols was not so much owing to the ROM (thought this differed at the start of the movement) but to the length of the muscle when the muscle group was experiencing the 'isometric hold' phase of the exercise. Nevertheless, future studies should aim to determine whether in making the internal loads comparable, the effects of training at the relatively elongated muscle length we describe here, still exhibit the advantage over training at a relatively shortened muscle length. The authors do also recognize that in covering a greater ROM, the muscle is loaded for a longer duration in LR (i.e. 0.25-0.50secs). However, previous studies (e.g.Ref (Adams et al., 2004)) have concluded that measures of work production during resistance training do not directly scale with the adaptation responses seen in skeletal muscle. Furthermore, it is difficult to give substance to a mechanism whereby such a small difference in duration of loading (i.e. load-time product) would lead to serial sarcomerogenesis and give rise to such striking differences in fascicle length.

In summary, following 8 weeks of resistance training and 4 weeks detraining over different ROM (and thus implied average muscle lengths), not only were there significant morphological differences between the two groups after training, but also, muscle strength was enhanced to a greater extent following training at a larger rather than a narrow ROM. Moreover, there was a significant difference between groups in muscle architecture, with fascicle length increments greater when training over a large ROM, supporting the notion that muscle length (or excursion) has a major influence on fascicle length. The implications of the results may be useful in athletic training, and also deter athletes from reducing their ROM during exercises to accommodate greater external loads.

PRACTICAL APPLICATIONS:

In the field of practice, when choosing a range of motion in which a resistance exercise should be performed, muscle mechanics must also be considered. We have shown that resistance training protocols that enforce a wider ROM enhance the muscle characteristics that influence force and power production to a greater extent than protocols where the range of motion is not as extensive. A common error in practice is allowing ROM to be compromised in order to accommodate a greater absolute external load, in an attempt to increase the stress of mechanical loading. Following this, it is important for the coach to reinforce a more complete ROM, even when absolute load maybe reduced, in order to provide a greater internal stress and more potent stimulus for adaptation. Optimization of training mechanics could therefore potentially reduce the time spent in the gym achieving sporting/ performance goals, as training time and exercise volume constraints are pivotal considerations in the periodization of training. Adherence to a greater ROM also provides a better long-term prognosis for retention of training adaptations e.g. following prolonged bed rest/ immobilization (caused by illness/ injury) or indeed during tapering.

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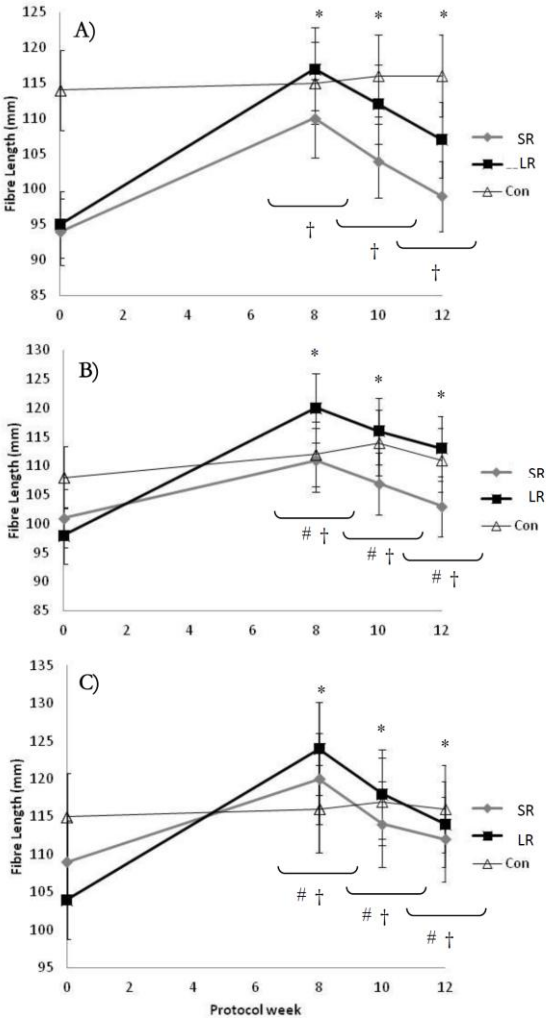
FIGURE LEGENDS

1. Figure 1. Graph showing relative changes in fascicle length (mm) during training and detraining. (A) Changes at 25%, (B) at 50% and (C) and 75%. *Significant relative change above baseline in both groups # Significantly different to other training group † Significantly different ($p<0.05$) from control group. (Mean ± S.E.)



TABLES

Group	Age (years)	Mass(Kg)	Height (m)
SR	19 ± 3.4	74.9 ± 10.1	174 ± 13.3
LR	19 ± 2.6	73.8 ± 14.9	171 ± 11.8
Con	23 ±2.4	77.9 ± 13.1	176 ± 9.5

Table 1. Participants' Physical Characteristics at baseline

Day 1	Exercise	Reps	Sets	Recovery (secs)	Intensity (1RM)
	BB Back Squat	10	3	90	80%
	Knee Extension	10	3	60	80%
	Bulgarian Split Squat	10	3	90	80%
	DL Sampson Chair	4 × 10 second holds	3	60	-
Day 2	BB Back Squat	10	3	90	80%
	Knee Extension	10	3	60	80%
	Leg Press	10	3	90	80%
	DB Lunges	10	3	60	80%
Day 3	BW Squats	30	3	90	-
	DL Sampson Chair	4 × 20 second holds	3	60	-
	BW Lunges	30	3	90	-
	SR Sampson Chair	5 × 5 second holds	3	60	-

Table 2. Resistance Training Program Outline. Exercises were carried out at 80% 1RM where appropriate or using body mass. BB = Barbell, DB = Dumbbell, DL = double-legged, SR = Single-legged, BW = Bodyweight.

