# THE ROLE OF EXERCISE AND AMINO ACID SUPPLEMENTATION IN DISUSE-INDUCED MUSCLE AND TENDON ATROPHY AND SUBSEQUENT ACTIVE RECOVERY

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## ABSTRACT

Skeletal muscle atrophy due to disuse is a widespread problem arising from many situations including clinical immobilisation due to injury or disease. As individuals age, the universally observed loss of muscle mass and function known as sarcopenia compounds this problem leading to a significant reduction in locomotor ability and quality of life for sufferers. Recently there has been much work investigating ways to mitigate muscle mass loss in immobilised limbs and to aid in recovery in the post immobilisation phase. Much focus has been placed on recovery protocols based upon either resistance training, nutritional supplementation, or both. In study 1 we report an extensive data set describing in detail skeletal muscle adaptations in structure and function in response to both disuse (ULLS) and retraining. The results indicate that, 1) the loss of muscle force with 3-week unloading in humans is mostly explained by muscle atrophy and a decrease in myosin content and, 2) all the neuromuscular changes induced by this model of disuse can be fully restored after a resistance training intervention of equal duration

Study 2 tests the hypothesis that increasing amino acid leucine availability by nutritional supplementation will increase muscle protein synthesis in immobilised muscle and result in mitigating the loss of lean muscle mass. In fact, we found that leucine supplementation alone was not sufficient to maintain muscle mass during 3 weeks of unilateral lower limb suspension. Study 3 examined the effect of leucine supplementation on muscle mass gains during an active recovery training program of muscles previously subjected to ULLS. Despite low participant numbers, the data

suggest that leucine supplementation had no effect on the extent to which muscle recovered post ULLS.

Study 4 investigates the role of training and immobilization in sarcopenia and discusses the potential of using ultrasound imaging to develop an image based biomarker of sarcopenia. Cross sectional data from both young and old individuals subjected to different loading conditions are analysed in this study. Life-long training appears to slow down the process of sarcopenia whilst periods of disuse due to injury or disease worsen the condition. The change in muscle fibre geometry exhibited in sarcopenia and disuse atrophy could potentially act as a convenient and inexpensive indicator of the onset of sarcopenia.

**Statement of Candidate's contribution**

This thesis was undertaken as part of the MYOAGE project, a large scale collaborative project funded by the European Commission under the 7th Framework programme with aim to identify the relative importance of muscle weakness and underlying mechanisms thereof to identify therapeutic strategies to prevent muscle loss and weakness in an aging population. The ultimate outcome of the work will be to provide the EU with cost-effective tools to understand and combat human age related muscle weakness, prevent dependency, and promote mobility of older European citizens.

The presented work was undertaken at the Institute for Biomedical Research into Human Movement and Health, Manchester Metropolitan University by PhD candidate Emma-Louise Campbell under the supervision of Prof. Marco Narici and Dr. Olivier Seynnes.

Participant recruitment and enrolling for the ULLS study was undertaken by the candidate through the production of promotional material distributed locally and contact with local media outlets such as newspaper articles and advertisements. Once enrolled, management of participants including screening, obtaining informed consent for testing procedures and scheduling of testing sessions was also the role of the candidate. The study design was already in place as per MYOAGE protocol. Preparation and dosage calculation of the amino acid and placebo supplements according to body mass was carried out by the candidate. All data collection including muscle function testing and ultrasound and MRI scanning took place at the

IRM facility by the candidate under instruction by Dr. Olivier Seynnes. Muscle biopsies were divided into several portions by Prof. Martin Fleuck and shared amongst collaborating MYOAGE laboratories. Single fibre CSA and myosin content measurements reported in this thesis were performed by Prof. Roberto Bottinelli. Data was analysed and prepared for dissemination by the candidate at the IRM.

The data for chapter 7 was collected in two sessions. One, at the 2010 European Veteran Athletics Championships in Nyiregyhaza, Hungary were muscle functional and structural data was gathered on master athletes aged 65yrs+. The ultrasound scanning and muscle functional data reported here were recorded by Dr. Jamie McPhee (IRM) and the candidate Emma-Louise Campbell. Data from recreationally active older individuals was collected as control subject data at the IRM by Dr. Jamie McPhee and the candidate. Participants were recruited through information session held at the University of the Third Age attended by Dr. Jamie McPhee and through a postal campaign aimed at individuals in the local area in the desired age group. Telephone screening interviews of potential candidates and arranging subsequent screening visits was undertaken by the candidate. At the testing sessions, data was collected by Dr. Jamie McPhee and Emma-Louise Campbell. The data reported in chapter 7 was analysed and presented by the candidate at the IRM.

# 1. Introduction

Skeletal muscle atrophy is a widespread problem arising as a result from many situations including immobilisation due to clinical unloading following injury, disuse due to inactivity and as a consequence of many illnesses. Although muscles of the whole body are affected, the weight bearing muscles of the lower limb are particularly susceptible. Accompanying the loss in skeletal muscle mass is a reduced functional capacity. Following periods of disuse, muscles produce maximum voluntary contractions (MVC) of much smaller magnitude than pre-disuse levels. Muscle weakness can have a drastic effect on quality of life with limited mobility posing a major problem for both young and old displaying characteristics of muscle atrophy. The loss of muscle mass and function following disuse can be slow to recover even in young healthy subjects. As older individuals display a normal age related loss of skeletal muscle mass and function known as sarcopenia, any period of disuse or reduced activity which would exacerbate the condition is especially significant. A change in muscle size, involving either hypertrophy or atrophy must involve a change in protein turnover (for a review of the literature see (Bajotto & Shimomura 2006). In general, reduced rates of protein synthesis and elevated protein degradation result in a net catabolic effect and loss of muscle bulk. Both physical activity and essential amino acid supplementation have been shown to effectively increase myofibrilar muscle protein synthesis (MPS) and are relatively easily manipulated. For this reason, much research into muscle atrophy countermeasures has centred around these two factors. The development of a more efficient post-operative rehabilitation procedure has the potential to improve post-operative muscle

function and may lead to significant improvements in the quality of life of individuals rehabilitating after disuse caused by injury or illness. While there is increasing evidence that both resistance training and nutritional supplementation with essential amino acids can initiate muscle hypertrophy and restore muscle function, current research exploring the synergistic effect of these interventions and their effect muscle atrophy in individuals subjected to severe disuse is limited.

# 2. LITERATURE REVIEW

## 2.1 Basic properties of Skeletal muscle structure and function

The human body is composed of four different types of tissue, connective, nervous, epithelial, and muscular, each composed of cells and structures connected by their similar shape or function. These four primary tissue groups contain many important sub-tissue groups, again with their own distinct form or function. Muscle, for example, consists of three types of different muscle tissues, each with their own unique structural and functional properties:

1. *Smooth muscle*, a non-striated muscle found in the walls of blood vessels and internal organs which is not under voluntary control.

2. *Cardiac Muscle*, a striated muscle found in the walls and myocardium of the heart which is not under voluntary control and,

3. *Skeletal muscle*, a striated muscle mostly attached to bones which is under voluntary control and responsible for locomotory control of the skeleton and the focus of this thesis.

### *2.1.1 Structure of skeletal muscle.*

Skeletal muscle is composed of two types of tissue, contractile and supporting. The contractile tissue is composed of numerous cylindrical cells or 'fibres' , each with a multinucleated sarcoplasm (the cell cytoplasm of the skeletal muscle cell) surrounded by the sarcolemma (skeletal muscle cell membrane). The sarcoplasm, as well as containing the nuclei houses many longitudinal rod-shaped units known as

myofibrils, each composed of numerous contractile units known as sarcomeres arranged in-series (Figure 2.1).

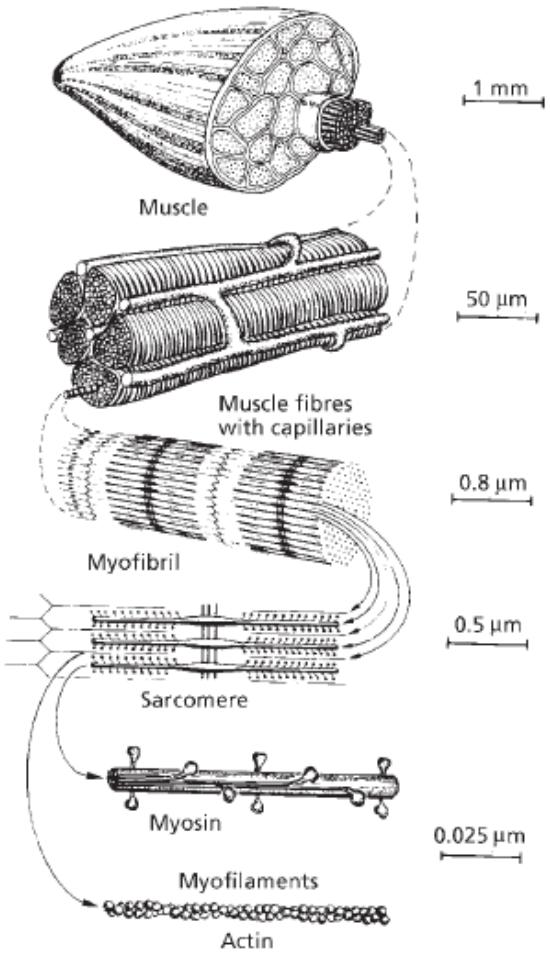


Figure 2.1 Diagrammatic representation of structural composition of skeletal muscle tissue, taken from (Billeter & Hoppeler 2003.).

It is the repeated arrangement of these banded sarcomeres which gives skeletal muscle its characteristic striated appearance, from which it takes its name (fig 2.2). The actin and myosin filaments of each sarcomere are bound between two neighbouring Z-discs, the boundaries of the sarcomere. Areas of the sarcomere where myosin filaments do not overlap the actin filaments are known as I-bands (isotropic) so named due to their appearance when viewed through an electron microscope. A-bands (anisotropic), which appear darker in microscopic images

represent the length of the thick myosin filaments. Towards the centre of the A-band is a paler region known as the H-zone in which the thin actin filaments do not overlap the myosin filaments.

The thin (actin) and thick (myosin) protein filaments of the myofibrils allow skeletal muscle to contract and generate force and motion via a process known as The Sliding Filament Theory of Muscle Contraction (HUXLEY & NIEDERGERKE 1954 )(HUXLEY & HANSON 1954). The cross-bridging of the myosin 'head' and the actin filaments create a rowing like action when the skeletal muscle contracts, the actin filament pulling the myosin inwards toward the H-zone of the sarcomere (contractile unit) to shorten the sarcomere and, therefore the entire muscle.

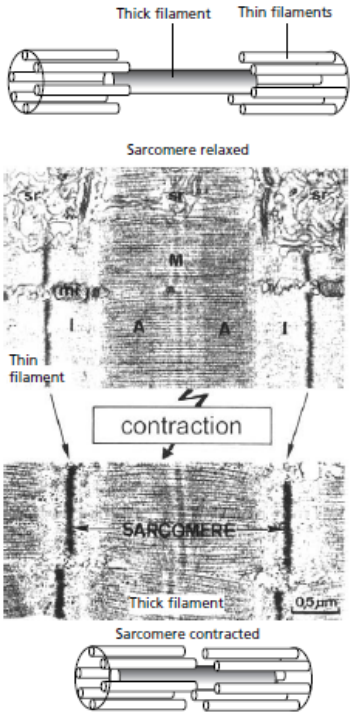


Figure 2.2 Electron micrographs demonstrating the sliding filament theory of and banding of sarcomeres (Alberts 1994).

## 2.1.2 Skeletal muscle fibre architecture.

There are approximately 640 skeletal muscles in the human body displaying huge variation in form and function. These muscles are classified according to the arrangement of the fibres, known as muscle architecture (Figure 2.3). Whilst in longitudinal parallel muscles like biceps brachii the fibres run parallel to the axis of force generation, in pennate muscles like vastus lateralis the architectural arrangement of fibres is more complex.

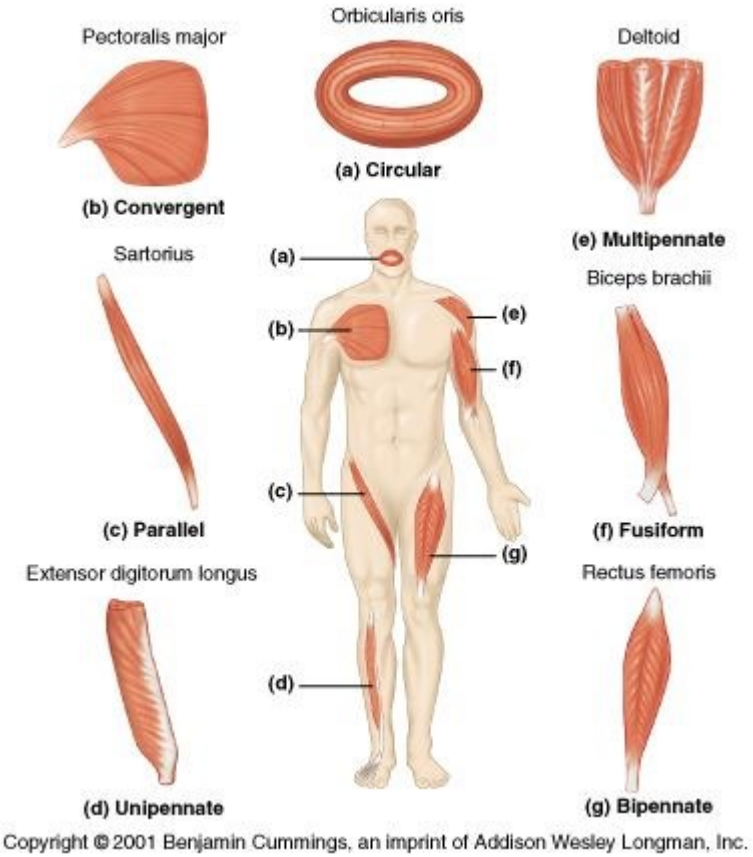


Figure 2.3. Classification of muscle according to fibre architecture

Pennate muscles have fibres which are positioned at an angle to the force generating axis, the pennation angle, (Θ). Whilst in parallel muscles the fibres attach directly to the tendon, pennate muscle fibres attach obliquely to a sheath of connective tissue called an aponuerosis, an extension of the tendon. As the fibres insert into the aponeurosis obliquely, more fibres (and hence sarcomeres) can be packed into the muscle in parallel, increasing the force producing capabilities of the muscle than if the fibres were to simply run longitudinally to the axis of force generation. The greater the pennation angle (up to 45º), the greater the number of sarcomeres in parallel and so any change in the pennation angle will represent a change in the force producing capabilities of the pennate muscle. As the sarcomeres are not packed parallel to the axis of force generation, the anatomical cross section of the muscle is not a reliable indicator of the number of contractile elements and thus the force producing capability in a pennate muscle. For this reason, the physiological cross sectional area is used which is calculated as PCSA = (V/t)·sin θ (Narici 1999) where V is muscle volume, t the distance between the two tendon aponeuroses and θ is the angle of pennation (figure 2.4). As mentioned above, a second significant architectural parameter is the length of the muscle fibres (L*f*) which represents the number of sarcomeres arranged in series. Fibre length is a determinant of the speed of contraction of the muscle ie. shorter fibres have less sarcomeres and hence have a slower shortening velocity.

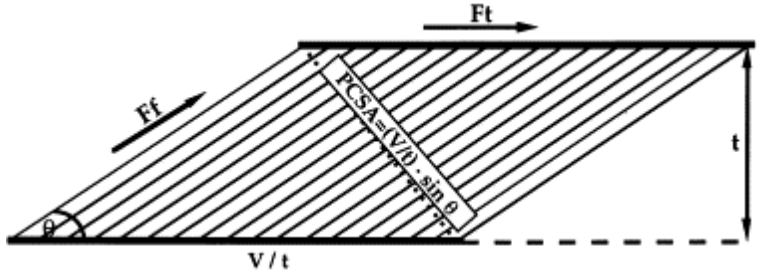

Figure 2.4 taken from Narici, 1999. Ft = tendon force. Ff = fascicle force. t = thickness. Θ = pennation angle. V = muscle volume.

### 2.1.3 Mechanical properties of skeletal muscle.

Explained above is the process by which individual sarcomeres contract and how their architectural arrangement can affect the force and speed of contraction of a skeletal muscle. When tendons of skeletal muscles are attached to bones, contraction of these muscles controls the movement of bones and body parts around the joint. Contracting muscles arranged in opposing groups around joints can either maintain the posture of the body or cause the bone to move and alter position, a basic principle of human movement. During a contraction, muscles work in synergy or antagonistically to the muscle or group of muscles responsible for the net movement around the joint. Whilst the contracting muscles produce linear forces, it is the resulting net rotational torque around the joint's centre of rotation which causes the action of the muscle. Generally, there are three skeletal muscle actions around a joint:

1) Eccentric contraction, when a muscle produces force during lengthening to overcome resistance resulting in motion,

2) Concentric contraction, when a muscle shortens during contraction to produce a torque larger than the resistive force to result in motion and

3) Isometric contraction, when the torque produced by a muscle or group of muscles is equal to the opposing resistive force and no movement is produced.

The net force generated depends on several factors and characteristics of the muscle itself notably the length and shortening velocity of contraction. As previously stated,

the length of a muscle fibre can affect the force produced during contraction due to varying number of sarcomeres in series, but the length of the sarcomeres themselves also have a significant effect on the muscle fibre's force producing capability. When a muscle is held at varying lengths, the sarcomere's overall lengths change according to the extent of overlap of the actin and myosin filaments (fig. 2.5). Maximum force will be generated when the number of actin-myosin cross bridges is at the maximum. If the sarcomeres are shortened or lengthened to reduce the number of active cross bridges the force produced decreases.

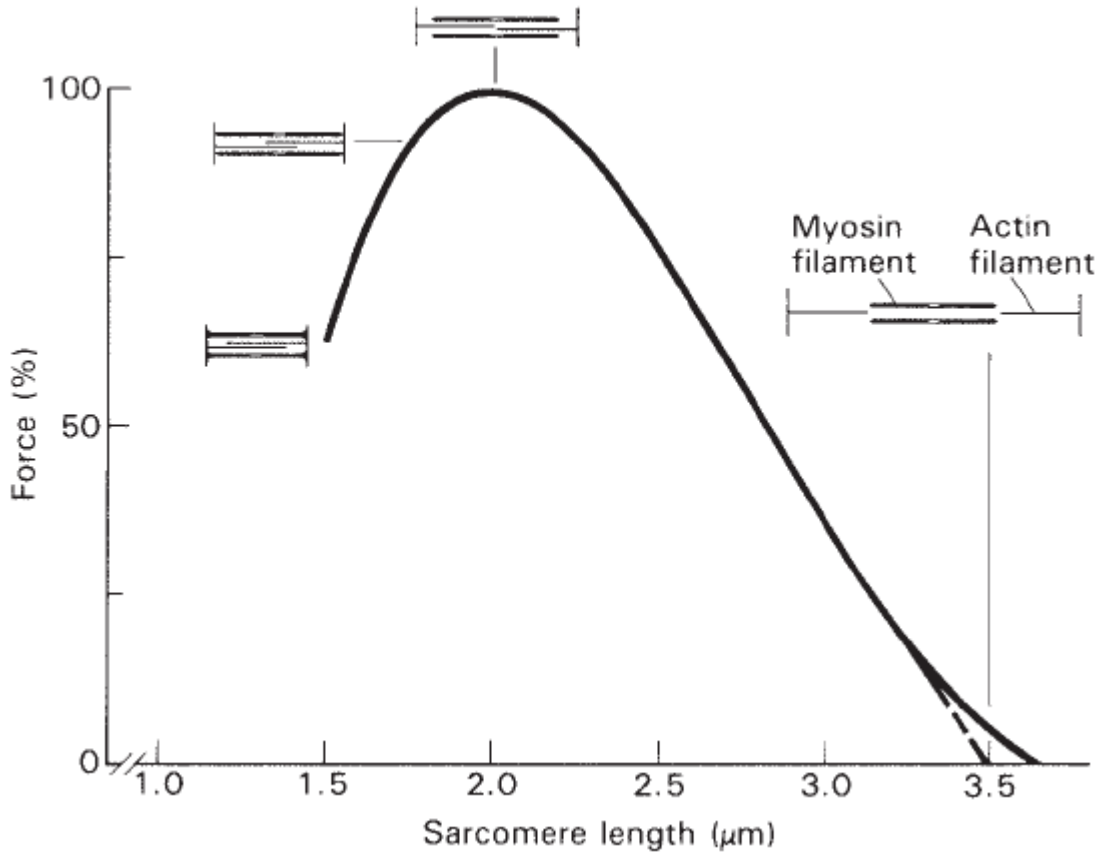


Fig. 2.5 length tension curve showing corresponding stages of filament overlap in contracting sarcomeres, taken from Edman & Reggiani 1987.

The resultant force however is not wholly dependent on the sarcomere architectural characteristics, but also the speed of velocity with which contraction occurs. Hill (1938)

studied the relationship between the force produced and velocity of contraction of frog sartorius muscles and showed that the relationship produced the characteristic hyperbolic curve known today as the force-velocity curve (fig 2.6). As can be seen from the figure, Hill demonstrated that the velocity with which a muscle shortens changes as the load changes. Maximum velocity (Vmax) occurs when the load is zero, inversely, the maximum force (P0) occurs when the muscle is stationary i.e. the velocity of shortening is zero.

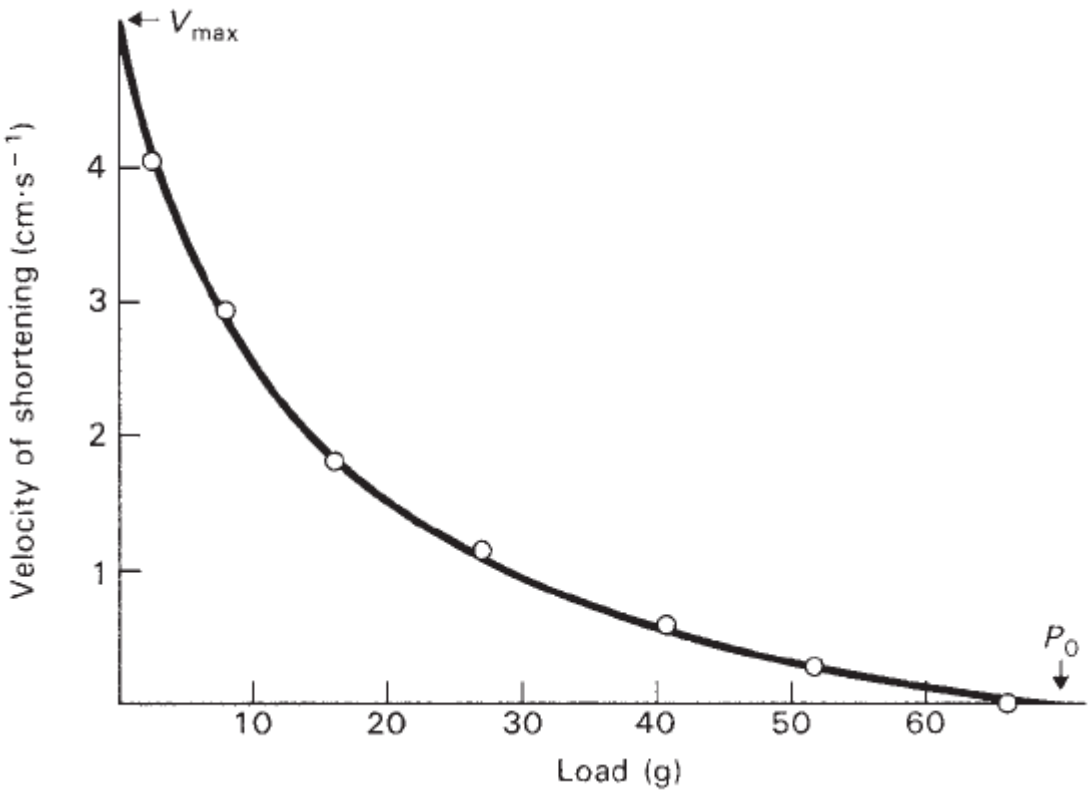


Figure 2 6. Hill's force velocity curve of whole frog sartorius muscle. Vmax = maximum velocity, P0 = Maximum force.

## 2.2 The effects of chronic unloading on skeletal muscle structure and function.

Declines in skeletal muscle function have been shown to be an outcome of chronic unloading conditions including spaceflight and earth-based models of simulated microgravity such as bed rest and Unilateral Lower Limb Suspension (ULLS) (Pavy-Le Traon et al. 2007). The main structural and functional adaptations that are reported are reductions in muscle mass and force and increased fatigability (DI PRAMPERO & NARICI 2003). Significant reductions in muscle strength have been reported in human skeletal muscle in response to disuse-induced atrophy due to ULLS. Seynnes et al. (2008) reported a reduction in plantar flexor maximum voluntary contraction of 10% after 23 days of ULLS, similar to some strength loss seen in the quadriceps femoris muscle, 14% in young males after 23 days of ULLS (Maarten D de Boer et al. 2007). Many other studies have reported strength losses of approximately 20% in human skeletal muscle following periods of disuse (Berg et al. 1991; Schulze et al. 2002; Clark et al. 2006). As muscle architecture is a primary determinant of skeletal muscle function (Cutts 1988), it is evident that changes in fascicle length ($L_f$) and pennation angle ($\theta$) constitute powerful indicators of alterations in muscle functional deficit in disuse (Koryak 2008). The decrease in fibre length and pennation angle resulting from disuse represents a reduction in the number of sarcomeres in series (fibre length) and parallel (pennation angle). The angle of pennation of skeletal muscle fibres significantly affects the transfer of forces from the contracting muscle to their tendons (Kawakami et al. 1993), the greater the pennation angle, the greater the force producing potential of the muscle and so the reduction in pennation angle seen in disuse, 7.6% after 23 days ULLS (de Boer et al. 2007) and as much as 10% after 90 days of bed rest (Reeves et al. 2002) will significantly contribute to the observed loss of muscle strength. Similarly, reduction

in fibre length, 7.7% after 23 days ULLS ( de Boer et al. 2007), 13% after 90 days bed rest (Reeves et al. 2002) has an effect on the force producing capabilities of skeletal muscle. A decrease in muscle fascicle length represents a loss of sarcomeres in series which, assuming tendon mechanical properties remain the same will result in an increased sarcomere excursion. The removal of sarcomeres causes the sarcomeres to operate further away from the optimum length which will result a decrease in sarcomere shortening velocity. A loss of sarcomeres in parallel, represented by a reduction in pennation angle and muscle CSA, and hence physiological cross section (PCSA), will impact upon the force producing capabilities of the muscle, with less in-parallel sarcomeres producing less force.

Reported decreases in the anatomical cross sectional area (ACSA) of the quadriceps femoris muscle average between 7% to 10% following 23 to 30 days of ULLS in young men (Berg et al. 1991; Maarten D de Boer et al. 2007). The reduction in muscle ACSA results from a reduction in muscle fibre cross sectional area and this morphological change inevitably affects skeletal muscle function and locomotor ability (Hudson & Franklin 2002). The disuse-induced reduction in muscle mass is accompanied by a loss in strength which far exceeds the loss in muscle mass, suggesting that many other factors including neurological factors and the loss of contractile proteins contribute to the observed strength decrease associated with disuse-induced skeletal muscle atrophy (Clark et al. 2006).

