Acute Disuse Muscle Atrophy in Health and Disease

An investigation of the timecourse, functional effects and a potential treatment strategy

By

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I dedicate this work, as I do everything, to Arthur, Rupert and Mabel.

Declaration

I hereby declare that the work presented in this thesis is my own work

Edward Hardy

* Statistical support and advice were provided by Dr Brett Doleman (PhD), especially with regard to the statistical aspects of meta-analysis.

Awards, Publications and Presentations

The following awards, publications and presentations have arisen from the work contained in this thesis:

Awards

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Publications

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Bass JJ, Hardy E, Inns T et al. (2021) Atrophy resistant vs atrophy susceptible skeletal muscle “aRaS” as a novel experimental paradigm to study the mechanisms of human disuse atrophy. Frontiers in Physiology, 12, https://doi.org/10.3389/fphys.2021.653060

Presentations

Podium presentations

Postoperative electrical muscle stimulation attenuates loss of muscle mass and function following major abdominal surgery
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Hardy E et al, Surgical Research Society, 2022

Atrophy resistant vs atrophy susceptible skeletal muscles: a novel experimental paradigm to study the mechanisms of human disuse atrophy and anabolic resistance to nutrition
Bass J et al, ECSS 2020

Poster Presentations

Postoperative muscle atrophy: an overlooked cause of morbidity.
Hardy E et al. ASGBI 2022,

Soup and sweet is not enough to eat: Postoperative nutritional intake is inadequate following open colorectal resection
Hardy E et al ASGBI 2022

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Bass J et al. BBEP 2022

Thesis Abstract

Purpose

Maintenance of skeletal muscle mass and function is essential for a healthy, long life. Episodes of reduced mobility may be associated with significant disuse muscle atrophy (DMA) and loss of function. It is increasingly suggested that the cumulative effect of these episodes through later life may be a major driver behind the development of sarcopenia. The purpose of this thesis was therefore to investigate the timecourse of DMA in a range of human lower limb muscles, how this varies over the period of immobilisation and between muscles, and its functional outcomes. Furthermore, the impact of associated ill health on these findings was investigated, as well as the effectiveness of neuromuscular electrical stimulation (NMES) in attenuating muscle loss.

Methods

Systematic review and meta-analysis of studies reporting measurement of muscle mass at repeated timepoints during a period of immobilisation, casting following bone fracture, and critical care admission was completed, with data synthesised to characterise the timecourse of DMA in leg muscles. Following this, two studies were conducted in which healthy young men were recruited to have unilateral lower limb immobilisation (ULLI) using a knee brace and ankle boot for five or 15 days. Measurement of muscle mass and function were completed at various timepoints. Measurements of muscle mass were made using MRI

(gold standard) and ultrasound, in order to validate the use of ultrasound in the detection and monitoring of DMA. Finally, a split leg randomised control trial of patients undergoing major abdominal surgery was completed. In this study, the control limb was used to characterise the degree of loss of muscle mass and function in an immobilised and unwell cohort, whilst the other limb underwent twice daily NMES to investigate its efficacy in attenuating loss of muscle mass and function.

Results

Systematic review and meta-analysis demonstrated rapid loss of muscle mass, with greater losses in individuals immobilised after bone fracture or due to critical illness. Rates of DMA appeared greatest during the first two weeks of immobilisation and to vary between muscles, however there were insufficient data to fully characterise these trends. Studies of healthy individuals undergoing two weeks of ULLI identified vastus lateralis (VL) and medial gastrocnemius (MG) as atrophy susceptible (aS) muscles, with significant decrease in muscle mass after just five days. Tibialis anterior (TA) was identified as an atrophy resistant (aR) muscle, with no significant change in muscle mass after 15 days of ULLI. Muscle strength and power also decreased in VL and MG, with a 20-30% reduction in maximum voluntary contraction (MVC) after 15 days, whereas no significant change was identified in TA MVC. Loss of VL CSA (-9.16%) and knee extension strength (-19.7%) in postoperative patients was more rapid than in healthy individuals. However, use of NMES reduced these

losses by approximately 50%, suggesting it is a promising therapeutic intervention to reduce muscle loss in individuals with reduced mobility.

Conclusion

During immobilisation there is rapid and significant loss of muscle mass and function. These losses are compounded in individuals who are unwell or recovering after surgery. The rate at which muscle mass and function is lost varies between muscles, with the greatest losses in functionally important muscles for standing and walking. NMES significantly attenuates the loss of muscle mass in postoperative patients and should be further investigated as a potential therapeutic option.

Contents

Tables

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Abbreviations

1-RM	1 repetition maximum
AA	Amino Acid
aDMA	Acute disuse muscle atrophy
AKT	Protein Kinase B
aR	Atrophy resistant
aS	Atrophy susceptible
AT	Anaerobic threshold
ATP	Adenosine triphosphate
ATS	American thoracic society
BL	Baseline
BMI	Body mass index
BR	Bed rest
CCC	Concordance correlation coefficient
CD	Capillary density
CON	Control leg
COP	Centre of pressure
CPET	Cardiopulmonary exercise testing
CSA	Cross sectional area
CT	Computed tomography
DMA	Disuse muscle atrophy
EAA	Essential Amino Acid
EC	Excitation contraction coupling
ECG	Electrocardiogram

ECMO	Extracorporeal membrane oxygenation
EMG	Electromyography
EMS	Electrical muscle stimulation
ERAS	Enhanced recovery after surgery
FL	Fibre length
FOXO	Forkhead box O
GI	Gastrointestinal
GSK3B	Glycogen synthase kinase 3b
HGS	Hand grip strength
IGF1	Insulin-like growth factor 1
iMOB	Immobilised leg
IRS1	Insulin receptor substrate 1
ITU	Intensive treatment unit
KE	Knee extension
KES	Knee extension strength
KF	Knee flexors
LEP	Leg extension power
LG	Lateral gastrocnemius
LoS	Length of stay
MG	Medial Gastrocnemius
MHC	Myosin heavy chain type
MP	Midpoint
MPB	Muscle protein breakdown
MPS	Muscle protein synthesis
MRI	Magnetic resonance imaging

MT	Muscle thickness
mTOR	mechanistic target of rapamycin
mTORC1 / 2	mTOR complex 1 / 2
MU	Motor unit
MUP	Motor unit potential
MURF1	Muscle RING-finger protein-1
MVC	Maximum voluntary contraction
NB	Net balance
NCAM	Neural cell adhesion molecule
NIBP	Non-invasive blood pressure monitoring
NMES	Neuromuscular electrical stimulation
NMJ	Neuromuscular junction
PA	Pennation angle
pCSA	Physiological cross sectional area
PI3K	Phosphoinositide 3-kinase
POD	Postoperative day
r	Pearson’s correlation coefficient
r_c	Linn’s concordance correlation coefficient
RCT	Randomised control trial
REML	Restricted maximum likelihood
RER	Respiratory exchange ratio
RET	Resistance exercise training
RF	Rectus femoris
RPM	Revolutions per minute
RVE	Resistance vibration exercise

SC	Satellite cells
SERCA	sarco-/endoplasmic reticulum Ca^{2+}-ATPase
Sol	Soleus
SPPB	Short physical performance battery test
SQRT	Square root of
SR	Sarcoplasmic reticulum
STIM	Leg randomised to undergo NMES
T-tubules	Transverse tubular system
TA	Tibialis Anterior
TBI	Traumatic brain injury
TS	Triceps Surae
TUG	Timed up and go
ULLI	Unilateral limb immobilisation
US	Ultrasound
USS	Ultrasound scanning
VL	Vastus Lateralis
VO_2MAX	Maximum ventilatory oxygen uptake
VO_2peak	Peak ventilatory oxygen uptake
Vol	Muscle volume

Prologue

Mrs X was a community dwelling, independent 78 year old lady, who underwent emergency surgery for a perforated bowel. After her operation she spent two days on the intensive care unit, before returning to the ward. She made a good recovery, and after a couple of weeks was declared medically fit to leave hospital. Unfortunately, by this time she had lost so much muscle mass and function that her mobility was not good enough to go home. She was admitted to a rehab ward, where she underwent regular physiotherapy. Whilst on the rehab ward she developed a chest infection and ended up being readmitted to an acute medical ward, where she was treated with a further week of antibiotics. After recovering from this she returned to a rehab ward, and eventually made it home with carers visiting three times a day. After only a few months at home she awoke one night to go to the toilet, and due to her poor mobility fell and fractured her hip. She was readmitted to hospital and underwent surgery to fix her hip, but despite intensive physiotherapy she never regained the mobility or confidence required to be return home and was discharged to a nursing home.

Any clinician working in a secondary care setting will be familiar with stories like this. The long term disability does not result from a loss of function in the cardiorespiratory, neurological, renal or digestive systems on which so much emphasis is focused, but because of a loss of skeletal muscle mass and function.

There is growing recognition of this phenomenon, and the syndrome of ‘acute sarcopenia’ is being increasingly discussed (Welch *et al.*, 2018). However, the speed with which muscle mass and function is lost remains poorly characterised. Furthermore, it is not known whether all muscles respond equally, or whether some are more susceptible than others to the effects of disuse and ill health. As a result few treatment strategies have been developed and the clinical scenario described above continues. The following chapters seek to deal with these issues

1 Introduction

Human skeletal muscle and the effects of disuse

1 Introduction

1.1 Skeletal muscle structure

1.1.1 Overall structure

On average skeletal muscle accounts for around 40% of total body mass, with approximately 25% of the body's total protein synthesis occurring in the muscular system (Morley et al., 2001). Individual muscles are surrounded by a dense layer of collagenous connective tissue known as the epimysium. Further layers of connective tissue, called the perimysium, run through the muscle dividing it into septa called fascicles. Fascicles contain bundles of approximately 20-80 individual muscle fibres which are surrounded by more connective tissue, the endomysium.

The nerves and blood vessels which supply the muscle run through these layers of connective tissue (the epimysium, perimysium and endomysium) to supply the individual fibres (Korthuis, 2011). Collagen in all 3 layers of connective tissue intertwines with the collagen of the tendons or aponeurosis to transmit the contractile force generated by the fibres (Biga *et al.*, 2019) (Figure 1.1).

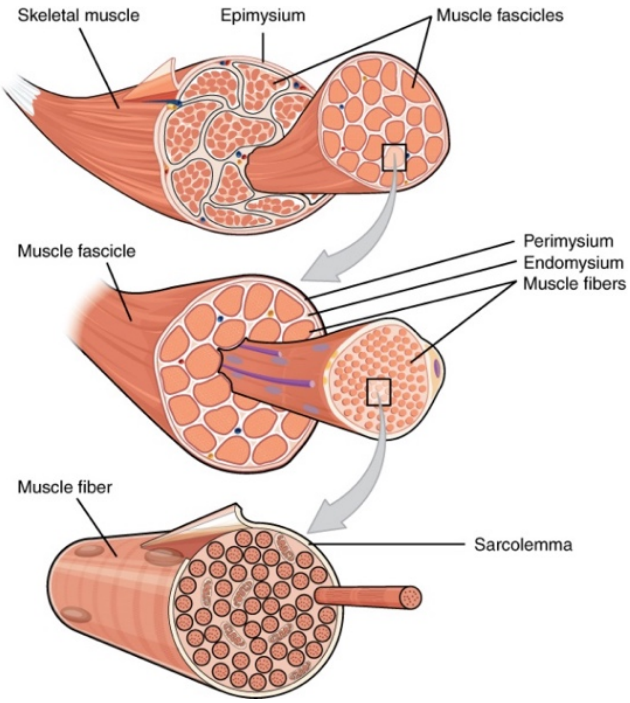

Figure 1.1: The Three Connective Tissue Layers. Bundles of muscle fibres. Fibres, Endomysium, Fascicles, Perimysium, Epimysium (Reproduced from Biga et al., 2019).

1.1.2 Muscle Architecture

The way in which the muscle fibres and fascicles are arranged within the muscle, relative to the axis along which it generates force, is described as muscle architecture (Lieber and Fridén, 2001). In simple depictions of muscle, fibres are shown as arising from a tendon and running in parallel along the axis of force generation to a distal insertion point (Figure 1.1). This arrangement is known as parallel or longitudinal architecture. However, most muscles of the human lower limb have their fibres arranged at an angle to the axis of force generation (Narici, 1999). This arrangement of fibres has an appearance similar to the barbs and rachis of a bird's feather and so muscles with this fibre arrangement are known as pennate ("penna" is the Latin for feather) (Azizi, Brainerd and Roberts, 2008). Muscles may be uni-pennate, bi-pennate or multi-pennate.

Pennate fibre arrangement allows for a greater number of muscle fibres to be arranged in parallel per unit of muscle volume, and thus allows greater force production relative to the overall volume of the muscle (Narici, Franchi and Maganaris, 2016a). This phenomenon is recognised and explained in the concept of the physiological cross-sectional area.

In general terms the ability of a muscle to generate a force is directly proportional to its cross sectional area (CSA) (Narici, Landoni and Minetti, 1992). However, this relationship does not actually depend on the CSA of the muscle but on the total CSA of all the fibres within the muscle, since it is these which generate force. In muscles with pennate fibre arrangement simple anatomical CSA will not truly represent CSA of all the muscle fibres and the calculation can be adjusted for the angle at which the fibres run in the muscle (the pennation angle). This is known as the physiological cross sectional area (pCSA) (Narici, Landoni and Minetti, 1992).

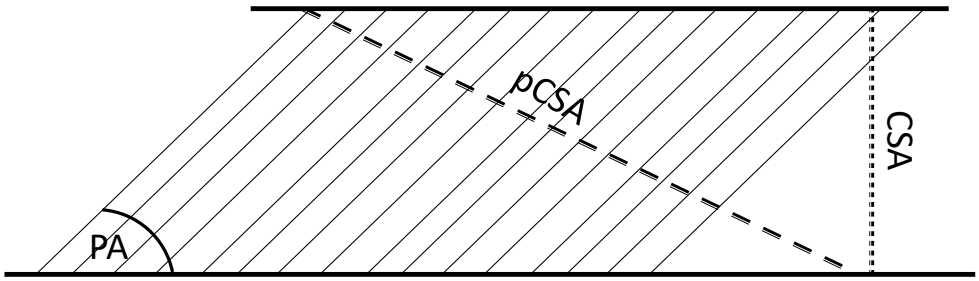

Figure 1.2: Scheme of a pennate muscle: showing the pennation angle (PA), the pCSA (dashed line) and aCSA (dotted line).

PCSA refers to the cross-sectional area directly perpendicular to the angle of the muscle fibres (Figure 1.2) A formula for its calculation was first described in 1984 (Powell et al., 1984) and it has been shown that for muscles with pennate fibre arrangement, calculation of pCSA gives a better estimation of the maximal force generating ability of the muscle (Lieber and Fridén, 2001).

PCSA takes into account two important factors in muscle architecture: pennation angle (PA) and fibre length (FL). PA is important as only the vector of fibre force generated along the line of action of the muscle will contribute to whole-muscle force generation. The total force generated by a muscle is therefore dependent on the angle at which the fibres insert into the aponeurosis (the PA) (Lieber and Fridén, 2001). Overall muscle shortening displacement and contraction velocity depend on the number of sarcomeres in series, and therefore fibre length. Pennate muscles have shorter fibre lengths compared to those with parallel fibre arrangement and shortening displacement and contraction velocity will therefore be lower in these muscles (Gans and Gaunt, 1991). pCSA, PA and FL vary between pennate muscles and contribute towards the variability in individual muscle functional characteristics. For example, muscles with a large pCSA, short FL and high PA (e.g. quadriceps) are capable of large force generation and are important for antigravity movements and posture (Lieber and Fridén, 2000). Conversely, muscles such as Tibialis Anterior have smaller pCSA, long FL, low PA and are better suited to rapid, low force movements, such as postural adjustment.