Site (% Femur L)	*Baseline*	*Week 8*	*Week 10*	*Week 12*
25 CSA	SR 2,877±338 LR 2,684±427 Con 3,201±253	SR 3,425±303* LR 3,592±303* Con 3,086±259	SR 3,361±306* LR 3,461±309* Con 3,079±240	SR 3,265±310 LR 3,328±295 Con 3,086±259
50 CSA	SR 3,033±289 LR 3,004±385 Con 3,326±354	SR 3,699±342* LR 3,545±339* Con 3,314±364	SR 3,526±334* LR 3,424±349* Con 3,294±357	SR 3,382±304* LR 3,233±338* Con 3,314±364
75 CSA	SR 1,081±175 LR 1,074±224 Con 1,366±165	SR 1,162±127* LR 1,505±217* # Con 1,370±185	SR 1,115±127* LR 1,369±200* Con 1,358±175	SR 1,037±119* LR 1,219±191* Con 1,370±185
25PEN	SR 10.1±0.3 LR 10.2±0.3 Con 11.9±0.2	SR 10.3±0.3* LR 11.2±0.6* Con 12.2±0.1	SR 10.0±0.7 LR 11.1±0.6 Con 12.0±0.1	SR 9.9±0.2 LR 11.0±0.6 Con 12.2±0.1
50PEN	SR 16.5±0.5 LR 15.9±0.2 Con 16.4±0.8	SR 17.2±0.3* LR 17.1±0.8* Con 16.4±0.7	SR 16.8±0.9 LR 16.6±0.8 Con 16.3±0.7	SR 16.4±0.4 LR 16.0±0.6 Con 16.4±0.7
75PEN	SR 16.0±1.4 LR 15.5±0.6 Con 19.4±0.7	SR 17.9±1.2* LR 17.5±0.7* Con 18.5±0.7	SR 16.8±0.9* LR 17.2±0.6* Con 18.4±0.6	SR 16.8±0.9 LR 16.3±0.5 Con 18.5±0.7

Table 3. Paired changes to (i) VL CSA and (ii) Pennation angle of fascicles at different femur lengths over the training and detraining phases. *Significantly above baseline # Significantly different to other training group. Values are mm^2 and degrees (mean ± S.E.)

Knee Flexion Angle (^{o})	**Torque (Nm) Baseline – Week 8**		**Relative Change (%)**	
	SR	**LR**	**SR**	**LR**
30	106-108	102-127	2±8	18±5*
50	167-175	157-193	5±10*	16±4*
60	191-197	186-213	4±5*	11±7*
65	207-216	194-231	5±4*	19±8*
70	212-225	202-247	6±2*	13±2*
75	218-215	193-232	-1±3	18±11*
90	216-218	161-208	1±2	30±5*

Table 4. Changes in quadriceps MVC over a range of knee flexion angles from baseline to week 8 (post-training). * Significantly above baseline measures

Muscular and endocrine adaptations to resistance training are stretch-mediated

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Running Title: Length modulates adaptation

List of Abbreviations:

CSA; cross-sectional area
IGF-I; insulin-like growth factor – 1
PI3K/Akt/mTOR; Phosphatidylinositol 3 Kinase/ Akt (protein kinase B)/ mammalian target of rapamycin
MAFBx; muscle atrophy F-Box
MuRF-1; muscle RING finger 1

ABSTRACT:

Introduction: Modulation of muscle characteristics was attempted through altering muscle stretch during resistance training. We also describe associated detraining responses. **Methods:** Participants undertook 8 weeks of quadriceps resistance training, loading the muscle in a shortened (SL-0-50^{o} knee flexion; n=10) or lengthened (LL- 40-90^{o}; n=11) position, followed by 4 weeks detraining. The controls (CON; n=10) were untrained. Quadriceps strength, *Vastus Lateralis* architecture, anatomical cross-sectional area (aCSA) and serum IGF-I were measured at weeks 0, 8, 10 and 12. **Results:** There were greater post-training increases in fascicle length (29±4% vs. 14±4%), distal aCSA (53±12% vs. 18±8%), strength (26±6% vs. 7±3%) and IGF-I (31±6% vs. 7±6%) in LL compared to SL ($p<0.05$). No changes were apparent in CON. Detraining decrements in strength and aCSA were greater in SL than LL ($p<0.05$). **Discussion:** Better improvements in muscle *in vivo* (and somewhat IGF-I) adaptations to resistance training are concurrent with muscle stretch inclusion within training.

Key Words: Detraining; Hypertrophy; Muscle Architecture; Range-of-Motion; Resistance Training.

INTRODUCTION:

Skeletal muscle architectural and morphological characteristics are important due to their direct influence on determinants of functional performance (i.e. strength and power). Therefore, key to optimising human function, is to understand the mechanical stimuli that induce alterations to the muscle's characteristics/ properties. In relation to muscle architecture, there are reports of increases in

fascicle length following resistance training (Reeves et al., 2009, Seynnes et al., 2007, Alegre et al., 2006), which impacts the force-velocity and force-length relationships of muscle, and also muscle excursion. An increase in fascicle length is thought to be brought about by in-series sarcomerogenesis. Muscle length (or passive tension/ stretch) and muscle excursion have been shown to be major regulators of serial sarcomerogenesis in animals (Koh and Herzog, 1998) and appears to be relatively independent of both muscle activation level and tension (Gajdosik, 2001). However in humans, conflicting evidence from studies on the major mechanical stimuli for such adaptation exists. In young adults, muscle contraction type (eccentric vs. concentric) was investigated as a possible primary candidate for fascicle length change (Blazevich et al., 2007). The authors concluded that other factors (possibly excursion of muscle during resistance training) were responsible as the main mechanical stimuli for changes in fascicle length. In contrast, in older individuals, Reeves et al.(Reeves et al., 2009) found that eccentric contractions (through enhanced training stimulus and associated greater muscle-tendon strain), were the driving force behind greater increases in fascicle length, compared to conventional weight training. Therefore, the primary constituent in resistance training for regulating fascicle length in humans remains ambiguous.

The inextricable link between muscle cross-sectional area (CSA) and strength has been known for many years. The two main mechanical signals that induce muscle hypertrophy (therefore increasing CSA) appear to be muscle force and/ or stretch. In animal models, muscle stretch (i.e. lengthening) combined with force generation seems to have an additive effect on protein synthesis and muscle size, over and above the effects of force generation/ stretch applied separately (Goldspink et al., 1992, Goldspink, 1999). The phenomenon of stretch-induced muscle hypertrophy has been demonstrated *in vitro* (Adachi et al., 2003) and has been associated with the PI3/Akt/mTOR signalling pathway for increasing protein synthesis(Sasai et al., 2010). In parallel to the mechanical stimulation of such signalling pathways, there is evidence that growth factors, such as IGF-I, mediates its effects through the aforementioned PI3/Akt/mTOR cascade, and precedes myotube hypertrophy (Rommel et al., 2001), whilst also preventing the expression of atrophy-induced ubiquitin ligases(Stitt et al., 2004). IGF-I mRNA has also been shown to increase to a much greater extent in response to stretch combined with electrical stimulation, compared to stimulation or stretch alone in adult skeletal muscle (Goldspink et al., 1995). However to our knowledge, no *in vivo* study in humans has systematically compared the effects of relatively high vs. low muscle stretch (where the degree of internal muscle loading is normalised in the two stretch conditions), on muscle characteristic responses to training (e.g. force generation capacity, morphology (CSA), architecture and circulating IGF-I levels).