Neural drive and muscle activation capacity appear to be affected in a degenerative manner by periods of prolonged disuse. In situations of chronic disuse the muscle's ability to fully activate during a maximal voluntary contraction is compromised. Bed rest studies have shown a decrease in the root mean square (RMS) of EMG of 6.5% after 20 days and 19% after 42 days (Kawakami et al. 2001). Similar results have

been observed in hind limb suspended rats (Edgerton & Roy 1994). Clark *et al.* (2006) investigated neuromuscular parameters after 4 weeks of ULLS and their findings showed a decrease of 30% in compound muscle action potential (CMAP), 10% decrease in evoked doublet force, 12% increase in twitch to doublet force ratio and an increase of 11% in post activation potential doublet ratio. CMAP is affected by muscle fibre conduction velocity which is directly proportional to fibre circumference and muscle membrane excitability level. Fibre atrophy is therefore likely to be a contributing factor to the reduction in neural drive observed during disuse (Clark *et al.* 2006) as well as a reduction in motor unit recruitment (Antonutto et al. 1999). Reductions in the activation capacity of muscle can result in a decrease in strength independent from decreases in muscle size or changes in architecture that may help explain the larger decrease in muscle strength relative to muscle atrophy in disuse.

Tendons play a vital role in the transmission of forces from contracting muscles to bones and therefore it is important to consider muscles and tendons as an in-series contractile element. By virtue of their mechanical properties, tendons affect maximum isometric and dynamic mechanical output. It follows that, at a given operating length, muscles which are attached to more compliant tendons will produce less force than when attached to stiffer tendons (Narici & Maganaris 2007). As well as the transmission of forces tendons have important elastic properties which can influence the interaction between muscle and tendon and allow energy storage during locomotion (Magnusson et al. 2008).

Tendons also display significant changes in their mechanical properties following periods of prolonged disuse. Various studies have demonstrated a significant decrease in tendon stiffness following disuse in both hind-limb suspended rats

Achilles tendons (Almeida-Silveira et al. 2000; Heinemeier et al. 2009) and human patellar tendons (Reeves et al. 2005; Maarten D de Boer et al. 2007; Kinugasa et al. 2010). After 23 days disuse (ULLS) in young men the stiffness and Young's modulus of the patellar tendon were reduced by 29.3% and 30.1% respectively (Maarten D de Boer et al. 2007).

Although in disuse both tendon stiffness and YM of the patellar tendon are significantly reduced, no change in tendon dimensions has been reported in response to unloading in either rat Achilles tendon or human patella tendon (Almeida-Silveira et al. 2000; Reeves et al. 2005; Kinugasa et al. 2010). This would suggest that changes in the intrinsic material properties of the tendon are responsible for the change in mechanical properties as opposed to changes in tendon dimensions. The change in mechanical properties is likely due to alterations in the quality or arrangement of collagen fibres. Such alterations may be the result of changes in the composition of the of the tendon matrix or the cross-linking of collagen fibres (Savolainen et al. 1988; Heinemeier et al. 2009). Savolainen *et al.* (1988) showed a decrease in cross-linking enzymes in rat Achilles tendon, although this is not a direct measurement of collagen fibre cross-linking. This could have an impact on tendon mechanical properties as cross-links are important in allowing tendons to withstand the high tensile forces which weight-bearing tissues are subjected to. Collagen fibre size and density in rat Achilles tendon have also been shown to decreased after periods of hind-limb unloading (Nakagawa et al. 1989). Another factor contributing to the change in tendon material properties in disuse recently reported is a decrease in the tendon collagen synthesis rate. The collagen synthesis rate in human patellar tendon has been shown to decrease by half after 10 days of disuse and to further halve after 21 days (Maarten D de Boer et al. 2007).

The decrease in tendon stiffness observed after periods of disuse is accompanied by an increase in tendon extensibility at a given force and so can affect the rate of torque development (RTD). This has been shown to be the case after 23 days of ULLS when RTD was decreased by 38% (Maarten D de Boer et al. 2007). Functional implications of decreased tendon stiffness include decreases in the rate of contractile force transmission which could have implications for fall prevention, increased likelihood of tendon strain injury and changes in the force-length relationship of the muscle.

The loss of muscle observed during periods of disuse is a result of an imbalance between muscle protein synthesis and breakdown. A net positive balance of protein synthesis will result in muscle hypertrophy whilst the opposite leads to muscle atrophy. Due to recent advances in methods of measuring MPS rates in humans, the mechanism of skeletal muscle atrophy has become clearer. It appears that the decrease in muscle protein synthesis makes a more significant contribution to decreases in muscle mass rather than an increase in protein breakdown. Many studies have shown little or no change to the rate of protein breakdown in immobilized muscle while muscle protein synthesis has been greatly reduced (Paddon-Jones et al. 2006). de Boer et al. (2007) showed myofibrillar protein synthesis in the vastus lateralis muscle to fall by 50% after 10 days of ULLS.

Previous research has shown that in response to atrophic stimulus the phosphoinositide-3 kinase (PI3K)/Akt kinase/mammalian target of rapamycin (mTOR) signal transduction pathway plays a vital role in the regulation of skeletal muscle cell size (Urso 2009). Ubiquitin ligases, FOXO transcription factors and various other genes are expressed in response to the disuse of skeletal muscle and have been shown to affect the PI3K/Akt/mTOR pathway (Urso et al. 2007;

Stevenson et al. 2003). Additionally, in response to chronic unloading the ubiquitin-proteosome system is responsible for the breakdown of myofibrilar proteins in skeletal muscle (Bodine et al. 2001; Lecker et al. 1999). The increasing investigation of the various signalling pathways associated with skeletal muscle atrophy in response to disuse shows that the conversion of decreased mechanical stimulus into molecular stimulus is a complicated process effected by many factors. As well as reintroducing mechanical stimuli through exercise, recent studies have shown that it is possible to affect these signalling pathways with nutritional factors. As exercise and nutrition are easily manipulated, there is much investigation into the application of nutrition and exercise training programs to counteract skeletal muscle disuse-induced atrophy.

### 2.3 The effect of ageing on skeletal muscle structure and function.

Sarcopenia is an ageing-related loss of muscle mass that has particularly important functional consequences and has come to be regarded as a major cause of frailty and reduced locomotor ability amongst the ever increasing elderly population (Narici & Maganaris 2006). Beyond the sixth decade, most individuals undergo changes in the nervous system, in hormonal status, in immune function and in dietary intake (Doherty 2003), all of which may result in atrophy and weakness of the musculoskeletal system (Roubenoff & Hughes 2000). As reductions in muscle mass and strength are observed even in master athletes who have trained throughout life, the process of sarcopenia is therefore universal with ageing (Roubenoff 2000). What is interesting is that in spite of its universal effect, there are currently no universally accepted criteria for the clinical diagnosis of sarcopenia. Generally, the term

sarcopenia is used to describe the progressive loss of muscle mass and function that occurs with ageing. But at what point is an individual said to be exhibiting sarcopenia? Many different approaches have been suggested as diagnostic criteria focusing on either the loss of muscle mass, function or both. However, such varying approaches lead to huge discrepancies in reported prevalence's of sarcopenia. In a recent review of methods used in the definition of sarcopenia, Bijlsma et al. (2013) assessed the variation between seven currently used diagnostic criteria based on muscle mass (measured by bioelectrical impedance analysis or DEXA) and handgrip strength. Their study highlighted the vast discrepancies that arise when applying different criteria for characterising sarcopenia to an identical population differing as much from 0% to up to 45% prevalence in elderly populations. In fact, only one participant out of 674 (0.2%) was classified as having sarcopenia according to all diagnostic criteria. What is ultimately needed clinically is a universally agreed set of criteria to objectively identify the onset of sarcopenia to optimise treatment and management of the condition. However, the data from Bijlsma et al.'s study suggests that cut off points, if they are to be used objectively need to be gender and population specific and derived from a large reference population. This relies on the quantitative measurement of lean skeletal muscle mass for comparison against reference populations in order to establish a cut off point for the loss of mass to be attributed to sarcopenia. Today many techniques are available for the assessment of lean muscle mass, all varying in availability and effectiveness. Bioelectrical impedance analysis (BIA) which determines lean body mass by passing an electrical current through the body, is a relatively simple and inexpensive way to measure lean muscle mass. The ratio of lean body mass to height squared has been used to define sarcopenia with some success using a cut-off point of a SM/height$^2$ ratio two

standard deviations below that of the mean of a population of young healthy adults (Baumgartner et al. 1998). However due to the nature of BIA this method has its limitations as results can be affected by fluid shift, temperature and oedema or inflammation, conditions which are prevalent in elderly populations making it a less than ideal method of measurement. In addition, BIA studies do not provide information on the distribution of lean mass throughout the body. In contrast, dual energy X-ray absorptiometry provides a method of quantifying lean mass whilst also determining where the lean muscle mass is located. This allows us to produce a ratio of appendicular skeletal muscle mass to height squared (skeletal muscle index, SMI) which is more relevant in terms of sarcopenia. Again, a cut-off point of two standard deviations from the average SMI of young adults is used to denote the onset of sarcopenia (Baumgartner et al., 1998). The SMI can also be calculated by measuring skeletal muscle volume using MRI and CT scanning. Although expensive and cumbersome, MRI scanning is considered the gold standard for measuring muscle volume. An additional advantage of these imaging techniques is that they allow us to take into account the infiltration of fatty tissue into lean mass which occurs in older muscle. Fat infiltration can introduce errors into lean muscle mass measurements from methods such as DEXA and although there are published methods of correcting DEXA lean muscle measurements, currently they remain population specific and are therefore of limited use in global application (Kim et al. 2002). In a clinical setting a compromise must be reached between affordability and availability and accuracy and reliability.

Although the process of sarcopenia is much more complex than that of disuse-induced atrophy, sarcopenic muscle displays many of the same characteristics. Recent work at the Institute for Biomedical Research into Human Movement and

Health (IRM) has shown that as well as the decrease in gross muscle size that accompanies old age (anatomical cross-sectional area ACSA and volume based on MRI scanning, (Morse et al. 2004), muscle architecture is also significantly altered in sarcopenia. Findings based on ultrasound scanning of older and younger individuals with similar daily energy expenditures have shown fibre fascicle length ($L_f$) and pennation angle (θ) of the gastrocnemius medialis (GM) of older men (age 70+ yr) measured *in vivo* to be 10% and 13% smaller than those of younger men (20-30 yr) respectively (Narici et al. 2003). The relatively smaller $L_f$ and θ seen in the skeletal muscle older individuals suggest that normal biological aging is associated with a loss of sarcomeres both in series and in parallel which may account for some of the loss of muscle force and power observed in older age. However, in aging, as in disuse, the loss of muscle force and power is greater than can be accounted for by the observed loss of muscle size and volume, even after considering age associated alterations in muscle architecture (Narici & Maganaris 2007). Factors such as changes in single-fibre specific tension and tendon mechanical properties also contribute to the decrease in muscle force and power that accompanies old age.

As with younger their younger counterparts, elderly populations display a marked reduction in MVC due to disuse resulting from immobilisation. In a population of 60-79yrs suffering unilateral hip osteoarthritis, it was shown that the MVC of the affected limb was 20% less than that of the unaffected side (Suetta et al. 2007). However, what makes this loss of function in elderly disused individuals more of a concern is the combination of disuse induced atrophy with the normal observed loss of with increasing age. This has been shown to be a reduction in strength of as much as 40% in the elderly (~70 yrs) compared to younger individuals (~20 yrs) (Roos et al. 1999; Lambertz et al. 2001; Macaluso et al. 2002). Compromised skeletal muscle

function resulting from disuse is an important environmental factor that worsens the effects of sarcopenia in elderly individuals who are likely to be already exhibiting decreased muscle strength and function. Elderly populations are also more likely to be exposed to the effect of disuse due to high levels of comorbidity and hospitalisation (Manton et al. 1993) and a slow recovery can have a dramatic functional effect on their quality of life. For reasons stated above, any method of quantifying sarcopenia should include a measure of skeletal muscle function as well as mass. Common measurements of muscle strength used in defining sarcopenia are those which reflect muscle functions performed in everyday life such as quadriceps MVC and handgrip strength. As well as their importance functionally, these groups of muscles are relatively easy to access and measure and so are extremely useful in a clinical setting.

The aetiology of sarcopenia seemingly consists of a complex interaction between many genetic and environmental factors (Roubenoff and Hughes 2000). A major contributor to the loss of muscle function in the elderly is the decrease in the number of alpha motor neurons present which can be in the region of 50% in individuals aged 70+ yrs (Doherty 2003). Whilst the remaining motor neurons try to compensate for the loss by recruiting denerved fibres, the resulting increase in motor unit size and decrease in numbers results in significantly reduced coordination of muscle function and an overall reduction in force production that is characteristic of elderly muscle (Doherty et al, 2003). While neuropathic processes leading to motor neuron death are important causes of the muscle atrophy associated with ageing (Roubenoff and Hughes 2000), changing hormonal and immunological statuses also play significant roles (Narici and Maganaris 2006). In particular, there appears to be an insulin resistance in elderly individuals which could be attributed to alterations in the

associated signalling pathways in aging cells (Guillet 2004) which may have an effect on muscle protein synthesis and hence muscle mass. Other hormones such as testosterone and growth hormone have been suggested to have a role in the decline in muscle mass in ageing individuals, namely due to their decreasing levels with advancing age. Studies in which growth hormone, insulin like growth factor-1 (IGF-1) or testosterone have been administered to elderly individuals have resulted in increased muscle mass which can be attributed to an increase in number and proliferation of satellite cells important in growth and repair of muscle fibres (Chen 2005). Of course these increases in muscle mass are a result of increases in net muscle protein synthesis which can only occur if there is adequate substrate present in the form of amino acids. This in itself can be a contributing factor to sarcopenia since elderly individuals exhibit decreased protein intake.

Unlike the neuronal changes, which are irreversible, environmental changes such as altered nutritional intake can be manipulated relatively easily and may present a means of minimising the age-related changes. For this reason, many studies focus on the availability of proteins, in particular essential amino acids as a method of potentially targeting the process of sarcopenia.

## 2.4 Countermeasures

As previously mentioned most studies into the mechanism of skeletal muscle atrophy resulting from periods of immobilization or disuse in humans have concluded that muscle wasting is determined by a net catabolism of muscle proteins. This imbalance in protein turnover is thought to come about due to a decline in muscle protein

synthesis rather than an increase in muscle proteolysis. As a result, investigations into countermeasures of disuse atrophy have been focused on methods of increasing muscle protein synthesis.

### **2.4.1** Resistance training

Changes seen in ageing and disuse can be attenuated and even reversed by increasing muscle chronic loading. Resistance training is a widely accepted and practised method of increasing muscle mass, strength and power. Many studies have demonstrated the increase in skeletal muscle mass resulting from resistance training programs. Seynnes et al. (2007) reported an increase in whole muscle quadriceps CSA measured by MRI scanning of 6.5% distally and 7.4% proximally after only 20 days of a 5-wk training period in young men and women. These reported increases in both whole muscle volume and muscle cross sectional area are largely understood to be due to an increase in single fibre hypertrophy i.e. an increase in muscle fibre CSA. In particular, type 2 muscle fibres (fast twitch) tend to increase to a greater extent than type 1 (slow) fibres (Deschenes et al. 2002). The hypertrophy observed in muscle fibres due to resistance training is essentially an increase in contractile material and is a result of increased muscle protein synthesis in response to the mechanical stimuli of increased muscle loading.

In addition to the changes observed at whole muscle level muscle architectural parameters such as VL fascicle length and pennation angle have been shown to increase by 2.4% and 9.9% respectively after 35 days training in young recreationally active volunteers (Seynnes et al. 2007). Increases in VL and $\theta$ demonstrate the addition of sarcomeres in both parallel and series to fibres which are subjected to loading stimuli through resistance training. These changes in the

morphological characteristics of muscles at both the architectural level and whole muscle volume and changes in patellar tendon moment arm will ultimately affect the force producing capabilities of a muscle by altering the physiological cross sectional area of a muscle (PSCA) and specific force. A nine week resistance training programme targeting the quadriceps femoris muscles resulted in an increase in PCSA of 5.5% and specific force of 20.1% in young untrained males between the ages of 18-39 (Erskine et al. 2010).

While the increases in muscle architectural parameters resulting from resistance training are considerable, the increases observed in muscle strength are generally much larger, 28.9% after 35 days (Seynnes et al. 2007) and 30% after 9 weeks resistance training (Erskine *et al.* 2010). This relative greater increase in muscle strength is largely attributable to neural adaptations, namely motor unit recruitment and firing, parameters which are known to have an important impact on muscle functional characteristics such as the rate of torque development and indeed maximum resultant force (Aagaard et al. 2002). These neural factors are thought to account for early gains in strength observed in resistance training programs which occur prior to observed changes in muscle hypertrophy.

The exact cellular and molecular mechanisms of skeletal hypertrophy in response to resistance training are still largely uncovered. What is known however is the importance of the role of satellite cells in the plasticity of skeletal muscle. Satellite cells are essentially muscle stem cells located on the surface of muscle fibres which upon activation differentiate into myoblasts and ultimately offer up nuclei to their neighbouring muscle cells via cell fusion. Thus, as the muscle fibre increases in size, due to satellite cell fusion so does the number of nuclei which helps to keep the myonuclear domain relatively constant in hypertrophying cells. Bellamy

et al. (2014) showed that in response to resistance training the available pool of satellite cells in skeletal muscle increase and reported a relationship between the acute temporal response of satellite cells to resistance training and the accretion of lean muscle mass. These data serve to highlight the important role of satellite cells in hypertrophy due to resistance training.

Another important aspect of hypertrophy to consider is the role of molecular regulators and signalling pathways. Recent studies have shown in the past thirty years that the protein kinase B/mammalian target of rapamycin, AKT/mTOR signalling pathway plays a major role in the regulation of skeletal muscle growth. Both AKT and mTOR are phosphorylated during muscle hypertrophy in response to load-induced training (Baar & Esser 1999; S. C. Bodine et al. 2001; Chen & Fang 2002; Urso 2009). The phosphorylation and activation of the AKT/mTOR pathway leads to the activation of $P70^{S6K,}$ which has been shown to be correlated with percentage increase in muscle mass after 6 weeks resistance training indicating that $P70^{S6K}$ phosphorylation is involved in the adaptation of skeletal muscle to chronic resistance training (Baar and Esser 1999). Interestingly, the AKT/mTOR pathway has also been shown to be down regulated during muscle atrophy due to both disuse and denervation (Pallafacchina et al. 2002), which suggests that activating the AKT/mTOR pathway during muscle disuse could provide a means of attenuating skeletal muscle disuse-atrophy.

Human tendon mechanical properties can also be modified by following resistance training protocols. In rabbits that underwent 40 weeks of exercise training, the load and energy absorbed at failure was much higher than in rabbits which had not underwent any exercise training (Viidik 1967). In vivo studies have shown that collagen synthesis in human Achilles' and patellar tendon to increase acutely after a

bout of endurance exercise and to remain at an elevated rate up to 72 hrs after exercise (Langberg et al. 2000, D. Miller et al. 2005). Human tendons also show in vivo biochemical changes resulting from exercise training. The fractional synthetic rate of tendon collagen synthesis has been shown to increase to a peak increase at 24 hrs post exercise of 1.7 times greater than that of resting tendon. After this time the collagen synthesis rate decreased but was still significantly elevated 72 hrs post–exercise. Tendons of both young and older individuals have shown increases in stiffness and Young's modulus in response to strength training indicating that resistance training as a countermeasure to muscle disuse atrophy and aging is important with regard to tendon as well as skeletal muscle.

### 2.4.2 Nutritional Intervention

Skeletal muscle is in a constant state of flux between anabolism and catabolism, and imbalances in this protein muscle turnover results in either the atrophy or hypertrophy of skeletal muscle mass (Nicastro et al. 2010) . Several studies have highlighted the stimulatory effect of anabolic amino acids on muscle protein synthesis. Reducing amino acid blood concentration to 50% of basal level in pigs by haemodialysis resulted in a corresponding reduction in muscle protein synthesis, with MPS rate returning to basal level when amino acids were replaced (Wolfe 2002). As the level of available amino acids was altered, so also was the rate of inward transport of amino acids resulting in no change in intracellular levels of amino acids suggesting that extracellular levels of amino acids may serve as signals to activate MPS. In addition, it has been suggested that atrophied muscle displays a reduced sensitivity to nutrients, especially protein, resulting in lower protein accretion than basal rates (Rennie et al. 2004). This relationship between availability

of amino acids and the rate of muscle protein synthesis serves as the basis for much research into nutritional countermeasures to disuse induced muscle atrophy.

The amino acid leucine has been the focus of much research into the attenuation skeletal muscle loss as it has been shown to have both anti-catabolic effects (Buse & Reid 1975; Zanchi et al. 2008) and be effective in promoting muscle protein synthesis (Paddon-Jones et al. 2004; Trappe et al. 2007; Glover et al. 2008). It is not completely understood how leucine contributes to skeletal muscle remodelling. It is believed to be a regulator of a number of cytoplasmic proteins known to be involved in initiating skeletal muscle protein synthesis and the insulin signalling pathway (Nicastro et al. 2010). Some investigators have suggested that membrane receptors and transporters sensitive to leucine may regulate the proteins involved in intracellular signalling pathways such as mTOR and, Akt (Glynn et al. 2010; Bohe et al. 2003; Hundal & Taylor 2009). This is an important statement as if this indeed does prove to be the case then leucine supplementation may serve as stimulator of the AKT/mTOR pathway during muscle disuse to counteract the down regulation seen in periods of muscle inactivity.

The literature that exists on human studies on leucine and skeletal muscle remodelling is so far inconsistent. Leucine rich essential amino acid supplemented feeding resulted in the amelioration of muscle disuse-induced wasting after 28 days bed rest in a study by Paddon-Jones *et al.* (2004) however this preservation of muscle mass was not enough to preserve muscle strength suggesting that some mechanical stimulus is required to preserve muscle strength in addition to the preservation of muscle mass. One study investigating the effect of leucine enriched meals on soleus muscle during bed rest found no structural changes between supplemented and controlled groups (Trappe et al. 2008), whilst another found that a

leucine supplemented group actually displayed a greater loss of muscle mass (~4%) of the thigh and calf muscles than a control group (Trappe et al. 2007).

Decreased muscle protein synthesis resulting from disuse is particularly problematic in elderly individuals who may already be exhibiting decreased protein synthesis due to insufficient protein intake so may display a greater loss of muscle bulk due to a net catabolism than their younger counterparts (Cuthbertson *et al.* 2005). Malnutrition is common among the elderly population due to factors such as a progressive loss of appetite and dietary shift towards sweeter foods (Tawa & Goldberg 1994). Studies have shown that ingestion of high protein meals can increase the postprandial muscle protein synthesis in old as well as young individuals (Symons et al. 2007; Paddon-Jones et al. 2008; Paddon-Jones & Rasmussen 2009; Rieu et al. 2006). A separate study showed that a single dose of essential amino acid mixture did not result in increased muscle protein synthesis in elderly but did in young individuals (Katsanos et al. 2005) however, this is likely due to the low dose of leucine in the amino acid mixture (1.72g). A later study by the same group showed that essential amino acid mixtures with different amounts of leucine resulted in different levels of postprandial muscle protein synthesis with higher doses of leucine resulting in higher protein synthesis (Katsanos et al. 2006). These results show that the availability of protein is a bigger problem for elderly individuals rather than the accretion of proteins and hence leucine supplementation is effective in increasing the rate of muscle protein synthesis in elderly individuals.

Whilst many studies have investigated the effects of mixtures of essential and non-essential amino acids on atrophied human skeletal muscle, there are few studies of humans which evaluate the isolated effects of a leucine supplement and even fewer in which the direct effects of leucine on muscle functional parameters have been

studied. It would be expected that while leucine supplementation may affect muscle mass, additional factors affecting muscle function e.g. neural drive would not be directly affected by amino acid supplementation and so the muscle function would not be directly affected by leucine per se.

Whilst skeletal muscle appears to be altered by protein intake, little is known about the response of tendon or tendon extracellular matrix to nutritional supplementation. The mechanical strength of a tendon depends primarily on the structure and intermolecular interactions of its collagen fibrils (Canty & Kadler 2002). However, the composition and structure of the ECM (e.g. macromolecules such as proteoglycans) also have a significant effect on tendon mechanical properties (Magnusson et al. 2003). The predominant proteoglycan in the tendon is Decorin, which belongs to a family of small leucine-rich proteoglycans (SLRP) so called due the presence of leucine-repeats in their structure. SLRP's have been shown to have a role in the regulation of collagen fibril formation and are thought to affect collagen fibril diameter by aligning collagens for successful cross-linking (Kalamajski and Oldberg 2010). The ECM including SLRP's can be altered by nutritional state (K Smith & Rennie 2007) indicating that an increase in leucine availability may increase the level of SLRP's in the tendon ECM which could have a positive effect on tendon mechanical properties.

Many investigators report that tendon collagen synthesis is not sensitive to feeding (Babraj et al. n.d.; Wackerhage & Rennie 2006; Ken Smith & Rennie 2007). However, a study by Barbosa et al. (2010) has shown that a leucine rich diet stimulates tendon collagen synthesis in malnourished rats, especially when in combination with physical activity and actually improved the biomechanical characteristics of the tendon (maximal load, displacement, stress and strain). This

recent evidence suggests that leucine intake may be an important factor in improving tendon mechanical properties or recovery and hence further research is necessary to explore this relationship.

## 3. Aims and Objectives

In this project healthy young males are used as test subjects to investigate the role of exercise and diet in disuse-induced skeletal muscle atrophy and subsequent recovery. A cross sectional study is also reported examining the effects of physical activity and periods of disuse on elderly sarcopenic individuals. Both muscle structural and functional parameters are reported upon. The aims of each study are outlined below.

1) To examine the structural and functional adaptations of the quadriceps femoris muscle and patellar tendon to a period of unloading and subsequent recovery through physical activity in young men.

2) To assess the role of physical activity and nutritional supplementation in improving the rate and extent to which muscle mass and musculoskeletal function is restored following a period of immobilisation.

3) To evaluate the response of elderly skeletal muscle to both increased physical activity and periods of immobilization and examine the role of physical activity in mitigating the effects of sarcopenia on elderly skeletal muscle.

The project has been broken down into 4 studies to achieve the *objectives* listed below:

**Study 1 Objective:** ***Skeletal muscle adaptations to physical inactivity and subsequent retraining in young men*****.** Skeletal muscle structure and function are markedly affected by chronic disuse. With unloading, muscle mass is lost at rate of about 0.4%/day but little is known about the recovery of muscle mass and strength

following disuse. Here we report an extensive data set describing in detail skeletal muscle adaptations in structure and function in response to both disuse (ULLS) and retraining.

**Study 2 Objective:** ***The effect of amino acid Leucine supplementation on disuse-induced atrophy in young subjects***. This study consists of an assessment of muscle disuse-atrophy in young, healthy volunteers undergoing unilateral lower limb suspension (ULLS) (Berg *et al.* 1991) with a test group of participants undergoing the same unloading procedure but receiving nutritional supplementation with the essential amino acid leucine to assess the effect of supplementation. A placebo group will take nutritional supplements containing the non-essential amino acid alanine.

**Study 3 Objective:** ***The effect of amino acid Leucine supplementation on the rate and extent of recovery following ULLS in young subjects.*** This study consists of an assessment of the time-course of the post-ULLS recovery of disuse-atrophy resulting from 3 weeks ULLS by a programme in which one group receives leucine supplementation plus resistance training while the other group receives only resistance training (and placebo alanine supplements).

**Study 4 Objective: I*nvestigating the role of life-long training in the prevention of sarcopenia and the development of an image based biomarker of sarcopenia.*** This study consists of an assessment of the quality and function in aged-matched elderly populations, a group of master athletes, sedentary control group and frailty due to hip-fracture surgery. Ultrasonic analysis of the changes in skeletal muscle fibre

architecture are used to propose an image based biomarker for the indication of the onset of sarcopenia.