1.1.3 Muscle Fibres

In healthy muscle, total muscle volume is determined mostly by the number of fibres, with each individual muscle fibre being a single muscle cell (Frontera and Ochala, 2015). Muscle cells have two unique properties; they are multinucleated (during embryonic development multiple myoblasts fuse (Draeger, Weeds and Fitzsimons, 1987)) and because of this they are post-mitotic. Each nucleus within the fibre controls the protein synthesised within its area of the cell. This area is termed the nuclear domain.

Muscle fibres themselves are surrounded by a specialised cell membrane called the sarcolemma. Around 80% of the contents are contractile, regulatory and cytoskeletal proteins, with each fibre containing thousands of myofibrils and billions of myofilaments (Frontera and Ochala, 2015). A more detailed description of these proteins follows in section 1.1.3.1.

As muscle cells are incapable of mitosis to replicate, adult muscle stem cells play an essential role in muscle growth, repair and regeneration (Hikida, 2011). These stem cells are called satellite cells (SC) as they sit surrounding muscle cells between the sarcolemma and the basal lamina (Mauro, 1961).

Other important intracellular structures of skeletal muscle include the sarcoplasmic reticulum, the transverse tubular system (T-tubules) and the mitochondrial network. T-tubules consist of invaginations of the sarcolemma which form a dense network throughout the fibre (Jayasinghe and Launikonis, 2013). The purpose of this network is to carry the excitation from the motor-neurone rapidly throughout the whole fibre to allow uniform Ca^{2+} release and contraction (Ibrahim *et al.*, 2011). In close contact with the T tubule system lies the sarcoplasmic reticulum (SR). This specialised cytoplasmic reticulum forms a continuous network surrounding the myofibrils. Around the T-tubules the SR forms into the terminal cisternae which act as the calcium store. When depolarisation occurs, this calcium is rapidly released into the cytoplasm causing contraction of the myofibrils. Calcium is then re-absorbed by the SR through sarco-/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) channels (Lamboley *et al.*, 2014).

Mitochondria in skeletal muscle also exist in a highly organised network with most densely packed into the space between myofilaments and in physical communication with the SR (Dahl *et al.*, 2015). This not only enables provision of the adenosine triphosphate (ATP) necessary for contraction (see section 1.1.3.3), but the close association with the SR also enables delivery of Ca^{2+} to the mitochondrial matrix, which then activates Ca^{2+} sensitive mitochondrial matrix dehydrogenases which enhances ATP production in a mechanism known as excitation-oxidative metabolism coupling (Eisner, Lenaers and Hajnóczky, 2014).

1.1.3.1 Contractile apparatus

The main component of muscle fibres are the highly organised contractile, cytoskeletal and regulatory proteins which make up the contractile apparatus. The myofibrils run along the longitudinal axis of the cells, and are linked to each other and the sarcolemma by cytoskeletal proteins including plectin, filamin and dystrophin (Trovato *et al.*, 2016). These cytoskeletal proteins are important in transferring the force of myofibrillar contraction to the sarcolemma and extracellular matrix to produce whole muscle contraction (Frontera and Ochala, 2015).

1.1.3.2 Myofibrillar structure

Myofibrils have a highly organised structure consisting of thick and thin filaments. The thick filament consists of hundreds of myosin molecules. Each myosin molecule consists of two identical subunits, which have a long tail segment and globular head region which is orientated at an angle to the tail. The tail segments are orientated towards the centre of the filament and intertwine with each other. The heads, which include the actin binding site and myosin ATPase region, protrude outwards in regular intervals and it is these which form the cross bridges between the thick and thin filaments (Gelfi, Vasso and Cerretelli, 2011). Thin filaments are made of three proteins. Spherical actin molecules bind to each other to form two intertwined strands. Running along these strands are the threadlike tropomyosin molecules, which are also bound to troponin. When viewing muscle fibres under light microscopy, skeletal muscles appear striated due to the specific arrangement of the light and heavy

chains. As seen in Figure 1.3, the dark A bands are formed by thick filaments, which overlap at each end with areas of light filaments. In the centre of the region only myosin heavy chains are present, the H zone. In the centre of this area is the M line which is formed by an arrangement of supporting proteins which bind neighbouring thick filaments to each other (Schiaffino and Reggiani, 2011a). The light I bands represent areas consisting of light fibres alone. At the centre of these regions, visible on electron microscopy, are dense Z lines. The Z line is a disc of cytoskeletal protein which connects neighbouring thin filaments together. Other cytoskeletal proteins such as desmin connect the Z disk to the sarcolemma and extra-cellular matrix to transfer contractile force. The area of myofibril between two Z-lines is known as the sarcomere, and this is the functional unit of skeletal muscle (Frontera and Ochala, 2015).

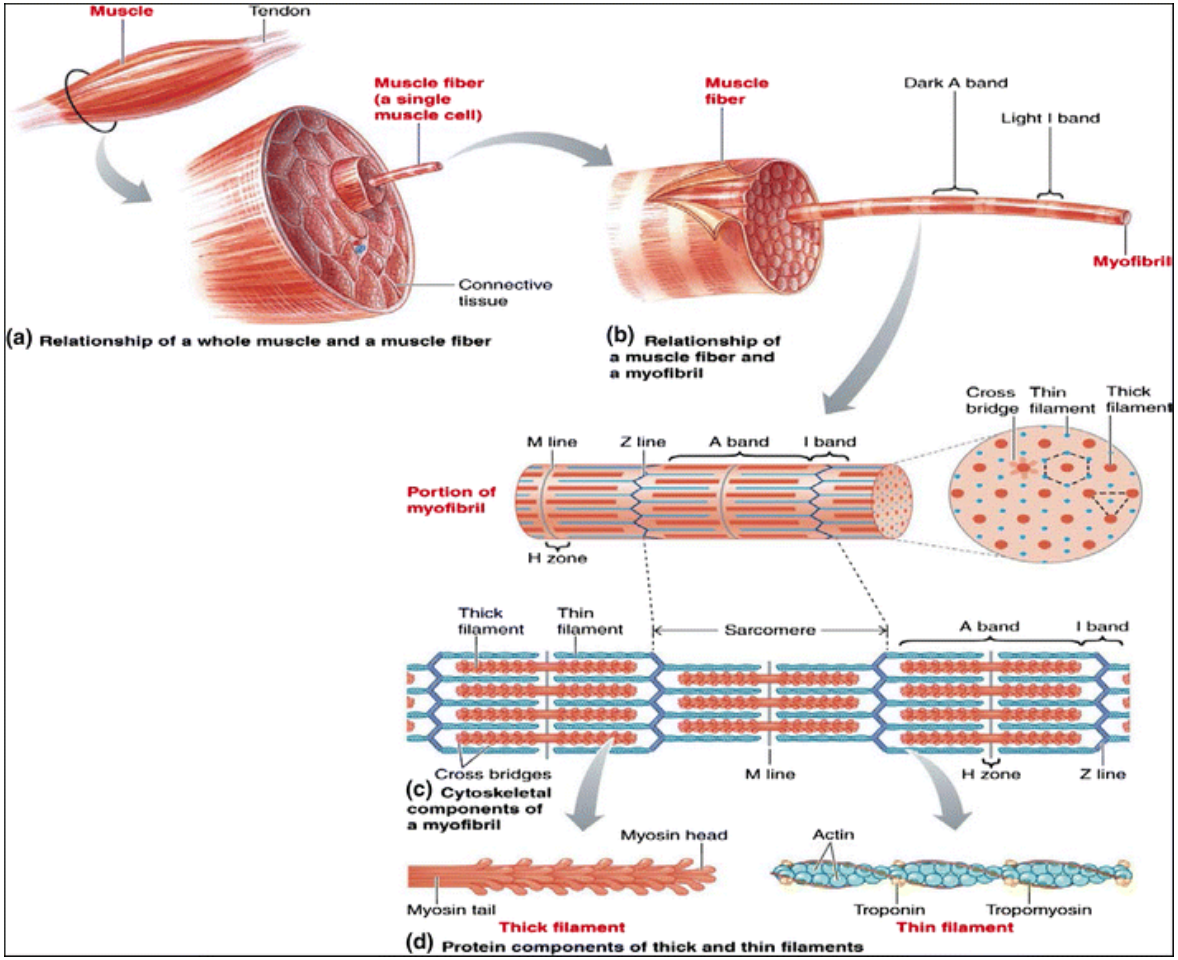

Figure 1.3: Organisation of skeletal muscle (taken from Sherwood L, 2012).

1.1.3.3 Excitation contraction coupling

Excitation-contraction (EC) refers to the interaction between actin and myosin fibres to form cross-bridges, which occurs following calcium release from the cisternae of the SR secondary to depolarisation from transmitted nerve stimulus (Frontera and Ochala, 2015). In brief, the depolarisation from the motor-neurone is transmitted to the sarcolemma, traveling along the t-tubules to the interior of the cell. The depolarisation opens dihydropyridine channels on the t-tubule causing an influx of calcium to the terminal cisternae. This in turn causes ryanodine receptors of the SR to open and Ca2+ influx to the sarcoplasm (Rebbeck *et al.*, 2014). The free calcium in the sarcoplasm binds to troponin triggering a transformation in its shape, which moves tropomyosin away from the myosin binding sites of the actin of the thin filament. This allows formation of cross bridges between actin and the actin binding site of the myosin head. Muscle contraction then occurs via myosin-actin cycling (Krans, 2010). The formation of the myosin-actin cross-bridge induces a change in conformation of the S1 region of the head of myosin, which slides the actin fibre over the myosin fibre and towards the H-zone and M-line. This is known as the ‘power stroke’ (Spudich, 2001). Hydrolysis of ATP at the ATPase region of the myosin head then causes release of the actin filament and a return of the myosin head to its original conformation. The actin-myosin cross-bridge then reforms at the next binding site along the actin filament, and the whole cycle repeats. The overall effect is the shortening of all sarcomeres along the length of the muscle fibre causing contraction of the muscle (Krans, 2010).

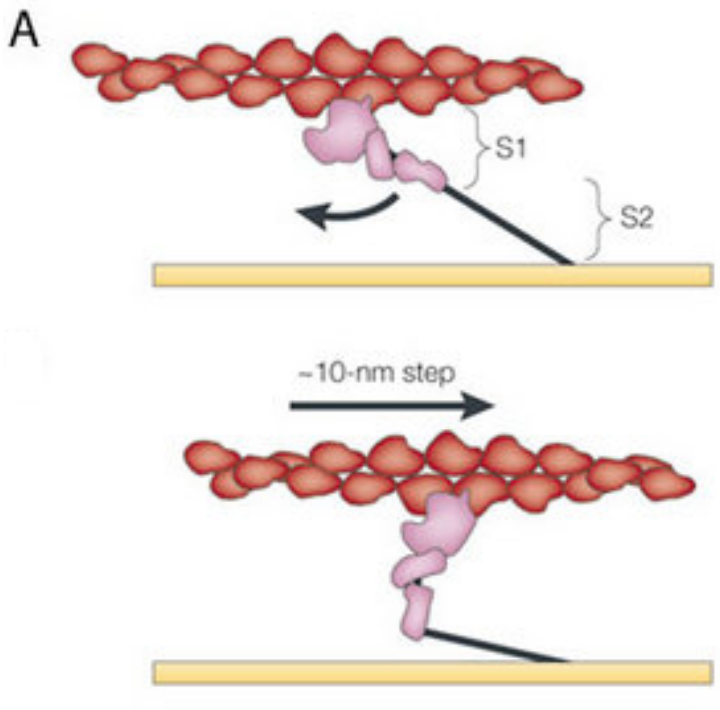

Figure 1.4: Change in conformation of myosin head during the power stroke causing sliding of light chain over heavy chain to cause shortening of sarcomere (taken from Spuldich J., 2001).

1.1.3.4 Muscle fibre heterogeneity

Different muscle fibres show significant variation in their characteristics, with different phenotypes of muscle fibres having been recognised since the nineteenth century. By 1873 Ranvier had identified a difference between red and white muscles and shown that white muscle had a fast twitch in response to a stimulus while red muscle showed a slower twitch (Needham, 1926). By the early 1900s different characteristics of these two groups had been further developed. Fast twitch fibres were shown to have greater explosive power but rapid fatigue, whilst slow twitch fibres were shown to exert less tension but with much greater endurance.

In an attempt to understand the basis for these different characteristics, muscle fibres have been further classified by their myosin heavy chain

(MHC) isoforms, histological and biochemical characteristics in the years that have followed (Frontera and Ochala, 2015). MHC isoforms appear to be the most appropriate and informative markers for fibre type classification (Pette and Staron, 2000). MHCs form the most essential part of the contractile machinery and so expression of MHC isoforms largely govern the contractile properties of muscle cells as they determine the maximum shortening velocity, maximum power generation and ATP consumption rate (He *et al.*, 2000).

Identification of different MHC isoforms can be accurately achieved through immuno-histochemistry using antimyosin antibodies or individual fibre sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE) (Pette, Peuker and Staron, 1999; Gelfi, Vasso and Cerretelli, 2011). Three forms of MHC have been identified as being expressed in adult human skeletal muscle: type I, type IIa and type IIx.

As muscle fibres are multinucleated with each nucleus controlling protein expression in its nuclear domain in response to localised stimuli, multiple isoforms of MHC may be expressed within each fibre (Gelfi, Vasso and Cerretelli, 2011). As a result, muscle fibres may be ‘pure fibres’, expressing just one MHC isoform, or may be ‘hybrid fibres’ containing a combination of MHC isoforms. The five most prevalent MHC isoforms in human skeletal muscles are I, I/IIa, IIa, IIa/IIx and IIx (Galpin et al., 2012).

Myosin ATPase hydrolysis rates vary between fibre types. Fibres with the fastest contraction time show the higher ATPase activity (Barany, 1967). Myosin ATPase can be histochemically stained, and fibres grouped based on the staining intensity. There are three main groups based on this classification (type 1, type 2a, type 2x), and these groups correlate well with the MHC classifications (see Figure 1.5). This is logical as the site that serves as ATPase is part of the globular head region of the myosin heavy chain (Pette, Peuker and Staron, 1999). Further intermediate groups (IC, 2C, 2AB etc) have been identified, and these correlate with the hybrid groups identified by MHC isoforms.

It is also possible to characterise fibres based around the dominant metabolic pathway involved in ATP generation in the cell, by staining for succinate dehydrogenase (SDH) activity to reflect potential for oxidative metabolism, or staining for glycolytic enzymes (Schiaffino and Reggiani, 2011a). It has been shown that in MHC type I fibres there are high levels of staining for SDH, whereas in type II fibres there is a spectrum, with type IIa fibres showing moderate oxidative metabolism whilst IIx show very little. Conversely, both type IIa and IIx fibres show high levels of glycolytic enzymes.