At the other end of the muscle loading spectrum, the response to diminished loading i.e. detraining, is the partial or complete loss of training-induced adaptations, in response to an insufficient loading stimulus. Significant decrements in strength, electromyogram amplitude, and mean fibre CSA have been reported to occur in as little as two weeks of detraining (Hortobagyi et al., 1993), with similar observations in chronic detraining periods (≥4wks) (Gondin et al., 2006, Kubo et al., 2010). However, the *in vivo* changes to muscle architecture during a relatively shorter period of time (≤4 weeks – such as that found in short-term injury, illness or tapering), to the author's knowledge, have not yet previously been described. Significant increases in fascicle length have been reported in as little as 10 days from the onset of resistance training (Seynnes et al., 2007). Counter-intuitively, Blazevich et al.(Blazevich et al., 2007) documented an increase in VL fascicle length during 3 months of detraining following 10 weeks of resistance training. Therefore following resistance training, the impact of detraining from the subsequent training appears to follow an unpredictable/unchartered pattern. In addition to its role in muscle mass accruement, IGF-I has been implicated in preventing the expression of the FOXO class of transcription factors and the mRNA increases of MAFbx and MuRF1 seen during muscle atrophy (Stitt et al., 2004). Therefore following detraining, it would therefore be interesting to describe the possible link between circulating IGF-I levels and the degree of muscle mass maintenance.

From the evidence of the current published literature, the aim of the present study was to investigate if performing resistance training at a longer muscle length (high muscle stretch condition) compared to a shorter muscle length (low muscle stretch condition) with identical load magnitude, would differentially modulate specific *in vivo* muscle responses including size, architecture and circulating IGF-I. In addition, we also questioned whether the magnitude of the preceding training responses would also influence the change in muscle parameters during detraining. It was hypothesized that the group training at longer (LL) muscle lengths (40-90^{o} excursion) would undergo a greater amount of skeletal muscle hypertrophy and fascicle lengthening compared to the group training at 0-50^{o} excursion (SL). Secondly, it was also hypothesized that the LL group would still have a larger muscle mass following detraining, probably due to greater initial gains and/or IGF-I mediated effects on protein degradation rate. Strength and fascicle angle related parameters were expected to follow a similar response as those associated with hypertrophy.

Materials & Methods:

Subjects

Thirty one volunteers were recruited from the local university campus, and gave written informed consent to participate in the study. All procedures and experimental protocols were approved by the local Ethics Committee. Exclusion criteria included the presence of any known musculoskeletal, neurological, inflammatory or metabolic disorders or injury. Participants took part in recreational activities such as team sports, and had either never taken part in lower limb resistance training or not within the previous 12 months. Team sports included rugby union and league, soccer, hockey and netball. Where several participants had the same sporting background, they were divided evenly and randomly allocated to a training group. All participants habitually took part in up to 3-5 hours of non-resistance based activity per week. Twenty one activity-matched participants were allocated to a training group – SL (shorter muscle length; 6 males, 4 females; aged 19±2.2 years, 1.76±0.15m, 75.7±13.2Kg) or LL (longer muscle length; 5 males, 6 females; 21±3.4 years, 1.75±0.14m, 74.9±14.7Kg). Ten participants (6 males and 4 females; 23±2.4 years, 1.76±0.09m, 77.9±13.1Kg) were assigned to the non-training control group (Con), and continued their normal habitual activity throughout the study period. A One-way ANOVA revealed that the population was homogeneous at baseline for all parameters of interest ($P>0.05$).

Study Design

The study design was convenience sampling, with random allocation to one of three groups. Following familiarisation with testing procedures at least one week prior to testing proper, participants were tested for muscle size, architecture, strength and serum IGF-I at baseline (week 0). This measurements were repeated, after 8 weeks resistance training (week 8), following two weeks of detraining (week 10) and finally after a further two weeks detraining (week 12). Blood sampling was always at the same time of day, whereas the *in vivo* tests were completed within 2 hours of the time-of-day of these tests when carried out at week 0 to minimise any impact of diurnal variability in muscle function.

Muscle Excursion

Total muscle excursion was set at that which occurred during 50^{o} range-of-motion (ROM), with this carried out at different portions of the knee angle-muscle length spectrum depending on the training group (Figure 1). With 0^{o} being full knee extension, SL followed an excursion from 0-50^{o} of knee flexion (i.e. shorter muscle lengths) and LL followed an excursion between 40-90^{o} (muscle loaded at a longer length). Therefore the work done, since external load were also made comparable, (force [see muscle force modelling below] × distance) was also closely matched.

[INSERT FIGURE 1 NEAR HERE]

Muscle Force Modelling

Due to the changing moment arm length of the patella tendon at discrete knee joint angles, differences in muscle force produced between the groups were accounted for. Thus quadriceps forces at the patella tendon were calculated as follows:

$Quad_{Force} = (Quad_{MaxTorque} + Ham_{CoTorque}) / Moment\ Arm_{PT}$ *Equation [1]*

where

$Ham_{CoTorque} = (Co\text{-}Con_{EMG} \times Flex_{MaxTorque}) /(Max\ BF_{EMG})$ *Equation [2]*

Where $Co\text{-}Con_{EMG}$ is co-contraction of the antagonist muscle group (using the biceps femoris as representative for the hamstrings), and Max BF_{EMG} is the maximum antagonist EMG(Reeves et al., 2003a). $Flex_{MaxTorque}$ is maximum flexion torque and Moment Arm_{PT} being the moment arm of the patellar tendon (values obtained from DEXA scans). Based on previous training data from our laboratory at end range-of-motion where a short isometric hold would take place, tendon forces produced at 90^o were on average ~32% greater than those produced at 50^o (to quantify the training load to apply, the torque of the mass of the external resistance (in Nm) is added to the left hand side of equation [1] above). Thus, it was calculated that whilst SL would exercise at a high-intensity of 80% 1RM (for a more detailed description see resistance training programme section), the LL group would train at a lower intensity of 55% 1RM to equate the absolute load seen by the tendon (i.e. at the joint centre of rotation) in the two groups.