## 4. Methods

The study was approved by Manchester Metropolitan University Faculty of Science and Engineering Research Ethics Committee (Ref. FAETC/08-09/13) and participants were excluded from the ULLS study if they suffered from or have ever suffered from previous stroke, uncontrolled cardiovascular disease, motor neurone disease, Parkinson's disease, medically diagnosed osteoporosis, type II diabetes, hypertension or myocardial infarction within the previous two years, acute febrile or systemic disease within the past two years or are currently taking beta blockers. ULLS participants were advised on early signs of deep vein thrombosis in the early sub-clinical phase and were monitored weekly using Doppler ultrasonography check for any signs of the possible occurrence of DVT.

*4.1 Amino Acid Supplementation.* Participants undergoing ULLS were randomly assigned to either a leucine intervention groups or an alanine placebo group. The recommended mean leucine requirement for adults is set at 39mg per kilogram of body weight (WHO/FAO/UNU, 2007). Based on previous literature surrounding leucine supplementation for exercise performance (Crowe et al. 2006; Mero et al. 1997), leucine dosage for the current study was set at was set at 50mg/kg body mass. To standardise the supplements the greatest body mass of the participants was used to calculate a dosage, which would ensure that all participants received a supramaximal dose of leucine. Each supplement contained either 6g of leucine of 6g or alanine and 0.5g of orange flavouring (Citric acid, dried glucose syrup, colour (E162, E160(a)) and sodium saccharin. For reference, figure 4.1 shows our supplement compared with the leucine content of popular high protein foods.

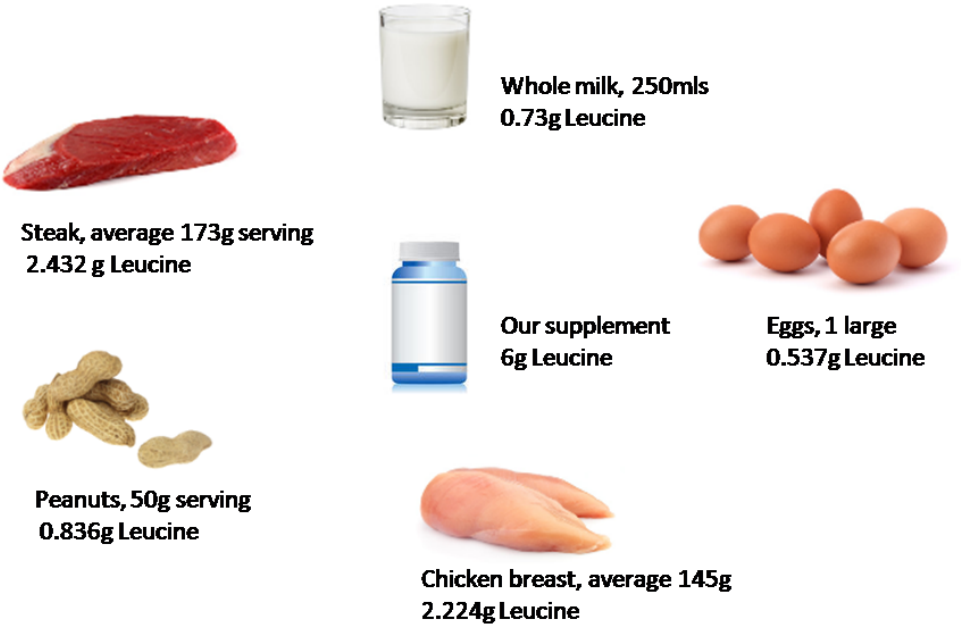


Figure 4.1 Leucine content of our nutritional supplement compared to popular high protein foods. Information from www.nutritionalvalue.org.

The non-essential amino acid alanine was chosen as a placebo supplement which contained the same flavouring as the active leucine supplements. Both supplements were visually indistinguishable and packaged in identical containers before distribution to participants. In contrast to leucine, non-essential amino acids like alanine do not have the capability to stimulate muscle protein synthesis after ingestion in humans (Tipton et al. 1999), hence its was selected as an isonitrogenous supplement that would not affect muscle protein synthesis in the control group.

Participants were instructed to take the supplement everyday throughout the ULLS period in the morning as soon as they awoke. Water was added to bottles containing pre-prepared mixtures of amino acid and flavouring to form a solution. Participants were instructed to ensure that all of the amino acid mixture was ingested i.e. no residue remained in the bottles.

*4.2 Unilateral Lower Limb Suspension procedure.* Participants underwent unilateral lower limb suspension (ULLS) (Berg et al.,1991) for three weeks to induce atrophy of the right limb. The left limb was fitted with a raised sole (8cm) and the right limb was kept in a slightly flexed position with the use of a sling which suspended the limb above the ground whilst the participants walked with crutches. The sling maintained the ankle joint at 0˚of flexion, with the foot at right angle to the tibia. Participants used the shoes, crutches and a sling for the duration of the three-week suspension period and were instructed to refrain from loading the right limb in anyway including driving vehicles.

*4.3 Ultrasonography.* Ultrasound scanning was used to assess muscle thickness, architecture at rest and during maximum voluntary contraction (MVC) and to measure patellar tendon elongation. With the subject lying in the supine position, images of muscle architecture (pennation angle θ and fascicle length L$f$) at rest of all four heads of the quadriceps femoris were recorded. Scans were taken at a level of approximately 40% of proximal femur length (rectus femoris scans were taken more proximally), (fig 4.2). The final position and orientation of the probe at the site were scans taken was recorded on an acetate sheet using moles and angiomas as markers to ensure all subsequent scans were taken at the same anatomical location for comparison. Scans taken during MVC were also recorded at the same anatomical location.

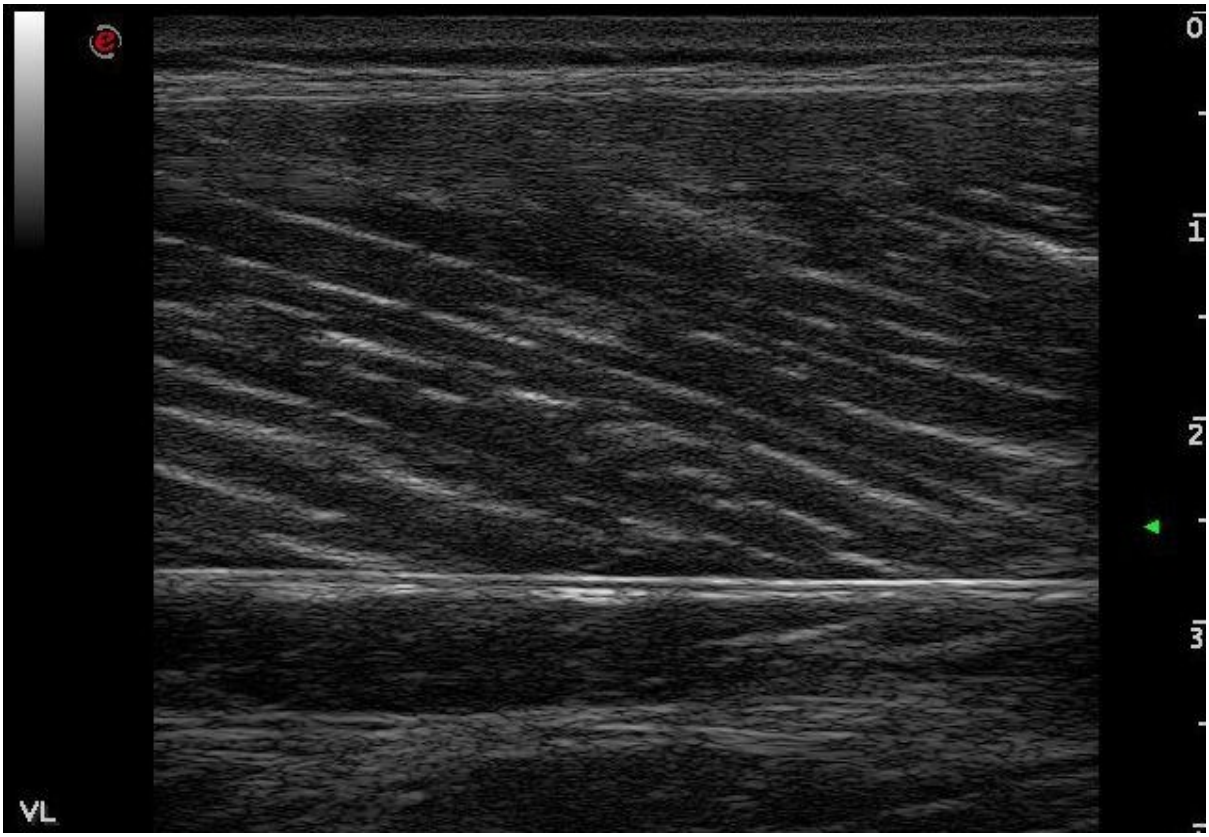


**Figure 4.2**. Ultrasound image of the vastus lateralis taken at rest at 40% proximal femur length.

The same anatomical positions were used for scanning the muscles during MVC and video clips were recorded as participants performed slow ramp contractions. Participants were instructed to start contracting after a verbal signal and to slowly reach maximum torque over a period of approximately four seconds

*4.4 Muscle volume.* Quadriceps muscle volume was measured using a 0.2 Tesla MRI scanner (E-Scan, Esaote Biomedica, Genova, Italy). Axial plane scans were acquired using a Turbo 3D T1 sequence with a slice thickness of 3.1mm. Oil filled capsules were secured to the skin along the length of the thigh as external markers at 7cm intervals with the distal border of the lateral femoral condyle (located using ultrasonography) used as a reference point (figure 4.3). Scans were performed with participants lying in the supine position with the knee fully extended.

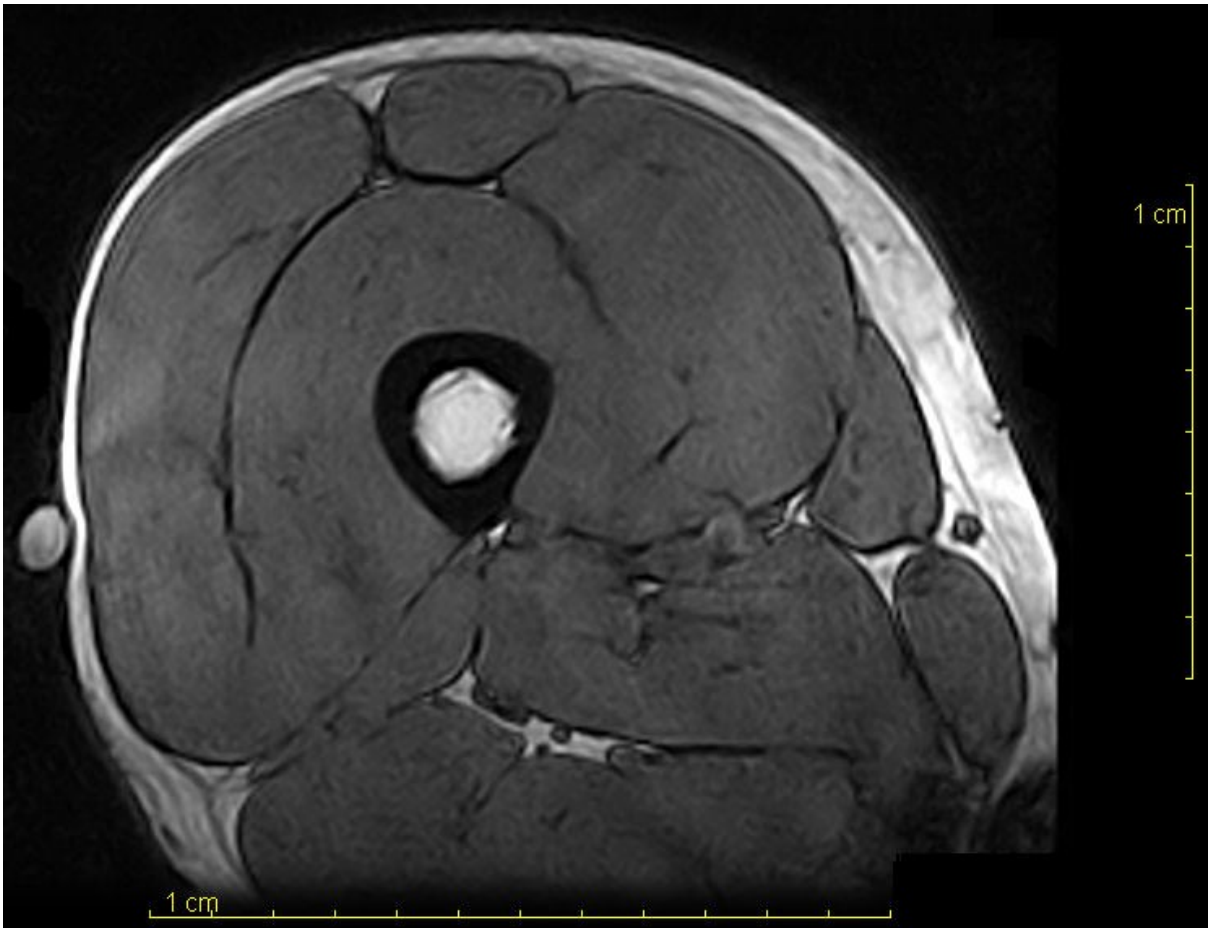


**Figure 4.3**. Axial MRI scan of the right quadriceps femoris muscle. Scans where taken pre-ULLS, post-ULLS and post RT. Oil filled capsules secured the skin along the lateral thigh were used as reference markers (pictured).

*4.5 Maximum Voluntary Contraction (MVC).* Maximum knee extension and knee flexion torque were measured using an isokinetic dynamometer at varying angles of knee flexion, 70˚, 80˚ and 90˚, with full knee extension corresponding to 0˚ flexion. Participants were seated in the chair and secured into position using straps to secure the hip and maintain the hip angle at 85˚ (full hip extension corresponding to 0˚). The position of the dynamometer and chair was adjusted to align the centre of knee joint rotation of the right limb with the centre of rotation of the dynamometer motor using a laser pointer. Participants performed two maximal knee extension contractions separated by a knee flexion contraction at each angle of knee flexion in a randomised order. Instructed to start contracting when a verbal signal was given

and to hold the maximum for 2-3 seconds until given a verbal signal to stop in order to produce a plateau at maximum contraction for analysis purposes.

*4.6 Electromyography.* Surface EMG of the Vastus Lateralis muscle (VL) and biceps femoris (BF) was recorded throughout the functional muscle test performed on the dynamometer. Skin was shaved and cleaned with an abrasive gel and alcohol swabs to ensure inter-electrode impedance was below 5kΩ. The position of the electrodes was recorded on acetate sheets using moles and angiomas as reference markers so as the electrodes could be placed in the same position at subsequent testing sessions. The reference electrode was placed on the skin covering the lateral femoral condyle. The raw EMG signal was acquired with a sampling frequency of 2000 Hz and processed with a multi channel analogue-digital converter (Biopac EMG 100B Systems, USA), with a 10-500Hz band pass filter. Root mean square (RMS) was calculated over 500 ms around the peak isometric torque. The BF co activation was estimated from RMS recordings during a maximal isometric knee flexion, assuming linearity between EMG activity and torque..

*4.7 Activation capacity.* The maximal M-wave of the VL was determined using electrical stimulation on the femoral nerve (model DS7, Digitimer stimulator, Welwyn, Garden City, UK). A hand-held monopolar cathode was placed on the skin in the femoral triangle. The anode was placed on the skin, under the posterior superior iliac spine. Small twitches were administered at various cathode positions to determine the optimal position to elicit highest twitch EMG and torque. Once the optimal position had been found, twitch intensity was increased until no further increase in the VL peak-to-peak M-wave was observed. This intensity was further increased by 50 mA to achieve supramaximal stimulation and this intensity was used throughout the testing procedure. The intensity of the supramaximal twitches was

reassessed and adjusted accordingly at each of the three testing sessions. Supramaximal doublets were administered during the plateau phase of MVC and at rest following MVC.

4.8 Patellar *Tendon Mechanical Properties.* Patellar Tendon (PT) dimensions were measured by transverse MRI scanning images of the tendon with the lower limb at anatomical position (knee joint at 0° of flexion) (figure 4.4). Tendon CSA was measured at 25%, 50% and 75% of the resting length of the PT. Three measurements were averaged at each site with the average CSA used for analysis purposes.

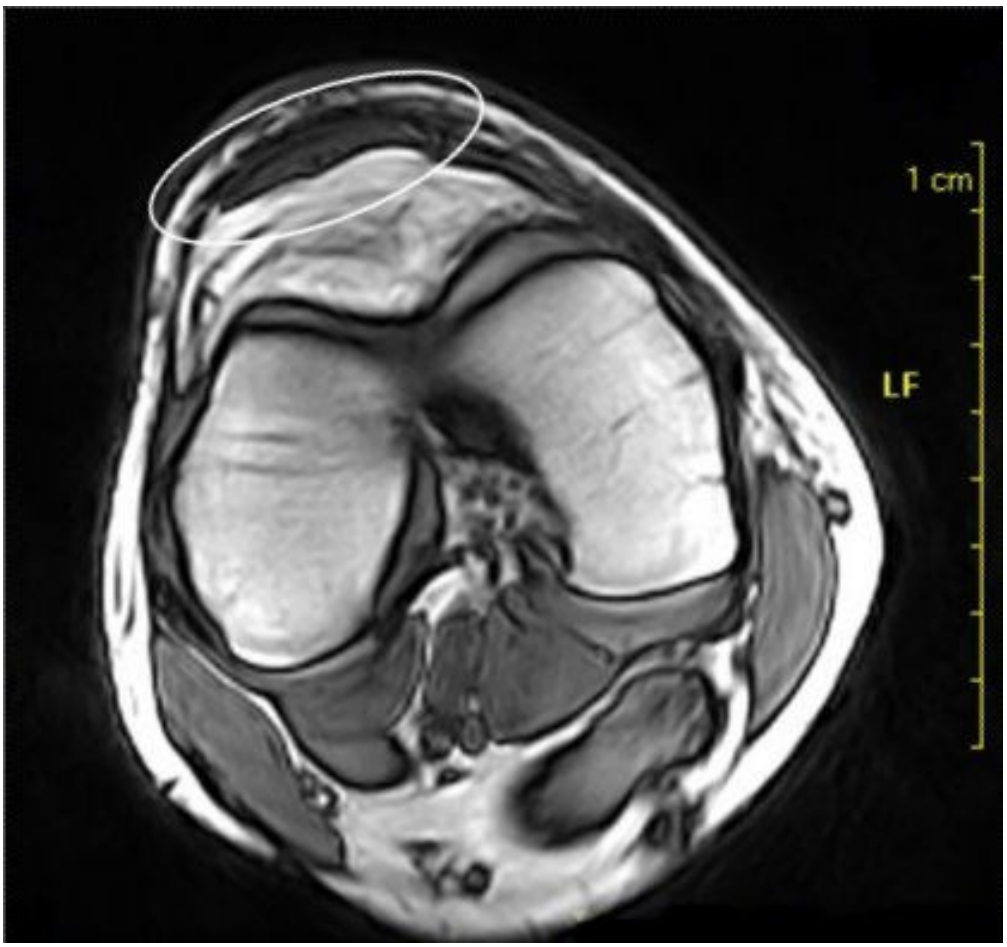


**Figure 4.4**. Axial MRI scan of the right knee showing patellar tendon (highlighted in white), used to measure patellar tendon dimensions.

PT moment arm was estimated form sagittal plane MRI scans of the PT at anatomical zero. The moment arm was determined as the perpendicular distance

from the PT to the tibio-femoral contact point (TFCP) which was defined as the midpoint on the plane connecting the medial and lateral tibio-femoral contact point (Tsaopoulos et al. 2006) (figure 4.5). As tendon mechanical properties were assessed at 90° flexion, the measured moment arm (at anatomical zero) was multiplied by the ratio of the moment arm lengths at 0º and 90° of knee joint flexion, as previously reported (Baltzopoulos 1995). PT force was calculated as net knee extensor torque (measured knee extensor torque + estimated antagonist knee flexor torque) divided by estimated PT moment arm.

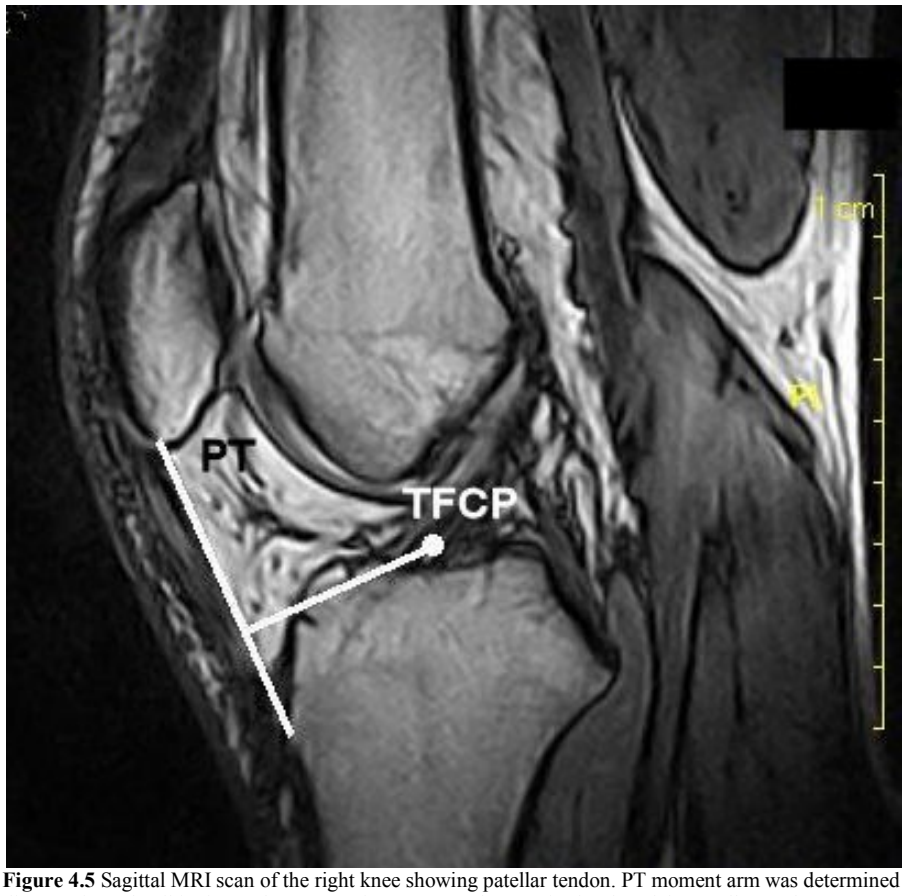


**Figure 4.5** Sagittal MRI scan of the right knee showing patellar tendon. PT moment arm was determined as the perpendicular distance from the tibio-femoral contact point, TFCP to the anterior border of the patellar tendon, PT.

Patellar tendon elongation was measured using ultrasonography. A 10 cm probe was placed on the skin above the tendon with the knee fixed at 90° flexion. Ultrasound clips were taken during slow ramp isometric MVC contractions with the tendon

elongation measured between the patella apex and the insertion of the tendon on the tibial tuberosity (figure 4.6). Ultrasound clips were synchronised with the torque and EMG data acquisitions. Tendon stress (MPa) was calculated by dividing tendon force by tendon CSA, tendon strain was calculated as the ratio of tendon displacement to the initial resting tendon length.

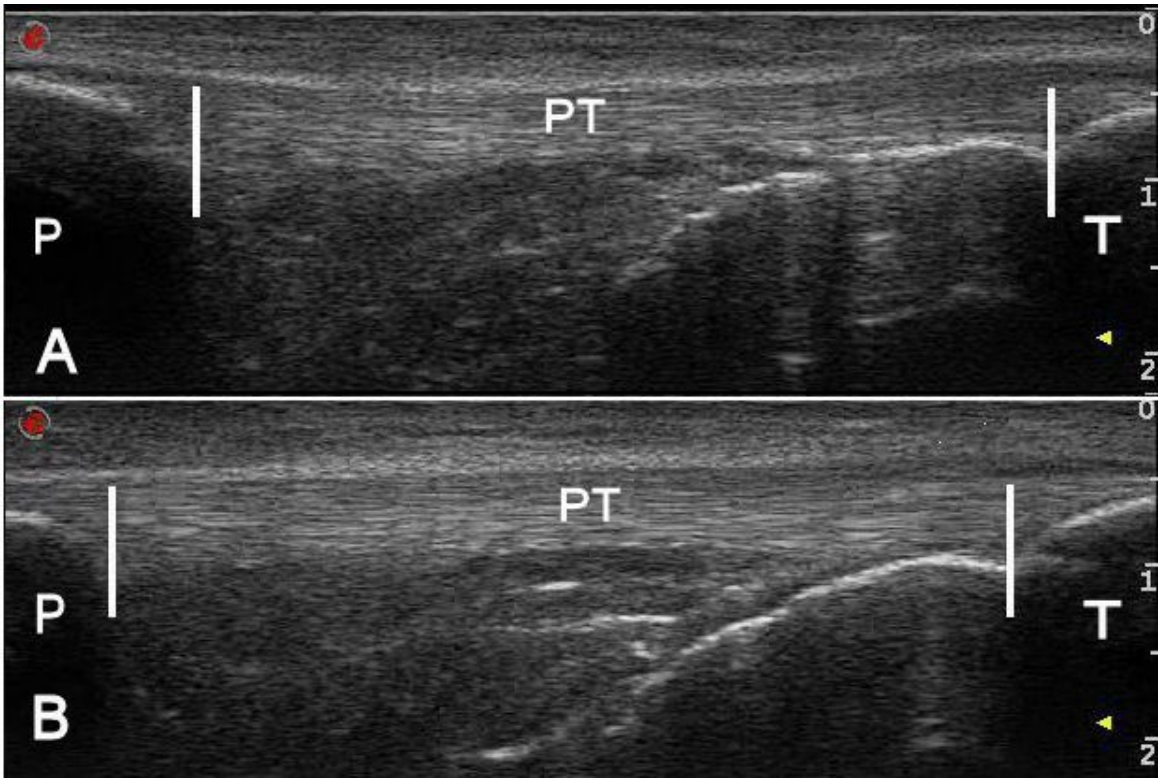


**Figure 4.6** Patellar tendon at rest (A) and during MVC (B). PT elongation was measured as displacement of the patella, P and the tibia, T.

Tendon stiffness was calculated between 90-100% of the maximum force level of the weakest participant over all testing sessions to ensure that stiffness was measured at the same absolute force levels in all subjects and testing sessions. Tendon stiffness (N-mm$^2$) was calculated as the gradient of the corresponding force-elongation. Young's modulus (GPa) over this interval was calculated by multiplying the stiffness values by the ratio of resting PT length over tendon CSA.

*4.9 Resistance training protocol.* Participants attended the IRM three days per week for a period of three weeks to undergo a resistance training (RT) program. Quadriceps extensions were performed at 80% of participants one repetition maximum (1RM). To find the 1RM, participants first warmed up with a series of contractions of a low resistance, after which the resistance was increased to find the maximum. Resistance was set at 80% of each participants 1RM at which three sets of ten contractions were performed during each training session. At the beginning of weeks two and three 1RM was reassessed and the resistance level was adjusted accordingly.

4.10 *Participants.*

Seventeen young men aged 18-53 were recruited into the ULLS study and assigned randomly to either a leucine or alanine supplementation group, Lue = 9, Ala = 8. Due to drop outs 9 participants (23±2.2 yrs : 76.4 ± 8.0 kg) completed the 3 week ULLS period, Leu = 6, Ala = 3. An additional 8 participants (24±0.6 yrs: 72.4 ± 8.0kg) were recruited separately to act as ambulatory controls and underwent the same testing procedures three weeks apart, without ULLS or supplementation.