The importance of other sarcoplasmic structures for the function of muscle fibres has already been outlined above. It has been found that both mitochondrial concentration and development of SR vary according to fibre type (Schiaffino, Hanzlíková and Pierobon, 1970). Mitochondrial

concentration is a measure of oxidative capacity (Mishra *et al.*, 2015), whilst highly developed SR is essential for rapid depolarisation and calcium release to allow fast contraction (Frontera and Ochala, 2015). In type 1 fibres there is a high mitochondrial concentration and poor SR development (Lamboley *et al.*, 2014). Type IIa fibres also show high mitochondrial concentration, but have well developed SR, whilst IIx fibres have low mitochondrial concentrations with well-developed SR (Schiaffino, Hanzlíková and Pierobon, 1970).

There is significant correlation between these methods of characterisation of fibre types. MHC type I fibres are slow twitch, oxidative, fatigue resistant fibres which have lower power generating capacity. MHC IIa fibres are fast twitch, oxidative glycolytic fibres, which have intermediate power generating capacity and fatigue resistance. MHC IIx fibres are fast twitch, glycolytic fibres which have high power generation but are rapidly fatigable. Hybrid fibre types show intermediate characteristics based on the two main isoforms of which they are comprised.

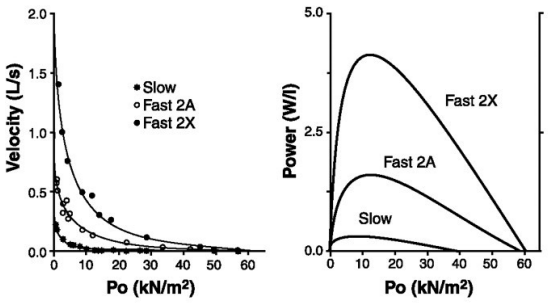

Figure 1.5: Characteristics of fibres based on MHC isoform classification, taken from Schiaffino and Reggiani 2011.

Expression of MHC isoforms by muscle fibres is highly plastic and can adapt to stimuli with the primary regulator of fibre type expression being physical exercise (Galpin *et al.*, 2012). For example, during periods of immobilisation a transition of fibres from a pure MHC I isoform to a MHC I / IIX hybrid has been observed (Demangel *et al.*, 2017).

1.1.4 Motor unit

Beyond its basic physical structure, the muscle can be seen as a collection of motor units. The motor unit consists of a single motor neurone with all the muscle fibres which it controls. The fibres within each motor unit have similar or identical properties in terms of structure and function (Schiaffino and Reggiani, 2011b). In 1887, Grützner first hypothesised that muscles were made up of a mixture of fibre types, and that the muscles characteristics were based on the proportions in which these fibres were present (Needham, 1926). It is now recognised that each muscle is made of tens or hundreds of motor units, each of which brings specific characteristics to the function of the muscle. Each motor unit acts as a single entity that represents the basic unit of control of movement (Heckman and Enoka, 2012). Investigation of adaptations in muscle in response to a stimulus must therefore consider each part of the motor unit when attempting to explain changes in function.

1.2 Skeletal muscle homeostasis

1.2.1 A dynamic balance

Skeletal muscle contractile proteins exist in a state of continual turnover with simultaneous synthesis and breakdown. This allows renewal and modification of their composition to adapt to differing circumstances and demands (Atherton, Phillips and Wilkinson, 2015). Depending on the long term net balance (NB) between muscle protein synthesis (MPS) and muscle protein breakdown (MPB), muscle fibres may increase or decrease in CSA, with the results of increasing or decreasing whole muscle volume (Frontera and Ochala, 2015). For most healthy people in day to day life muscle exists in a dynamic equilibrium, with rates of MPS equalling MPB (Brook *et al.*, 2016a).

1.2.2 Signalling pathways

Rates of MPS and MPB are controlled by complex and interlinked intracellular signalling pathways (see Figure 1.6) which respond to various anabolic and/or catabolic drivers such as amino acid supply, exercise, circulating growth factors (GF), inflammation, disease and oxidative stress (Mukund and Subramaniam, 2020). These pathways are complex, still not fully defined and a subject of some controversy. A full discussion of these pathways is therefore beyond the scope of this chapter. However, the main pathways have been well-defined and may be largely categorised as anabolic or catabolic (Egerman and Glass, 2014).

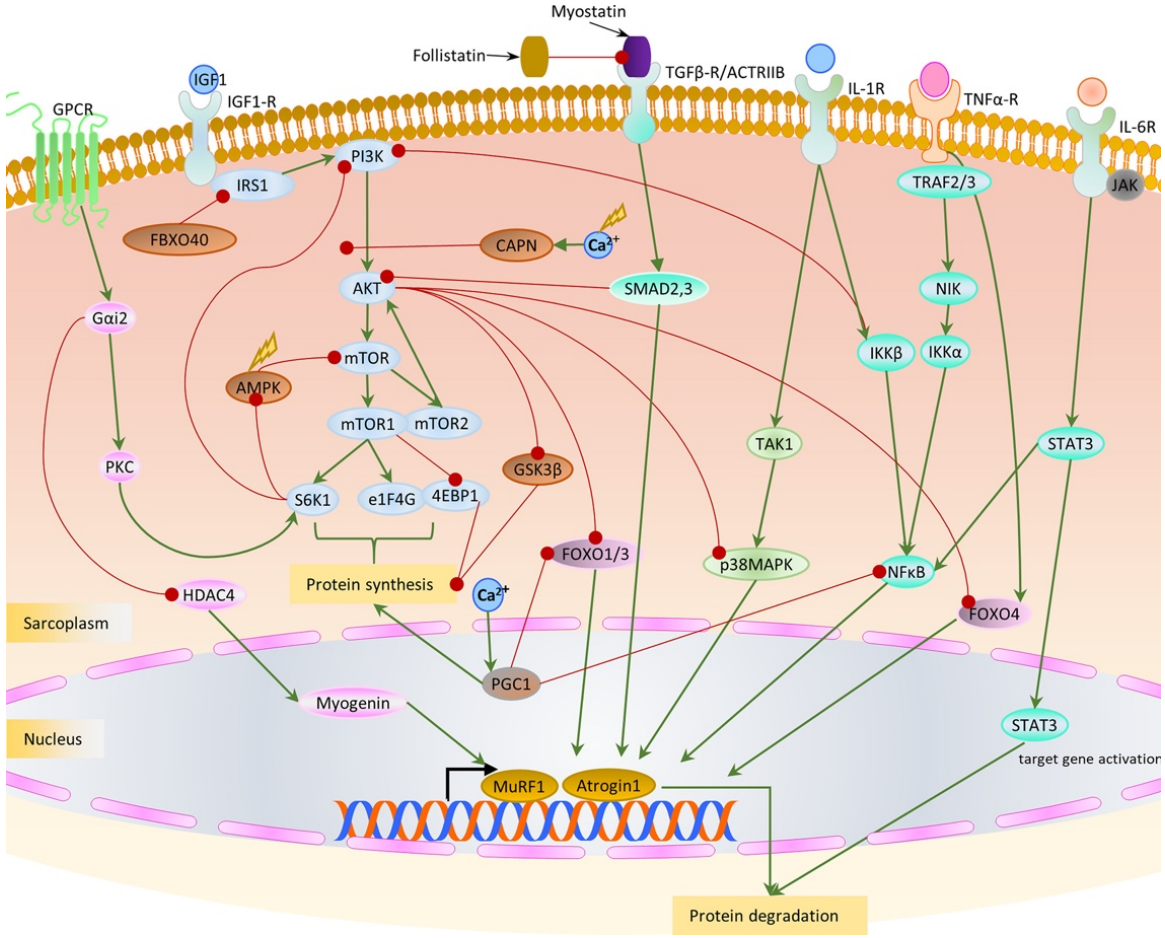

Figure 1.6: Schematic representation of major signalling pathways controlling MPS / MPB rates. Reproduced from Mukund K and Subramaniam S, 2020.

1.2.2.1 Anabolic signalling pathways

The primary signalling pathway responsible for increasing MPS is the insulin-like growth factor 1 (IGF1) / phosphoinositide 3-kinase (PI3K) / Protein Kinase B (AKT) / mechanistic target of rapamycin (mTOR) pathway. Binding of IGF1 to its receptor causes recruitment of insulin receptor substrate (IRS1), which activates PI3K, which in turn activates AKT. The primary activity of AKT is to phosphorylate mTOR, which may then complex with RPTOR to form mTOR complex 1 (mTORC1) or RICTOR to form mTOR complex 2 (mTORC2). mTORC1 upregulates the activation of ribosomal protein S6 kinase beta-1 (S6K1, also known as p70S6k) and downregulates the inhibitor of eIF4E-4EBP1 leading to increased MPS (Mukund and Subramaniam, 2020). AKT also acts independently of mTOR to increase MPS by inhibiting glycogen synthase kinase 3b (GSK3B) and decrease MPB by inhibiting Forkhead box O (FOXO) (Schiaffino and Mammucari, 2011) and increasing myoblast differentiation by inhibiting myostatin (Glass, 2010).

1.2.2.2 Catabolic signalling pathways

Whist there is one major anabolic cell signalling pathway, there are multiple catabolic pathways. The ubiquitin-proteasome system, through the action of ubiquitin E3 ligases, such as Atrogin-1 and MURF1, is considered the most significant regulator of muscle protein breakdown (Mukund and Subramaniam, 2020). Levels of Atrogin-1 and MURF1 are dependent on the activation of FOXO transcription factors, which are in turn regulated by AKT as mentioned above (Sandri *et al.*, 2004), with

reduced activation of AKT therefore increasing activity of ubiquitin-proteasome system. However, Atrogin-1 and MURF-1 expression may also be upregulated through alternative pathways. Inflammatory mediators interleukin 1 (IL-1) and Tumour Necrosis Factor (TNF) α act through the p38MAPK and NFκB pathways, whilst IL-6 acts via phosphorylation of STAT3 (Mukund and Subramaniam, 2020). TNFα has also been shown to independently activate FOXO4 (Moylan et al., 2008) and thereby increase activation of ubiquitin E3 ligases.

Further catabolic signalling pathways and processes have been described in response to inflammatory mediators. Increased activation of lysosomal protease activity in skeletal muscle, with the result of destruction of cellular organelles, has been shown to be dependent on TNFα and FOXO3 signalling pathways (Penna et al., 2013), and is important in muscle loss during starvation, cancer cachexia and disuse. Calpains, calcium regulated proteases, and caspases have also been shown to increase MPB, although the factors controlling these pathways are less clear (Skipworth et al., 2006).

Whilst long-term net imbalance between MPS and MPB leads to the gain or loss of muscle mass respectively, the actual protein balance of a muscle varies throughout the day, controlled by the signalling pathways outlined above.

1.2.2.3 Impact of nutrition on muscle protein balance

In relation to nutrition, during the post-absorptive (i.e. fasted) state MPS rates range from 0.03-0.06%/h^{-1} (Mittendorfer *et al.*, 2005; Kumar *et al.*, 2009), with MPB rates of 0.08-0.10%/h^{-1} (Phillips *et al.*, 1999), effectively resulting in a net loss of muscle protein. However, periodic increases in MPS rates during postprandial periods occur throughout the day to preserve a net balance (Brook *et al.*, 2016a). MPS rates rise significantly (around three time fasted levels) for a short time (approximately two hours) following the ingestion of food (Atherton *et al.*, 2010) (Figure 1.7). The most potent driver of this increase in MPS rates are increased levels of circulating amino acids (AA) with the branched chain AAs isoleucine, valine and in particular leucine being central to these effects (Wilkinson *et al.*, 2013).

The increase in MPS in response to free AA is driven via the mTOR pathway, and although it is known not to be via IGF-1, the activation of this pathway in response to AA is still not clearly defined (Han *et al.*, 2012). It seems likely that increased free intracellular AA levels causes activation of an alternate signalling pathway which also results in activation of mTOR/ mTORC1, the leucyl tRNA synthetase (LRS) having been identified as a key upstream mediator (Han *et al.*, 2012; D'Hulst *et al.*, 2020).

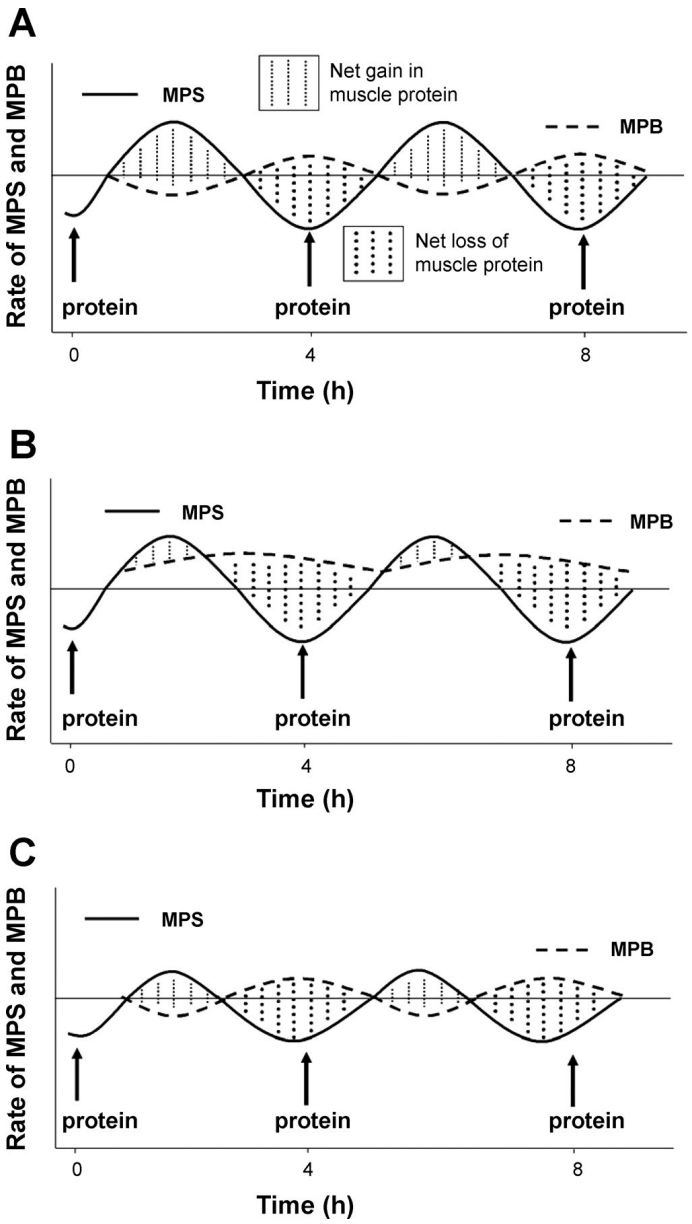

Figure 1.7: Balance between MPS and MPB. A) net balance of MPS and MPB, B) elevated MPB causing net loss of muscle protein c) reduced MPS causing net loss of skeletal muscle (Reproduced from Phillips SM, Glover EI and Rennie MJ 2009).

Carbohydrates also play an important role in the anabolic response to dietary intake. Increases in plasma insulin levels have been shown to decrease MPB rates through downregulation of ubiquitin ligases (Børsheim *et al.*, 2004; Greenhaff *et al.*, 2008), a process that is not seen with elevated AA levels alone.

Interestingly the anabolic effects of increased levels of AA appears to be time-limited, with MPS rates decreasing to baseline levels despite an ongoing supply of free essential AA (EAA). This process is known as the muscle full effect (Atherton *et al.*, 2010). The pathways involved in regulating this process are still not fully understood, but it has been suggested that protein levels are returned to a set point so muscle protein stores are maintained at a constant level (Brook *et al.*, 2016a).