Patella Tendon Moment Arm Measurement

The patella moment arm was estimated from sagittal scans of the right leg of each participant using a single-energy DEXA (Dual Energy X-Ray Absorptiometry) scan (Hologic QDR, Vertec, Reading, UK), with the knee placed at 90^o of knee flexion. The patella tendon moment arm was defined as the perpendicular distance between the tibiofemoral contact point and the mid-portion of the patella tendon. DEXA imaging has been used to estimate moment arm previously in other anatomical sites, with good reliability (Wang et al., 2009). The single energy scanning method has also been compared with MRI ((0.2-Tesla Magnetic Resonance Imaging (MRI) scanner (E-scan, Esaote Biomedica, Genoa, Italy)) images (taken in the sagittal plane using a spin-echo TI half fourier (HF) sequence with a slice thickness of 8mm, inter-slice gap of 0.6mm and the parameters time to repetition/echo time/number of excitations (TR/TE/NEX), 420/18/1; field ofview, 160·160mm; matrix, 256·256 pixels)) in our lab and does indeed provide externally valid measurements. Indeed we have systematically measured the knee moment arms of four participants (2 males and 2 females) using both pieces of equipment. This revealed a non-significant (Wilcoxon signed-rank test: Z-score=-1.826, 2-tailed $p>0.05$) trend for the moment arm values using the DEXA to be 7.5±1.5% (or 3.2±0.6mm) greater than those obtained using an MRI scan.

Estimation of Co-Contraction from Electromyographical Activity

A pair of self-adhesive Ag-AgCl electrodes 15 mm in diameter (Neuroline 720, Ambu, Denmark), was placed on clean, shaved, and previously abraded skin, in a bipolar configuration with an inter-electrode distance of 20 mm, at 50% of femur length, in the mid-sagittal plane of the biceps femoris muscle (BF). The reference electrode (Blue sensor L, Ambu, Denmark) was placed on the lateral tibial condyle. The raw EMG signal was preamplified (MP100, Biopac Systems Inc., USA), amplified (MP100, Biopac Systems Inc., USA), bandpass filtered between 10-500 Hz (Biopac Systems, USA), and sampled at 2000 Hz. All EMG and torque signals were displayed in real time in AcqKnowledge software (Biopac systems Inc., USA) via a PC (iMac, Apple Inc., USA). Two maximal flexion contractions were carried out to obtain the EMG at maximal flexion torque. The root mean square (RMS) EMG activity was averaged for a 500ms period which coincided with the plateau of peak torque.

As mentioned above, the EMG of the long head of the biceps femoris muscle was measured to ascertain the level of antagonist muscle co-contraction during the required isometric knee-extension performances. The biceps femoris torque during a knee-flexion contraction was calculated by the biceps femoris EMG activity during knee extension being divided by the biceps femoris peak flexor EMG at 70^o knee flexion; the maximal flexor torque is then multiplied by this value to determine co-contraction torque. The co-contraction torque values are used to correct the voluntary knee-extension torques (and hence the

forces during the ramped contractions) using the following formula:

$$CT = OT + CcT \qquad \textit{Equation [3]}$$

where CT represents corrected knee-extensor torque, OT is the observed knee-extensor torque, and CcT is the calculated hamstrings torque during knee extension (i.e. antagonist co-contraction torque).

Resistance Training Programme

Resistance training was performed 3 times per week (twice supervised and one home-based session) by both SL and LL training groups for 8 weeks, using a combination of free, machine (Technogym, UK) and body weights. Exercises for the knee extensors included bilateral barbell squat, seated leg press, seated knee extension, Bulgarian split squat and the Sampson chair. Throughout the training period, participants performed 3-4 sets of 8-10 reps (depending on stage of program and exercise) at 80% (SL) or 55% (LL) 1 repetition maximum (1RM), defined by the maximum load that could be lifted throughout the entire designated range-of-motion. 1RMs were re-assessed every two weeks for each of the exercises and training loads adjusted accordingly. A generalised warm-up was completed at 70-75% age-predicted maximum heart rate on a treadmill for 5 minutes, after which a goniometer was attached (using double-sided sticky tape) to the centre of rotation of the knee. As the participant performed squat exercises, the goniometer rotated. The investigator/training partner confirmed from the scale that they had reached 50^{o} or 90^{o} of knee flexion (depending on the training group) during the eccentric phase and therefore could hold the load steady over 2 seconds, before beginning the concentric phase of movement and return to the starting joint angle (i.e. either 0^{o} or 40^{o}). As the participants performed the leg press and knee extension exercises, the concentric movement was performed firstly followed by a hold, and then the eccentric phase was performed back to the starting joint-angle, which again was confirmed and timed by the investigator/training partner. The short isometric hold over 2 seconds was to emphasise the stretch at the end of the excursion. Therefore, the vast majority of quadriceps time-under-tension in SL was spent at shorter muscle lengths close to and including 50^{o}, whereas LL group's quadriceps was predominantly at longer muscle lengths close to and including 90^{o}. (NB. The load was removed in LL prior to the subject straightening up between 40^{o} -0^{o} degrees at the end of each set). All exercises involved eccentric and concentric contractions, except for the Sampson chair, which was isometric loading, with LL holding the position with the knee at 90^{o} and SL with the knee at 50^{o} angle. Timing of contractions was controlled using a metronome (1 second eccentric, 2 second isometric hold, 1 second concentric). The subjects completed 2 familiarisation sessions at 70% (SL) and 40% (LL) of 1RM prior to commencing the resistance training programme.

Muscle architecture and Muscle Length

Architecture was measured at rest with each participant seated in an upright position on an isokinetic dynamometer (Cybex, Phoenix Healthcare Products, UK). Following equipment calibration, each participant was positioned with a hip angle of 80^{o} (straight back 90^{o}) and knee at 90^{o} knee flexion (straight leg 0^{o}). All muscle architectural measurements were determined at rest using real-time ultrasonography (7.5-MHz, 40-mm linear array, B-mode ultrasound probe, AU5, Esaote Biomedica, Italy) at rest, with images captured using a digital video recorder (Tevion, UK). Vastus Lateralis fascicle pennation angle (θ) was measured as the angle of fascicle insertion into the deep aponeurosis (Rutherford and Jones, 1992). Images were obtained perpendicular to the dermal surface of the VL and orientated along the plane of the muscle fascicles. Images were taken at 25% (proximal), 50% (central) and 75% (distal) of total femur length (as described below) and 50% of muscle width at each point (where 50% muscle width is defined as the mid-point between the fascia separating the VL and Rectus Femoris, and fascia separating the VL and Biceps Femoris muscles). Fascicle length was defined as the length of the fascicular path between the deep aponeurosis and superficial aponeurosis of the VL. The majority of fascicles extended off the acquired image, where the missing portion was estimated by linear extrapolation. This was achieved by measuring the linear distance from the identifiable end of a

fascicle to the intersection of a line drawn from the fascicle and a line drawn from the superficial aponeurosis. This method has been shown to produce reliable results previously (Blazevich et al., 2007). All images were analysed and measured using Image J (Wayne Rasband, National Institute of Health, USA).