Due to a further dropout only 8 of the original recruits to the ULLS study (23 ± 2.5 yrs ; 76.9 ±10.9 kg) completed the resistance training recovery period, Leu = 6, Ala = 2.

For chapter 7, Data was collected from a total of 92 individuals including 24 young untrained (YU) males 18-35 yrs (24 ± 2.6 yrs ; 73.3 ± 8.2 kg) and 27 older recreationally active males and females (OA) 65yrs+ (70.9 ± 9.9 yrs) from the local population. The 24 YU males included the original baseline measurements taken from the control and ULLS groups for studies 1 and 2 before any intervention. Twenty-four male and female master athletes (70.9 ± 9.9 yrs) competing at the

2010 European Veteran Athletic Championships (MA) were also assessed as well as 17 older frail males and females (OF) awaiting hip arthroplasty surgery 65yrs+.

All participants signed a written informed consent and the study was approved by the Manchester Metropolitan Faculty of Science and Engineering Ethics Committee. The same participants were used in chapter 6 to study the effect of amino acid supplementation on immobilisation-induced atrophy. Participants were randomly assigned to one of two test groups each taking a supplement of essential amino acid leucine (Leu) intervention group or non- essential amino acid alanine (Ala) group. While both groups showed adaptations in response to ULLS, amino acid supplementation was shown to have no effect of the loss of muscle mass and strength associated with disuse-induced atrophy (c.f. chapter 6). Subsequently, the data was pooled together and analysed with the purpose of examining the skeletal muscle adaptations to ULLS and subsequent retraining.

*Statistics.* Normal distribution of the data was confirmed by Shapiro-Wilks test for normality. Data were analysed using a Mixed ANOVA as we have both a within-subject factor (the repeated measure time, Pre and Post ULLS measurements) and a between subject factor (supplementation, Leucine or Alanine). Where Mauchley's test of sphericity was violated Greenhouse-Geisser corrected F ratios were used for significance level. Pre and post test for the control subjects were analysed using student's t test. Significance level was set at $p<0.05$.

# 5. Study 1. Skeletal muscle adaptations to physical inactivity and subsequent retraining in young men.

## 5.1 Summary

Skeletal muscle structure and function are markedly affected by chronic disuse. With unloading, muscle mass is lost at rate of about 0.4%/day but little is known about the recovery of muscle mass and strength following disuse. Here we report an extensive data set describing in detail skeletal muscle adaptations in structure and function in response to both disuse and retraining.

Nine young men (23±2.2 yrs: body mass: 76.4 ± 10.4 kg) underwent 3 weeks of unilateral lower limb suspension (ULLS) followed by a 3-week resistance training recovery program. Knee extensor (KE) isometric torque, voluntary activation (VA), quadriceps femoris (QF) muscle volume ($QF_{vol}$), fascicle length ($L$f) and pennation angle ($\theta$), physiological cross-sectional area (PCSA) of all four heads of the QF muscle, were measured before, after ULLS, and post-ULLS-resistance training. Needle biopsies were taken from the vastus lateralis muscle of a subgroup (n=6) of the same subjects and cross sectional area of individual muscle s and myosin content of muscle samples were determined.

Following 3 weeks of ULLS, isometric torque decreased by 26%, PCSA by 3%, $QF_{vol}$ by 10%.$L$fand θ of all four heads of QF significantly decreased ($P \leq 0.05$). Following the 3-week retraining period, isometric torque, PCSA, $QF_{vol}$ ,$L$f and θ of all four heads of QF were all fully restored to pre ULLS values. CSA of individual muscle fibres and myosin content of muscle samples decreased by 26% and 35%

respectively (post-ULLS) and recovered to almost pre-ULLS values following retraining. There were no significant changes in voluntary activation of the quadriceps muscles in response to either ULLS or subsequent retraining. These results indicate that, 1) the loss of muscle force with 3-week unloading in humans is mostly explained by muscle atrophy and by a decrease in myosin content and, 2) all the neuromuscular changes induced by this model of disuse can be fully restored after a resistance training intervention of equal duration.

## 5.2 Introduction

Deficits in skeletal muscle mass and function are primary outcomes of chronic unloading, with antigravity muscles such as the knee extensors (KE) being particularly affected (Narici & de Boer 2011). An extensive knowledge of these adverse conditions is paramount, for they are inevitably linked to clinical situations such as prolonged best rest or the use of crutches due to bone fracture or musculoskeletal injury. Several models including bed rest, limb cast immobilization and unilateral lower limb suspension (ULLS) have been used to investigate the effects and recovery from disuse (Adams et al. 2003; M D de Boer et al. 2007). ULLS mimics the standard clinical practice of joint unloading commonly practiced following musculoskeletal injuries or surgery. In addition, this model offers the advantage of confining atrophy to the musculoskeletal system of the unloaded limb.

Reported reductions in KE torque in response to ULLS range 15% to 21 % for durations of 14 to 28 days, 19% for mid-term, 15-28 days, and 21% for long-term, 28+ days, ULLS (Narici and De Boer, 2011).

Reported decreases in muscle size, represented by both anatomical cross sectional area (ACSA) and muscle volume of the quadriceps femoris muscle, average about 10% following 10 days to 5 weeks of ULLS, or approximately 2.6% per week (Narici and De Boer, 2011). The disuse-induced loss of muscle strength far exceeds that of muscle mass, indicating the contribution of other factors, such as neural drive, changes in muscle architecture (De Boer et al. 2007) and a decrease in single fibre specific tension (Danton et al., 2003).

Because muscle architecture is a main determinant of muscle function (Cutts 1988; Gans & Bock 1956; Lieber & Friden 2001), changes in fascicle length ($L_f$) and pennation angle (θ) represent powerful indicators of alterations in functional deficit induced by disuse (Narici & Cerretelli 1998). Pennation, a key-parameter of muscle architecture, is a natural strategy to pack more contractile elements in to a given muscle volume. A decrease in pennation angle (θ), due to muscle atrophy, is commonly interpreted as a decrease in the number of fascicles in parallel (Kawakami et al. 1993) . Reductions in θ seen after only short durations of ULLS or bed rest (~8-13% in the vastus lateralis muscle after 23 to 35 days, (de Boer et al. 2007 & 2008) indicate that substantial muscle remodelling occurs with few days of muscle unloading.

In line with the decrease in pennation angle, a reduction in muscle fascicle length of~8% was also found after 23 days ULLS (de Boer et al., 2007). Such a decrease implies a loss of sarcomeres in series and a consequential increase in sarcomere excursion during shortening of the whole muscle, shifting the operating muscle length towards a less favourable portion of its length-tension curve. Moreover, a reduction in the number of sarcomere in series also predicts a decrease in maximum shortening velocity. Hence, beyond gross atrophy, disused muscles are

affected by subtle, yet substantial, architectural alterations, resulting in losses of force, velocity and power. Information regarding the reversibility of these changes during active recovery is currently lacking.

Neural drive and muscle activation capacity appear to be reduced by periods of prolonged disuse, as reflected by the decreased ability of muscles to fully activate during a maximal voluntary contraction. Bed rest studies have shown a decrease in the root mean square (RMS) of EMG of 6.5% after 20 days (Kawakami et al. 2001) and 19% after 42 days (Edgerton et al. 2001). Nevertheless, the contribution of reduced neural drive to the loss of muscle strength associated with disuse seems still an unresolved issue as several studies showed no change in maximal EMG activity in both vastus lateralis(de Boer et al., 2007) and plantar flexor muscles (Seynnes et al. 2008) after ULLS, whilst others (Clark et al. 2006) showed a 30% decrease in compound muscle action potential (CMAP).

Despite the importance of effective recovery strategies in clinical settings, little is known about the resilience of muscle functional properties following periods of disuse (Hanson et al. 2010) . Disuse atrophy resulting from short periods of immobilization, ~10 days seems to be reversed rather quickly through passive recovery i.e. returning to normal weight bearing. However, without a rehabilitation program full recovery from longer periods of disuse seems to require longer durations. For instance, (Berg & Tesch 1996) reported a 13% decrease in KE torque following 10 days of ULLS, which returned to baseline levels after only 4 days of passive recovery. In a longer ULLS study which lasted4 weeks and resulted in a 22% decrease in KE strength, muscle torque remained 11% below baseline levels after 4 days of passive recovery, eventually returning to normal 7 weeks following the ULLS period (Berg et al. 1991). Similarly, another study of 2-week immobilization

showed that the KE torque could return to pre-ULLS levels within 4 weeks of active recovery (Suetta et al. 2009) .The results of this study are noteworthy for they imply that early active recovery may not only improve patient functional status but is likely to have a greater impact on quality of life, thereby reducing post-treatment hospitalisation times.

Here we report an extensive data set describing in detail skeletal muscle adaptations in structure and function in response to both disuse and active recovery. Knee extensor (KE) isometric and dynamic torque, voluntary activation (VA), contractile properties (Mwave and twitch response), volume ($QF_{vol}$), fascicle length ($L_f$) and pennation angle ($\theta$) of all 4 heads of the quadriceps femoris were measured before and after 3 weeks ULLS and after 3 weeks active recovery. We hypothesised that substantial remodelling of skeletal muscle architecture would be induced by the 3 weeks of unloading and, unlike passive recovery, a rehabilitation program of equal duration would effectively restore all the muscle structural and functional parameters affected during disuse.

## 5.3. Methods

*Participants.*

Seventeen young men aged 18-53 were recruited into the ULLS study and assigned randomly to either a leucine or alanine supplementation group, Lue = 9, Ala = 8. Due to drop outs 9 participants (23±2.2 yrs : 76.4 ± 8.0 kg) completed the 3 week ULLS period, Leu = 6, Ala = 3. An additional 8 participants (24±0.6 yrs: 72.4 ±

8.0kg) were recruited separately to act as ambulatory controls and underwent the same testing procedures three weeks apart, without ULLS or supplementation.

Due to a further dropout only 8 of the original recruits to the ULLS study (23 ± 2.5 yrs ; 76.9 ±10.9 kg) completed the resistance training recovery period, Leu = 6, Ala = 2.

All participants signed a written informed consent and the study was approved by the Manchester Metropolitan Faculty of Science and Engineering Ethics Committee. The same participants were used in chapter 6 to study the effect of amino acid supplementation on immobilisation-induced atrophy. Participants were randomly assigned to one of two test groups each taking a supplement of essential amino acid leucine (Leu) intervention group or non- essential amino acid alanine (Ala) group. While both groups showed adaptations in response to ULLS, amino acid supplementation was shown to have no effect of the loss of muscle mass and strength associated with disuse-induced atrophy (c.f. chapter 6). Subsequently, the data was pooled together and analysed with the purpose of examining the skeletal muscle adaptations to ULLS and subsequent retraining.

Participants underwent three weeks of disuse with the unilateral lower limb suspension (ULLS) model (see section 4.2). Following the ULLS, participants attended the laboratory three days per week for a period of three weeks to undergo a resistance training (RT) program (see section 4.9). Three needle biopsies were taken from vastus lateralis muscle of a subset of 6 subjects: one pre-suspension (pre-ULLS), one post suspension (post ULLS), one post exercise recovery (post RT) (section 4.10). Single fibre CSA measurement (section 4.11) and Myosin content (section4.12) were measured on the muscle biopsy samples.

Ultrasound scanning was used to assess muscle architecture at rest and during maximum voluntary contraction (MVC) (section 4.3). Maximum knee extension and knee flexion torque were measured at three angles of knee flexion, 70˚, 80˚ and 90˚ (section 4.5). Surface EMG of the Vastus Lateralis muscle (VL) and biceps femoris (BF) were recorded throughout the functional muscle test performed on the dynamometer (section 4.6). Quadriceps activation was also assessed with the twitch interpolation technique using electrical stimulation applied to the femoral nerve (section 4.7).

*Statistics.* The normal distribution of all data sets was verified with a Shapiro-Wilks test. Student's *t* tests for independent samples were applied to compare baseline values between the suspended and control groups. To determine the effect of the suspension period on the investigated variables, a one-way ANOVA for repeated measures was conducted with a Bonferroni adjustment for post hoc analysis. Statistical significance was set as $\alpha$ =0.05. Unless stated otherwise, results are means ± standard error of the mean.

## 5.4 Results

Statistical tests performed on baseline measurements showed no difference between the ULLS and control groups in any of the variables measured.

*Muscle strength and activation.* Maximum voluntary contraction of QF decreased by 26% following 3 weeks of ULLS ($p<0.005$), but increased during active recovery to give post-RT strength levels just 2.5% below baseline values (n.s. from baseline). Voluntary activation decreased by 5.1% during ULLS but this decline did not reach significance. However, a mirrored trend was observed after active recovery, when

the voluntary activation capacity increased by 6.6% ($p<0.05$), (table 5.1, Fig. 5.1).

Control subjects showed no difference in MVC from baseline to post-ULLS testing.

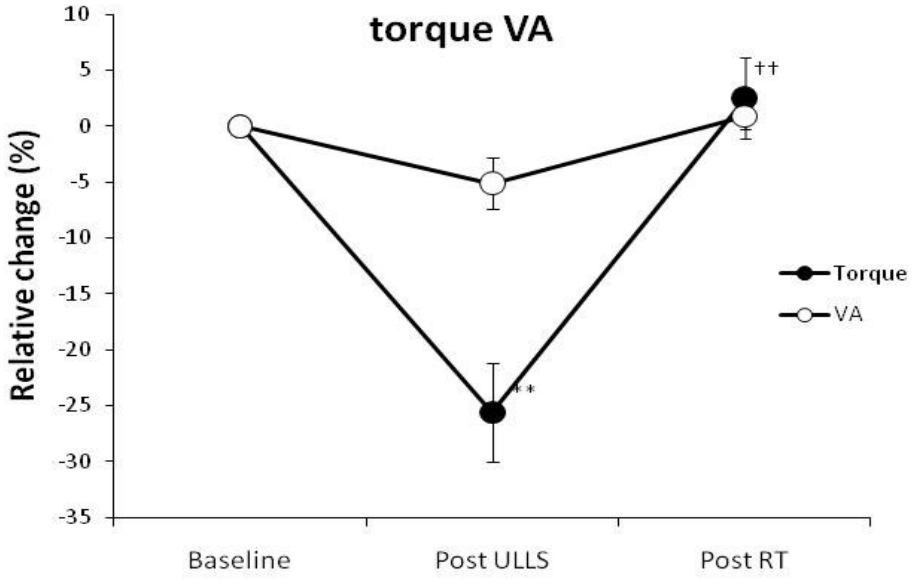


Figure 5.1. Percentage change in MVC torque and voluntary activation (VA) capacity of the quadriceps femoris at baseline, following 3 weeks suspension and after 3 weeks subsequent active recovery. Data are means ± SEM. No changes in VA were statistically significant. ** Statistically significant from baseline P<0.005, †† significantly different from post-ULLS values P<0.005

**Table 5.1 Quadriceps MVC (maximum voluntary activation),VA ( voluntary activation capacity), PCSA (physiological cross sectional area) and SF (specific force ) and baseline, post-ULLS and post-RT**. Data are means±SEM *significantly different from baseline p<0.05 **significantly different from baseline p<0.005 †significantly different from post-ULLS p<0.05 ††significantly different from post-ULLS P<0.005

| | ULLS (n=8) | | | Control (n=8) | |
|---|---|---|---|---|---|
| | Baseline | 3 wksPost-ULLS | 6 wksPost-RT | Pre | Post |
| MVC Nm | 299±14 | 221±14** | 291±14†† | 311±21 | 294±26 |
| VA % | 87±3.5 | 83±3.5 | 88±3 | -- | -- |
| $EMG_{RMS/M\text{-}wave}$ | 0.073±0.02 | 0.0614±0.02 | 0.073±0.02 | 0.070±0.01 | 0.070±0.01 |
| PCSA mm2 | 208±6 | 203±8 | 218±14 | 199.98±18.0 | 202.66±21.7 |
| SF Nm | 32±2 | 24±1* | 35±3† | 38.0±4.4 | 37.1±6.3 |

*Muscle volume.* Whole quadriceps muscle volume decreased by 10% during ULLS ($p<0.05$) but muscle mass was restored following 3 weeks of active recovery, with post-RT muscle volume being 2% higher than baseline measurements (Figure 5.2). At single muscle level, atrophy of the rectus femoris did not reach significance in response to suspension, -5% (n.s.). However, RF volume increased by 12% from post ULLS to post-RT values($p<0.05$) to increase post RT volume by 6% above baseline (n.s.). The volume of the 3 remaining heads of the quadriceps femoris all decreased during ULLS, VL and VI by 12% ($p<0.05$) and VM by 9% ($p<0.005$), and all muscles returning to pre ULLS values following active recovery (Figure 5.3). A one-way ANOVA of the percentage change in muscle volume of each of the four heads of the QF showed no significant differences in the individual responses of each heads during both ULLS and RT (Figure 5. 3). Control subjects showed no difference in QF muscle volume from baseline to post-ULLS testing.

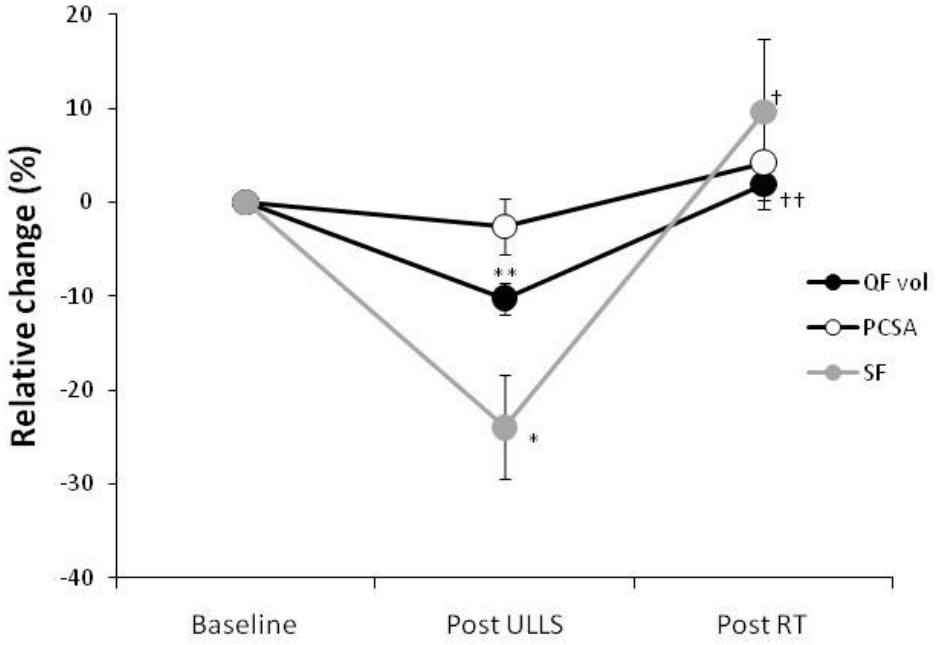


**Figure 5.2. Relative change from baseline of whole QF volume, PCSA and specific force following 3 weeks suspension and after 3 weeks subsequent resistance training.** Data are means ±

SEM. No changes in PCSA were statistically significant. *Statistically significant from baseline P<0.05, ** statistically significant from baseline P<0.005, †statistically significant from post-ULLS P<0.05, ††statistically significant from post-ULLS P<0.005.

*Resting twitch characteristics.* Although there was some tendency for a change in the twitch characteristics at rest in response to both ULLS and retraining, none of the differences in twitch properties were significant (Table 5.2).

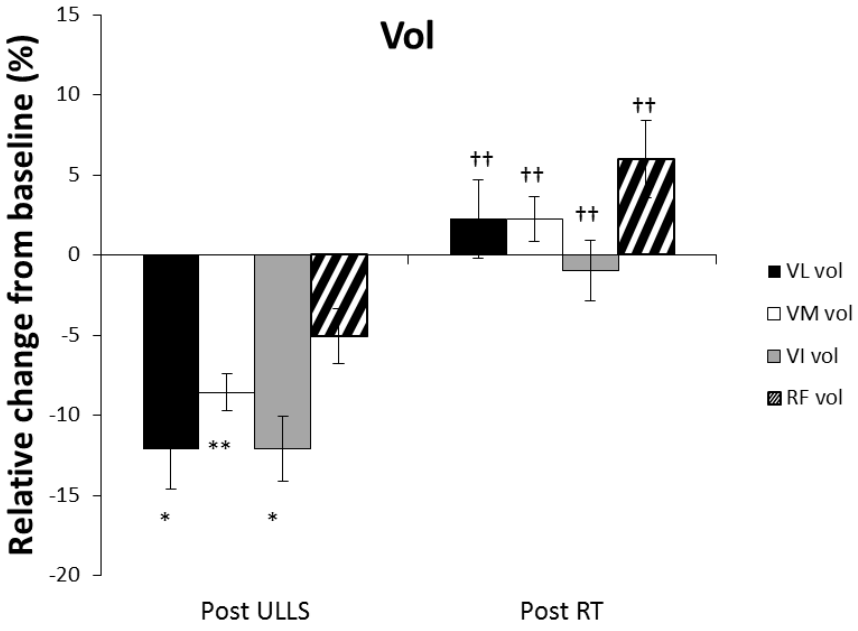


**Figure 5.3.Relative change from baseline of muscle volume of the all 4 heads of quadriceps following 3 weeks suspension and after 3 weeks subsequent active recovery.** Data are means ±

SEM. *Statistically significant from baseline P<0.05, ** statistically significant from baseline P<0.005, † statistically significant from post-ULLS P<0.05, †† statistically significant from post-ULLS P<0.005.

*Muscle architecture.* Resting fascicle length decreased in all four heads of the quadriceps femoris following suspension, VL by 9% (p<0.005), VM by 6% (p<0.05), VI by 11% (p<0.005) and RF by 9 % (P<0.05). This parameter was fully restored to baseline values in all four muscles, following active recovery (Figure 4.4). Similarly, pennation angle at rest decreased in all four heads, in VL by 10% (p<0.005),in VI and RF by 7% (P<0.05) and by 8.6% for VM (n.s.) (Figure 5.4). There was no inter-muscle difference in the magnitude of changes in L*f* or θ after ULLS or subsequent resistance training. Control subjects showed no difference in L*f* or θ from baseline to post-ULLS testing.

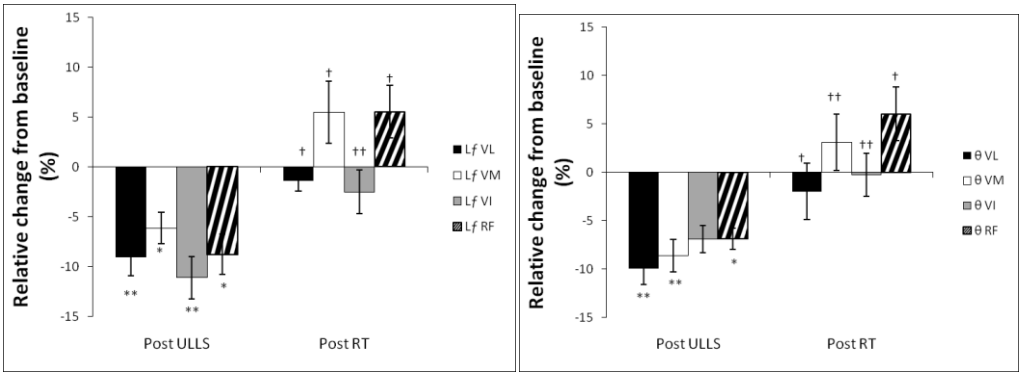


**Figure 5.4. Relative change from baseline of fascicle length and pennation angle of the all 4 heads of quadriceps following 3 weeks suspension and after 3 weeks subsequent active recovery.** Data are means ± SEM. *Statistically significant from baseline P<0.05, ** statistically significant from baseline P<0.005, † statistically significant from post-ULLS P<0.05, †† statistically significant from post-ULLS P<0.005

**Table 5. 2 Resting twitch characteristics at baseline, post-ULLS and post-RT**. Data are means±SEM all data are not statistically significant. Time to peak tension (TPT) and 1/2 relaxation time shown in milliseconds, peak twitch in Nm. dT/dt is rate of torque development, Tw/MVC is peak tension normalised to MVC.

| | Baseline | 3 wksPost-ULLS | 6 wksPost-RT |
|---|---|---|---|
| PTw | 66±4 | 58±6 | 63±4 |
| TPT | 58±2 | 62±2 | 60±1 |
| 1/2RT | 71±6 | 78±9 | 72±4 |
| dT/dt | 1.15±0.1 | 0.96±0.1 | 1.06±0.1 |
| Tw/MVC | 0.23±0.02 | 0.27±0.03 | 0.22±0.02 |

*Physiological cross sectional area and specific force.* Quadriceps PSCA did not change significantly across the study, despite a 3% reduction after ULLS, and a 5% increase above baseline values after re-training. Specific force did decrease significantly in response to suspension by 24% ($p<0.05$) and increased 10% above baseline values (n.s.). Control subjects showed no difference in PCSA or specific force from baseline to post-ULLS testing (Table 5.1, Figure 5.2).

*Single fibre cross sectional area.* Figure 5.5 shows the mean values of single muscle fibre cross sectional area. Single muscle fibres went through significant atrophy (-26%) following ULLS and recovered almost pre-ULLS size following retraining.

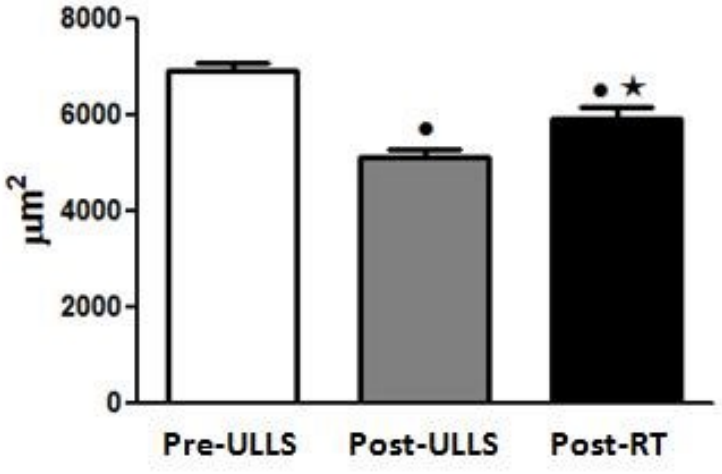


**Figure 5.5. Single fibre CSA of vastus lateralis fibres following 3 weeks suspension (Post ULLS) and after 3 weeks subsequent resistance training (Post-RT).**Data are means ± SEM. Statistically significant from Pre ULLS P<0.05,★ statistically significant from Post ULLS P<0.05

*Myosin content.* Fig 5.6 shows the percentage values of myosin content. Myosin content was significantly lower in post-ULLS than in pre-ULLS(-35%). After active recovery, myosin content was significantly higher than post-ULLS, although it did not reach the levels observed before suspension.

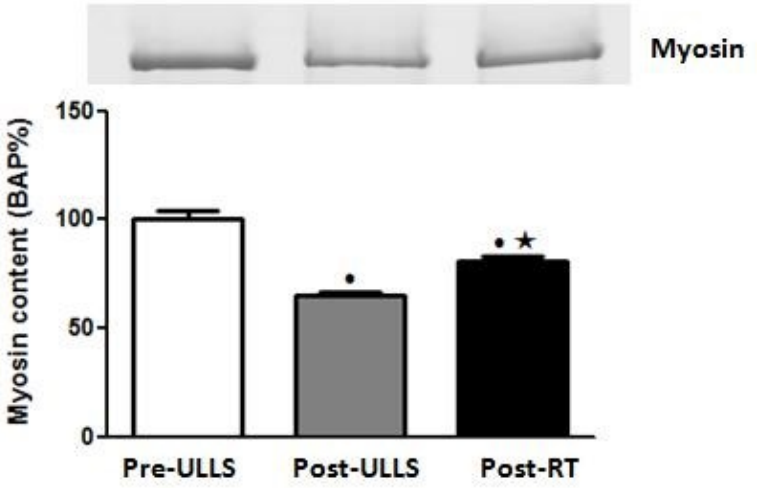

**Figure 5.6. Percentage values of myosin content following 3 weeks suspension and after 3 weeks subsequent resistance training.** Data are means ± SEM. ˙Statistically significant from Pre ULLS P<0.05,★ statistically significant from Post ULLS P<0.05.