1.2.2.4 Impact of exercise

Mechanotransduction is the term used to describe the conversion of mechanical stress into a biochemical signal that triggers anabolic intracellular signalling and MPS (Pasiakos, 2012) and is key for the maintenance and/or growth of skeletal muscle.

Whist it is clear that load bearing is a definite stimulus of MPS, the exact mechanism of exercise-induced muscle hypertrophy remains unclear (Bamman *et al.*, 2001). There is evidence that exercise increases activity of the muscle IGF-1 system, which results in increased activity of the PI3K/ AKT pathway (Bamman *et al.*, 2001), with this further supported by other studies which show that exercise results in increased muscle production of IGF-1. which then triggers the PI3K/Akt pathway (DeVol *et al.*, 1990). However other studies suggest that exercise acts independently of IGF-1, with suggested alternative pathways including activation of the mechanosensitive tyrosine kinase, focal adhesion kinase (FAK) (Gao *et al.*, 2018). Mechanical activation of FAK leads to activation

of phospholipase D1 and the production of phosphatidic acid which directly actives mTOR (O'neil *et al.*, 2009).

Whatever the upstream signalling cascade most studies agree that MTORC1 is the central determinant of MPS in response to exercise (Goodman *et al.*, 2011). However, mTORC1 activation and the increase in MPS rates in response to exercise varies depending on the type and intensity of exercise performed. For example, mTORC1 activation and MPS rates have been shown to vary dependant on volume of exercise, load placed on the muscle (Burd *et al.*, 2010), and the time that the muscle is under tension (Shepstone *et al.*, 2005). There is also evidence that exercise may also result in an increase in muscle fibre number independently of mTOR, as when rapamycin is used to block this pathway in participants undergoing resistance exercise training (RET), some increase in muscle CSA are still observed (Goodman *et al.*, 2011). Exercise has also been shown to temporarily increase circulating levels of anabolic hormones such as testosterone, growth hormone and cortisol (Hansen *et al.*, 2001).

Exercise also appears to cause a decrease in MuRF-1, Calpin-1 and Calpain-2 production, and thereby leads to a reduction in MPB, although again the signalling pathway involved in this is not clear. Whilst this may seem confusing, it is likely that the many different pathways identified through which exercise affects muscle protein balance reflects the central role which exercise plays in modulating the activity of other signalling

pathways and thereby controlling muscle homeostasis. This is evident in the effect that exercise has on muscle response to increased AA intake, where exercise before AA intake has been shown to both prolong and amplify the anabolic response to free AA and thereby increase overall MPS (Brook *et al.*, 2016a).

1.2.3 Blood flow

Skeletal muscle contains a dense capillary network that provides the supply of oxygen and nutrients and removal of waste that is essential for optimal muscle health and function (Hendrickse and Degens, 2019). The two key factors in governing muscle blood flow (MBF) are the density and distribution of capillaries, with greater capillary density (CD) and more homogenous fibre distribution leading to increased flow, improved oxygenation (Goldman, Bateman and Ellis, 2006) and fatigue resistance (Hauton *et al.*, 2015). The CD of muscle is, like many features of skeletal muscle, highly adaptable and is known to be increased with endurance exercise training (Saltin *et al.*, 1977).

Capillary density also closely correlates with muscle fibre size and increases in CD and fibre growth are coupled (Hendrickse and Degens, 2019). Is has not been established whether changes in CD are responsible for changes in fibre size, or both CD and fibre size respond simultaneously to the same stimuli, or CD changes in response to an alteration in fibre size. Evidence from a single study in rats showed that CD changes lag behind loss of muscle fibre CSA following denervation,

suggesting that CD changes are responsive to loss of muscle mass rather than driving them, but to date, this has not been studied in human subjects (Paudyal *et al.*, 2018).

To illustrate the scope of variation in MBF requirements, during strenuous acute exercise MBF may rise to 100-times baseline levels (Poole *et al.*, 2011), with a 50-80% increase following food ingestion mediated by insulin (Wagenmakers *et al.*, 2016). In older age this increase in muscle blood flow following feeding is diminished and as a result, some suggest that the nutrient dependent depression of MPB rates seen in healthy young muscle is also lost (Phillips *et al.*, 2015). As such, reductions in MBF responses to known vasodilatory stimuli (e.g., nutrition and acute exercise) may be responsible for alterations in muscle protein metabolism and subsequent losses of muscle mass.

1.2.4 Stem Cells

As introduced in section 1.1.3, in adult skeletal muscle stem cells mostly exist as SCs, so called because of their location at the periphery of muscle fibres in a ‘niche’ beneath the basal lamina (Mukund and Subramaniam, 2019). It is these SCs which give the muscle its ability to regenerate following injury. There is evidence that SCs play a key role in maintenance of muscle mass and muscle hypertrophy (Blaauw and Reggiani, 2014). For example, adult muscle nuclear domains remain consistent in size even following hypertrophy, suggesting new nuceli are produced during hypertrophy. Furthermore, in both animal (rat)

(Schiaffino, Pierobon Bormioli and Aloisi, 1976) and human studies (Mackey *et al.*, 2009; Macaluso and Myburgh, 2012) there is increased SC activation during RET-induced muscle hypertrophy, with this activation of SCs preceding muscle hypertrophy (Bruusgaard *et al.*, 2010). The signalling pathways which trigger this process of increased SC activity are not fully characterised, but it has been suggested that mechanotransduction and raised pressure in the SC niche may play an important role in their activation (Blaauw and Reggiani, 2014).

1.3 Skeletal muscle function

Due to the unique structure and cellular processes of skeletal muscle it has multiple functions important for whole body health, maintenance of independence, and survival.

1.3.1 Mechanical function

The primary function of skeletal muscle is mechanical, acting as the engine for movement of the skeleton by converting chemical energy to mechanical energy, through the processes already described. This results in generation of force and power with the result of producing movement or maintaining posture and therefore allowing activity. The importance of this function cannot be underestimated as it allows participation in social and occupational activities and independence with activities of daily life (Frontera and Ochala, 2015). Maintenance of this muscle function is therefore essential in maintaining vitality and mental wellbeing into older age (Goodpaster *et al.*, 2006a).

The complexity and variety of mechanical tasks undertaken by skeletal muscles requires a variety of characteristics. Some tasks require short periods of high force generation, whilst others need repeated, slow, low force movements. The mechanical function of muscle can therefore be assessed under three main categories: strength, power and fatiguability.

Strength is the peak torque that can be developed against a load and is a measure of the ability of a muscle to generate force. It is a direct product of the force of contraction generated by muscle fibres, and depends on the number of sarcomeres acting in parallel and the force per sarcomere (Mitchell *et al.*, 2012). Changes in size and number of fibres will therefore have a direct effect on muscle strength (Metter *et al.*, 1997).

Power is a measure of the work performed by a muscle per unit of time. It is a measure of the product of velocity and force of contraction. It depends not only on generation of force, but also coordination of movement. It is known that power is lost faster than strength in relation to declines in muscle function (Skelton *et al.*, 1994).

Performance fatigue describes the exercise induced reduction in the ability of a muscle to exert power (Mitchell *et al.*, 2012). Fatigability has many contributors, including contraction type and task intensity and varies between muscle groups (Avin and Law, 2011). As outlined above, predominance of type 2 fibre types makes muscles more fatigue susceptible due their increased reliance on glycolytic metabolism.

1.3.2 Metabolic function

The second function of muscle, beyond locomotion, is to act as a metabolic organ. Skeletal muscle is a major site of insulin mediated glucose uptake (DeFronzo and Tripathy, 2009) and a large reservoir of protein. As the process of MPS and MPB are both constantly in action, muscle is a dynamic and plastic organ and can respond to various physiological and pathological stimuli (Bonaldo and Sandri, 2013), enabling control of blood glucose level and supply of AAs essential to function of other organs (Spargo, Pratt and Daniel, 1979). Both of these roles are essential in the body's ability to respond to stress and chronic illness (Frontera and Ochala, 2015).

1.3.2.1 Blood glucose control

Following a meal, the rise in blood insulin levels stimulates the rapid translocation of glucose transport proteins (the primary from of which in human skeletal muscle is GLUT4), into muscle cell membranes (Hirshman *et al.*, 1990), and absorption of blood glucose into skeletal muscle cells. The majority of this glucose is then converted to glycogen (Sinacore and Gulve, 1993). Muscle glycogen acts primarily as a source of fuel for the muscle itself during periods of fasting, and only helps to maintain blood glucose levels by reducing the requirement for muscle uptake of blood glucose during fasting (Wasserman, 2009). If a state of starvation continues, following the consumption of glycogen stores (which takes around 24 hours in an adult) the liver switches to gluconeogenesis and produces glucose using circulating AAs. These

AAs are liberated from skeletal muscle and skeletal muscle thus acts as a key fuel for periods of physiological stress (Sinacore and Gulve, 1993).

The insulin sensitivity of skeletal muscle and its ability to contribute to blood glucose level control have been shown to be dependent on muscle blood flow (Baron *et al.*, 1994), and can be improved with physical training (Holten *et al.*, 2004; Turcotte and Fisher, 2008). It is now well established that deficits in skeletal muscle insulin sensitivity are key in the development of problems with whole body blood glucose control, such as metabolic syndrome, non-alcohol fatty liver disease and type 2 diabetes mellitus (Petersen *et al.*, 2007).

1.3.2.2 Protein store

As already discussed, in post-absorptive periods when there is no supply of additional EAA being absorbed, the rate of MPB outweighs that of MPS (Brook *et al.*, 2016a). As a result, there is a net breakdown of muscle protein and release of AAs into the blood. The released EAAs are then distributed to other tissues and organs to allow synthesis of essential proteins and enzymes (Wolfe, 2018). In the postprandial period, MPS rates are restored, and muscle stores of AA are replenished. Skeletal muscle can therefore be seen as a dynamic store of AAs which supplies other organs in times of fasting.

Muscles also play a key role in providing AAs at times of physiological stress. Following burns, trauma, sepsis, or surgery, patients may enter a state of hypercortisolaemia and hypoandrogenemia, such that MPB rates exceed MPS even after intake of dietary proteins (Ferrando et al., 2001). Furthermore, transmembrane transport of AA out of muscle cells is increased, whist inward transport is slowed (Biolo *et al.*, 2002). This release of AA is essential for the production of acute phase proteins, inflammatory mediators and proteins required for wound healing (Hasselgren, 2012).

1.4 The importance of maintaining a normal muscle mass

Skeletal muscle plays an essential role in maintenance of independence, health and vitality throughout life and into older age. It is the largest body compartment in most adults and loss of muscle is a key marker of frailty (Heymsfield *et al.*, 2014). Indeed, low skeletal muscle mass, in any age group is an independent risk factor for mortality (Goodpaster *et al.*, 2006a). Maintaining a healthy lean muscle mass is crucial for providing energy to vital organs during stress conditions (Bonaldo and Sandri, 2013) and aiding recovery during periods of ill health. In periods of physiological stress such as trauma, sepsis and cancer there is a great demand on AAs liberated from muscles to supply the increased demand for AA precursors for synthesis of inflammatory proteins, proteins involved in wound healing and acute phase proteins in the liver (Biolo *et al.*, 2002). The lack of protein reserve arising from low skeletal muscle mass results in worse outcomes, with even recurrence of malignancy

being increased in those with low lean muscle mass (Kadar *et al.*, 2000). These effects are further compounded by low muscle function (specifically strength and power), which results in even worse recovery times from severe illness and prolonged hospitalisation (Wolfe, 2006).

1.5 Muscle atrophy

Atrophy is defined as the decrease in size of a tissue or organ as a result of cell shrinkage, due to loss of organelles, cytoplasm or proteins (Bonaldo and Sandri, 2013). In muscle there are many pathological processes which cause atrophy. Specific medical conditions such as cancer, diabetes, renal failure, heart failure, neurodegenerative diseases and systemic inflammatory response syndrome (SIRS) which may be caused by sepsis, burns, surgery or major trauma (Gao *et al.*, 2018) all cause muscle atrophy. Amongst the general population not affected by these specific conditions muscle atrophy also occurs secondary to both ageing and disuse (Oikawa, Holloway and Phillips, 2019).

As outlined already, muscle relies on the interaction of nutrition and contraction to induce increases in MPS and suppression of MPB to maintain the net protein balance required for muscle mass maintenance. Disuse may therefore compound any loss of muscle mass due to innate muscle aging or underlying conditions and has been postulated to act as a significant driver of muscle atrophy across the life-course, playing a crucial role in the muscle atrophy observed with advancing age

(McCormick and Vasilaki, 2018; Oikawa, Holloway and Phillips, 2019)(McCormick and Vasilaki, 2018).

1.5.1 A sedentary and ageing population

Current UK national guidelines advise moderate intensity exercise for 150 minutes per week with additional muscle strengthening exercise for all adults (Bull, 2010). However, recent figures show that 60% of people are unaware of these requirements (British Heart Foundation, 2017), with 44% of adults never doing any moderate physical activity and 39% (equal to 20 million people) being identified as inactive. In addition, levels of inactivity have been shown to increase with advancing age (British Heart Foundation, 2015). As one of the main anabolic stimuli for maintenance of muscle mass is contractile activity (i.e. exercise), based on these statistics people in the UK are at risk of low lean muscle mass and the associated health implications with this risk increasing with age.

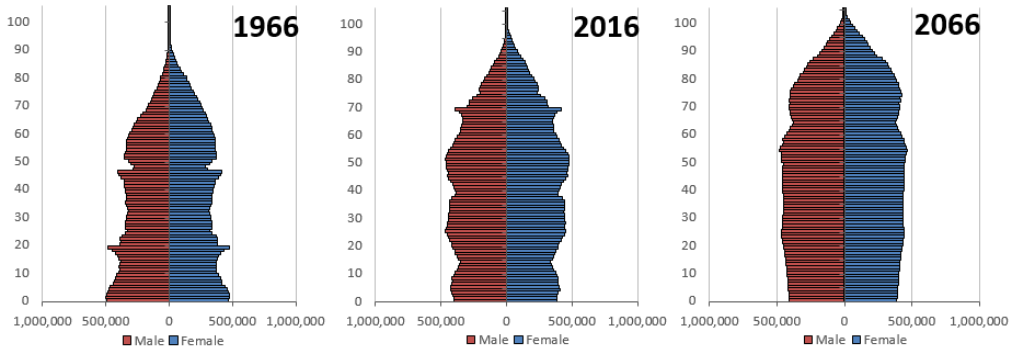

Figure 1.8*: Population pyramids, 1966, 2016 and 2066 (principal projection), UK. From the ONS Report: Living longer: how our population is changing and why it matters.*

In common with other western countries, the population age of the UK is rapidly increasing. By 2066 it is predicted that 26% of the population will be over 65 years, with the fastest increase seen in the over 85 years age-group (Office of National Statistics, 2018) (Figure 1.8). As a result, an

ever-increasing proportion of the population are expected to be inactive and have a low skeletal muscle mass.

1.5.2 Muscle ageing and sarcopenia

As part of the aging process “there is probably no decline in structure and function more dramatic than the decline in lean body mass or muscle mass over the decades of life” (Rosenberg, 1997). Age-associated loss of muscle mass and function are well characterised (Metter *et al.*, 1997), with a rapid increase in the rate of decline beyond the 60th year of life (Mitchell *et al.*, 2012; Dodds *et al.*, 2014). Furthermore, there is consistent evidence of more rapid loss of muscle function than muscle mass. For example, there is a loss of approximately 1% of lean leg muscle mass per year in adults over 70 years of age, but a threefold greater loss of leg strength (Goodpaster *et al.*, 2006a). The greater change in muscle function compared to size indicates that in addition to reduced muscle mass, declines in muscle quality (Goodpaster *et al.*, 2006a), and likely neuromuscular function (Borzuola *et al.*, 2020) also contribute to age-associated reductions in muscle function.