VL muscle lengths were also determined using ultrasound in the mid-sagittal plane. This was determined as the length from the myotendinous junction of the VL and patellar tendon to the point where VL adjoins to the *Tensor Fascia Latae* and *Rectus femoris* muscle. These points were marked on the skin and measured with standard anthropometric measuring tape.

Muscle aCSA

VL muscle anatomical cross-sectional area (aCSA) was measured using real-time ultrasonography at rest. aCSA was measured with the ultrasound probe in the transverse plane at three sites; 25%, 50% and 75% of total femur length. Femur length was defined as the line passing from the greater trochanter to the central palpable point of the space between the femur and tibia condyles when the knee was flexed at 90^{o}. Echo-absorptive tape was placed at regular intervals (~3cm) along the muscle width at each site so that when the probe was placed on the leg, two distinct shadows were cast on the ultrasound image. Therefore each ultrasound image provided a section of VL within the boundaries set by the two shadows and fascia surrounding the muscle. Individual images were reconstructed using the femur and superficial markers as reference points, with the total aCSA measured using image J. The validity and reliability of this technique has been shown previously (Reeves et al., 2004a). It should be noted here that all sonographs (and other muscle parameters) were taken/ measured ~3-4 days post-training to avoid osmotic fluid shifts that may confound architectural or morphological measurements (Berg et al., 2008).

Circulating Insulin-Like Growth Factor -1 (IGF -I) levels

At each of the designated testing intervals (i.e. baseline, weeks 8, 10 and 12) and following an overnight fasting period (~10 hours for all participants), participants reported to the laboratory. A 21-gauge 1-inch ultra thin wall needle (Terumo Medical Corporation, New Jersey, USA) was inserted into the antecubital vein of the forearm. Using a vacutainer assembly and serum separator tubes (Monovette, Sarstedt, Numbrecht, Germany), 5 mL blood samples were collected. After being kept on an ice bed for up to 2-hours (or a minimum of 30 mins as per the ELISA kit's manufacturers recommendations), the sample was then centrifuged at 4°C for 10 min at 4,800 rpm, with the supernatant being removed and stored in eppendorfs at −20° Celsius for later analysis. The IGF-I (R&D Systems Europe, UK. Sensitivity of 0.026 ng/mL; intra-assay variability of 4.0%, manufacturers' data) was analysed using standard enzyme-linked immuno-sorbent assay procedures.

Strength measurement

Maximal isometric knee extension torque was measured with the knee at 70° knee flexion (full knee extension = 0°) on the right leg of all participants. After a series of warm up trials consisting of ten isokinetic contractions at $60°{\cdot}s^{-1}$ at 50-85% maximal effort, participants were instructed to rapidly exert maximal isometric force against the dynamometer (Cybex, Phoenix Healthcare, UK) lever arm. Participants were given both verbal and visual encouragement/feedback throughout their effort. Joint torque data were displayed on the screen of a MacBook Air computer (Apple Computer, Cupertino, CA, USA), which was interfaced with an A/D system (Acknowledge, Biopac Systems, Santa Barbara, CA, USA) with a sampling frequency of 2000 Hz. Isometric contractions were held for ~2 s at the plateau with a 60 s rest period between contractions. Peak torque was expressed as the average of data points over a 200 ms period at the plateau phase (i.e. 100 ms either side of the instantaneous peak torque). The peak torque of three extensions was used as the measure of strength in each participant.

Statistics

Data were analysed using IBM SPSS v19 (IBM Inc, USA). The Shapiro-wilk and Levene's tests revealed the data sets to be parametric and these were therefore analysed using a mixed-design repeated

measures 3×4 ANOVA. The within-group factor was the phase of training (i.e. week 0, 8, 10 and 12) and the between-group factor was training group (i.e. SL, LL or Con). Post-Hoc contrast analyses with bonferroni corrections were used to compare data to baseline ('within' factor) and to control group ('between' factor). All data are presented as mean ± standard error of the mean (SEM). Statistical significance was set with alpha at ≤ 0.05. The average statistical power of the measured muscle parameters (CSA, pennation angle, fascicle length and strength) was statistically adequate at beta ≥ 0.86.

Results:

Measurements precision:

A pilot study was conducted at the onset of the study on a similar population (i.e. age and physical characteristics). Repeated measures of VL muscle anatomical CSA, architecture and strength on a group of 5 individuals (2 males, 3 females) were collected on three separate occasions (spanning consecutive 7 days). Within-day coefficients of variation (CV in %) of 1.5%, 1.9%, 3.3%, 2.2% and 0.8%, and between day CVs of 2.6%, 2.1%, 3.6% and 1.8% were yielded for aCSA, fascicle length, fascicle pennation angle, DEXA moment arm (within-day only – measurements all preformed on same day) and strength respectively. Therefore the repeatability of the measurements was considered acceptable.

Quantification of muscle-tendon complex stretch

In order to measure the extent of lengthening (or passive stretch) at each training joint-angle compared to full extension, the VL muscle length was measured as the distance between the two myotendinous junctions of the muscle, with the results shown in Table 1. At 90^o knee flexion, the VL was significantly ($p=0.03$) lengthened compared to full extension, but not at either 40^o or 50^o.

[INSERT TABLE 1 NEAR HERE]

Architecture

Fascicle Length: There were significant relative increases in VL fascicle lengths as a result of the training protocol in both groups post training and detraining compared to baseline, at all three measurement sites (see Figure 2, $p<0.001$). Post-training, fascicles increased in length to a greater extent at all sites in LL compared to SL (Δ 27±3mm; Δ21±3mm; Δ 24±3mm; vs. Δ18±4mm; Δ9±6mm; Δ 12±5mm; $p = 0.02$ proximal, $p<0.01$ central and distal respectively). This significant main effect of group was retained through the entire detraining period at all sites ($p<0.01$). The control group did not display any significant changes in fascicle length during the training and detraining periods (averaged over 3 sites Δ 3±4mm; $p>0.05$).

Fascicle pennation Angle: Both training groups experienced significant increases in fascicle pennation angle post-training at proximal (SL; 9.9±0.4^o to 10.7±0.4^o Δ9±4%, and LL; 9.0±0.4^o to 10±0.3^o Δ14±7%, $p=0.034$), central (SL; 16.2±0.5^o to 17.2±0.4^oΔ7±2%, and LL; 15.3±0.4^o to 16.2±0.5^o Δ6±3%, $p=0.041$) and distal (SL; 16.5±1.2^o to 18.1±1.0^o Δ11±4% and LL; 18.1±0.9^o to 19.2±0.8^o Δ7±3%, $p=0.003$) sites. Fascicle pennation angle remained elevated compared to baseline ($p<0.05$) at week 10 at all three sites, but not at week 12 in both training groups at any measurement site. There was no difference ($p>0.05$) between SL and LL groups in fascicle angle at any stage. The control group displayed no changes in fascicle angle over the 12 week period (averaged over 3 sites - 15.9±0.5^o to 15.7±0.5^o Δ 1±1%, $p>0.05$).