## 5.5 Discussion

Data from the current study show that significant changes in skeletal muscle structure and function occur in response to unloading, but these changes can be reversed with active recovery with muscle function fully restored 3 weeks post disuse. A previous study showed that young men displaying muscle atrophy following 2 weeks of immobilisation regained both quadriceps MVC and muscle volume to reach baseline levels after 4 weeks of resistance training (Suetta et al., 2009). Our study shows that with resistive training performed in the early post-disuse phase recovery from a longer immobilisation period is possible in a shorter recovery timescale.

*Muscle structure and function.* Following 3 weeks of ULLS, MVC of the knee extensors decreased by 26%, corresponding to a daily rate of loss of 1.24%. With such a dramatic decrease in force one would expect a corresponding reduction in PCSA of the quadriceps femoris muscle, however PCSA in the present study only slightly decreased by 3%. This surprisingly small reduction in PCSA is simply due to the fact that PCSA is the ratio of muscle volume to fascicle length and since in this study $QF_{vol}$ decreased by 10% and Lf by 9%, this produced virtually no change in PCSA. For this reason, PCSA calculated in this way seems an unreliable indicator

of muscle atrophy and a poor predictor of force loss with unloading. Instead, muscle volume seems to be a more reliable indicator of muscle atrophy, and our study suggests that the significant decrease in maximum isometric torque was largely accounted for by quadriceps atrophy. This contention is also supported by the results of the analysis of the CSA of individual muscle fibres which clearly indicates fibre atrophy (-26%) resulting from the 3 week ULLS.

Because of the paradoxically small reduction in PCSA, a large decrease in quadriceps specific force, -24% was found. Although, in principle a decrease in force per cross sectional area is consistent with previous observations on unloading (Berg et al. 1997; D'antona et al. 2003), we believe that a 24% reduction is an overestimation due to the non-significant change in PCSA. Using a more conservative approach by which specific force is calculated using anatomical rather than physiological CSA, the resulting decrease in F/CSA is 19%. The muscular origin of this loss of specific force seems confirmed by the fact that in this experiment voluntary activation did not change during the ULLS period suggesting that single fibre changes are responsible for this phenomenon. Previous studies have shown that myosin content is a main determinant of single fibre specific tension (D'Antona et al., 2003). Hence the large decrease in myosin content (-35%) observed as a result of the ULLS points to a decrease in single fibre specific tension, a likely contributing factor to the loss of specific force observed at whole quadriceps level.

Other candidates that could have contributed to the decrease in whole muscle F/CSA are changes in the structural integrity of the extracellular matrix as this is thought to largely contribute to the force transmitted by the contracting fibres to the tendon and, a decrease in fascicle length. No study has yet specifically investigated the role of changes in the extracellular matrix in the loss of muscle force/CSA. However

studies examining the ECM components in immobilised human skeletal muscles have shown that within 48hrs of immobilisation mRNA for matrix metalloproteinase's and ECM structural components such as collagen are down-regulated (Urso et al., 2006). As the ECM is important in regulating cell behaviour via membrane permeability and interaction with growth factors and signal transduction pathways, any alterations in ECM composition is likely to affect normal cell function.

As for the contribution of a decrease in fascicle length to the loss of force/CSA, our study showed an average 9% decrease in Lf for all four heads of the QF. This reduced muscle fascicle length represents a loss of sarcomeres in series which predicts an increase in the sarcomere excursion required to achieve a given whole muscle shortening. Therefore, assuming tendon mechanical properties remain the same, just a small decrease in L$f$ may cause the fibres to operate at a less optimum portion of the length-tension curve. However, a previous similar study by our group observed a decrease in patellar tendon stiffness of -29%following 23 days ULLS (de Boer, *et. al.,* 2007). A more compliant tendon should theoretically cause a leftward shift of the length-tension curve and may to some extent compensate for the shift due to decreased fibre length (Narici and Maganaris 2007). Therefore, decreased fibre length seems an unlikely cause of the decrease in F/CSA.

*Preferential atrophy.* Vastus lateralis is often used as a representative muscle of how the quadriceps muscles as a whole react to various alterations in loading conditions. In the present study changes in muscle volume, L$f$ and θ in response to unloading and active recovery were assessed individually in all four heads of the quadriceps.

Whole quadriceps muscle volume as measured *in vivo* by MRI scanning showed a decrease of 10% following 3 weeks ULLS, equating to a daily rate of loss of 0.48%. This finding is of considerable clinical value since it highlights that disuse-atrophy is an extremely fast process requiring countermeasures from the very first few days of unloading. VL, VI and VM showed similar extents of atrophy. However RF muscle volume decreased by only 5% and failed to reach significance, in line with previous observations of Capodaglio & Narici (1998). This is likely explained by the fact that the RF is a bi-articular muscle crossing both the knee and hip joints. In our model of immobilisation, the knee joint was kept as fixed as possible (within limits of the model). However, the hip joint was free to extend and flex during normal activity. Rectus femoris' role in stabilising the hip joint and aiding in hip flexion appears to be enough to preserve muscle mass and prevent atrophy to the extent displayed by the other QF muscles which act solely on the knee joint.

*Effects of active recovery.* The effect of active recovery on skeletal muscle after periods of immobilisation is not widely reported upon, however data exists to support our findings that full recovery from prolonged unloading is possible after 4 weeks retraining in young men (Suetta et al. 2009; Hortobagyi et al. 2000). Our results show that resistance training in the very early stages of recovery following periods of disuse can reverse both the structural and functional changes resulting from skeletal muscle atrophy in as little as three weeks post-immobilization. Our data on muscle volume, architecture and strength following resistance training show that muscle recovery was complete after 3 weeks of active recovery immediately following ULLS. While PCSA and specific force resulted in post RT levels which were also not significantly different from baseline, PCSA and specific force attained values slightly higher than baseline. These data show that both increased PCSA and

specific force contribute to this increase of MVC in disused muscle as it does in healthy individuals undergoing resistance training programs (Erskine et al. 2010).

*Preferential hypertrophy.* Post RT muscle volume changes with respect to baseline a show RF to appear to be more responsive to resistance training. However, on closer inspection it can be seen that all 4 heads have a similar response to active recovery following disuse, however due to the fact that RF displayed less atrophy during disuse, the similar increases in muscle mass result in a higher than baseline muscle mass for RF after 3 weeks active recovery. It could be hypothesised that if training were to continue the remaining 3 muscles, VL, VI and VM would eventually approach similar post-RT to baseline increases in muscle mass as RF. The 3 week suspension period also resulted in changes in L$f$ and θ in line with whole QF muscle volume decrease. The difference in RF muscle atrophy was not accompanied by similar preservation on RF individual architectural parameters. In fact, all four heads of the QF displayed similar adaptations in L$f$ and θ with no statistical differences between responses.

*Voluntary activation and neural drive*. The observed changes in net KE torque could not be accounted for by changes in maximum voluntary activation or neural drive since neither VA or VL activation ($EMG_{rms/M\text{-}wave}$) changed with ULLS or subsequent retraining. These findings are in line with previous findings (de Boer et. al. 2007&Seynnes at.al., 2010) but at odds with others (Berg et al. 1997; Koryak 2008). As EMG data was only recorded for VL as a representative of quadriceps femoris activation, comment cannot currently be made on the individual responses in VM, VI and RF neural activation. Similar results would have been expected for the

VM and VI too, perhaps with the exception of RF which, being a bi-articular muscle, is likely to have been more activated that the other three heads of the quadriceps during the ULLS period. This hypothesis seems supported by the lesser atrophy displayed by this muscle in response to ULLS.

*Muscle twitch characteristics.* Previous studies have shown a decrease in human triceps surae muscle TPT (7%) and 1/2RT (2%, n.s.), in response to plaster cast immobilisation (Davies. et.al,. 2008). However, these authors found an increase in PT with immobilisation, contrary to our finding of a decrease in PT in the quadriceps muscle. An increase in PT has been attributed to a decreased reuptake of calcium by the sarcoplasmic reticulum during immobilisation leading to increased levels of Ca2+ and therefore to twitch potentiation (Thom et al. 2001). However, significant decreases, -22% in PT of quadriceps of young individuals in response to disuse have been observed (Suetta, 2009) whilst in the same study TPT was not affected by immobilisation or retraining. The results of Suetta's study appear to be more in accordance with the results of the present study than with those of the previous two group of authors (Davies. et.al,. 2008). Whilst in our study no significant changes were observed in resting twitch characteristics, there was a trend in the decrease of the PT in response to ULLS, -12%, with PT subsequently increasing at 6 weeks post ULLS to 4.5% below baseline following resistance training protocol. Also, no significant changes in TPT were found in the present study.

The above conflicting results seem to indicate that different muscle groups respond differently to immobilisation. The lack of change in quadriceps twitch characteristics may also be due to the fact that ULLS is a less drastic method of disuse than immobilisation. For instance, this difference is reflected by more pronounced spinal and supra-spinal neural adaptation induced by immobilisation when compared to

ULLS (Clark, 2009). The lack of significant changes in muscle twitch characteristics found in the present study seems consistent with the absence of changes in VL myosin heavy chain composition in the participants of this ULLS study (Bottinelli, personal communication 2012).

*Implications of the findings for the recovery of muscle in young and older individuals following period of disuse.* The results of this work strongly suggest that early rehabilitation is an effective strategy for recovering skeletal muscle mass and function following a period of immobilisation in young individuals. The fast recuperation of muscle mass observed with RT suggests that overloading of skeletal muscle of young individuals during the recovery period, quickly overcomes the anabolic blunting of protein synthesis caused by inactivity (Glover et al. 2008) . This fast recovery of muscle mass seems also due to the 'muscle memory' phenomenon, described by (Liu & Jorgensen 2011), which shows that myonuclei of muscle fibres are preserved during a period of inactivity and are thus readily available for supporting fibre hypertrophy when muscle is reloaded again(Liu & Jorgensen 2011). However, the same does not appear to hold true for older individuals. A previous study has shown that after 2 weeks ULLS young individuals restored MVC, muscle volume and architecture to baseline levels by following a resistance training protocol for 4 weeks. Older individuals following the same unloading procedures and training protocol did regain pre-immobilization MVC levels however muscle volume and architecture did not fully recover (Suetta *et. al.,* 2009). The reduced capacity for skeletal muscle recovery from disuse atrophy observed in older individuals may be attributed to a combination of age related changes in skeletal muscle physiology. In particular, a decrease in the anabolic response of aged skeletal muscle to both resistance exercise and protein availability (Drummond et al. 2008) could explain the

slower recovery of older individuals from disuse atrophy. In addition, it is thought that older individuals not only have a decrease number of satellite cells, but that the remaining pools of satellite cells exhibit reduced activation capacity (Gallegly et al. 2004). With periods of immobilisation more common in the elderly population due to illness and injury, further research is necessary to fully understand the observed reduced plasticity of aged skeletal muscle and the exploration of potential countermeasures.

# *6. STUDY 2. THE EFFECT OF AMINO ACID SUPPLEMENTATION ON IMMOBILISATION-INDUCED ATROPHY IN YOUNG MEN*.

## 6.1 Summary

It is well known that chronic unloading conditions result in declines in skeletal muscle structure and function. The loss of muscle mass and strength can be very slow to recover from, even in young healthy subjects. While resistance training during immobilization has been shown to somewhat mitigate skeletal disuse atrophy, this is not always possible in clinical situations due to pain resulting from injuries or the need to immobilise joints for recovery purposes. This study examines the efficacy of supplementation with the amino acid leucine during immobilization to

nutritionally counteract the net negative balance in protein synthesis and breakdown in response to decreased mechanical stimulus. Nine young men (23±2.2 yrs: 76.4±10.4kg) underwent 3 weeks of unilateral lower limb suspension (ULLS) and were randomly assigned to a leucine intervention group (Leu) or an alanine placebo group (Ala). Muscle structure and functional parameters were recorded at baseline and following 3 weeks ULLS. While both groups showed adaptations in response to ULLS, Leu supplementation did not appear to alleviate the loss of muscle mass and strength associated with disuse-induced atrophy.

## 6.2 Introduction

Declines in skeletal muscle function have been shown to be an outcome of chronic unloading conditions including spaceflight and Earth-based models of simulated microgravity such as bed rest and unilateral lower limb suspension (ULLS) (Pavy-Le Traon et al. 2007). Reported reductions in KE torque in response to ULLS range from 15% to 21 % for durations of 14 to 28 days, (Ploutz-Snyder et al. 1996; Berg & Tesch 1996; Clark et al. 2006; Maarten D de Boer et al. 2007; Cook et al. 2007). Anatomical cross sectional area (ACSA) of the quadriceps femoris muscle decreased by 7% to 10% following 23 to 30 days of ULLS in young men (Berg et al.,1991, de Boer et al.,2007), much less than the accompanied loss in strength suggesting that many other factors including neurological and the loss of contractile proteins contribute to the observed strength decrease associated with disuse-induced skeletal muscle atrophy (Clark et al.,2006). Additionally, as tendons play a key-role in the transmission of forces from contracting muscles to bones it is important to consider muscle and tendons as an in-series contractile element. Human tendon tissues are

susceptible to disuse displaying a decline in their mechanical and material properties when subject to conditions of chronic unloading. Patella tendon stiffness and Young's modulus decreased by -29.3% and -30.1% respectively after 23 days of ULLS (de Boer *et al.*,2007), a finding consistent with stiffness reduction of +58% and -65% reported in bed rest studies of 20 and 90 days (Kubo et al.,2000, Reeves et al.,2005).

Loss of muscle mass and strength following disuse in clinical situations can be very slow to recover, even in young healthy and active subjects (Rutherford et al. 1990) therefore much research has been directed towards the development of an effective protocol to mitigate disuse-induced atrophy and improve recovery. As muscle atrophy is determined by a net catabolism of muscle proteins brought about by reduced rates of protein synthesis and in the early stages of disuse increased protein degradation (Paddon-Jones 2006; Phillips et al. 2009) much research into limiting disuse-induced muscle atrophy have focused on methods to increase muscle protein synthesis in unloaded muscle. The essential amino acid (EAA) leucine has been has received a lot of attention in the literature due to both its anti-catabolic effects (Buse & Reid 1975; Zanchi et al. 2008) and effectiveness in promoting muscle protein synthesis (Paddon-Jones et al. 2004; Glover et al. 2008; Trappe et al. 2007). It is not completely understood how leucine contributes to skeletal muscle remodelling but it is believed to be a regulator of a number of cytoplasmic proteins known to be involved in initiating skeletal muscle protein synthesis and the insulin signalling pathway (Nicastro et al. 2010). Some investigators have suggested that membrane receptors and transporters sensitive to leucine may regulate the proteins involved in intracellular signalling pathways such as mTOR and, Akt (Glynn et al. 2010; Bohe et al. 2003; Hundal & Taylor 2009). This is an important statement as if this indeed

does prove to be the case then leucine supplementation may serve as stimulator of the AKT/mTOR pathway during muscle disuse to counteract the down regulation seen in periods of muscle inactivity. Indeed, Leucine rich EEA supplementation during 28 days bed rest did preserve skeletal muscle mass and myofibrillar protein synthesis, however this was not enough to preserve muscle strength suggesting that some mechanical stimulus is required to preserve muscle strength in addition to the preservation of muscle mass (Paddon-Jones *et al.*,2004). Incongruously, in a study of 60 days of bed rest no effect of leucine enriched meals was observed in structural changes of soleus muscle between supplemented and controlled groups in a study by Trappe et al. (2008). A similar study found that a leucine supplemented group actually displayed a greater loss of muscle mass (~4%) of the thigh and calf muscles than a control group (Trappe et al. 2007). In addition to the conflicting results of the effects of mixtures of essential and non-essential amino acids on atrophied human skeletal muscle, there are few studies of humans which evaluate the isolated effects of a leucine supplement and even fewer in which the direct effects of leucine on muscle functional parameters have been studied.

Whilst skeletal muscle appears to be altered by protein intake, little is known about the response of tendon or tendon extracellular matrix to nutritional supplementation. Functional implication of decreases in tendon stiffness include reductions in the rate of contractile force transmission which could have implications for fall prevention, increased likelihood of tendon strain injury and changes in the force-length relationship of the muscle. The mechanical strength of a tendon depends primarily on the structure and intermolecular interactions of its collagen fibrils (Ganty and Kadler, 2002). However, the composition and structure of the ECM (e.g. macromolecules such as proteoglycans) also have an effect (Magnusson et al. 2003).

The predominant proteoglycan in the tendon, Decorin, belongs to the family of small leucine-rich proteoglycans (SLRP) so called due the presence of leucine-repeats in their structure. SLRP's have been shown to have a role in regulating of collagen fibril formation and are thought to affect fibril diameter by aligning collagens for successful cross-linking (Kalamajski and Oldberg 2010). Many investigators report that tendon collagen synthesis is not sensitive to feeding (Babraj et al. n.d.; Wackerhage & Rennie 2006; Ken Smith & Rennie 2007). However, a recent study by (Barbosa et al. 2010) has shown that a leucine rich diet stimulates tendon collagen synthesis in malnourished rats, especially when in combination with physical activity and actually improved the biomechanical characteristics of the tendon (maximal load, displacement, stress and strain). This recent evidence suggests that leucine intake may be an important factor in improving tendon mechanical properties or recovery and hence further research is necessary to explore this relationship.

In the light of the considerations above, the aim of this study was to investigate the hypothesis that atrophy associated with situations of chronic unloading could be mitigated to an extent by the administration of a daily Leucine essential amino acid supplement (Leu) compared to a non-essential amino acid Alanine placebo supplemented group (Ala) in young men undergoing 21 days of unilateral lower limb suspension (ULLS).

### 6.3 Methods

The study was approved by Manchester Metropolitan University Faculty of Science and Engineering Research Ethics Committee (Ref. FAETC/08-09/13) and participants were excluded from the study if they suffered from or have ever suffered

from previous stroke, uncontrolled cardiovascular disease, motor neurone disease, Parkinson's disease, medically diagnosed osteoporosis, type II diabetes, hypertension or myocardial infarction within the previous two years, acute febrile or systemic disease within the past two years or are currently taking beta blockers. Participants were advised on early signs of deep vein thrombosis in the early sub-clinical phase and were monitored weekly using Doppler ultrasonography in order to detect any signs of the possible occurrence of DVT.

The ULLS test group consisted of 17 participants who were randomly assigned to an essential amino acid leucine (Leu) intervention group (n=9) and non- essential amino acid alanine (Ala) group (n=8), however due to participant withdrawal from the project for various reasons (see appendix 1), nine participants completed the three week suspension period and subsequent testing (Leu n=6; Ala n=3). Eight subjects were recruited separately to act as control subjects and underwent the same testing procedures three weeks apart but did not undergo ULLS and did not take any nutritional supplements. Control participants (n=8) were recruited separately specifically for control data collections primarily due to the difficulties associated with recruitment of people willing to undergo ULLS.

Prior to the testing and suspension period participants visited the IRM to be familiarised with the procedures involved in the testing protocol. Participants were shown how to perform maximum voluntary contractions (MVC) and slow ramp contractions (SRC) and were introduced to electrical stimulation used in M-wave and interpolated twitch procedures. All baseline data was collected prior to the ULLS period. Blood samples were taken in a fasted state before 11am and prior to any testing involving exercise. During the testing sessions ultrasound and MRI scans were taken before dynamometer testing to rule out any slight changes in muscle

structure or size due to swelling after exercise. Once the scans were complete participants were transferred to dynamometer to perform the strength and neurophysiological tests.

*Amino Acid Supplementation.* Participants undergoing ULLS were randomly assigned to either a leucine intervention group or an alanine placebo group.

*Unilateral Lower Limb Suspension procedure.* Participants underwent unilateral lower limb suspension (ULLS) (Berg et al.,1991) for three weeks to induce atrophy of the right limb.

Ultrasound scanning and MRI were used to assess muscle architecture and volume respectively. Maximum knee extension and knee flexion torque were measured using an isokinetic dynamometer at varying angles of knee flexion. Surface EMG of the Vastus Lateralis muscle (VL) and biceps femoris (BF) was recorded throughout the functional muscle test performed on the dynamometer.

Patellar Tendon Mechanical Properties using both ultrasound scanning and MRI techniques.

*Statistics.* Nine participants completed the ULLS period, six participants remaining in the Leucine supplement group and three in the Alanine placebo group. Due to the particularly small number of participants in the control group, it was deemed inappropriate to perform statistical analysis as any calculations would not be sufficiently robust to confirm statistical significance between the supplementation groups. Consequently, we report on experimental trends and observations between the leucine and alanine groups.

## 6.4 Results

*Quadriceps muscle volume and architecture*. After 3 weeks of ULLS quadriceps muscle volume decreased considerably in both groups. However there appeared to be no difference in response to ULLS between supplementation groups with 11% decrease in Leu and 9% in Ala after 3 weeks. Similar changes were observed in Vastus lateralis (VL) architecture with a clear decrease in Lʃ and θ due to disuse. However the responses between supplementation groups did not differ, Lʃ decreased by 9.6% and 8.3% in Leu and Ala respectively and θ by 10.0% in Leu and 11.3% in Ala groups. No changes were seen in the quadriceps muscle volume or VL architecture of the control groups (Figure 6.1).

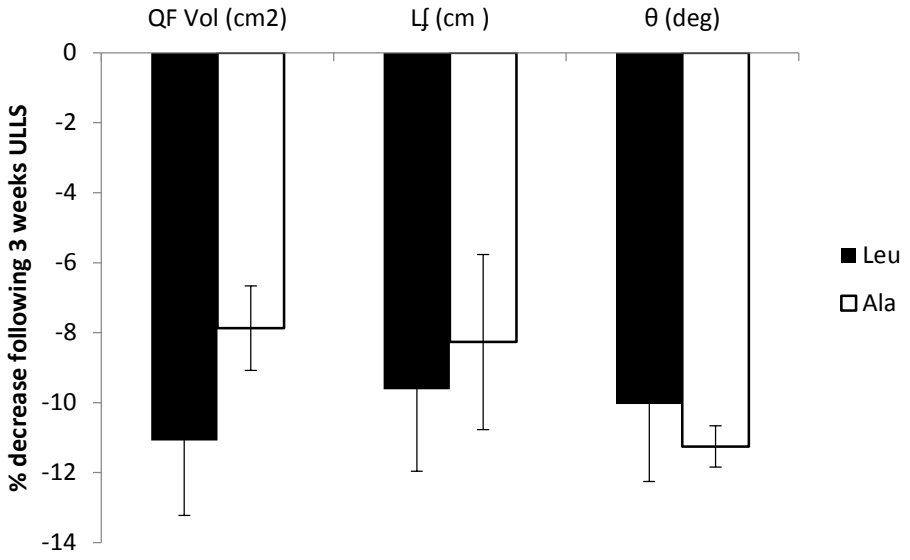


**Figure 6.1** Percentage decreases from baseline values to 3 weeks post-ULLS of QFvol Lʃ and θ. Data are means±SEM

*Muscle Function.* Maximal isometric knee extension strength (MVC) decreased by 29% and 14% in Leu and Ala groups respectively following the ULLS period. Although both groups showed a clear decrease in strength. The alanine

supplementation group appeared to have lost more strength during ULLS but this is most likely an artefact due to both a low sample size and a lower baseline MVC value in the Ala group. Voluntary activation ratio did not reach statistical significance and again there was no observed effect of supplementation. VL activation based on Root Mean Square EMG normalised to M-wave decreased by 13% in Leu 17% in Ala groups but supplementation appeared to have had no effect (Figure 6.2).

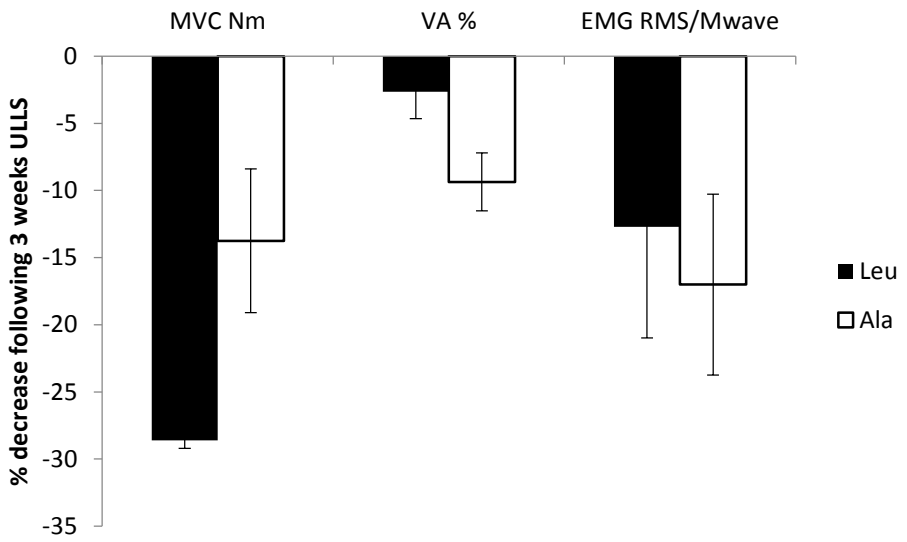


**Figure 6.2**. Percentage decreases from baseline values to 3 weeks post-ULLS of KE MVC, quadriceps voluntary activation (VA) and Vastus Lateralis EMG root mean square normalised to maximal M-wave. Data are means±SEM

PSCA did not change significantly in either Leu or Ala groups in response to ULLS. Specific force decreased 28% on Leu and 7% in Ala. While it appears that the Leu group had greater losses in SF, no statistical significance was found between groups.

*Patellar tendon dimensions and mechanical properties.* No changes were observed in patellar tendon dimensions, CSA or resting length in either supplementation group in response to ULLS nor was there a change in patellar tendon elongation. Stiffness also decreased similarly between Leu and Ala, by 26% and 24% respectively with no difference in response between supplementation groups (Table 6.2).

**Table 6. 3**. Tendon properties before and after ULLS

| | **ULLS (n=9)** | | | | **Control (n=8)** | |
|---|---|---|---|---|---|---|
| | Leu (n=6) | | Al (n=3) | | | |
| | Baseline | Post ULLS | Baseline | Post ULLS | Baseline | Post 3 wk |
| CSA $mm^2$ | 106.5±2.0 | 106.6±2.2 | 118.8±2.8 | 118.3±2.0 | 1835.2±186.4 | 1870.5±192.3 |
| Resting length mm | 47.3±1.6 | 47.2±1.9 | 51.7±1.8 | 52.1±1.1 | 1835.2±186.4 | 1870.5±192.3 |
| Elongation mm | 4.56±0.2 | 4.97±0.4 | 3.4±0.2 | 3.89±0.02 | 1835.2±186.4 | 1870.5±192.3 |
| $Max^m$ force N | 5033.5±244.0 | 3616.4±377.9 | 4935.2±309.8 | 3488.4±479.9 | 7.2±0.5 | 7.8±0.4 |
| Stiffness $Nmm^-$ | 1601.5±93.9 | 1188.0±0.6 | 1751.5±175.2 | 1345.1±224.5 | 17.1±0.5 | 16.5±1.0 |
| YM GPa | 0.71±0.05 | 0.52±0.04 | 0.76±0.08 | 0.59±0.10 | 1835.2±186.4 | 1870.5±192.3 |
| Stress MPa | 47.4±2.7 | 34.2±4.0 | 41.4±1.6 | 29.4±3.5 | 1835.2±186.4 | 1870.5±192.3 |
| Strain % | 9.75±0.6 | 10.53±0.7 | 6.59±0.2 | 7.75±0.1 | 1835.2±186.4 | 1870.5±192.3 |

Young's modulus decreased by -26% and -23% in Leu and Ala groups respectively with no difference in response between the two groups. Tendon stress also decreased but with no difference in the decreases of the supplementation groups, -29% in Leu and -30% in Ala. There was no observed change in patellar tendon strain in response to ULLS (Table 6.3, Figure 6.3).