Age-related changes in muscle and the mechanisms underpinning muscle aging are still not fully understood. Many factors are likely to contribute to the net loss of muscle mass and function observed with aging. An increase in markers of senescence amongst skeletal muscle fibres and satellite cells in older skeletal muscle (Zhang *et al.*, 2022) is accompanied by a reduction in satellite cell number and impaired satellite

cell activation (ME *et al.*, 2009), with muscle fibre atrophy and a reduction in total muscle fibre numbers (Lexell, Taylor and Sjöström, 1988; Wilkinson, Piasecki and Atherton, 2018). There is also evidence of an accumulation of a greater proportion of non-contractile tissue in aged muscle, which may be secondary to increased muscle fibrosis due to fibro-adipogenic progenitor dysfunction (ME *et al.*, 2009) and muscle fibre denervation (Piasecki, Ireland, David A Jones, *et al.*, 2016). The fibre type composition of muscle may also be altered with advancing age. Studies suggest that in older males there is a shift towards more 'slow twitch' type 1 fibres as detected by electromyography (EMG) (Vaillancourt, Larsson and Newell, 2003) and preferential denervation of fast type fibres (Einsiedel and Luff, 1992). This is accompanied by a decrease in number and function of muscle mitochondria (Peterson, Johannsen and Ravussin, 2012).

There is also some evidence of a decline in intracellular events related to contractile function with ageing (Krivickas *et al.*, 2001), such as a decrease in calcium released by the SR (Delbono, O'Rourke and Ettinger, 1995) which would impair muscle activation and may be due to fragmented SR (Weisleder *et al.*, 2006). All these factors may account for the loss of muscle mass and function (especially strength and power) which is seen with ageing.

Interestingly, resistance exercise has been shown to be an effective countermeasure against many of these processes, highlighting the role

disuse plays in accelerating skeletal muscle aging (Frontera, Zayas and Rodriguez, 2012; Peterson, Johannsen and Ravussin, 2012).

1.5.2.1 Sarcopenia

Sarcopenia was first defined by Rosenberg in 1989 and is derived from the Greek terms for flesh *("sarx")* and loss *("penia")* (Rosenberg, 1989). The definition of sarcopenia has been through many iterations, with the most recent definition from the European Working Group on Sarcopenia in Older People (EWGSOP) defining sarcopenia as 'low muscle strength, accompanied by low muscle mass with or without low physical performance' (Cruz-Jentoft *et al.*, 2019).

This is significantly different to the previous EWGSOP definition whereby the primary characteristic was low muscle mass (Alfonso J Cruz-Jentoft *et al.*, 2010). Table 1 depicts the operational definition of sarcopenia based on the 2018 report.

Criteria	Operational Rules
	Probable sarcopenia is identified by Criterion 1. Diagnosis is confirmed by additional documentation of Criterion 2. If Criteria 1, 2 and 3 are all met, sarcopenia is considered severe.
1	Low muscle strength
2	Low muscle quality or quantity

3	Low physical performance

Table 1.1: Operational definition of sarcopenia (Cruz-Jentoft et al., 2019).

It has been demonstrated that osteoporosis, insulin resistance, obesity and arthritis are each more common amongst those with sarcopenia, and these individuals are also more susceptible to accidental falls and fractures (Deschenes, 2004). Furthermore, individuals with sarcopenia are at increased risk of death compared with age-matched individuals who are not sarcopenic (Alfonso J Cruz-Jentoft *et al.*, 2010), and the degree of sarcopenia serves as a significant predictor of all-cause mortality (Metter *et al.*, 2002).

Estimated annual costs of the sequel of sarcopenia are $18 billion in the US (Clark and Manini, 2010), and £2.5 billion in the UK (Pinedo-Villanueva *et al.*, 2019). Each individual with sarcopenia has excess healthcare expenditure costs of around £2707 (Pinedo-Villanueva *et al.*, 2019), and just a 10% reduction in the prevalence of sarcopenia would result in savings of millions of pounds (Janssen *et al.*, 2004)..

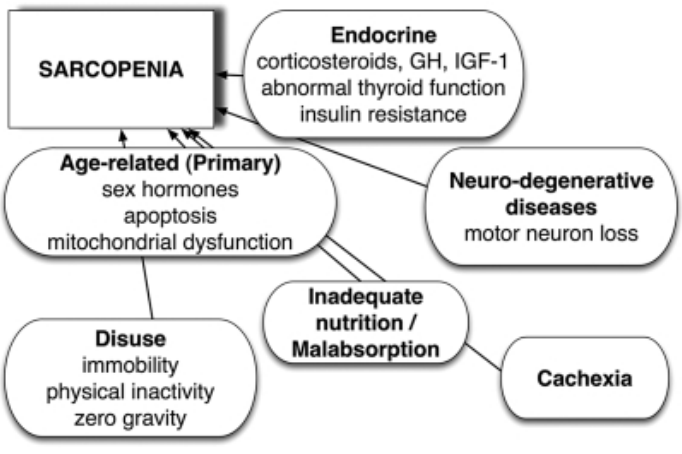

Figure 1.9: Mechanisms of Sarcopenia (from Cruz-Jentof et al, 2010).

Although the development of sarcopenia is multifactorial and its true aetiology is not yet defined, it has been suggested that age-related changes in muscle likely accounts for 30-40% of declines in strength, with the remaining decrease explained by a combination of reduced physical activity, endocrine disturbances and motor neurone degeneration/loss (Dhillon and Hasni, 2017) (Figure 1.9). Furthermore, it is often the case that age-related declines in muscle function initiate a downward spiral, leading to reduced physical activity, which results in further decreases in muscle mass and function (Oikawa, Holloway and Phillips, 2019).

1.5.2.2 The role of disuse in age-related muscle loss

One important aspect in the development of sarcopenia may be muscle atrophy induced by short-term periods of muscle disuse, with suggestions that multiple short-term periods of muscle disuse (due to injury or sickness) contribute considerably to the net muscle loss observed with ageing (Tudorascu *et al.*, 2014) (Figure 1.10). Situations such as recovery from illness and injury, hospitalisation, or even poor weather can result in short periods of muscle disuse in older adults. These short periods (<10 days) accumulate, often increasing with age and as such contribute to age-related muscle loss (Wall, Dirks and van Loon, 2013).

There is also evidence that short periods of inactivity have a much greater effect on aged muscle. For example, Suetta and colleagues found that following 2 weeks of immobilisation, older adults were not able to fully

recover losses of skeletal muscle mass, despite receiving the same intensive RET that led to full recovery in young adults (Suetta *et al.*, 2009). In addition, in a longitudinal study of muscle mass and function, it was found that in older adults, gains of lean mass were not accompanied by strength maintenance or gain (Goodpaster *et al.*, 2006a). Furthermore, aged muscle has a reduced MPS response to AA intake and contraction, and this phenomena termed *'anabolic resistance'* has been suggested to result from periods of muscle disuse (Oikawa, Holloway and Phillips, 2019).

Seemingly innocuous, short-term periods of immobilisation may therefore play an important role in the development of sarcopenia, causing rapid drops in muscle mass and function from which individuals never recover.

Figures from one NHS trust in the UK indicate that over 30% of the population aged over 75 years are hospitalised each year (Information Services Division, 2018), with a separate study showing that this patient age group took an average of just 740 steps per day during hospitalisation (Fisher *et al.*, 2011). To contextualise this number, studies have shown that a daily step count of 2000 steps or less elicits the same loss of lean leg mass, aerobic capacity, strength and muscle fibre volume as total bed rest (Arentson-Lantz *et al.*, 2019a). This would indicate that up to 1/3 of older adults in the UK undergo a period of immobilisation comparable to complete bed rest each year.

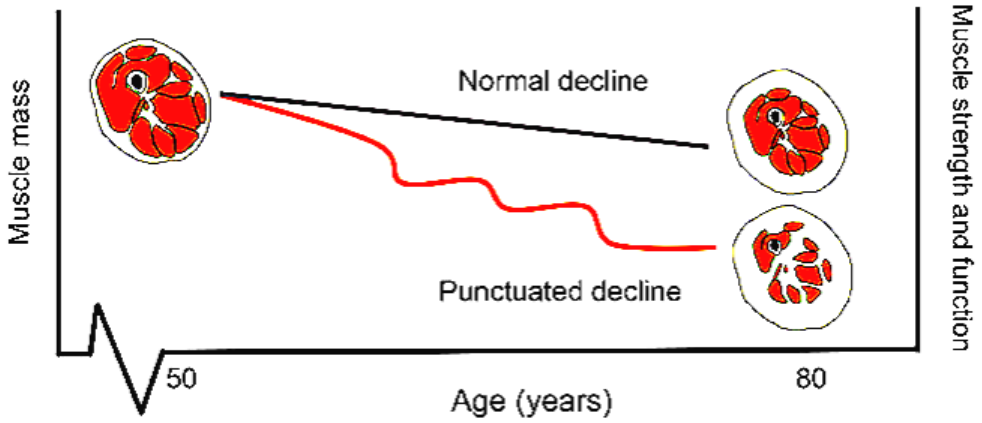

Figure 1.10: Punctuated decline in muscle mass due to short periods of reduced mobility (reproduced from Oikawa S, Holloway T and Phillips S, 2019).

Whilst this explanation of the role of disuse in the development of sarcopenia makes sense, there remain some contradictions. For example, in aging muscle a shift in balance of muscle fibre types towards a greater proportion of MHC type 1 fibres has been an observed (Vaillancourt, Larsson and Newell, 2003), whereas after periods of disuse there is a shift towards a greater proportion of type 2 fibres (Demangel *et al.*, 2017). Further studies are required to provide a definitive answer to this, however it is possible that the acute changes after a period of disuse may undergo remodelling over time, with the rapid loss of type 1 fibres after disuse followed by slower remodelling and transition of type 2 fibres back to type 1.

1.6 Disuse muscle atrophy

Muscle atrophy solely attributable to disuse is often seen in the context of limb immobilisation following fracture (Psatha *et al.*, 2012a), major surgery (Hardy *et al.*, 2022), spaceflight (Juhl *et al.*, 2021), or during a period of reduced step count or bed rest (Nunes *et al.*, 2022). However, disuse may also act alongside other causes of muscle atrophy such as critical illness (Puthucheary, Rawal, McPhail, Connolly, Ratnayake, Chan, Hopkinson, Padhke, Dew, Paul S Sidhu, *et al.*, 2013), sepsis (Lee and Kim, 2010), disease (e.g cancer)(von Haehling, Anker and Anker, 2016) and ageing (Goodpaster *et al.*, 2006a). This may be in a negative feedback cycle whereby muscle loss due to ill health or aging leads to reduced physical activity and this disuse then drives further muscle loss.

Alternatively, as previously explored (see section 1.2.2.4), physical activity (or lack of) as a major modulator of muscle protein balance may act synergistically with another modulator to drive muscle atrophy. For example, in situations of critical illness or following surgery, it is possible that an inflammatory response driving catabolism may couple with reduced step count or bed rest limiting anabolism (Hardy *et al.*, 2022). Determining the extent to which each factor is driving muscle atrophy is not always possible, however it is likely that disuse is a contributor to many cases of muscle atrophy, and this is especially true in the healthcare setting.

1.6.1 Timecourse of disuse muscle atrophy

Many studies have been carried out to assess the degree of muscle loss associated with disuse muscle atrophy (DMA), using a number of different models including space flight, bed rest, limb immobilisation and step count reduction. Those associated with space missions reported a loss of ~10% of quadriceps muscle volume and 17% loss of gastrocnemius following 100-200 days of microgravity (LeBlanc *et al.*, 2000). However, a similar degree of muscle loss was reported after just 2 weeks of microgravity with 15% loss of muscle volume in knee extensors and 8% loss in plantar flexors (Akima *et al.*, 2000), suggesting that the impacts of DMA may occur early in the process, similar to the hypertrophy observed with RET (Brook ref 2015). Head-down bed rest is purported to model the microgravity of spaceflight (Gao *et al.*, 2018) and has produced similar results to that seen after a space mission with a ~17% loss of vastus lateralis (VL) muscle volume occurring after 84 days of head down bed rest (Trappe *et al.*, 2004).

In single leg immobilisation, the rate of muscle loss has been reported to be ~0.4% per day in the quadriceps and 0.36% in the plantar flexors when studied over a 42 day period (Hackney and Ploutz-Snyder, 2012). However, when a single leg was immobilised for just 7 days in a group of healthy young men, a 6% loss of quadriceps volume was observed, with a 3.5% loss in hamstrings and a 7.2% loss in the VL (Kilroe *et al.*, 2019). This corresponds to an atrophy rate of over double that expected when atrophy is measured over a longer period, again supporting the

suggestion that the DMA predominates early during immobilisation. Other studies also support the idea of greater muscle atrophy during initial immobilisation than would be expected from longer term studies, with reports of a 3.5% loss of quadriceps CSA after 5 days (Dirks *et al.*, 2014; Wall *et al.*, 2014a) which progress to an 8% reduction in CSA by 14 days (Wall *et al.*, 2014), but only progress to 17% by 6-weeks (Rudrappa *et al.*, 2016a).

As DMA studies utilise different methods and time periods of immobilisation, in different muscle groups and various populations, it is difficult to establish the true, muscle specific differential rate of DMA from the data available (Gao *et al.*, 2018). However, it would appear there may be a period of acute DMA (aDMA) with more rapid muscle loss in the first 14 days of disuse (and perhaps even sooner), following which the rate of atrophy plateaus and eventually muscle mass stabilises. This more rapid phase may be driven by different atrophy mechanisms than those observed in later phases of DMA.

This proposition of an aDMA phase is supported by evidence from a study in intensive treatment unit (ITU) patients, who were admitted comatosed following a stroke or traumatic brain injury without any other concurrent medical conditions. The results of this study therefore represent atrophy driven almost entirely by disuse. This study showed that there was a 11.8% muscle loss of anterior thigh muscle CSA from baseline by 7 days, 22.5% by 14 days, 28.7% by day 21, 33% by day 28

and 34.8% loss by day 42. That equates to a rate of ~ 1.7%/day during the first and second week, slowing to 1.14%/day in the third week, 0.88%/day in the fourth week, and just 0.18%/day in the weeks thereafter (Figure 1.11).