[INSERT FIGURE 2 NEAR HERE]

Anatomical Cross-Sectional Area (aCSA):

Changes to aCSA are shown in Table 2 and Figure 3. VL aCSA increased significantly ($p<0.0001$) relative to baseline following training at all sites in both training groups, which was still evident at the conclusion of the detraining period in both training groups at proximal, central and distal sites ($p<0.01$) . There was also a trend for LL to exhibit greater relative gains in aCSA compared to SL at all sites at week 8 ($P<0.06$), but only significantly so distally. Here there was a main group effect ($p=0.030$) with LL exhibiting a 53±12% increase compared to SL

showing 18±8% increment in VL aCSA. The superior adaptations were retained distally at both week 10 (LL; 45±13% vs. SL; 11±10%, p=0.043) and week 12 (LL; 32±9% vs. SL; 2±7%, p=0.022). There was no notable VL aCSA change over the 12 week period for the controls (0±2%, 4±6%, 3±4% proximal, central and distal respectively; p>0.05).

[INSERT TABLE 2 NEAR HERE]
[INSERT FIGURE 3 NEAR HERE]

Insulin-Like Growth Factor -1 (IGF - I)
Changes in IGF-I are shown in Figure 4. IGF-I levels increased significantly as a result of training in LL group but not SL group at week 8 (SL, 407 ± 25 ng/mL – 429 ± 30 ng/mL, 7±6%; p=0.438; LL, 375 ± 18 ng/mL – 489 ± 29 ng/mL, 31±6%; p=0.033), with a significant between group effect (p<0.001). LL group maintained greater IGF-I levels compared to baseline (p=0.006) and SL group (p=0.013) at week 10, but had returned to baseline levels by week 12 with no main group effect evident at the conclusion of detraining (p>0.05). The control group showed no significant change in the circulating levels of this hormone at any stage of the study period (p>0.05).

[INSERT FIGURE 4 NEAR HERE]

Muscle Strength:

Both SL and LL groups increased strength at 70^{o} knee flexion as a result of the training protocols (p<0.001), with LL experiencing a significantly (p=0.015) greater increase from 223±30Nm to 268±28Nm (26±6%) compared to SL 273± 37Nm from pre-training torque of 253±32Nm (7±3%). The strength improvements in LL were retained to a greater extent throughout the detraining phase (week 10; p=0.008, week 12; p=0.011) compared to SL, although by week 12 SL had returned to baseline strength measures, whereas LL still remained elevated relative to week 0 (p<0.05). The control group's strength did not significantly alter during the 12 week training and detraining period (p>0.05).

Discussion:

The aim of the current investigation was to identify the *in vivo* effects of dynamic resistance training at two distinct average muscle-tendon unit lengths (shorter versus longer), and determine the time-course of any reversibility during detraining on morphological, architectural and functional properties of the VL and IGF-I levels. It was hypothesized that, due to a greater internal physiological stress (i.e. higher activation and metabolic demands of producing force at longer muscle lengths compared to shorter muscle lengths even when forces are normalized (Kooistra et al., 2006, de Ruiter et al., 2005) and stretch on the VL muscle when training at longer muscle lengths compared to shorter muscle lengths, the LL group would have superior adaptations to SL in terms of size and function. The results of this study showed that although both SL and LL groups displayed marked increases in muscle structural and mechanical characteristics compared to a control group, in general, the effect of training at longer muscle lengths showed greater adaptation in many of the measured parameters, thus partially confirming our hypotheses. In addition, we theorized that adaptations would be retained by the group training at a longer muscle length (due to the greater adaptations of the preceding training) during a subsequent period of detraining, and this was also confirmed in our observations.

In vivo changes to muscle architecture following a length-restricted resistance training protocol, to the author's knowledge have not yet been reported. Therefore we would contend that this is the first study to demonstrate superior increases in VL fascicle length following an extended period of loading at longer muscle lengths. The relative increases in fascicle length were greater in the LL group than those of SL post-training at each of the three measured sites. Observations from animal models have demonstrated the importance of stretch in modulation of sarcomere number (Tabary et al., 1972, Williams and Goldspink, 1973) and is associated with an increase in protein synthesis(Loughna et al., 1986). Table 1 shows that by placing the leg at 90^{o} of knee flexion, that the VL muscle was stretched to a greater degree than any other training knee joint angle. By performing load bearing exercise with the muscle-tendon complex in a relatively lengthened position (i.e. 40-90^{o} in LL

group), an enhanced mechanical stimulus is experienced (i.e. greater stretch), thereby augmenting fascicle length increase by an increase in the number of in-series sarcomeres to allow each sarcomere to work at optimal length in line with the length-tension relationship of this unit. It should be pointed out here that, although fascicle length was measured at 90^{o} of knee flexion and the SL group did not encounter this joint-angle during training specifically, they would still have experienced this joint angle during their normal daily routine including sit-to-stand transitions. Therefore when measuring fascicle length in the laboratory, the in-series and parallel elastic components should not be sufficiently 'stiffer' in this group to justify the large between group changes we describe here.

Excursion range has been suggested to be important for regulating sarcomere number in the rabbit (Koh and Herzog, 1998), although our results suggest that an excursion with the muscle in a predominantly lengthened position, that is the major regulator of fascicle length *in vivo*. This is due to the fact that both groups performed the same degree of excursion of 50^{o}, yet had different levels of adaptation. Additionally, in favour that excursion offset hypothesis, is that muscle forces at the tendon were also matched during resistance training, therefore no additional mechanical stimulus for fascicle length change (i.e. greater force) was present in the LL group, which is in agreement with previous work (de la Tour et al., 1979).