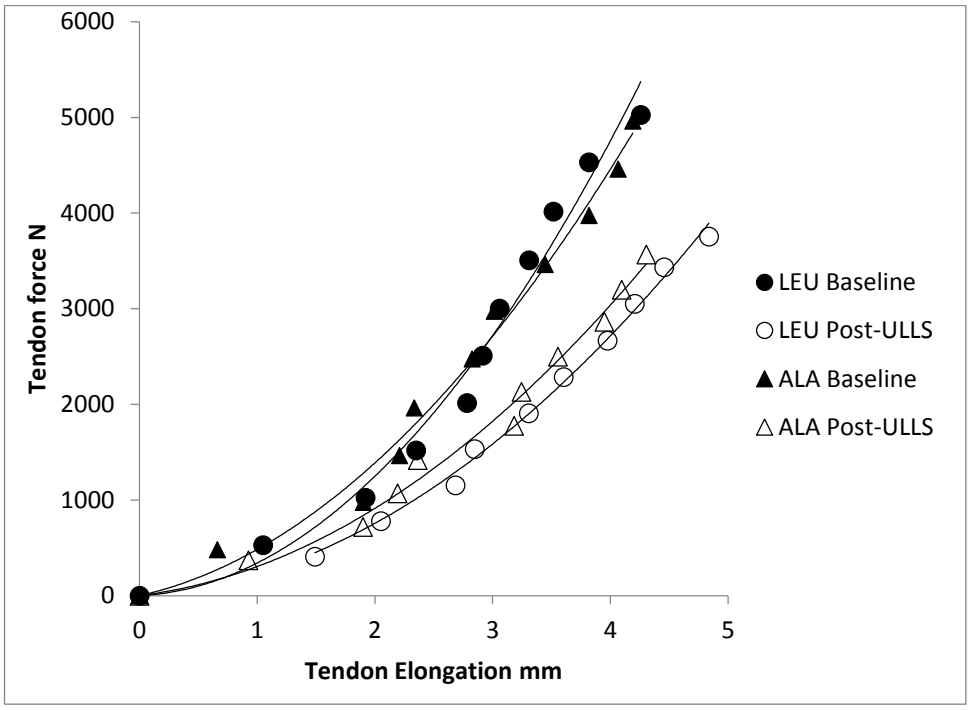


**Figure 6.3**. Patellar tendon force-elongation relationships of Leu and Ala groups at baseline and post 3 weeks ULLS

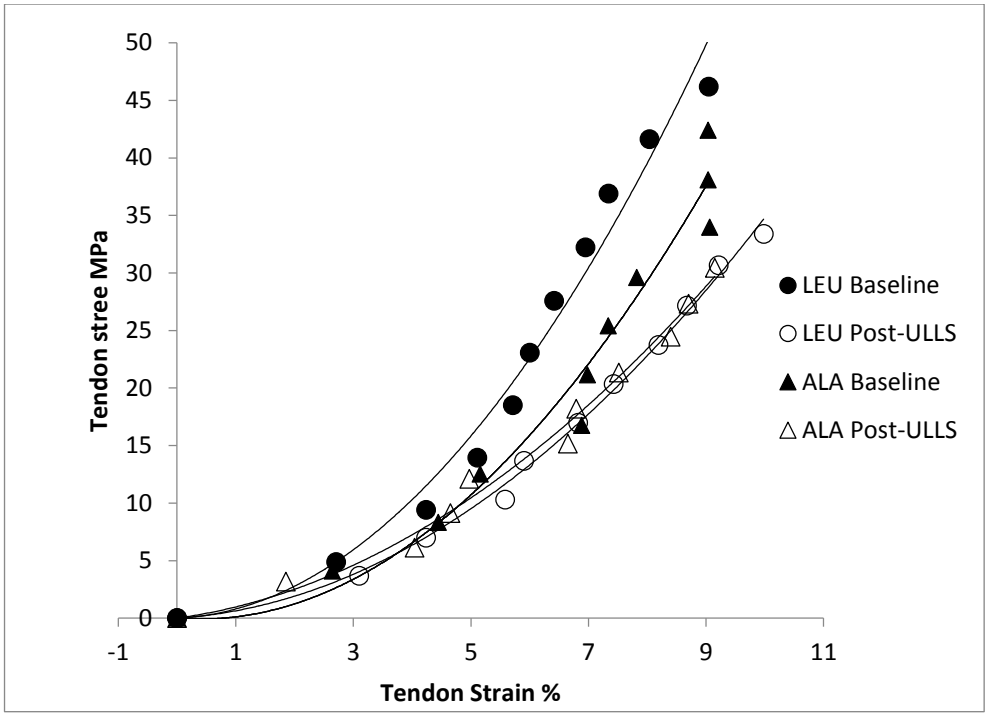


**Figure 6.4**. Patellar tendon stress-strain relationships of Leu and Ala groups at baseline and post 3 weeks ULLS

## 6.5 Discussion

The primary objective of this study was to determine whether leucine supplementation had a mitigating effect on skeletal muscle atrophy and decline in function associated with periods of immobilisation. The data shows that both groups exhibited reduced muscle function and a decline in muscle quality, however there appeared to be no significant differences in response to ULLS between LEU and ALA groups. A secondary objective was to investigate whether leucine supplementation would mitigate the decline in tendon stiffness and Young's modulus associated with disuse, by attenuating the decline tendon collagen synthesis observed during ULLS.

**Effect of Leucine supplementation on skeletal muscle disuse-induced atrophy.**

Both groups showed a change in quadriceps structure in response to the three-week ULLS period. Both groups displayed reductions in fascicle length and angle of the vastus lateralis, demonstrating a loss in sarcomeres in series and in parallel. This loss is also seen in the change in whole quadriceps muscle volume with both groups losing muscle mass after three weeks of ULLS. These results are consistent with the losses in muscle L$f$, θ and mass seen in previous limb immobilisation and disuse studies (de Boer et al.,2007, Reeves et al.,2002, Berg *et al.*,1991). As with muscle mass and architecture, there appeared to be no differences in muscle functional parameters between Leu and Ala groups. Although there appears to be a trend for a greater reduction in MVC torque in the Leu than Ala groups, this is likely to be due to the small number of participants in the Ala group (n=3) than to any actual effect of the supplementation, particularly given that no difference was observed in the structural parameters of the muscles between the groups after ULLS. Additionally, as shown in table 6.4 the change in characteristics of our Leu intervention group do not appear to differ from the results of other ULLS studies in which no nutritional intervention was involved. It is also important to note that biopsies were taken for biochemical analysis at testing sessions, and while every effort was taken to reduce their effect on the functional testing of the muscles, some residual pain or nervous damage may have persisted in individual cases and may have contributed to a discrepancy in extent of loss of muscle strength between supplementation groups that cannot be explained by similar changes in structural properties and neural activation.

*Table 6.3. Changes in quadriceps femoris characteristics following ULLS of 21-35 days. CSA is included as an indicator of loss of muscle mass where QF vol was not measured.*

| Study | Present | deBoer 2007 | Haus 2007 | Shulze 2002 |
|---|---|---|---|---|
| Days ULLS | 21 | 23 | 35 | 21 |
| Δ MVC | 29% | 21% | 24% | 17% |

| | | | | |
|---|---|---|---|---|
| Δ Vol | 11% | - | 9% | - |
| Δ CSA | - | 10% | - | 7% |

The current study indicates that the daily dose of leucine ingested by participants in the Leu group may have had little or no effect on muscle protein turnover and was insufficient to preserve muscle mass, structure and function during the ULLS period. A possible explanation for the lack of observed effect of Leu supplementation is that the disused muscle may become resistant to essential amino acid (EAA) supplementation. It has been shown that focal adhesion kinase, FAK phosphorylation decreases in immobilised human quadriceps muscle associated with reduced mechanotransduction (de Boer et al.,2007b). This decrease in FAK phosphorylation in immobilised muscle appears to be unaffected by feeding with EAA and may contribute to the relationship between reduced mechanical loading and decreased MPS rates (Glover et al.,2008). Also disused skeletal muscles of young subjects displayed similar AKT-mTOR pathway responses due to EAA feeding in that little increase in mTOR phosphorylation is observed with increases in EAA availability. In this state of anabolic resistance, fed-state gains in MPS rate which would normally balance the fasted state deficits are reduced resulting in a net decline in MPS rate. As a result, muscle protein breakdown becomes dominant and muscle disuse atrophy is not mitigated by enhanced amino acid availability (Phillips et al.,2009). Our findings support those of Brooks et al. (2010) in which amino acid supplementation alone was not sufficient to mitigate muscle loss during bed rest. However AA supplementation coupled with resistance training was much more effective suggesting that some mechanical stimulus is needed for disused muscle to use the ingested AA. Their work showed that patients receiving both AA supplementation and RT myostatin transcription was reduced with transcript levels

of the AA only group twice that of the AA plus RT group (Brooks et al.,2010). Essential amino acid may only work with a minimal level of mechanical stimulus suggesting that rehabilitation programs involving combined EAA supplementation and resistance training may be effective in reversing muscle disuse atrophy.

**Effect of Leucine supplementation on changes in Patellar tendon dimensions and properties in response to disuse-induced atrophy.**

Various studies have demonstrated significant alterations in tendon mechanical and material properties following disuse in both hind-limb suspended rat Achilles tendons (Almeida-Silveira et al. 2000; Heinemeier et al. 2009) and human patellar tendons ( de Boer et al. 2007; Kinugasa et al. 2010; Reeves et al. 2005). The results of our study supports these findings with marked reductions in patellar tendon stiffness and Young's modulus observed following three weeks of ULLS. These alterations are possibly due to changes in the structure and arrangement of collagen fibrils within the tendon tissues. Our group has previously shown that 3 weeks of ULLS induces a marked decrease in patellar tendon collagen synthesis, with a halving by day 10 and a further halving by day 21 (deBoer, 2007b). The subjects in the previous study continued with their normal diet and hence any reduced collagen synthesis experienced was a result of immobilisation rather than amino acid availability. Decorin, an SLRP abundant in the extracellular matrix related to water content and flexibility in tendon plays an important role in the formation of collagen fibrils and the regulation of fibre diameter and hence is likely to influence tendon properties. The theory behind Leucine supplementation as a means to preserve tendon mechanical properties in disuse relies upon the assumption that dietary Leucine acts as a raw material for the production of SLRP's such as decorin. It has been previously shown that a Leucine rich diet can improve tendon mechanical

properties in malnourished rats, especially in combination with exercise (Barbosa et al. 2010), however in the current study supplementation with dietary Leucine did not maintain tendon properties throughout the period of disuse. The supplementation of further Leucine in addition to normal dietary intake does not appear to increase collagen synthesis rates to a level capable of maintaining the homeostatic balance of tissue remodelling necessary for tendon functional stability, particularly in the absence of physical activity. Despite the previously shown decrease in tendon collagen synthesis in response to ULLS (de Boer et al. 2007) the present study showed no change in patellar tendon dimensions.

On consideration of the above results it appears that the daily dose of leucine had no substantial effect on muscle or tendon protein turnover and was not sufficient to preserve structure or function during the ULLS period. However, given the low number of participants in the Ala supplementation, further investigations with larger sample sizes are required to estimate the placebo effect of supplementation with more accuracy.

# 7. STUDY 3. THE EFFECT OF AMINO ACID SUPPLEMENTATION ON RESISTANCE TRAINING BASED RECOVERY OF IMMOBILISATION-INDUCED ATROPHY IN YOUNG SUBJECTS.

## 7.1 Summary

The previous study showed that Leu supplementation alone was not enough to increase MPS in disused skeletal muscle. In this study, we investigated the hypothesis that leucine supplementation coupled with resistance training improves the rate and extent of recovery following ULLS in young subjects. Eight participants that had undergone three weeks of ULLS participated in the study that was included in the ethical approval for study 1 (Ref. FAETC/08-09/13). Participants underwent three weeks of resistance training targeting the quadriceps of the limb suspended in study one. Participants were assigned to either a Leu supplementation group or an Ala placebo group. Due to dropouts, the Ala had a small sample size of 2 and therefore the utilisation of robust statistical testing was not possible. However, here we report the results and comment on observed trends.

## 7.2 Introduction

Most studies into the mechanism of skeletal muscle atrophy resulting from periods of immobilization or disuse in humans have concluded that muscle wasting is caused by a net catabolism of muscle proteins. This imbalance in protein turnover is thought to come about due to a decline in muscle protein synthesis rather than an increase in muscle proteolysis. As a result, investigations into countermeasures of disuse

atrophy have been focused on methods of increasing muscle protein synthesis with particular focus on resistance exercise and/or nutritional supplementation. The present study assesses the efficacy of a combined resistance training protocol and amino acid (Leucine) supplementation on the active recovery of atrophied skeletal muscle and tendon following disuse.

**Resistance Training**

Changes seen in ageing and disuse can be attenuated and even reversed by increasing muscle chronic loading. Resistance training is a widely accepted and practised method of increasing muscle mass, strength and power. Muscle architectural parameters such as VL fascicle length and pennation angle have been shown to increase by 2.4% and 9.9% respectively after 35 days training in young recreationally active volunteers (Seynnes *et al.* 2007). Increases in the morphological characteristics of muscles at both the architectural level and whole muscle volume and changes in patellar tendon moment arm will ultimately affect the force producing capabilities of a muscle by altering the physiological cross sectional area of a muscle (PSCA) and specific force. Following a nine week resistance training programme targeting the quadriceps femoris muscles resulted in an increase in PCSA of 5.5% and specific force of 20.1% in young untrained males between the ages of 18-39 (Erskine *et al.* 2010).

While the increases in muscle architectural parameters as a result of resistance training are considerable, the increase seen in muscle strength are generally much larger, 28.9% after 35 days (Seynnes *et al.* 2007) and 30% after 9

weeks resistance training (Erskine *et al.* 2010). This relative greater increase in muscle strength is largely attributable to neural adaptations. Mirroring the direction of changes in muscle EMG activity seen in disuse, an increase is observed following resistance training programs, by as much as 30% in young subject undergoing 30 days of resistance training (Seynnes *et al.* 2007).

The exact cellular and molecular mechanisms of skeletal hypertrophy in response to resistance training are still largely uncovered. However, recent studies have shown in the past thirty years that the protein kinase B/mammalian target of rapamycin, AKT/mTOR signalling pathway plays a major role in the regulation of skeletal muscle growth. Both AKT and mTOR are phosphorylated during muscle hypertrophy in response to load-induced training (Baar & Esser 1999; Bodine et al. 2001; Urso 2009). The phosphorylation and activation of the AKT/mTOR pathway leads to the activation of $P70^{S6K,}$ which has been shown to be correlated with percentage increase in muscle mass after 6 weeks resistance training indicating that $P70^{S6K}$ phosphorylation is involved in the adaptation of skeletal muscle to chronic resistance training (Baar and Esser 1999). Interestingly, the AKT/mTOR pathway has also been shown to be down regulated during muscle atrophy due to both disuse and denervation (Pallafacchina et al. 2002), which suggests that activating the AKT/mTOR pathway during muscle disuse could provide a means of attenuating skeletal muscle disuse-atrophy.

It is through its interaction with the AKT/mTOR pathway that Leucine has become the subject of many studies examining means of increasing muscle protein synthesis (MPS). Many studies have reported increased in MPS due to increased Leucine availability in both humans and animal models (Koopman et al. 2005; Wolfe 2002; Paddon-Jones et al. 2004; Trappe et al. 2007). Human tendon

mechanical properties can also be modified by following resistance training protocols. In rabbits that underwent 4 weeks of exercise training, the load and energy absorbed at failure was much higher than in rabbits which had not underwent any exercise training (Viidik 1967). In vivo studies have shown that collagen synthesis in human Achilles' and patellar tendon increase acutely after a bout of endurance exercise and to remain at an elevated rate up to 72 hrs after exercise (B. F. Miller et al. 2005). Human tendons also show in vivo biochemical changes as a result of exercise training. The fractional synthetic rate of tendon collagen synthesis has been shown to increase to a peak increase at 24 hrs post exercise of 1.7 times greater than that of resting tendon. After this time, the collagen synthesis rate decreased but was still significantly elevated 72 hrs post–exercise (Miller *et al* 2005). Tendons of both young and older individuals have shown increases in stiffness and Young's modulus in response to strength training indicating that resistance training as a countermeasure to muscle disuse atrophy and aging is important with regard to tendon as well as skeletal muscle.

Whilst many studies have investigated the effects of mixtures of essential and non-essential amino acids on atrophied human skeletal muscle, there are few studies on human subjects which evaluate the isolated effects of a leucine supplement and even fewer in which the direct effects of leucine on muscle and tendon functional parameters have been studied. It would be expected that while leucine supplementation may affect muscle mass, additional factors affecting muscle function e.g. neural drive would not be directly affected by amino acid supplementation and so the muscle function would not be directly affected by leucine per se.

### 7.3 Methods

Eight participants that had undergone three weeks of ULLS participated in the study that was included in the ethical approval for study 1 (Ref. FAETC/08-09/13). Participants underwent three weeks of resistance training targeting the quadriceps of the limb suspended in study one.

The study was approved by Manchester Metropolitan University Faculty of Science and Engineering Research Ethics Committee (Ref. FAETC/08-09/13) and participants were excluded from the study if they suffered from or have ever suffered from previous stroke, uncontrolled cardiovascular disease, motor neurone disease, Parkinson's disease, medically diagnosed osteoporosis, type II diabetes, hypertension or myocardial infarction within the previous two years, acute febrile or systemic disease within the past two years or are currently taking beta blockers. Participants were advised on early signs of deep vein thrombosis in the early sub-clinical phase and were monitored weekly using Doppler ultrasonography in order to detect any signs of the possible occurrence of DVT.

The ULLS test group consisted of 17 participants who were randomly assigned to an essential amino acid leucine (Leu) intervention group (n=9) and non- essential amino acid alanine (Ala) group (n=8), however due to participant withdrawal from the project for various reasons (see appendix 1), nine participants completed the three week suspension period and subsequent testing (Leu n=6; Ala n=3). A further drop out prior to training protocol resulted in a test group of 8 participants (23±2.5 yrs: 76.9±10.9 kg), 6 in the leucine group and 2 in the alanine group.

Prior to the testing and suspension period participants visited the IRM to be familiarised with the procedures involved in the testing protocol. Participants were

shown how to perform maximum voluntary contractions (MVC) and slow ramp contractions (SRC) and were introduced to electrical stimulation used in M-wave and interpolated twitch procedures. All baseline data was collected prior to the ULLS period. Blood samples were taken in a fasted state before 11am and prior to any testing involving exercise. During the testing sessions ultrasound and MRI scans were taken before dynamometer testing to rule out any slight changes in muscle structure or size due to swelling after exercise. Once the scans were complete participants were transferred to dynamometer to perform the strength and neurophysiological tests.

*Amino Acid Supplementation.* Participants undergoing ULLS were randomly assigned to either a leucine intervention groups or an alanine placebo group.

*Unilateral Lower Limb Suspension procedure.* Participants underwent unilateral lower limb suspension (ULLS) (Berg et al.,1991) for three weeks to induce atrophy of the right limb.

Ultrasound scanning and MRI were used to assess muscle architecture and volume respectively. Maximum knee extension and knee flexion torque were measured using an isokinetic dynamometer at varying angles of knee flexion. Surface EMG of the Vastus Lateralis muscle (VL) and biceps femoris (BF) was recorded throughout the functional muscle test performed on the dynamometer.

Patellar Tendon Mechanical Properties using both ultrasound scanning and MRI techniques.

Participants underwent a three week resistance training protocol.

***Statistics***

Normal distribution of the days was confirmed by Shapiro-Wilks test for normality. Data were analysed using a Mixed ANOVA as we have both a within-subject factor (the repeated measure time, Pre and Post RT measurements) and a between subject factor (supplementation, Leucine or Alanine). Where Mauchley's test of sphericity was violated Greenhouse-Geisser corrected F ratios were used for significance level. Due to drop outs, the remaining small sample size of the alanine group prevented robust statistical analysis between supplementation groups. However, below we report the data and comment on the observed trends.

### 7.4 Results

*Quadriceps muscle volume and architecture*. Following 3 weeks of RT active recovery quadriceps muscle volume increased significantly in both supplementation groups. However, there did not appear to be a difference in the extent of response to RT between supplementation groups with 13% increase in Leu and 10% in Ala after 3 weeks active recovery. Vastus lateralis muscle fibre architecture displayed a similar pattern with an observed significant increase in Lʃ and θ of both groups following active recovery. However the responses again were not observed to differ, Lʃ increasing by 8.5% and 5.2 in Leu and Ala respectively and θ increasing by 5.5% and 12.4% in Leu and Ala (Figure 7.1).

**Table 7.1**Quadriceps muscle volume and VL architecture at Post ULLS and Post-RTin both Leu and Ala groups. Data are means±SEM *significantly different between Leu and Ala groups $p<0.05$ ** significantly different between Leu and Ala $P<0.005$

| | Leu (n=6) | | Al (n=2) | |
|---|---|---|---|---|
| | Post-ULLS | Post-RT | Post-ULLS | Post-RT |

| | | | | |
|---|---|---|---|---|
| Whole QF vol ($cm^2$) | 1754.8±103 | 2024.2±163 | 1818.9±121 | 2011.8±137 |
| VL Lʃ (cm) | 7.7±0.4 | 8.4±0.2 | 7.7±0.6 | 8.1±0.9 |
| VL θ (degrees) | 11.3±0.6 | 11.9±0.6 | 12.2±0.002 | 14.0±0.7 |

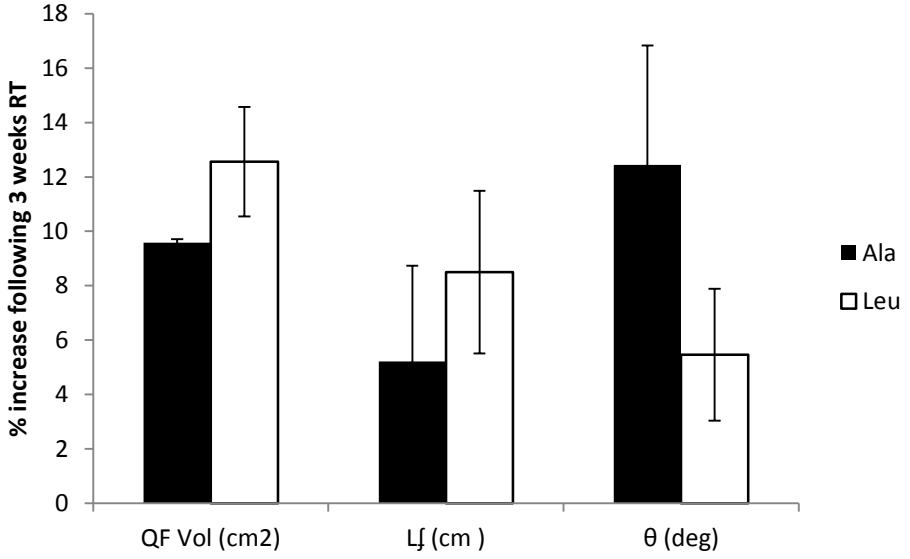


**Figure 7.1**. Percentage increases of post-ULLS and post-RT values of QF vol Lʃ and θ. Data are means±SEM *significantly different between Leu and Ala groups $p<0.05$

*Muscle Function.* Maximal isometric knee extension strength (MVC) increased by 24% and 22% in Leu and Ala groups respectively following in response to the resistance training protocol. Although both groups showed a significant increase in strength there was no significant difference in the strength gains of Leu and Ala supplementation groups). Changes in voluntary activation ratio did not reach statistical significance in either supplementation group). VL activation based on Root Mean Square EMG normalised to M-wave increased by 13% in Leu17% in Ala groups but supplementation appeared to have no effect (Table 7.2, Figure 7.2).

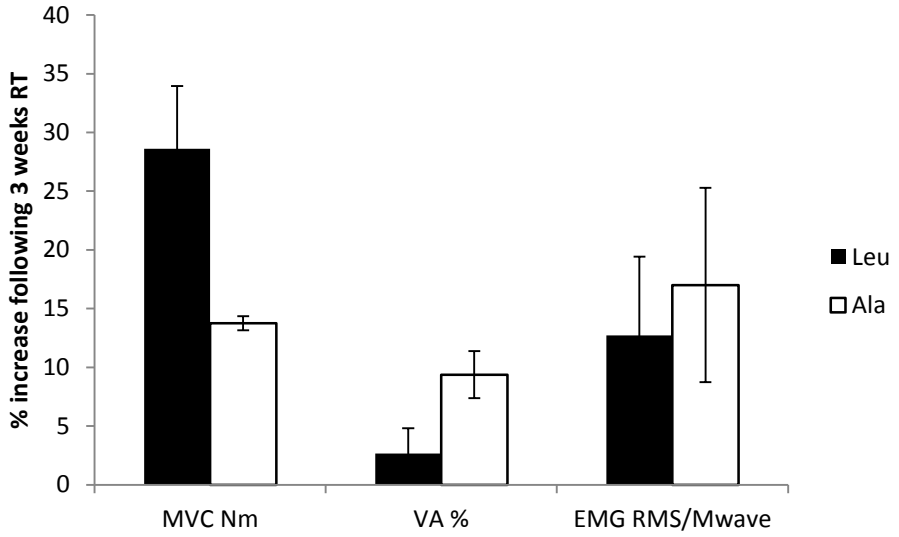


**Figure 7.2**. Percentage increases of post-ULLS and post-RT values of MVC, VA and EMG. Data are means±SEM *significantly different between Leu and Ala groups $p<0.05$

**Table 7.2** Muscle Strength and activation Post ULLS and Post RT in both Le and Al groups. Data are means±SEM *significantly different between Leu and Ala groups $p<0.05$

| | Leu (n=6) | | Al (n=2) | |
|---|---|---|---|---|
| | Post-ULLS | Post-RT | Post-ULLS | Post-RT |
| MVC (Nm) | 225±19 | 297±18 | 212±14 | 271±22 |
| Voluntary activation (%) | 82±5 | 86±4 | 84±3 | 94±0.3 |
| RMS/M-Wave | 0.05±0.02 | 0.07±0.02 | 0.09±0.04 | 0.09±0.02 |

*Patellar tendon dimensions and mechanical properties.* No changes were observed in patellar tendon dimensions, CSA or resting length in either supplementation group in response to the RT protocol. The same was observed for elongation of the patellar tendon. Patellar tendon stiffness increased significantly in Leu and Ala, by 20% and

21% respectively with no difference in response between supplementation groups (Table 7.3,Figure 7.3).