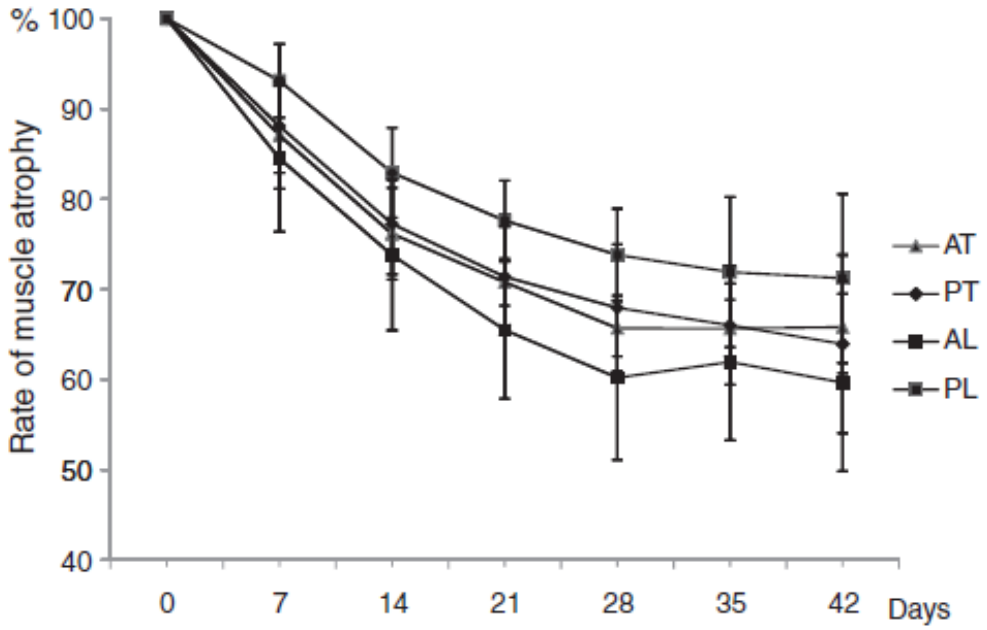

Figure 1.11: Degree of muscle atrophy in ITU patients with isolated brain injury resulting in coma and therefore total immobilisation (From Hirose et al., 2013). AT = anterior thigh, PT= posterior thigh, AL=anterior leg, PL=posterior leg

1.6.2 Possible mechanisms

As reviewed in section 1.2.2.4, exercise or contractile activity causes an increase in net protein balance through various and complex intracellular signalling cascades, and as such is essential for muscle mass to remain neutral on a day-to-day basis.

Decreases in rates of MPS is the most well-established change associated with disuse (Maarten D. de Boer *et al.*, 2007) and this is mainly as a result of decreased activation of the AKT/mTOR pathway (Gao *et al.*, 2018), most probably due to decreased activation of FAK (Glover *et al.*, 2008).

Many studies, where the focus has been on DMA during longer periods of disuse, have suggested that muscle loss is primarily due to a reduction in baseline MPS rates and reduced MPS responsiveness to AA intake, with minimal contribution from MPB (Glover *et al.*, 2008; Wall and van Loon, 2013). However, whilst decreases in MPS remains likely to be the major driver of DMA, there is growing evidence of increased activation of catabolic pathways during the early (first 14 days) phase of DMA. Expression of MAFbx, MuRF1 (Jones *et al.*, 2004a; Wall *et al.*, 2014a) and Atrogin-1 (Suetta *et al.*, 2012) levels all rise rapidly following immobilisation and peak in the first 5 to 14 days (Murton, Constantin and Greenhaff, 2008), suggesting that an increase in MPB may play a role in the early rapid DMA phase. Disuse does not only lead to a net negative protein balance and resultant reduction in fibre CSA, but also causes muscle remodelling. It would appear that type 1 muscle fibres are lost preferentially during disuse with a transition in fibre balance from slow to fast observed in muscles following DMA (Gao *et al.*, 2018).

It must however be noted that the increase in expression of MuRF1/MAFbx does vary from muscle to muscle (Lecker *et al.*, 2004). As discussed in more detail in section 1.7.3, different muscles atrophy at

different rates and to different extents following immobilisation, and so differences in the mechanisms of DMA between muscles would not be surprising. Increases in expression of MuRF1 and MAFbx follow the same pattern as rates of DMA, with a more significant increase in gastrocnemius compared to tibialis anterior, mirroring the greater and more rapid DMA seen in the gastrocnemius (Gao *et al.*, 2018).

1.7 Investigating acute disuse muscle atrophy

1.7.1 Methods of immobilisation

Microgravity, bed rest (BR) (with or without head down tilt), single limb immobilisation (also known as unilateral limb immobilisation (ULLI)) and step reduction are all models which have been used to study the effects of disuse on skeletal muscle. It is accepted and reported in the literature that each model has its own advantages and disadvantages (Gao *et al.*, 2018) and there is seemingly no consensus as to which model is best.

Microgravity studies may reveal interesting and important information regarding the timecourse and mechanisms of DMA, but they are most applicable to space travel and the physiological implications for astronauts (which are numerous and include significant impacts on skeletal muscle structure and function (Fitts, Riley and Widrick, 2000)), since these conditions are not encountered on earth.

Whilst step-reduction addresses the impact of sedentarism (which is topical given the low uptake and adherence to physical activity (British Heart Foundation, 2017), it may be argued that by its nature this cannot be a true model of disuse and is rather a model of less use (Bowden Davies *et al.*, 2019). Muscle atrophy is apparent with step-reduction, but the magnitude of these losses are relatively small compared to both BR and ULLI, and take much longer to be realised (Gao *et al.*, 2018).

Some studies report that ULLI results in a greater degree of muscle loss than BR (Clark, 2009), with the suggestion that whilst BR prevents weight bearing some movement is still allowed, whereas in single limb immobilisation no movement is possible in a select group of muscles (Reggiani, 2015). In contradiction of this however, it has been shown through use of EMG that motor neurone activity does continue in both limbs even during ULLI (Schiaffino and Reggiani, 2011a). Furthermore a number of studies report no significant difference between muscle loss during BR or single limb immobilisation (Dirks *et al.*, 2016).

Bed rest also induces greater morbidity. For example, even if leg muscle loss is exaggerated with single limb immobilisation, BR confers a greater overall muscle loss with 1.5kg of whole body lean mass lost following just 1 week of BR, compared to a loss of just 220g following a week of single limb immobilisation (an almost 7x greater total loss of skeletal muscle) (Dirks *et al.*, 2016). BR also carries the risk of pressure sores and a

greater degree of inconvenience for the participants, in addition to much higher research costs.

As such, the most practical and therefore commonly used (Campbell *et al.*, 2019) technique for the investigation of DMA amongst healthy volunteers is unilateral limb immobilisation.

1.7.2 Duration of immobilisation

Most studies of disuse atrophy have focused on long periods of immobilisation, generally over 2 weeks, with some for more than 3 months (Paddon-Jones *et al.*, 2004; Wall *et al.*, 2014a). This reflects the past focus of much DMA research being space exploration (Lang *et al.*, 2017). However, other than for limb casting following fracture, such prolonged periods of immobilisation are not often clinically relevant.

Average length of stay (LoS) for hospital admissions in the UK is 6 days, with this figure remaining largely consistent over the last decade (Stewart, 2019). This also represents the average LoS for patients undergoing major abdominal surgery, with a median admission duration for elective colorectal cancer resection of 7 days (Aravani *et al.*, 2016). Indeed, even in situations where patients are unwell after surgery and therefore experience a prolonged stay, this is often little more than 2 weeks. For example following emergency laparotomy the median length of stay was 16.3 days (Hussain *et al.*, 2017).

As described above (see section 1.7.2), a number of studies have shown that significant muscle loss occurs after just five days (Suetta *et al.*, 2012; Dirks *et al.*, 2014; Demangel *et al.*, 2017), and that DMA may occur rapidly during early immobilisation which then slows (Rudrappa *et al.*, 2016a). This thesis will therefore focus on this 'acute' phase of disuse muscle atrophy (0-14 days), as it is most clinically relevant and muscle loss appears to be most rapid during this time period.

1.7.3 Comparison of different muscles

Muscle is not a uniform tissue, with muscle fibre types differing between muscles and even between motor units of the same muscle. In a similar way, there appears to be differing atrophy responses to disuse between muscles even within the same limb, and possibly even within different regions of one muscle. Two separate studies totalling 19 male subjects who underwent 60 days of head down BR demonstrated that muscles of the posterior calf (including soleus and gastrocnemius) and the Vasti muscles of the thigh showed the greatest degree of atrophy, with ankle dorsiflexors (i.e., tibialis anterior (TA)) and the hamstring muscles showing the least atrophy (Belavý *et al.*, 2009; Miokovic *et al.*, 2012). These results are also reflected in studies utilising single leg immobilisation (Kilroe *et al.*, 2019), whereby it has been demonstrated that muscles of the anterior thigh atrophy more rapidly than those of the posterior thigh, even over five days of immobilisation.. There is also some evidence that muscles atrophy at differing rates along their length, with

the greatest loss not necessarily occurring at the area of greatest bulk (Miokovic *et al.*, 2012).

Various explanations for the heterogeneity in DMA have been suggested. It is possible that these differences are explained by the degree of usage reduction caused by immobilisation. For example, the Vasti are recruited far more than the hamstring muscles during activities of daily life (ADL) such as walking and rising from a chair (Takahashi *et al.*, 1994; Richardson, Frank and Haseler, 1998). Immobilisation therefore leads to a greater relative reduction in activity in the Vasti, which may then be reflected in the greater degree of atrophy observed in the Vasti.

Heterogenous DMA may also reflect the differing balance of muscle fibre types between muscles, as disuse has been shown to cause a relatively greater reduction in MHC I fibres (Gao *et al.*, 2018). Human autopsy studies of whole muscles have shown MHC type I fibre to make up over 50% of the total in medial gastrocnemius muscle (MG) (Jennekens, Tomlinson and Walton, 1971), whilst only 30% of fibres in the in the Vastus lateralis (VL) are MHC type I (Edgerton, Smith and Simpson, 1975). Studies reporting fibre type ratios for the TA in both rats and rabbits show just 5% MHC I fibres in the TA (Lexell *et al.*, 1994; Tasić *et al.*, 2003). The proportions of MHC I fibre type observed correlate with the rate of DMA in the corresponding muscle, with those muscles with a higher MHC type I fibre proportion atrophying more quickly.

It is also possible that the differing rates of DMA between muscles reflects different activation of intracellular processes. For example, comparison of different muscles during acute disuse has shown that expression of MuRF1/MAFbx genes (involved in MPB cell signalling pathway, section 1.2.2.2) increased to a significantly greater degree and for longer in MG than in TA, reflecting the greater and more rapid losses of muscle mass seen in MG (Gao *et al.*, 2018).

1.7.4 Assessment of muscle mass

Many techniques may be employed in the estimation of whole-body muscle mass, from simple anthropomorphic measurements (such as height, weight, body mass index (BMI), skin fold thickness and limb circumference) to cross sectional imaging (such as magnetic resonance imaging (MRI) and computed tomography (CT)).

Each different technique for the assessment of muscle mass varies not only in sensitivity and accuracy, but also in how easy and expensive it is to perform. The best technique to employ will therefore depend on the setting, with easy, inexpensive, quick but less accurate methods more suited to whole population screening, and more time-consuming, expensive, but more accurate measures being better suited to smaller cohort research projects.

1.7.4.1 Body composition

The definition of skeletal muscle mass may vary depending on the level at which it is being considered. The composition of the body may be assessed based on the most simple model of fat and fat free mass, through to considering the body as multiple different but interacting levels, including the atomic, molecular, cellular, tissue-organ system levels and finally whole body measurements (Wang, Pierson and Heymsfield, 1992).

Anthropomorphic measures such as BMI and skin fold thickness give an idea of whole-body composition and may be used to roughly estimate the ratio and relative size of different tissues. Cross sectional imaging may provide measurements of muscle at a tissue level, but these measurements often include intramuscular fat, blood vessels and nerves. Molecular and even atomic measurements may also be made and used to estimate whole body muscle mass, based on the known molecular make up of muscle.

1.7.4.2 Whole body measures

Early estimates of whole body skeletal mass were based on the observation that creatine, a substance derived from skeletal muscle was excreted in urine at an almost constant rate (Heymsfield *et al.*, 2014). Over 100 years ago it was first calculated that urine creatinine excretion of 1g per 24 hours equated to 22.9kg of skeletal muscle (Bürger, 1919). Other similar techniques that have been used in the past include

determination of total body potassium by counting of K^+ isotopes to determine fat free mass (Novak, 1972) and neutron activation analysis to assess whole body nitrogen levels (Knight *et al.*, 1986) which may then be used to estimate total body protein mass. However, these types of measures are often time consuming and have low accuracy (Ellis, 2000).

More recently, the D3-creatine dilution method has been developed. This involves the oral ingestion of deuterated creatine, which is then absorbed and incorporated into the existing contractile apparatus within muscle fibres. A single, spot urine sample taken 48-96 hours after ingestion allows measurement of creatinine (which is derived from the conversion of muscle creatine to creatinine) and the ratio of labelled to unlabelled creatinine in these samples can be calculated (Cegielski *et al.*, 2021). From this, the size of the whole body muscle creatine pool can be estimated (Stimpson *et al.*, 2012) and then the total whole-body skeletal muscle mass calculated, assuming that there is 4.3 g creatine per kg of muscle. This technique has been shown to have a strong correlation with whole-body muscle mass assessment using MRI (Clark *et al.*, 2014).

In the 1960s the concept that all tissues in the body conduct electricity, with different levels of resistance based on their water and fat content, lead to the development of bioelectrical impedance analysis (BIA) as a way of estimating body composition (Heymsfield *et al.*, 2014).

BIA works by using a various number of electrodes to measure the degree of voltage drop of a weak current passed through the body. More recently a new technique known as bio-impedance spectroscopy (BIS) has been introduced, which allows the body to be divided into segments, and the fat free mass (FFM) of each compartment estimated (Ellis, 2000). Whilst both of these measures are quick, relatively cheap and non-invasive, several factors including hydration status, ethnicity, phase of menstrual cycle and the machine used (Dehghan and Merchant, 2008) can influence its calculation, resulting in a variability of estimated lean body weight of up to 10% (NIHT, 1996), limiting its accuracy and utility in the detection of small changes in muscle mass.

In a similar way to how BIA uses impedance of electrical flow through the body, dual-energy x-ray absorptiometry (DXA) utilises the differing attenuation of x-rays as they pass through different tissues including bone, lean tissue and fat. In clinical settings DXA is primarily used for the measurement of bone density (BMD) (Bobo Tanner and Moore, 2012) and subsequent diagnosis of skeletal conditions such as osteoporosis. However, the use of two x-ray beams of different energies makes it possible to separate soft tissues into fat and lean tissue based on the differing attenuation they cause (Heymsfield *et al.*, 2014). Not only can DXA estimate fat and fat free mass, but it can also isolate different body regions and therefore be used to assess skeletal muscle mass of the legs or arms in isolation (Fuller, Laskey and Elia, 1992).

When compared to cadaveric models, DXA estimates of body composition showed good correlation (r^2 0.98) (Ellis *et al.*, 1994). The standard error of estimate for whole body lean tissue mass with DXA is just 2.7 kg, and DXA has been shown to accurately measure changes in lean tissue mass (Svendsen *et al.*, 1993). In addition, although recent reports have found DXA to overestimate lean mass (Bredella *et al.*, 2010), this error is consistent and so will likely have little impact on the assessment of change within an individual.

Due to its clinical role in the estimation of BMD, DXA is widely available and relatively cheap to perform (beyond initial equipment outlay). Furthermore, the radiation exposure associated with DXA is minimal at 0.01mSv, a dose that is often describe as comparable to three days background radiation or eating a handful of walnuts. It is therefore a useful tool in the estimation of muscle mass in different body regions and how these change with various interventions. However, DXA is unable to differentiate intramuscular adipose tissue, and can be affected by extracellular fluid accumulation in muscle, which may result in over estimation of lean mass and variability of results depending on hydration status (Chianca *et al.*, 2021).