The relationship between muscle aCSA and strength is well established. The LL group had greater relative increases in aCSAs at all three measured sites along the VL following the training period, however, only statistically so distally. A heterogeneous hypertrophic response following knee-extensor resistance training has been reported previously in the VL muscle (Narici et al., 1996, Blazevich et al., 2007). A lack of intramuscular homogeneity in stress in the knee extensors may be due to one or a combination of several factors, to provide "mechanical stability" (Leeuwen and Spoor, 1992)(the latter referring here to the ability to efficiently use the combined properties of muscle strength, architecture, proprioception and tendon characteristics to effect movement about a joint (Ryan, 1994)) force generation and/ or force transfer (Blazevich et al., 2006). It has been shown that muscle experiences both serial and parallel force distribution, and that depending on muscle length, can exhibit a proximo-distal difference in force transmission(Huijing and Jaspers, 2005). The difference in the VL muscle's distribution of force due to alteration in its length may be one possible reason for observed distal hypertrophy in LL group compared to SL. The increase in strength following the training period measured by isometric MVC was greater in LL group than SL, and maybe reflective of the changes in aCSA and not in changes to fascicle angle of pennation (Figure 3). Nevertheless, we do acknowledge here that aCSA may not be as strongly correlated with force compared to pCSA, as aCSA taken at right angles will therefore not pass through all the fibres of a highly pennate muscle. Nonetheless Bamman et al. (2000) found aCSA to be as effective as pCSA in estimating strength. The changes in strength reported in the current study for the LL group are similar to those reported in other training studies e.g.(Jones and Rutherford, 1987, Narici et al., 1996, McMahon et al., 2012), but those of SL are less (we propose that the seemingly low training responsiveness of SL is due to a combination of lower training duration, volume and chronic muscle activation (Kooistra et al., 2006) compared with that encountered in the work of Jones & Rutherford(Jones and Rutherford, 1987). Additionally, we would point out that not only was the range of motion similar between the training groups, but also that the isometric strength test angle was 20^{o} from the end range of motion of each group (i.e. 50^{o} and 90^{o}), thereby conferring a high degree of equity to the assessment of strength. Additionally, in a similar study, we have recently shown that there are significantly greater increases in isometric MVC of the entire measured torque-angle relationship (30^{o}-90^{o} knee flexion) in wide range of motion training group compared to that in a short range of motion training group(McMahon et al., 2012). It is also possible that following length-specific training that optimal angle of force production could have changed due to varying changes in fascicle length and as an adaptive process to the functional length of the muscle-tendon unit. However to gain an insight into changes in optimal angle would also need scrutiny of

changes in tendon mechanical properties, and from the current data set would be difficult to predict.

Here, we outline some of the possibilities that may have given rise to a generally greater hypertrophy following length specific training. Firstly, it has been shown that VL activation and oxygen consumption are significantly greater when performing isometric MVCs at longer (60^o and 90^o) muscle lengths compared to shorter (30^o) muscle lengths, with the greatest activation and oxygen consumption at 90^o (Kooistra et al., 2006, Kooistra et al., 2008). In conjunction with these results, one of the major findings was that IGF-I levels in our current study were significantly greater in LL post training compared to SL (31% vs. 7% increment). *In vivo* increases in IGF-I following resistance training in humans been reported previously(Borst et al., 2001), with extensive *in vitro* research showing that IGF-I is an important regulator of muscle mass *in vivo*, and that experimental manipulation of IGF-I levels can cause a tremendous increase in muscle mass and protein synthesis (Goldspink et al., 1995, Lee et al., 2004). *In vivo* data from adult skeletal muscle has shown that, although electrical stimulation failed to increase levels of growth or protein synthesis, static stretch of muscle increased IGF-I mRNA 12-fold with a concomitant increase in fractional (138%) and total (191%) protein synthesis rates. Furthermore, stretch combined with electrical stimulation increased IGF-I mRNA concentration 40-fold , with fractional and total protein synthesis rates increasing by 345% and 450% respectively in this condition (Goldspink et al., 1995). The above evidence suggests that by training at longer muscle lengths (i.e. LL group), muscle is activated to a greater degree, experiences greater metabolic stress, and also a greater mechanical stress. In response, there is a co-ordinated activation of probably several signalling cascades, which may be mediated, at least in part, by the activity of IGF-I. Combination of these factors may explain the larger hypertrophic response of the LL group compared to SL.

The temporal response of *in vivo* changes to architecture during a short period of detraining to the authors' knowledge, have not been reported previously. During a period of 3 months detraining, fascicle length was previously shown to increase following the removal of the training protocol (Blazevich et al., 2007). In the current study, our results show that in both SL and LL groups, fascicle lengths were reduced during the detraining period and these reductions were fairly linear in each group. For example, there was a Δ-6% (14% to 8%) and Δ-5% (29% to 24%) reduction (averaged across the three sites) compared to baseline in SL and LL groups respectively at week 10, with almost identical reductions at week 12 (SL; Δ5%, 8% to 3%, LL; Δ6%, 24% to 18%). This data would indicate that although there appears to be no difference in the rate of loss of adaptation between groups, the LL group's greater initial increases to fascicle length during training resulted in retention of increased fascicle lengths following 4 weeks detraining.

From the results of the effect of detraining on VL aCSA, there were similar magnitudes of relative losses in aCSA and fascicle length (parallel and serial sarcomere number) in SL (week 10; Δ-4% and week 12; Δ-5% relative to baseline averaged over three sites). However in the LL group, the magnitude of decrements in aCSA was more pronounced compared to fascicle length especially in the latter stages (week 10; Δ-6% and week 12; Δ-13%), however the LL group still retained greater overall aCSA values relative to baseline at week 12 (+21±6%) compared to SL (+11± 4%). It has been previously shown that IGF-1 has been implicated in preventing the expression of the FOXO class of transcription factors and the mRNA increases of MAFbx and MuRF1 seen during muscle atrophy (Stitt et al., 2004). The LL group continued to have significantly greater IGF-I levels than SL at both week 8 and week 10. Although not a chronic increase in IGF-I levels, the LL group's responses certainly lasted longer than the transient increase in IGF-I observed with acute bouts of resistance exercise. Therefore, there may have been a protective role played by IGF-I allowing greater retention of muscle mass in LL. In support of this notion is the observation that the LL group IGF-I levels at week 12 had returned to baseline levels, and that this period also coincided with the relatively larger magnitude of aCSA decrement in LL group at the conclusion of the detraining phase. The above (i.e. larger initial gains of muscle mass) may also provide a rationale for generally greater strength

retention in LL at the end of the detraining period, through the positive correlation between mass and strength. In addition, the VL (and *vastus medialis*) muscle has been shown to be activated to a greater extent at longer muscle lengths (Kooistra et al., 2006), therefore if the muscle is activated chronically to a larger extent, the magnitude of neural drive is likely to be retained thereby sustaining muscle strength gains during at least the first 4 weeks of detraining.