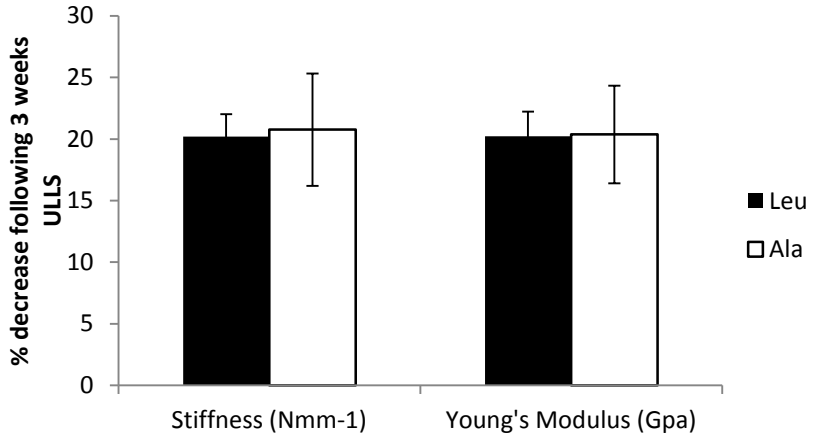


**Figure 7.3**. Percentage increases from baseline values to 3 weeks post-RTS of patellar tendon stiffness and Young's modulus. Data are means±SEM

**Table 7.3** Tendon properties in both Leu and Ala groups after ULLS and after RT

| | Leu (n=6) | | Al (n=2) | |
|---|---|---|---|---|
| | Post-ULLS | Post-RT | Post-ULLS | Post-RT |
| CSA $mm^2$ | 106.9±2.2 | 107.3±2.2 | 118.3±2.5 | 119±2.5 |
| Resting length | 47.2±1.9 | 47.5±2.0 | 52.1±1.4 | 52.1±1.8 |
| Elongation mm | 4.8±0.4 | 4.1±0.4 | 3.9±0.02 | 3.7±0.04 |
| Stiffness $Nmm^{-1}$ | 1188±91 | 1488±103 | 1345±275 | 1683±250 |
| Young's modulus GPa | 0.52±0.04 | 0.66±0.05 | 0.63±0.12 | 0.74±0.11 |
| Stress (MPa) | 34.2±4.0 | 44.0±3.4 | 29.4±4.3 | 36.6±2.6 |
| Strain (%) | 10.5±0.7 | 8.7±0.9 | 7.5±0.2 | 7.1±0.3 |

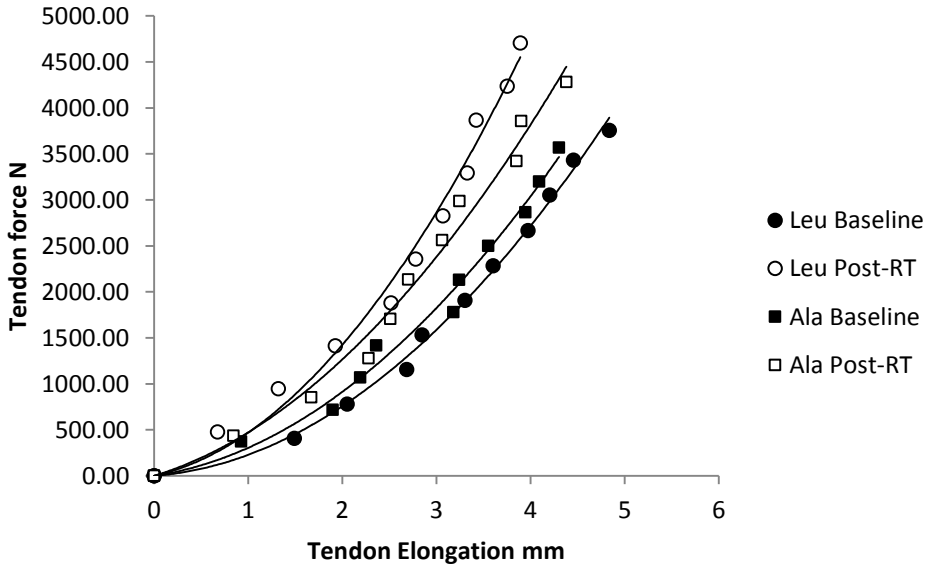


**Figure 7.4**. Patellar tendon force-elongation relationships of Leu and Ala groups at baseline and post 3 weeks RT

Young's modulus increased by 20% in both Leu and Ala groups. Patellar tendon stress also increased by 19% and 16% in Leu and Ala groups respectively but with no difference in the decreases of the supplementations groups, -29% in Leu and -30% in Ala.

While there was an observed decrease in patellar tendon strain in both supplementation groups it did not reach statistical significance (Table 7.3, Figure 7.5).

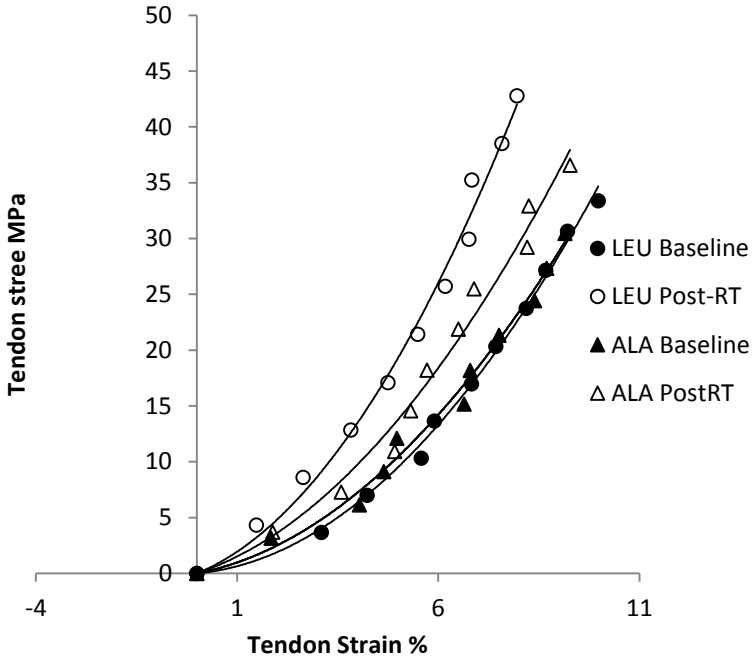


**Figure 7.5**. Patellar tendon stress-strain relationships of Leu and Ala groups at baseline and post 3 weeks RT

## 7.5 Discussion

We have previously reported that all the neuromuscular parameters affected during the ULLS model of disuse can be fully restored after a resistance training intervention of equal duration (Campbell et al. 2013). The present study was designed to determine if active recovery through resistance training coupled with leucine supplementation had a greater effect on post-immobilisation recovery than resistance training alone. The data shows that while both Leu and Ala supplementation groups regained muscle mass and function similar to pre-

immobilisation levels, there did not appear to be any difference in the response observed between supplementation groups.

**Effect of combined Leucine supplementation and resistance training on skeletal muscle structure and function.**

The data clearly show that 3 week resistance training protocol resulted in significant increases in whole quadriceps muscle volume and L$f$ and θ of vastus lateralis. Muscle strength was also recovered to pre-immobilisation levels in both Ala and Leu supplementation groups. What is also evident however, is that there is no significant difference in the responses of the different supplementation groups to recovery through resistance training for any of the variables measured. The previous study shows that supplementation with Leu during a 3 week period of disuse was not sufficient to counteract the atrophic effect of lower limb immobilisation. It is well known that physical activity during recovery increases muscle protein synthesis and improves sensitivity to anabolic stimulus (Tipton 2013). This study examined the hypothesis that with this increase in anabolic sensitivity, Leu supplementation could further increase the recovery of skeletal muscle structure and function seen in active recovery programs utilising resistance training alone.

As previously demonstrated, full recovery of skeletal muscle structure and function following short-term unloading via resistance training is possible in young men (Campbell et al. 2013; Suetta et al. 2009). To accomplished this, net MPS rates must be increased from the reduced rates seen in atrophic muscle. Resistance exercise is a well-documented method of increasing MPS in healthy individuals (Biolo et al. 1995) although increasing MPS does not solely rely upon anabolic stimulus but is also dependent upon adequate substrate availability as

protein deficient meals do not stimulate MPS in the manner observed following the ingestion of balanced meals (Yoshizawa et al. 1998; Phillips et al. 1997). As the removal of Leu can prevent stimulation of MPS by amino acid ingestion, is not merely through availability that amino acids stimulate MPS. Essential amino acids and Leucine in particular, act as both substrates and signals for muscle protein synthesis. Leucine has been shown to enhance the exercise-induced phosphorylation of enzyme P70-S6 kinase, a ribosomal protein important in muscle hypertrophy (Blomstrand et al. 2006). Leucine has been shown to effect MPS through the activation of mTOR and through insulin-dependent mechanisms (Stipanuk 2007). This would suggest that Leu supplementation would increase the recovery response of skeletal muscle to RT following the immobilisation period however in the present study this was not the case. The combination of RT and EAA supplementation appears to have a greater effect on individuals with a decreased energy intake then just EAA alone (Brooks et al. 2008). However, in our study participants did not alter their normal dietary habits and subsequently any Leu ingested was likely to have been in excess to the protein requirements of skeletal muscle.

There is no doubt nutrition, particularly protein intake is important in recovery, but the literature surrounding the relationship between protein intake and resulting effects on skeletal muscle protein synthesis is inconsistent. It would succeed that there exists a limit as to how much protein the body can synthesise in response to increase amino acid availability and our study appears to show that amino acid supplementation does not further increase skeletal muscle hypertrophy due to RT when protein requirements are met via normal dietary protein intake. This hypothesis is in concordance with the results of a previous study examining the effect on MPS after the ingestion of whey protein. Forty-five to 90 minutes

following the ingestion of a whey protein bolus, myofibrilar MPS increased threefold before rapidly returning to basal levels despite continuing increased intramuscular and plasma leucine levels (Atherton et al. 2010). It would appear that skeletal muscles are not able to exponentially increase the MPS rate and that a maximum threshold does exist. Any amino acids ingested above this threshold are counterproductive in terms of increasing muscle mass and improving function.

***Effect of Leucine supplementation on changes in Patellar tendon dimensions and properties in response to resistance training based recovery of immobilisation-induced atrophy in young subjects.***

Unlike the quadriceps muscle the patellar tendon did not display any change in dimensions in response to the 3 weeks resistance training protocol. Previous studies have shown region specific increases in patellar tendon CSA however these results were in response to much longer training programs, 9 – 12 weeks. It is not surprising that the patellar tendon displayed much less adaptation in terms of dimensions and volume than the quadriceps muscle given their lower metabolic rate and reduced vascularity. However, plasticity of human tendons means that the mechanical properties can be modified by following resistance training protocols. This has been demonstrated in recent studies in young individuals in which observed increases in PT stiffness range from 15% - 24% following prolonged resistance training protocols of at least 9 weeks (Kongsgaard, 2007). Our results show that disused tendons can recover mechanically relatively

quickly when subject to active recovery through resistance training. Although stiffness and Young's modulus of the patellar tendon did not fully recover to baseline

levels after 3 weeks resistance training, significant increases of 20% and 21% of stiffness were found in Leu and Ala supplementation groups respectively. Young's modulus also increased by 20% in both supplementation groups. However, none of the tendon functional parameters measured showed any difference in response between the supplementation groups. Despite the knowledge that healthy tendons respond well to resistance training, little is known about the effect of resistance training on deconditioned tendons in the early post-immobilization phase. Following consideration of our results it would appear that there is benefit in commencing active recovery sooner rather than later as significant improvements are evident in just 3 weeks post-immobilisation. This would however be limited to injuries or surgery not directly involving the tendon itself where there is no need to limit movement to prevent stretching of the repaired tendon or allow scar tissue to form. Nevertheless, additional research is needed to investigate further the potential of early post-operative resistance training on tendon recovery post immobilisation.

The literature surrounding the response of tendon and other connective tissues is very conflicting, however most investigators report that human tendons are not sensitive to feeding (Babraj *et al.*; Wackerhage and Rennie 2006; Smith and Rennie 2007). Although the results of the present study show no difference in response of human patellar tendon to resistance training in either Leu or Ala supplementation groups, it would be presumptuous to state that essential amino acid availability has no absolute effect on tendon responses to training given the relatively short active recovery period of 3 weeks. It is also feasible that like skeletal muscle, tendon tissues also have a maximum threshold for increased collagen synthesis. It has been shown that in malnourished states leucine, in combination with increased insulin plasma levels inhibits proteolysis and stimulates muscle protein synthesis in rats (Ventrucci

et al. 2004). Additionally, it is thought that leucine might act as a substrate for the production of decorin which is important in the cross-linking of tendon collagen fibrils. Subsequently a leucine rich diet can improve tendon biomechanical properties in the tendons of malnourished rats (Barbosa, 2010) and it would be reasonable to assume that the same would hold true for human tendons in malnourished or protein deficient individuals. Given that our participants were not subject to malnourishment or reduced protein intake, it is possible that if indeed there is a maximum threshold for increasing tendon collagen synthesis via increased amino acid availability it may have already been reached by normal dietary intake of essential amino acids. To that end, any increase in tendon collagen synthesis and subsequent improvements in mechanical properties are likely due to the increased physical activity rather than amino acid supplementation which is reflected in the results above.

# 8. Investigating the role of training and immobilization in sarcopenia and the development of an image based biomarker.

## 8.1 Summary

Sarcopenia is an ageing-related loss of muscle mass that has particularly important functional consequences and has come to be regarded as a major cause of frailty and reduced loco motor ability amongst the ever increasing elderly population (Narici and Maganaris 2006). Beyond the sixth decade, most individuals undergo changes in the nervous system, in hormonal status, in immune function and in dietary intake (Doherty 2003), all of which may result in atrophy and weakness of the musculoskeletal system (Roubenoff and Hughes 2000). As reductions in muscle mass and strength are observed even in master athletes who have trained throughout life, the process of sarcopenia is therefore universal with ageing (Roubenoff 2000).

In this study, we look at cross sectional data from both young and old individuals subjected to different loading conditions. Life-long training appears to slow down the process of sarcopenia whilst periods of disuse due to injury or disease worsen the condition. The change in muscle fibre architecture in sarcopenia is affected by altered loading conditions. The resulting change in muscle fibre geometry could potentially act as a convenient and inexpensive indicator of the onset of sarcopenia.

## 8.2 Introduction

Sarcopenia is a difficult condition to diagnose as no broadly accepted clinical definition exists (Cruz-Jentoft *et al.* 2010). Current guidelines by the European

Working Group on Sarcopenia in Old People, EWGSOP recommends that assessment of the condition to reference both low muscle mass and impaired muscle function. Appendicular skeletal muscle mass corrected for body height, the Skeletal Muscle Index, is a generally accepted diagnostic marker for sarcopenia. An SMI two standard deviations below the mean SMI of young healthy reference groups is used as a cut-off point for the diagnosis of sarcopenia (Baumgartner et al. 1998). To include impaired muscle function in the diagnosis of sarcopenia is difficult as most individuals will not have been previously tested by means of determining muscle function clinically e.g. dynamometry or EMG, which means they have no baseline measurements for comparison. Comparing to an age group mean is slightly misleading as no account is made for previous training or circumstances earlier in life which my skew the comparison, for example a life-long training athlete may have a marked reduction in muscle function yet still be capable of MVC's much higher than the average MVC of an age matched population. However, as strength does not soley depend on muscle mass, a diagnosis based on just one factor alone is too narrow for clinical use and hence muscle mass and function must both be considered. As declined physical function is usually the more prominent and obvious effect of sarcopenia, a more practical method of determining declined skeletal muscle function is the use of everyday tasks incorporated into a short physical performance battery (SPPB). Developed by the National Institute of Health, the SPPB assesses gait speed, balance, and the chair stand, three manoeuvres which are important components of everyday activities and have a great impact upon quality of life and the ability to live independently. A major benefit of the SPPB over more technical methods of muscle assessment such as dynamometry is that it can be

performed without the need for expensive, highly specialised equipment in both the clinic and the patients home.

In contrast, most current methods of estimating skeletal muscle mass rely on cumbersome techniques requiring specialised equipment. Body imaging techniques such as magnetic resonance imaging (MRI), computed tomography (CT) and dual energy X-ray absorptiometry (DXA) are considered 'gold standards' when estimating skeletal muscle mass due their ability to precisely differentiate between different tissues. However, high costs and limited availability of equipment make them unsuitable for routine clinical assessment, particularly with the elderly who are less able to travel to specialised centres and unable to get in and out of the cumbersome scanners. Portable methods of estimating skeletal muscle mass that can be performed without specialist machinery in GP practices or even patients home would allow clinicians or health care professionals to assess both muscle function via SPPB and skeletal muscle mass together in a short period of time with minimum disruption to patients. Currently bioelectrical impedance and anthropometry can be performed quickly however, both are not without error. For instance, bioelectrical impedance tests can be affected by altered hydration statuses of individuals which can influence estimation of the ASM and may also overestimate fat free mass in heavier individuals (Roubenoff et al. 1997). Anthropometric estimation is vulnerable to changes in fat deposition which are known to occur with age as well as changes in hormonal status and are therefore not considered accurate enough for a clinical diagnosis.

As well as the decrease in muscle mass that accompanies old age (anatomical cross-sectional area ACSA and volume based on MRI scanning), (Janssen et al, 2000, Morse *et al.*, 2004, Narici and Maganaris ), muscle architecture is also

significantly altered in sarcopenia. Findings based on ultrasound scanning of older and younger individuals with similar daily energy expenditures have shown fibre fascicle length ($L_f$) and pennation angle ($\theta$) of the gastrocnemius medialis (GM) of older men (age 70+ yr) measured *in vivo* to be 10% and 13% smaller than those of younger men (20-30 yr) respectively (Narici *et al.*, 2003). The relatively smaller $L_f$ and $\theta$ seen in the skeletal muscle older individuals suggest that normal biological aging is associated with a loss of sarcomeres both in series and in parallel which may account for some of the loss of muscle force and power observed in older age. Skeletal muscle architecture is assessed using ultrasonography, a process which is much quicker and less expensive than other body imaging techniques e.g. MRI. Additionally, the availability of small portable ultrasound machine enable skeletal muscle architecture to be assessed anywhere and allows the patient to remain in comfortable surroundings e.g. in bed at hospital or at home.

In this study VL muscle architecture assessed by ultrasonography ($VL_{Lf}$, $VL_{\theta}$ and $VL_t$) is analysed to determine if changes in VL architecture can be used as a quick and inexpensive diagnostic marker of sarcopenia. Data from different age groups and athletic training capabilities were analysed to compare architectural parameters of individuals displaying reduced functional capacity due to sarcopenia of normal ageing and sarcopenia exacerbated by injury or disuse. Master athletes were also assessed to examine differences in elderly recreationally active individuals to those who engage in regular physical training.

## 8.3 Methods

Data was collected from a total of 92 individuals including 24 young untrained (YU) males 18-35 yrs and 27 older recreationally active males and females (OA) 65yrs+ from the local population. Twenty-four male and female master athletes competing at the 2010 European Veteran Athletic Championships (MA) 65yrs+ were also assessed as well as 17 older frail males and females (OF) awaiting hip arthroplasty surgery 65yrs+.

Ultrasound scanning was used to assess muscle architecture at rest with the subject lying in the supine position. Images of muscle architecture (pennation angle θ and fascicle length L$f$) at rest of all four heads of the quadriceps femoris were taken distally, at distance corresponding to 40% of femur length (rectus femoris scans were taken more proximally, at 50% of femur length). Images were analysed offline to assess muscle architecture. Muscle thickness was defined as the shortest distance between the superficial and deep aponeurosis. Pennation angle was measured as the angle between fascicles and the deep aponeurosis and fascicle length was measured by outlining the course fascicles between aponeuroses (figure 4.2, chapter 4). For the latter, any fascicles which were not fully visible within the width of the ultrasound probe were extrapolated as a straight line (Seynnes, 2008). An average of three measurements was calculated for each architectural parameter and retained as the final value.

**Statistics**. Data are reported as means ± standard deviation and were analysed by a one-way ANOVA followed by Tukey's post hoc test where homogeneity of

variances was met and Games-Howell where it was not, for further analysis. Statistical significance level was set at $p<0.05$.

## 8.4 Results

### Changes in muscle structure and function between young and older groups.

**Table 8.1** Vastus lateralis thickness, fascicle length and pennation angle and quadriceps MVC in YU, MA, OA and OF groups

| | **Young Untrained** | **Master Athletes** | **Older Active** | **Older Frail** |
|---|---|---|---|---|
| $VL_t$ cm | 2.12±0.1 | 1.75±0.1 | 1.61±0.1 | 1.09±0.1 |
| VL L$f$ cm | 8.12±0.2 | 7.57±0.2 | 7.24±0.2 | 5.89±0.2 |
| VL θ | 14.56±0.6 | 13.15±0.5 | 11.3±0.5 | 9.68±0.4 |
| MVC Nm | 276.52±11.5 | 177.15±9.0 | 161.4±8.7 | -- |

*Muscle Thickness*. VLt was 17.5% lower in MA than in YU ($p=0.01$) compared with 24.1% lower in OA ($p<0.001$). VLt in OA was 8% lower than MA ($p=0.417$). The OF group VLt was significantly lower than all other groups ($p<0.001$), OA by 32.3%, MA by 37.7% and YU by 48.6% (Figure 8.1).

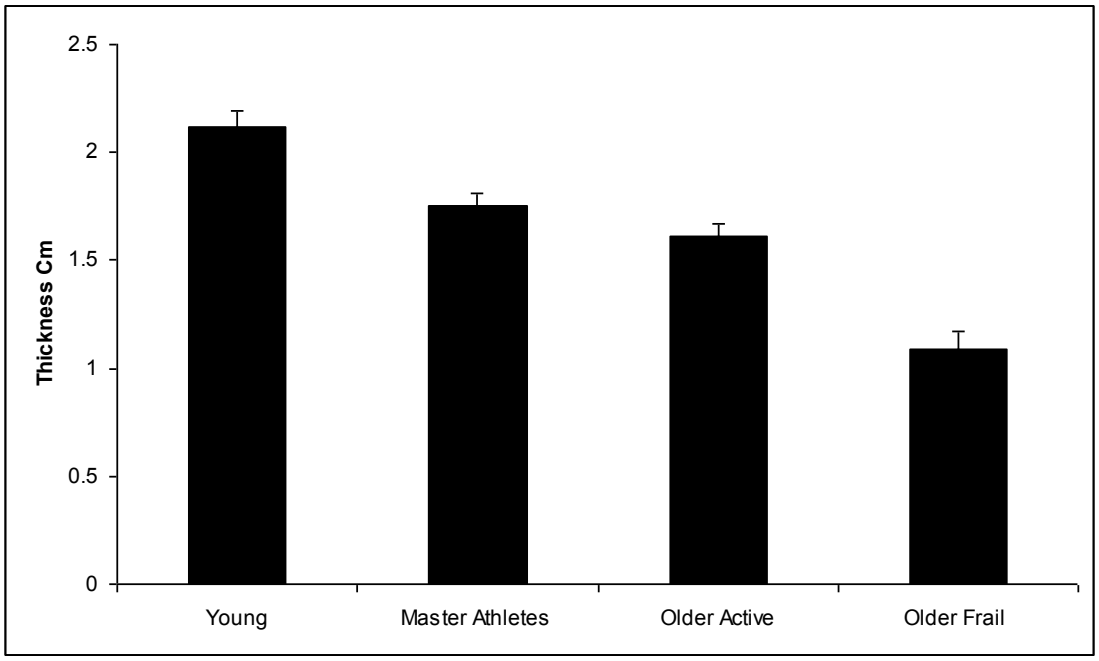


**Figure 8.1** VL muscle thickness in YU, MA, OA and OF. Data are means±SEM.

*Fascicle Length*. VLL*f* was 6.8% lower in MA than in YU (p=0.295) compared with 10.5% lower in OA (p<0.021). VLt in OA was 4.4% lower than MA (p=0.675). The OF group VLt was significantly lower than all other groups, OA by 8.9% (p=0.005), MA by 22.2% (p=0.001) and YU by 27.5% (p<0.001) (Figure 8.2).

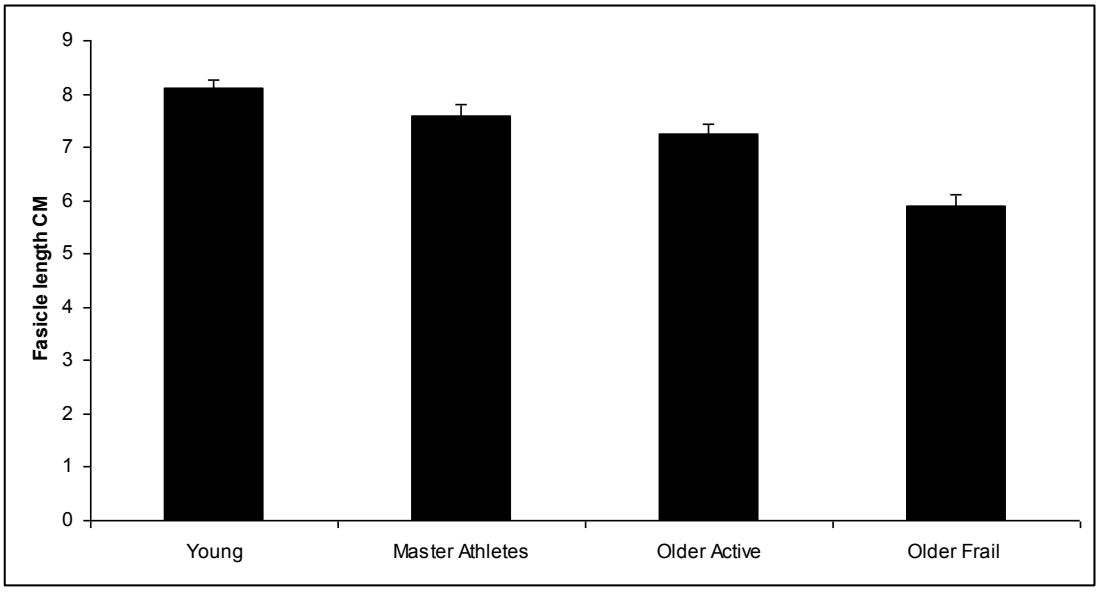


**Figure 8.2** VL fascicle length in YU, MA, OA and OF. Data are means±SEM

*Pennation Angle.* $VL_\theta$ was 9.7% lower in MA than in YU (p=0.299) compared with 22.3% lower in OA (p=0.001). $VL_\theta$ in OA was 14% lower than MA (p=0.063). The OF group $VL_\theta$ was significantly lower than MA by 26.4% (p<0.001) and YU by 33.5% (p<0.001). The difference in $VL_\theta$ between OF and OA failed to reach statistical significance, a difference of 14.3% (p=0.057) (Figure 8.3).