1.7.4.3 Muscle / muscle group specific measures

The whole body measures outlined in the section above provide estimates of whole body, or whole limb muscle mass, derived from formulas containing assumptions regarding tissue composition. Cross sectional imaging methods such as CT or MRI allow direct, in vivo measurements of individual muscles and their CSA, and as a results are considered the gold standard for in-vivo measurements of muscle mass (M. V Franchi *et al.*, 2018).

MRI uses large magnetic forces to cause hydrogen protons within tissues to align to the magnetic field. Radiofrequency energy pulsed in a perpendicular direction to the magnetic field is then used to cause the protons to 'spin' into a new alignment and once this is withdrawn measurements are made of the intensity of the energy released and time taken for its release (Ellis, 2000). Mathematical analysis of these results can then be computed to produce images showing slices through the body at the level the scan was performed.

MRI was pioneered by Sir Peter Mansfield in Nottingham in 1978 (Morris, 2021) and has since become an important clinical imaging modality, especially for the assessment of soft tissue structures (Kalmar *et al.*, 1988). Muscle MRI can (with the correct scan card and analysis software) provide swathes of information beyond muscle CSA or VOL, such as fascicle pennation angle, estimation of body composition, assessment of muscle disruption, oedema, myosteatosis and myofibrosis (Fischer *et al.*,

2014). In addition, MRI scans do not subject participants to any radiation exposure, increasing its utility as a research tool. However, use of MRI involves high costs, large static machinery, and lengthy acquisition time (Chianca *et al.*, 2021), and as a result is not practical or suitable in all settings.

CT utilises x-rays which are collimated to give a fan-shaped beam which is passed through the body to detectors on the other side. The x-ray source (+/- detector) is rotated completely around the body, and the results then reconstructed to give a cross sectional image of the body similar to an MRI. In contrast to MRI however, CT gives true measurements of tissue density, based on x-ray attenuation, with each pixel of the image having a precise value in Hounsfield units (named after Godfrey Hounsfield who pioneered the use of CT in the 1970s) (Heymsfield *et al.*, 2014). CT can therefore not only be used to measure individual muscle volumes but also muscle density. Reduced muscle density may represent fat infiltration and therefore reduced muscle quality, and has been shown to be associated with adverse clinical outcomes (Herrod *et al.*, 2019).

Many studies have tried to establish the reliability of both MRI and CT acquired measurements of muscle. A number of studies report a coefficient of variance of 1-2% for the assessment of muscle mass, indicating a high level of precision; with measurement of quadriceps CSA having a maximum error of approximately 2% for an individual and less

than 1% for the true mean of a group (MacDonald, Greig and Baracos, 2011). Both CT and MRI are similarly accurate when validated against human cadavers with a maximum difference of just 0.6cm^2 for of muscle area (r^2 of 0.99 for both MRI and CT) (Mitsiopoulos *et al.*, 1998).

Whilst both MRI and CT represent the gold standard for the in vivo measurement of skeletal muscle mass, they are not without their drawbacks. CT involves a large radiation dose, making repeated measures unsuitable for healthy research volunteers and not possible in some patient cohorts, whilst MRI is slow (e.g., Tesla 1 (T1- low field) imaging of whole legs takes approximately 30 minutes), expensive and often inaccessible.

Ultrasound scanning (USS), in particular B mode ultrasound, has also been used in the assessment of skeletal muscle, gaining more attention and becoming more widely used in recent years (Stringer and Wilson, 2018; Casey *et al.*, 2022). Unlike MRI and CT, USS is relatively inexpensive, easily accessible, portable and quick to perform (Nijholt *et al.*, 2017). It has also been shown to produce good intra- and inter-rater reliability in the measurement of muscle thickness (MT) (Tillquist et al., 2014; Pardo et al., 2018) with good accuracy (MacGillivray *et al.*, 2009). When compared to a gold standard, USS and MRI measures of CSA in VL muscles had a mean difference of just 0.53cm^3 (MacGillivray *et al.*, 2009) and a concordance correlation coefficient (CCC) of 0.78 (Scott *et al.*, 2017). Furthermore, changes in US measured MT of the quadriceps

in response to strength training have been shown to correlate with changes seen in MRI (measured muscle CSA and volume r=0.82 and 0.73, respectively) (M. V Franchi *et al.*, 2018). This suggests that US is a reliable measure of dynamic muscle changes in response to a hypertrophic stimulus.

Ultrasound has also been used, albeit less, to assess DMA via measurements of simple muscle layer depth in biceps, forearm and thigh (Segaran et al., 2017), and CSA in biceps and quadriceps (ten Haaf et al., 2017), with DMA observed via USS in each of these studies. Panoramic USS to measure CSA has also been used to detect DMA over a 70 day period in both the quadriceps and gastrocnemius, with this measurement also validated against MRI with a sensitivity of 73.7% and 83.1% for the different muscles respectively (Scott et al., 2017).

USS also has the ability to assess muscle architecture and is the primary imaging modality used to do this in vivo (Franchi *et al.*, 2018), facilitating measures of FL and PA. When combined with MT via USS and muscle volume via MRI, the measurement of FL and PA enables calculation of pCSA (Narici, Franchi and Maganaris, 2016), a concept earlier discussed in more detail in section 1.1.2. In relation to the ability if USS to assess changes in muscle architecture, in healthy individuals USS was able to show differential adaptations in muscle architecture with eccentric versus concentric RET (Franchi *et al.*, 2014), and in critically unwell patients USS has shown dynamic changes in muscle architecture (Turton et al.,

2016). However, it must be noted that these observations were not validated against MRI or CT in either study. US is also highly user dependent, with the accuracy of repeated measures depending on exact replication of probe position, angle and pressure. Furthermore, recent exercise can affect results, possibly through acute intramuscular fluid shifts (Welch *et al.*, 2021).

1.7.5 Summary

In summary, human skeletal muscle is a complex and highly specialised organ. Maintenance of muscle mass and proper functioning of muscle is important not only for personal independence and quality of life via locomotion, but also for whole body health. In addition, low levels of skeletal muscle are a key predictor of poor survival during times of physiological stress such as major surgery.

There are many different causes of muscle atrophy, but one of the most common, and therefore important, causes is disuse. Disuse may also play a contributory role in other disease processes which result in further muscle loss. Even relatively short periods of immobility can lead to significant losses of muscle mass and function, and there is evidence that these losses are at their most rapid in the “acute phase” of immobilisation. Hospitalisation and surgery are two major causes of immobilisation and as a result DMA often contributes to healthcare related morbidity. The timecourse of aDMA, its variability between different muscle groups and the functional effects of these changes are still not well characterised.

Clarification of these issues may provide useful experimental models to allow identification of the pathways and mechanisms which control this process, and development of potential therapeutic interventions.

1.8 Thesis aims

The aims of this thesis are therefore to:

assess the degree of ‘acute’ DMA in a range of human lower limb muscles and characterise the timecourse of this atrophy

assess the magnitude of ‘acute’ DMA in different muscles and explore the underlying physiological features of any observed differences

assess the difference in rate of aDMA in the leg muscles of immobilised, but otherwise healthy individuals and individuals immobilised during periods of ill health

assess the functional outcomes of aDMA in different muscles and explore the underlying physiological features of any observed differences

investigate the utility of a potential therapeutic intervention to curtail the loss of muscle mass and function during a period of immobilisation

2 The timecourse of disuse muscle atrophy of the human lower limb in health and disease: A systematic review and meta-analysis.

Chapter abstract

Background

Short, intermittent episodes of disuse muscle atrophy (DMA) may have a negative impact on age related muscle loss. There is evidence of variability in the rate of DMA between muscles and over the duration of immobilisation. As yet this is poorly characterised. This review aims to establish and compare the time-course of DMA in immobilised human lower limb muscles in both healthy and critically ill individuals, exploring evidence for an acute phase of DMA and differential rates of atrophy between muscle groups.

Methods

MEDLINE, Embase, CINHAL and CENTRAL databases were searched from inception to April 2021 for any study of human lower limb immobilisation reporting muscle volume (Vol), cross sectional area (CSA), architecture, or lean leg mass over multiple time points. Risk of bias was assessed using ROBINS-I. Where possible, meta-analysis was performed using a DerSimonian and Laird random effects model with effect sizes reported as mean differences (MD) with 95% confidence intervals (95%CI) at various time-points. A narrative synthesis of results was performed when meta-analysis was not possible.

Results

Twenty-nine studies were included, 12 in healthy volunteers (total n=140), 14 in patients on an Intensive Therapy Unit (ITU) (total n=422) and 3 in patients with ankle fracture (total n=39). Rate of quadriceps atrophy over the first 14 days was significantly greater in the ITU patients (MD -1.01 95%CI -1.32, -0.69), than healthy cohorts (MD -0.12 95%CI -0.49, 0.24) ($p<0.001$). Rates of atrophy appeared to vary between muscle groups (greatest in triceps surae, followed by quadriceps, then hamstrings then foot dorsiflexors). Rates of atrophy appear to decrease over time (day 0-14 vs day 0-28 in healthy quadriceps and triceps surae, day 0-2 vs day0-7 in ITU quadriceps).

Discussion

There appears to be variability in the rate of atrophy between muscle groups, and more rapid atrophy during the earliest period of immobilisation. Rates of atrophy are greater amongst critically unwell patients. Early intervention is required to prevent muscle loss in clinical patients. Any intervention should consider variability in atrophy between muscle groups and target those muscle groups worst affected. Evidence of variability in atrophy rates may indicate different mechanisms being dominant at different time points and in different muscle. Overall evidence is limited, and existing data has wide variability in the measures reported. The majority of included studies are at moderate risk of bias. Further work is required to fully characterise the timecourse of DMA in both health and disease.

2.1 Introduction

As discussed in chapter one, maintenance of adequate skeletal muscle mass is essential for a healthy, long life (Mitchell *et al.*, 2012). Sarcopenia (low skeletal muscle strength and mass, with or without low physical performance (Cruz-Jentoft *et al.*, 2019)) is associated with an increased risk of falls, need for long term care (Deschenes, 2004), prevalence of chronic health conditions (Srikanthan, Hevener and Karlamangla, 2010)) and a worse age-matched mortality (Alfonso J Cruz-Jentoft *et al.*, 2010). Whilst some muscle loss inevitably occurs with age, not all older people become sarcopenic, and identification of the factors which drive individuals into this state is therefore a topic of considerable interest (Narici and Maffulli, 2010).

Maintenance of muscle mass is known to depend on anabolic responsiveness to exercise and nutrition (Brook *et al.*, 2016b). Disuse combined with anabolic resistance (McKendry *et al.*, 2021) may therefore be a significant contributor. Loss of muscle mass and function occurs after just a few days immobilisation (Dirks *et al.*, 2014). It is theorised this may be followed by reduced habitual activity levels, which in turn increases the risk of further episodes of disuse and may lead to a vicious cycle of repeated episodes of dramatic loss of muscle mass and function after short periods of disuse accelerating the usual age-related loss of muscle, and driving people below the sarcopenic threshold (Oikawa, Holloway and Phillips, 2019).

Much of the existing research into disuse muscle atrophy (DMA) is in the context of space exploration (Lang *et al.*, 2017) and consequently reports the effects of immobilisation for many weeks or even months (LeBlanc *et al.*, 2000; Paddon-Jones *et al.*, 2004). However, comparing the results of several studies suggests variability in DMA. For example, rates of DMA may vary over the duration of immobilisation, with early rapid losses after which the rate of atrophy slows and eventually plateaus (Rudrappa *et al.*, 2016a). For example, after 17 days microgravity a 7.4% loss of quadriceps muscle volume (Vol) was reported, which increased to just 12.4% loss after 23 weeks (LeBlanc *et al.*, 2000). The same phenomenon is observed over shorter periods of time with corresponding changes in muscle function (Wall *et al.*, 2014b; Rudrappa *et al.*, 2016b) . There is also evidence that the rate of DMA may differ between muscles (Miokovic *et al.*, 2012), with some muscles appearing to be atrophy susceptible (aS) whilst others are atrophy resistant (aR). Furthermore, given the catabolic impact of illness and infection (Ferrando *et al.*, 2001), acute muscle loss in hospitalised, and therefore often relatively immobile patients, may be more rapid and severe than that seen in healthy volunteer studies.

If demonstrated to be true, this variability in the rate of DMA may have several implications. From a scientific standpoint, if the rate of atrophy varies over time, then different cellular mechanisms and pathways may be active (or dominant) at different periods. Investigation of the difference in response to immobilisation between aR and aS muscles may yield important information into the mechanisms through which DMA is

controlled and potentially mitigated. From a clinical perspective, if muscle is lost most rapidly at the start of immobilisation it is essential that strategies and therapies to counter this are introduced early during hospital admissions. Consideration of aS and aR muscles will be important in optimising the design of any therapies introduced to combat DMA.

The primary aim of this systematic review and meta-analysis is therefore to characterise and compare the time-course of DMA in muscles of the human lower limb during immobilisation in both healthy and critically ill individuals. It will explore the evidence for non-linear muscle loss, including an acute phase of DMA, and differential rates of muscle loss in different leg muscles and muscle groups.

2.2 Methods

2.2.1 Study design

This systematic review was registered prospectively with PROSPERO (registration number 106495) and carried out in accordance with the PRISMA statement (Page *et al.*, 2021). Any study reporting data on human lower limb muscle changes during immobilisation or admission to ITU over multiple time points was included. The minimum outcome reporting required for inclusion was measurement of at least one of muscle Vol, CSA, lean leg mass or muscle architecture (muscle thickness (MT), fibre length (FL), pennation angle (PA)) at baseline and a minimum of two timepoints following immobilisation or admission to ITU. Studies which did not report muscle measures at multiple time points during immobilisation, did not involve full immobilisation, bed rest (BR), or critical care admission, did not report leg muscle changes, or did not report the measures listed above were excluded.

2.2.2 Literature search

Literature searches were completed by a trained Clinical Research Librarian using the following databases: MEDLINE, Embase, CINHAL and CENTRAL (all searched from their inception to 01/04/2021). No language or date restrictions were applied to the searches. Previous systematic reviews of related topics were also searched for relevant studies. References of identified and potentially relevant studies were hand-searched for further relevant studies. Finally, all studies citing the

included studies identified on Google Scholar were screened for inclusion. Example search strategies can be found in Appendix A.

Abstracts were screened independently by two authors with the aid of Rayyan systematic review software (2016, Qatar Computing Research Institute, Doha, Qatar) (Ouzzani *et al.*, 2016) and were considered for full text review if either author deemed them to be potentially relevant. A grey literature search as described above was also completed. Full text versions of all potentially relevant primary studies were then independently screened against the inclusion and exclusion criteria by two authors and agreement for inclusion reached by consensus. If there was disagreement as to whether a study should be included the opinion of the project's supervising professor was sought.

2.2.3 Data extraction

Study characteristics and outcome data were extracted by one author (EH). Where studies reported the outcomes of interest in graphical form only, relevant data were extracted using WebPlotDigitiser (Rohatgi, 2020). Studies not published in English were translated using Google Translate to allow identification of the relevant data. For studies reporting data as percentage change only, attempts were made to contact the authors to get original data. Risk of bias for included studies was assessed using the Risk of Bias In Non-randomized Studies of Interventions (ROBINS-I) assessment tool, with overall risk of bias decided as per the ROBINS-I critera (Sterne *et al.*, 2016).