Conclusion

Previous evidence in young humans (Blazevich et al., 2007) demonstrated that eccentric training was not the major determinant for fascicle length adaptations. However in the current study, greater increases in fascicle length of the VL were present following longer muscle length training, despite the overall degree of excursion and muscle forces at the tendon being identical. We present for the first time to our knowledge, that in humans, muscle length controlled excursion is the major mechanical stimuli for changes to fascicle length; although the exact mechanisms underpinning such adaptations are complex and have yet to be elucidated. A previous study (Kubo et al., 2006a) investigating isometric training at longer and shorter muscle lengths, found no differences between training groups in muscle morphology. Here, we present evidence that prolonged dynamic resistance training at predominantly longer compared to shorter muscle lengths, resulted in more marked improvements in strength and regional muscle hypertrophy, and that these adaptations may be in part mediated from a greater hormonal response elicited. Furthermore, with muscle architecture and strength being major influences on the determinants of functional performance, from athletes to the elderly, the above results suggest that length specific training should be implemented. Additionally, with adaptations retained more successfully over 4 weeks of detraining with the muscle lengthened, such training could also be used to offset deleterious effects of detraining or hypoactivity.

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Tables:

Knee Joint angle (^{o} knee flexion)	VL Muscle Length (cm)	Muscle Length to Femur Length Ratio
0^o (Full extension)	32.8±1.1	0.74 : 1
40^o	34.7±0.9	0.78 : 1
50^o	35.3±0.9	0.80 : 1
90^o	37.0±1.1*	0.83 : 1

Table 1. Resting *Vastus Lateralis* length at various knee-joint angles. NB. In this population, the average femur length was 44.6±1.0 cm (*n*=6). * Significantly different to full extension (p<0.05).

Figure Legends:

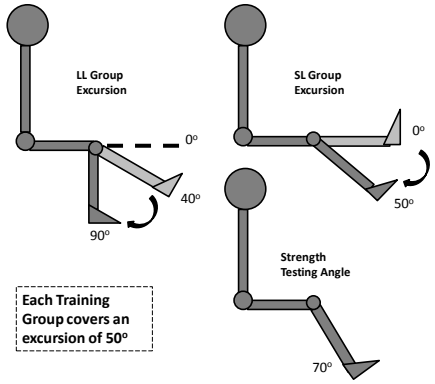

Figure 1: Diagram of training excursions and testing joint angle.

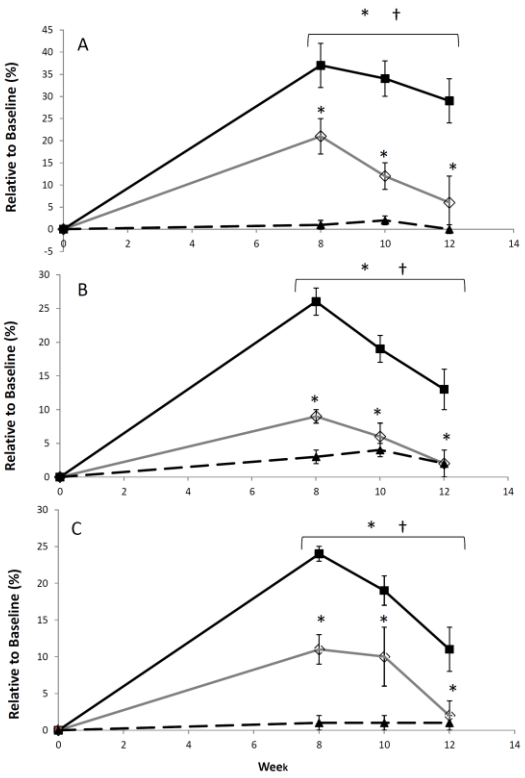

Figure 2: Relative changes to fascicle length following training and detraining in SL (open squares), LL (black squares) and control (dashed line) groups at A) proximal, B) central and C) distal sites of the VL muscle. * Significant difference compared to baseline ($p<0.05$) † Significant difference between groups ($p<0.05$).

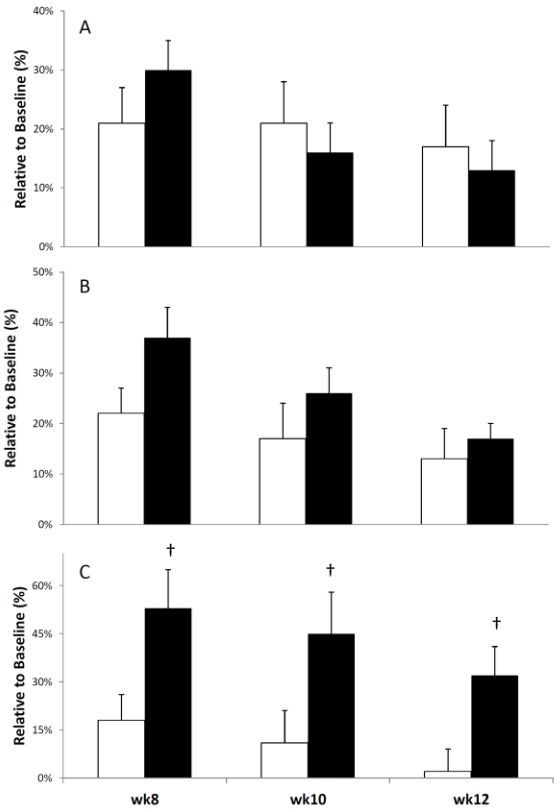

Figure 3: Changes following training and detraining to aCSA in SL group (white bars) and LL group (black bars) at (A) proximal, (B) central and (C) distal sites of VL muscle. † Significant group difference in aCSA ($p<0.05$).

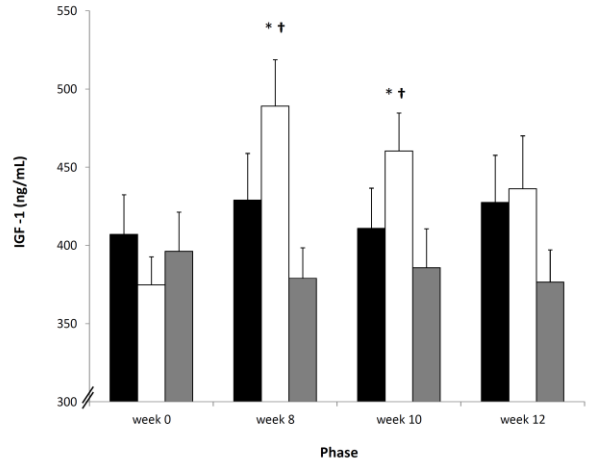

Figure 4: Changes in IGF-I following training and detraining in the SL (black bars), LL (white bars) and control (grey bars) groups. * Significant difference compared to baseline ($p<0.05$) † Significant difference between groups ($p<0.05$).