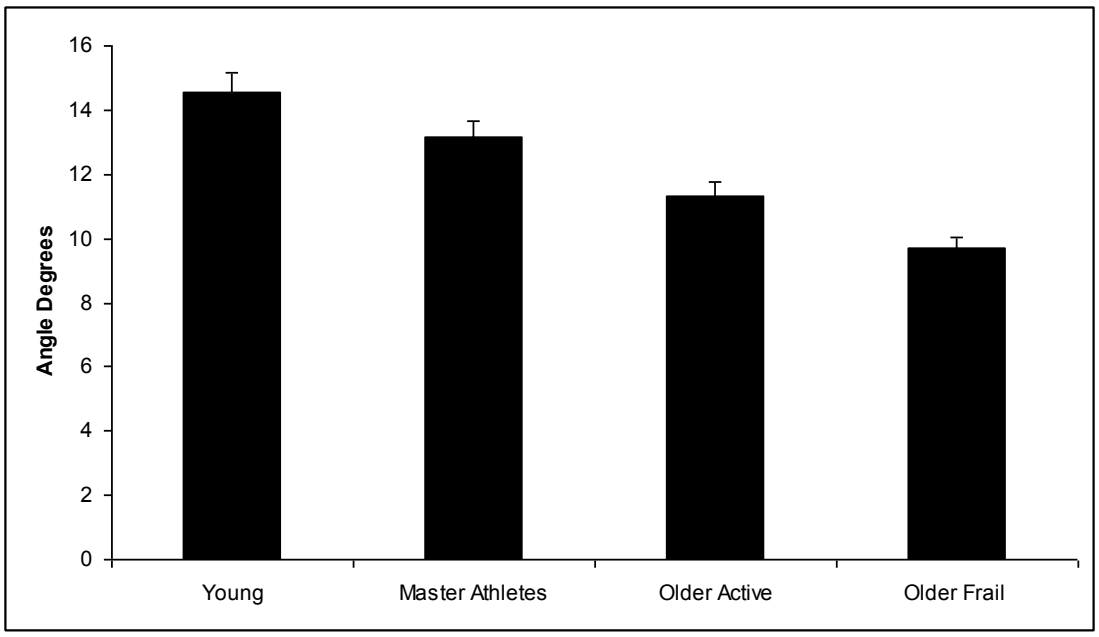


**Figure 8.3** VL pennation angle in YU, MA, OA and OF. Data are means±SEM

*Maximum Voluntary Contraction strength.* MVC was 35.9% lower in MA than in YU (p=<0.001) compared with 41.6% lower in OA (p<0.001). $VL_\theta$ in OA was 8.9% lower than MA (p=0.01) (Figure 8.4).

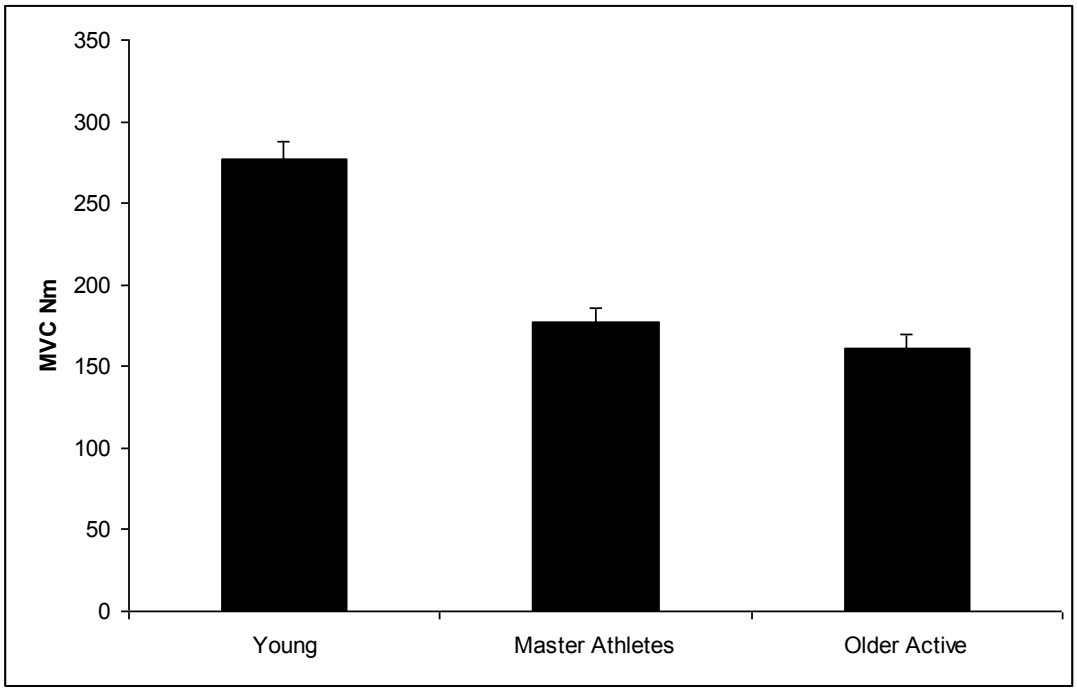


**Figure 8.4** MVC in YU, MA, OA and OF. Data are means±SEM

## Changes in muscle architecture in old age as a biomarker of sarcopenia.

Differences between the $VL_t$ and $VL_{Lf}$ of the YU, MA, OA and OF groups are reported above and are summarised in the table below. Also reported in the table is the ratio of VL fascicle length to muscle thickness $Lf/t$ (Table 8.2).

**Table 8.2** $VL_t$, $VL_{Lf}$ and $Lf/t$ ratio in YU, MA, OA and OF groups. Data are means ±SEM

| | Young Untrained | Master Athletes | Older Active | Older Frail |
|---|---|---|---|---|
| $VL_t$ cm | 2.12±0.1 | 1.75±0.1 | 1.61±0.1 | 1.09±0.1 |
| $VL_{Lf}$ cm | 8.12±0.2 | 7.57±0.2 | 7.24±0.2 | 5.89±0.2 |
| $Lf/t$ | 3.93±0.1 | 4.38±0.1 | 4.58±0.1 | 5.68±0.3 |

As can be seen from figure 7.5 the ratio of fascicle length to muscle thickness L*f*/t increases with normal ageing. L*f*/t was 16.5% higher in the OA group than in the YU group, p=0.009. L*f*/t is exaggerated by a decline in physical activity as is seen in the OF group. L*f*/t was 16.5% higher in the OA group than in the YA group, p=0.009, whilst the MA group was only 10% higher than YU and did not reach statistical significance, p=0.142. The OF group had a L*f*/*t* ratio 44.5% higher than the YA group, p<0.001, and was also significantly higher from both the MA, p=0.004, and OA, p=0.014 (figure 8.5).

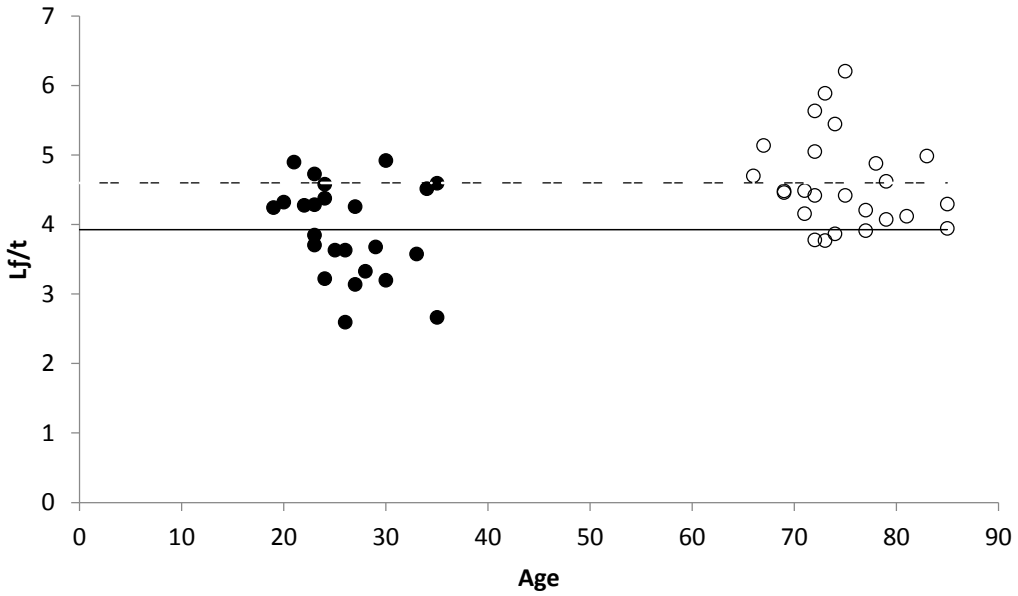


**Figure 8.5** Chart showing effect of normal ageing on L*f*/t ratio in YU (□) and OA (o). YU mean value represented by complete line, OA mean represented by broken line.

## 8.5 Discussion

It is a well-known and observed fact that the muscles of older individuals are intrinsically weaker than those of their younger counterparts, as represented in the results from the present study. What is also evident is that periods of inactivity due to injury or disuse drastically exacerbates the age-related deterioration in muscle quality and function. The above results show that the characteristic changes in muscle architecture seen in sarcopenia, a reduction in number or sarcomeres in parallel represented by pennation angle and muscle thickness and a reduction in number of sarcomeres in series represented by fascicle length can be somewhat mitigated by lifelong training. Muscle function in this case represented by strength also appears to be preserved in elderly individuals who lead an active lifestyle. This is not particularly surprising as individuals engaging in lifelong training generally maintain a much higher level of physical activity than their aged matched counterparts and are less likely to succumb to periods of prolonged activity effectively counteracting some very important factors contributing to sarcopenia.

An important factor contributing to age-related declines in muscle structure and function in the sharp decrease in the number of functioning motor neurons in individuals 70 years and over (Brown et al. 1988). Tibialis anterior muscles of recreationally active older men ~66yrs have been shown to contain 40% less motor units than those of young active young men ~25 yrs. A second group of older men ~82 yrs were shown to have an even greater loss of 60% less motor units than the young active groups (McNeil et al. 2005). This reported pattern of age-related motor unit loss suggests that motor unit number is dependent upon both age and physical activity level and mirrors the observed patterns in age-related losses of skeletal muscle structure and function. Hence the maintaining of motor unit numbers through

physical activity is likely a contributing factor to the preservation of structure and function in older individuals leading an active life.

Although lifelong training does not fully mitigate the progressive loss of muscle function with age, our results combined with those of previous studies show that the effects of sarcopenia can be slowed to an extent by physical activity. This is an important finding given that functional parameters of skeletal muscle including rate of force development and maximal power are important in the prevention of falls and reduction of frailty in older individuals. Improved muscle function in elderly individuals due to increased physical activity will help reduce the likelihood of falls and injury which are highly prevalent in today's ageing population leading to a general improved quality of life and reduced morbidity (Narici *et al.*, 2003). However, as individuals age, a variety of factors make physical exercise increasingly difficult. Stiffness, aches and pains and a loss of balance result in the perception that individuals are less capable of physical exercise and tends to result in elderly individuals being encouraged to 'take it easy'. Early assessment of the characteristics of sarcopenia could help to assess to what extent limited mobility is resultant from normal aging and how much is a symptom of compounding factors such as underlying injury or disease. This information would be valuable in tailoring exercise programs focusing on improving skeletal muscle function and, in particular, everyday activities linked to mobility and independent living. The relatively smaller $L_f$ and $\theta$ seen in the skeletal muscle older individuals suggest that normal biological aging is associated with a loss of sarcomeres both in series and in parallel which may account for some of the loss of muscle force and power observed in older age. However, in aging, as in disuse, the loss of muscle force and power is greater than

can be accounted for by the observed loss of muscle size and volume, even after considering age associated alterations in muscle architecture (Narici and Maganaris, 2007). Factors such as changes in single-fibre specific tension and tendon mechanical properties also contribute to the decrease in muscle force and power that accompanies old age. However, as skeletal muscle retains its capacity for adaptation into old age, there is increasing evidence that older individuals can limit and even reverse to a certain degree age-associated muscle weakness by inducing neuromuscular and tendinous adaptations with resistance training (Narici and Maganaris, 2006). Studies have shown it possible to increase muscle ACSA (13%) and maximal isometric muscle strength (24%) (Suetta *et al.*, 2004) as well as increasing $L_f$(9%) and θ (30%) (Reeves *et al.*, 2004) in elderly individuals by following RT programs.

The results show that the loss of sarcomeres in series and in parallel do not occur at the same rate. The greater reduction in fascicle pennation angle with advancing age suggests that sarcomeres in parallel are lost at a faster rate than sarcomeres in series. This imbalance between the changes of muscle architectural parameters results in a change in the geometric arrangement of muscle fibres, as seen in the change of ratio of fascicle length to muscle thickness (L*f*/t ) of the vastus lateralis muscle. The L*f*/t ratio increases with advancing age and is exaggerated by periods of disuse as seen in the much higher L*f*/t of the OF group compared to the OA group. While L*f*/t of MA is larger than that of the YU, the difference is not significant unlike that of the YU and the OA group.

This data suggests that the L*f*/t ratio as determined from ultrasound scanning may be useful as an indication of sarcopenia and could be used to aid in the detection and diagnoses of the onset of the condition when more robust methods such as MRI for

skeletal muscle mass are unavailable or impractical. As mentioned earlier an SMI of two standard deviations below the mean SMI of young healthy reference groups is used as a cut-off point for the diagnosis of sarcopenia (Baumgartner et al. 1998). Figure 8.7 shows percentage of each test group which could be considered as displaying characteristics of onset of sarcopenia according to their L$f$/t ratios. Of the OA group 17% had L$f$/t ratios of 2 standard deviations or more above the YU mean whilst the OF group had considerably more individuals with L$f$/t ratios above the 2SD cut off point, 61%. The MA group had only 4% individuals displaying L$f$/t ratios higher than the 2SD cut off point (Figure 8.7)

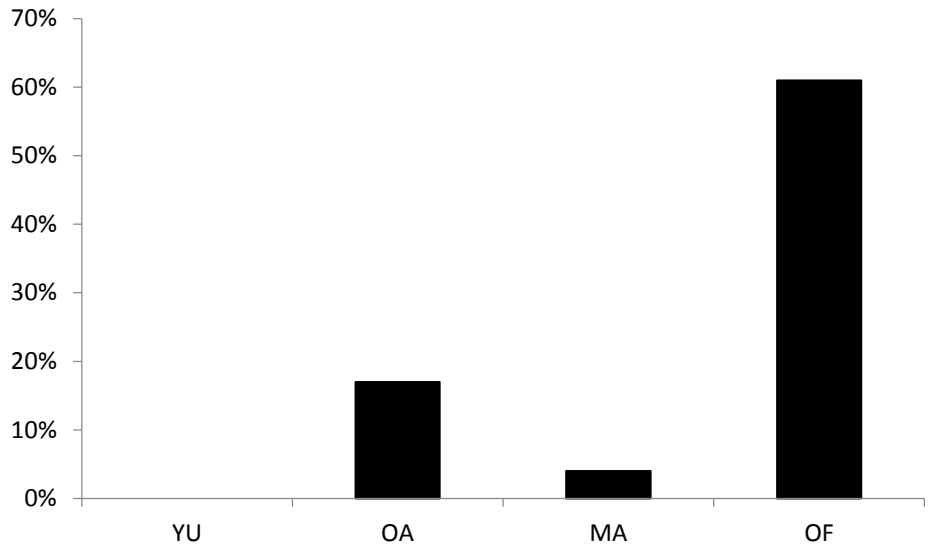


**Figure 8.7** Percentage of test groups with L$f$/t ratios higher than the 2SD cut off point

*8.6 Conclusion.* The above data supports the hypothesis that sarcopenia, although a universal process can be mitigated to some extent by sustained physical exercise. As physical activity becomes increasingly difficult as individuals age early evaluation of skeletal muscle quality and function could aid in the development of tailored exercise programs for the elderly focusing on everyday tasks important for independent living and falls prevention. Ultrasound assessment of the geometric

arrangement of muscle fibres appears to be a reliable and inexpensive indicator of the sarcopenic status of an individual. While body imaging techniques like MRI and CT scanning remain the gold standard for skeletal muscle assessment, ultrasound imaging is better suited to elderly patients as portable devices can be used to assess muscle architecture with minimal disruption to patients.

## 9. Conclusions

This thesis centred around skeletal muscle atrophy in response to both aging and disuse. We reported the effects of ULLS on young healthy males and conducted a resistance training program on the afore mentioned volunteers to assess the extent and rate of recovery of disused muscle when subject to different nutritional conditions. A cross sectional study was carried out to explore the role of physical exercise on aging human skeletal muscle. The geometric changes in muscle fibre architecture were examined to establish its potential as an image based biomarker of the onset of sarcopenia.

### 9.1 Skeletal muscle adaptations to physical inactivity and subsequent retraining in young men

When subject to 3 weeks of unilateral lower limb suspension, the suspended leg underwent dramatic adaptations in response to unloading. Both structural and functional parameters of skeletal muscle measured before and after ULLS showed significant changes due to disuse atrophy. Through 3 weeks of subsequent active recovery via resistance training, the quadriceps muscle of the previously suspended limb regained muscle mass, architecture and function to pre ULLS levels. This is a relatively short period of recovery compared to previous studies. The results show that in cases where it is possible, post immobilization recovery programs that begin in the early post -disuse phase can be a very effective strategy for skeletal muscle recovery. The increased loading experienced during resistance training appears to overcome the anabolic blunting experienced in the skeletal muscle as a result if decreases mechanical stimulus. Re-introducing mechanical stimulus elevates protein synthesis level seen to decrease in atrophic situations restoring muscle mass,

architecture and function. This study also concluded that the four heads of the quadriceps femoris muscle responded differently to unloading, a finding that is significant given that in a majority of the literature concerning muscle atrophy and hypertrophy vastus lateralis is used as a representative of the quadriceps femoris as a whole.

## 9.2 *THE EFFECT OF AMINO ACID SUPPLEMENTATION ON IMMOBILISATION-INDUCED ATROPHY IN YOUNG MEN*.

In this study, young healthy men undergoing unilateral lower limb suspension were randomly assigned to either a nutritional intervention group (leu) or an alanine placebo group (Ala). The purpose of this investigation was to assess if Leu supplementation during periods of disuse was enough to stimulate MPS levels and prevent the loss of skeletal muscle mass as a result from negative protein synthesis/breakdown balance. While the quadriceps femoris muscle of both supplementation groups underwent significant detrimental alterations in response to disuse, there was no difference in the response between the Leu and Ala groups.

Leucine has been shown to stimulate muscle protein synthesis in various unloading conditions, however it does not seem to have any anabolic effect on disused muscle.

It is likely that disused skeletal muscle develops an anabolic resistance to protein synthesis substrate meaning any additional protein given may be counterproductive in preserving skeletal muscle structure and function during immobilization periods. As expected, the patellar tendon did not display sensitivity to amino acid feeding with no difference observed in the response of either supplementation group to

unloading of human skeletal muscle. However, given the low number of participants in the Ala supplementation, further investigations with larger sample sizes are required to estimate the placebo effect of supplementation with more accuracy.

## 9.3 THE EFFECT OF AMINO ACID SUPPLEMENTATION ON RESISTANCE TRAINING BASED RECOVERY OF IMMOBILISATION-INDUCED ATROPHY IN YOUNG SUBJECTS.

In this study, eight men who underwent ULLS in study 2 were subjected to a 3 weeks active recovery program consisting of either resistance training and Leu supplementation or resistance training only (Ala placebo). While both groups regained muscle structure and function, the supplementation with Leu did not appear to have any effect on the extent of the recovery. However, with such small number in for the Ala sample size it is impossible to establish of the Leucine supplementation had no absolute effect upon the recovery of disused skeletal muscle. In our study the participants did not alter their normal dietary intake. The results would show that, at least during rehabilitation normal dietary protein intake appears to be sufficient for the growth and repair of skeletal muscle. Any superfluous Leu supplied is likely to be in excess and cannot be utilised by the disused muscle as either protein synthesis signal it substrate. In order to obtain more accurate and clinically relevant data, the experiment should be repeated an allow a much bigger sample size in order to elucidate if there is indeed some meahcnostransduction pathways that exists which can be stimulated by amino acid ingestion in disused muscle.

This dissertation has also investigated the effect of Leucine supplementation on changes in Patellar tendon dimensions and properties in response to disuse-induced atrophy. The study showed that daily supplementation with leucine during the

immobilisation period may have had little or no effect on the maintence of patellar tendon properties. Itis probable that due to the fact that our participants were not malnourished, but rather maintaining a normal dietary intake throughout the trial, that levels of dietary leucine present as raw materials for SLRP production were already sufficient for the reduced level of collagen synthesis resulting from immobilisation. Any additional Leucine ingested in supplement form was surplus to the requirements of the tendon in the absence of physical activity. The same was true for the active recovery period (chapter 6). Leucine supplementation did not improve the rate at which tendon mechanical properties improved. The observed increases in stiffness and Young's modulus are therefore due to the increased physical activity itself rather than the incerase in Leucine availability. As above, normal dietary Leucine intake levels appear to be sufficient to facilitate the increased collagen synthesis of tendon subject to resistance training.

A positive finding of the study in relation to tendon is that the reduction in mechanical properties, reduced stiffness and Young's modulus observed following three weeks of ULLS can be reversed in a relatively short time frame through a program of resistance training. Our participants acheived significant increases of 20% and 21% of stiffness were found in Leu and Ala supplementation groups respectively, with Young's modulus also increasing by 20% in both supplementation groups after just 3 weeks of active recovery.

Although the current study is based on a small sample of participants, the findings suggest that that with such significant increases in mechanical properties in this short recovery time period, where possible, it would be beneficial to begin rehabilitation early in the post-operative period.

## 9.4 INVESTIGATING THE ROLE OF PROLONGED PHYSICAL ACTIVITY AND IMMOBILIZATION PERIODS IN THE PROCESSES OF SARCOPENIA.

This consisted of a cross sectional study of 92 individuals including 24 young untrained (YU) males 18-35 yrs and 27 older recreationally active males and females (OA) 65yrs+ from the local population. Twenty-four male and female master athletes competing at the 2010 European Veteran Athletic Championships (MA) 65yrs+ were also assessed as well as 17 older frail males and females (OF) awaiting hip arthroplasty surgery 65yrs+. Life-long training appeared to mitigate the loss of skeletal muscle from the weight bearing quadriceps of master athletes. The characteristics of sarcopenia are also exacerbated by periods of disuse which are very common within the elderly population. As skeletal muscle architectural parameters do not decrease at equal and uniform rates in response to aging, there occurs a change in the skeletal muscle fibre geometry as an individual ages. This geometry also appears to be affected by disuse. As ultrasound imaging is cheap and relatively easily accessed, the use of ultrasound assess ratio of fascicle length to muscle thickness could be used as a potential biomarker for the indication of the onset of sarcopenia. Further work on larger sample groups should be carried out to examine the robustness of the proposed marker.

## 9.5 LIMITATIONS AND FUTURE DIRECTIONS

There are limitations to the body of work which warrant discussion. In chapter 4, the initial phases of study design included a three day food diary which participants were required to keep for three days (to include both weekdays and weekends). The purpose of this was to analyse participants' dietary intake including entire calorie and protein intake. Ideally, participants would complete the diary three days prior to ULLS and the same diary three days during ULLS. This information would have allowed us to determine in the first instance if subjects' dietary intake was altered due to ULLS as a change in energy expenditure would be expected due to participants walking with the aid of crutches. In reality, the food diaries were poorly completed by participants presumably due to the complexity of the ULLS procedure and the huge adjustment to daily life that participants had to undergo. It was decided that it was more important for participants to concentrate on properly fulfilling the ULLS protocol and adhering to the supplementation requirements and the diaries were withdrawn from future trails. The supplement and placebo were designed to be iso-caloric in attempt to limit any difference change in dietary intake within the supplementation groups but without solid data it is impossible to say if this was the case.

With respect to the ULLS procedure itself, whilst we were satisfied that all completing participants complied with the unloading protocol, we did not have in place an objective way of monitoring ULLS compliance. Compliance was monitored through daily phone contact to talk to participants and weekly visits to the IRM to ensure correct unloading procedure was still adhered to. However, these methods required complete honesty on the participants' part and are not quantifiable. As there is no heel strike in the suspended limb during ULLS, the dynamics of the suspended

limb are drastically different to typical walking dynamics. It is therefore theoretically possible to apply techniques used in biomechanical investigation to monitor compliance objectively. One such method would be to use pressure sensitive insoles to measure foot pressure. Assuming that the insole was worn throughout the entire ambulatory period of ULLS any weight bearing activity of the suspended limb would be recorded. To date this method of compliance does not appear to have been investigated. A method that has been trialled is the use of two-dimensional accelerometery of the suspended limb (Cook et al. 2005). Accelerometers were attached to participants left ankles and reading were taken during both walking and during suspensions due to ULLS for comparison. Subjects were also asked to perform tasks designed to simulate normal daily activity including sitting, lying and standing up. Participants were asked to walk both with and without crutches for 30 steps at self-selected slow, medium and fast paces. The investigators recorded both numbers of steps and the mean peak axial acceleration (MPAA), the speed at which the ankle swings immediately prior to heel strike. Although during the ULLS no actual steps were taken, the software algorithms did detect some quantified 'steps' however the MPAA of these 'steps' were significantly lower than those of normal walking at all speeds and can hence be differentiated from actual steps. The authors concluded that this method could be used to monitor ULLS compliance with 97% compared with visual confirmation. Further studies should be carried out to assess the effect of variables such as the tension on the sling suspending the limb, angle of suspension and subject characteristics such as height and weight. In conclusion, possibly the best method of compliance monitoring may include aspects of both accelerometer and pressure monitoring to provide the most accurate quantifiable measurement of compliance. Trips and stumbles as participants become accustomed

to walking with crutches would of course need to be considered when applying a threshold level of compliance.

Sample size was a problem for the ULLS studies particularly for regarding supplementation. There was a high dropout rate for the ULLS procedure and unfortunately most dropouts had been previously randomly assigned to the alanine placebo group leaving an Ala supplementation group of just three on completion of the study. With such a low sample size, it was impossible to perform robust statistical analysis to confirm the lack of effect of amino acid supplementation on disuse and retraining. Ideally the study would be repeated using participants from the same demographic group to increased completed sample sizes to confirm our hypotheses.

In chapter 8 we used master athletes were used to examine the effect of lifelong training on the age-related loss in muscle mass and function known as sarcopenia. Measurements of quadriceps femoris were used to as an indicator of muscle volume and architecture. It should be noted however, that the group of master athletes consisted of athletes from all disciplines and there may be variations in the quality of thigh muscles between the disciplines. A more in depth study should be conducted to examine and quantify any potential effect individual disciplines may have upon thigh muscle structural and functional parameters and the subsequent effect this could have the progression of sarcopenia.

# 10. Appendices

Appendix 1. ULLS Participant Completions.

| | PHASES COMPLETED | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | BASELINE TESTING | 1ST BIOPSY | ULLS | POST-ULLS TESTING | 2ND BIOPSY | RESISTANCE TRAINING | POST-RT TESTING | 3RD BIOPSY |
| ULLS10_MF | √ | √ | √ | √ | √ | √ | √ | √ |
| ULLS10_GB | √ | √ | √ | √ | √ | √ | √ | √ |
| ULLS10_DB | √ | √ | √ | √ | √ | √ | √ | √ |
| ULLS10_JR | √ | √ | √ | √ | √ | √ | √ | √ |
| ULLS10_AB | √ | √ | √ | √ | √ | LEFT MANCHESTER | | |
| ULLS10_JS | √ | √ | DID NOT WANT TO GIVE FURTHER BIOPSIES | | | | | |
| ULLS11_PC | √ | √ | DID NOT WANT TO GIVE FURTHER BIOPSIES | | | | | |
| ULLS10_SV | √ | √ | NON COMPLIANCE | | | | | |
| ULLS10_AA | √ | √ | NON COMPLIANCE | | | | | |
| ULLS10_AM | √ | √ | NON COMPLIANCE | | | | | |
| ULLS10_KS | √ | DID NOT WANT FURTHER TESTING | | | | | | |
| ULLS10_DBR | √ | PERSONAL PROBLEMS | | | | | | |
| ULLS11_SB | √ | √ | DID NOT WANT TO GIVE FURTHER BIOPSIES | | | | | |
| ULLS11_RL | √ | √ | √ | √ | √ | √ | √ | √ |
| ULLS11_ML | √ | √ | √ | √ | √ | √ | √ | √ |
| ULLS11_DC | √ | √ | √ | √ | √ | √ | √ | √ |
| ULLS11_MG | √ | √ | √ | √ | √ | √ | √ | √ |

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