2.2.4 Statistical analysis

Effect sizes are reported as mean differences (MD) with 95% confidence intervals (CI). Change standard deviations (SD) were calculated by using the baseline SD and final SD and assuming a correlation coefficient of 0.7 using formulae from the Cochrane Handbook. Missing SD values were estimated from other studies using a summary statistic level imputation method (Weir *et al.*, 2018). A DerSimonian and Laird random-effects model was used. Statistical heterogeneity was assessed using the I^2 statistic. We attempted to conduct tests for publication bias but the small number of studies precluded this. When studies from different cohorts measured the same outcome at similar timepoints, we performed a subgroup analysis and report the p value from the subgroup differences ($p<0.05$). All meta-analyses were conducted using Stata Version 16.

Where there was insufficient original data to allow formal comparison, or comparison was not possible due to mathematical constraints (e.g. between % change), pooled means were calculated from original data.

2.3 Results

A total of 3684 potentially relevant abstracts were screened for inclusion, of which 3303 were unique. Of the unique abstracts screened 45 were found to be possibly relevant and underwent full text review. Following full text review, a further 14 were excluded with the remaining 31 studies found to be relevant for inclusion in this review (Figure 2.1).

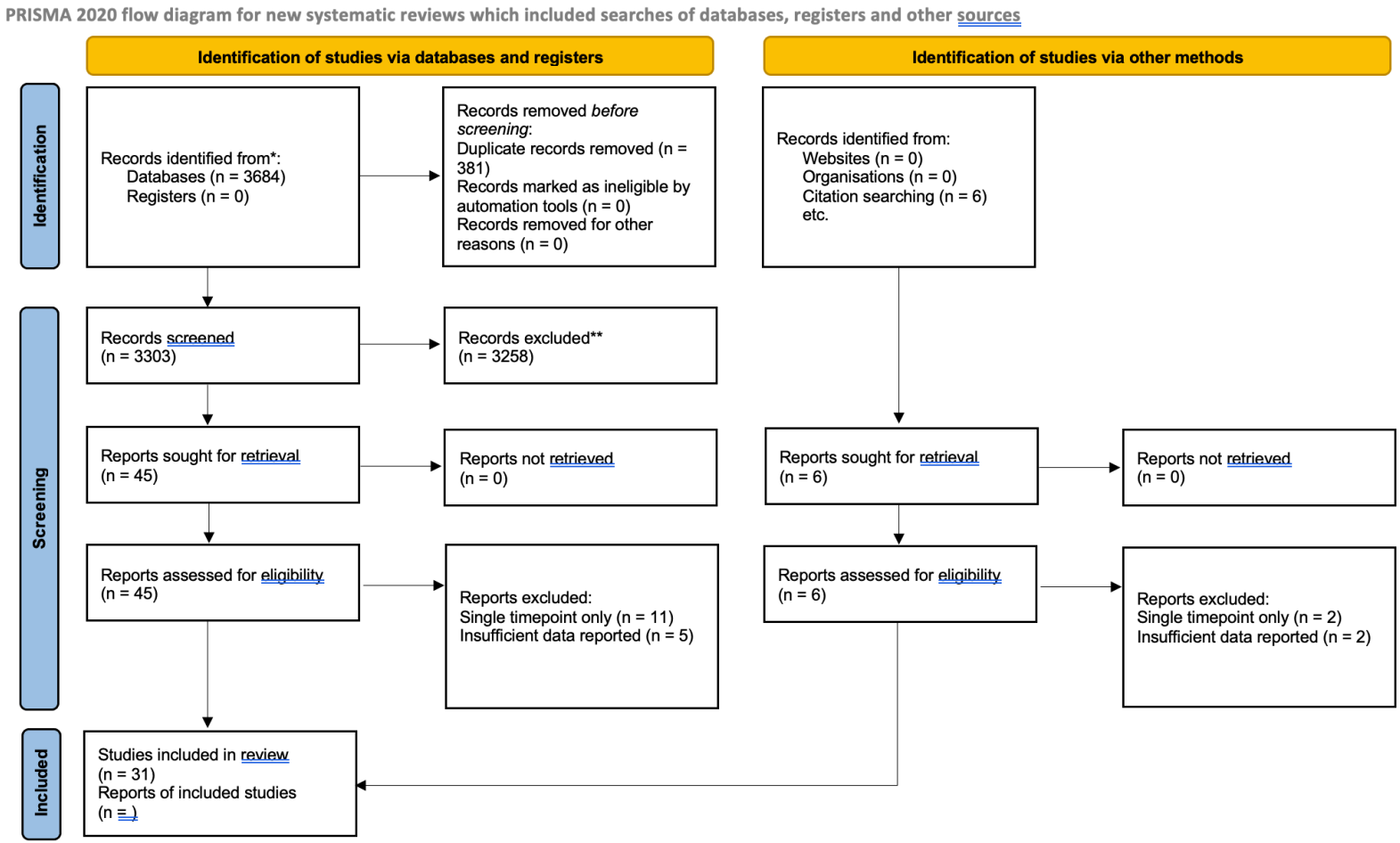

Figure 2.1: PRISMA flow diagram (Page et al., 2021)

2.3.1 Study characteristics

Date of publication ranged from 1997 to 2020. Twenty-eight studies were full texts, one was a correspondence and two were conference abstracts. All studies were published in peer reviewed journals. (Table 2.1)

Twelve studies reported results from healthy volunteer studies. Three studies used unilateral lower limb immobilisation (ULLI), whilst the remainder used BR. Eight were cohort studies, and for the remaining four data were taken from the control limb of a randomised control trial (RCT). Sample sizes ranged from 6 to 20. Time to first measurement ranged from 2 to 28 days, and final measurement ranged from 7 to 88 days.

Fourteen studies reported results from patients admitted to an ITU. Twelve were cohort studies, whilst data from the control limb of one case control study and one RCT were also collected. Ten studies contained a mixture of all ITU admissions, two were of traumatic brain injury (TBI) patients, one was of septic shock patients and one was of extracorporeal membrane oxygenation (ECMO) patients. Sample sizes ranged from 11 to 100. Time to first measurement ranged from 2 to 10 days, and final measurement ranged from 5 to 42 days.

Three studies reported results from patients immobilised following ankle fracture. All were cohort studies. Sample size ranged from 1 to 20. Time to first measurement ranged from 7 to 14 days, final measurement ranged from 14 to 43 days.

Study	Type of study/ publication	Setting	Mechanism of immobilisation	N	Muscles studied	Imaging modality	Measurement	Measurement timepoints (day)
Abe T (1997)	Cohort. Full paper	Healthy	Bed Rest	8	Ant thigh (grouped), Calf (grouped), VL, MG	USS	Muscle Thickness (mm)	7, 14, 20
Akima (1997)	Cohort. Full paper	Healthy	Bed Rest	10	Knee Extensors (grouped), Knee Flexors (Grouped), VL VM, VI, RF, MG, LG, Sol, TA	MRI	Vol(cm3), CSA(cm2)*	10, 20
Armbrecht (2010)	RCT. Effect of resistive vibration exercise (RVE). Full paper	Healthy	Bed Rest	20	Whole leg	DXA	Lean Mass (g)	12, 31, 44, 55
Belavy (2009)	RCT. Effect of RVE. Full paper	Healthy	Bed Rest	20	TA, MG, LG, Sol, Peroneals, Tib Post, FDL, Quads, Hamstring, RF, Vastii, Adductors	MRI	Vol (% change)	14, 28, 42, 56
Boer (2007)	Cohort. Full paper	Healthy	ULLI	17	Quads (grouped), VM, VL, VI, RF	MRI, USS	CSA, FL, PA	14, 23
Borges (2019)	Cohort. Full paper	ITU (sepsis)	Critical care	37	RF	USS	CSA (cm2)	2, 4, 6, 10
Hayes (2018)	Cohort. Full paper	ITU (ECMO)	Critical care	25	RF, VL, VI, RF, Quads (grouped)	USS	CSA (cm2), MT (cm), Echogenicity	10, 20,

Hirose (20013)	Case control vs Electrical muscle stimulation (EMS). Full paper	ITU (CVA / TBI)	Critical care	11	Ant Thigh (grouped), Post Thigh (grouped), Ant lower leg (grouped), Post Lower leg (grouped)	CT	CSA (cm2)	7, 21, 28, 35,42
Kawashima (2004)	Cohort. Full paper	Healthy	Bed Rest	10	Adductors (grouped), AM, AL, AB	MRI	CSA (cm2)	10, 20,
Kilroe (2020)	Cohort. Full paper	Healthy	ULLI	13	Quads (grouped)	MRI	Vol (cm3)	2, 7
Katari (2018)	Cohort. Full paper	ITU (all causes)	Critical care	100	RF	USS	MT (cm)	3, 5, 7, 10, 14, 21
Menids (2009)	RCT, vs. RVE. Full paper	Healthy	Bed Rest	6	Iliacus, Psoas, Iliopsoas, Sartorius, RF	MRI	CSA (cm2)	14, 28, 42, 56
Mikovic (2012)	Cohort. Full paper	Healthy	Bed Rest	9	AB, AL, AM, Pec, Gracilis, Sart, RF, Vasti (grouped), MH, LH, EDL, TA, Perioneals, FDL, FHL, TP, LG, MG, Sol	MRI	Vol (cm3)	28, 56
Mulder (2006)	RCT, vs RVE. Full paper	Healthy	Bed Rest	10	Quad (grouped)	MRI	CSA (cm2)*	Low
Nakanishi (2017)	Cohort. Full paper	ITU (all causes)	Critical care	28	RF	USS	CSA (% change), MT (% change)	3, 5, 7

Pardo (2018)	Cohort. Full paper	ITU (mixed)	Critical care	29	Quads (grouped)	USS	MT (cm)	3, 5, 7, 10, 21
Parry (2015)	Cohort. Full paper	ITU (mixed)	Critical care	22	RF, VL, VI	USS	CSA, MT, PA (all % change)	3, 5, 7, 10
Psatha (2012)	Cohort. Full paper	Ankle Fracture	ULLI (below knee cast)	18	TA, Sol, GM, GL, Tricep Surae (grouped)	MRI	CSA (%change)*	8, 15, 29, 43
Putuchery (2017)	Cohort. Correspondence	ITU (all causes)	Critical care	43	RF	USS	CSA, MT (all % change)*	7, 10
Rittweger (2005)	Cohort. Full paper	Healthy	Bed Rest	9	Calf Muscles (combined)	CT	CSA (% change)*	28, 43, 56, 68, 88
Segaran (2017)	Cohort. Full paper	ITU	Critical care	44	Bicep, Forearm, Thigh (average of all)	US	MT (cm)	7, 14
Seynnes (2008)	Cohort. Full paper	Healthy	ULLI	8	Sol, MG, LG	MRI, USS	Vol (cm3), FL, PA	14, 28
Silva (2019)	Cohort. Full paper	ITU (TBI)	Critical care	30	TA, RF	USS	MT (cm)*	3, 7, 14
Stevens (2004)	Cohort. Full paper	Ankle fracture	ULLI (below knee cast)	20	LG, MG, Sol, Ant lower leg (combined), Post lower leg (combined)	MRI	CSA (cm2)	7, 14
Shin (2016)	Cohort. Full paper	ITU (all causes)	Critical care	27	Upper arm, Thigh, Calf	Tape	Limb circumference	3, 5
Toledo (2017)	Cohort. Abstract only	ITU (all causes)	Critical care	20	Quads (combined)	USS	MT (cm)	3, 7

Turton (2016)	Cohort. Full paper	ITU (all causes)	Critical care	22	MG, VL	USS	MT, FL, PA	5, 10
Vendenborne (1998)	Cohort. Full paper	Ankle Fracture	ULLI (below knee cast)	1	MG, LG, Sol	MRI	CSA (cm2)	14, 28
Wapel (2018)	RCT vs EMS. Abstract only	ITU (all causes)	Critical care	15	Thigh muscle (combined)	CT	CSA (%change)	7, 14

Table 2.1: Summary of included studies. *=data only presented in graphical format and extracted using web plot digitiser. Abbreviations: RCT= Randomised control trial, RVE = resistive vibration exercise, EMS = electrical muscle stimulation, ITU = Intensive Care Unit, ULLI= unilateral lower limb immobilisation, VL/M/I = vastus lateralis, medialis, intermedius (respective), RF= rectus femoris, M/LG = Medial / Lateral Gastrocnemius, Sol = Soleus, TA =. Tibialis Anterior, Vol = volume, CSA = cross sectional area, MT = muscle thickness

2.3.2 Risk of Bias

Risk of bias was assessed using the ROBINS-I tool (Table 2.2). Overall, 29 studies were found to be at moderate risk of bias, and 2 studies were found to be at low risk of bias.

Of those studies found to be at moderate risk of bias, all were at moderate risk in measurement of outcomes due to a lack of blinding in assessors performing or analysing the scans. Three studies performed in ITU patients were at moderate risk of bias due to patient selection, because of varying time from start of intervention (ITU admission) to baseline scans.

Seven studies were at serious risk of bias due to missing data. Six of these studies were in ITU patients, with a loss of patients as time progressed, and one was in patients following ankle fracture with not all patients attending for scans at all timepoints.

One healthy volunteer study was at moderate risk of bias due to deviation from intended intervention, as immobilised patients performed tests of maximum voluntary contraction (MVC) at two timepoints during their immobilisation.

Study	Confounding	Selection	Classification of intervention	Deviation from intended intervention	Missing data	Measurement of outcomes	Reported result	Overall
Abe T (1997)	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Akima (1997)	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Armbrecht (2010)	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Belavy (2009)	Low	Low	Low	Low	Low	Low	Low	Low
Boer (2007)	Low	Low	Low	Moderate	Low	Moderate	Low	Moderate
Borges (2019)	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Dillon (2018)	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Hayes (2018)	Low	Moderate	Low	Low	Low	Moderate	Low	Moderate
Hirose (2013)	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Kawashima (2004)	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Kilroe (2020)	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Kitari (2018)	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Menids (2009)	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Mikovic (2012)	Low	Low	Low	Low	Low	Low	Low	Low
Mulder (2006)	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Nakanishi (2017)	Low	Low	Low	Low	Serious	Moderate	Low	Moderate
Pardo (2018)	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Parry (2015)	Low	Moderate	Low	Low	Serious	Moderate	Low	Moderate
Psatha (2012)	Low	Low	Low	Low	Serious	Moderate	Low	Moderate
Putuchery (2013)	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Rittweger (2005)	Low	Low	Low	Low	Low	Moderate	Low	Moderate

Segaran (2017)	Low	Moderate	Low	Low	Serious	Moderate	Low	Moderate
Seynnes (2008)	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Silva (2019)	Low	Low	Low	Low	Serious	Moderate	Low	Moderate
Stevens (2004)	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Toledo (2017)	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Turton (2016)	Low	Low	Low	Low	Serious	Moderate	Low	Moderate
Vendenborne (1998)	Low	Low	Low	Low	Low	Moderate	Low	Moderate
Wapel (2017)	Low	Low	Low	Low	Low	Moderate	Low	Moderate

Table 2.2: ROBINS I Risk of Bias assessment for included studies

2.3.3 Healthy volunteer studies

2.3.3.1 Whole leg

One study (Armbrecht *et al.*, 2010) reporting whole leg changes as assessed by DXA in 20 immobilised healthy participants showed that lean mass changed by -3.5% at day 12 (D12), -5.4% at D31, -7.0% at D44 and -8.3% by D56.

2.3.3.2 Hip Flexors

One study (Dilani Mendis *et al.*, 2009), in which six healthy young males had 56 days of BR, reported the results of immobilisation on the CSA of iliacus and psoas as assessed by MRI. CSA remained stable across all timepoints. Compared to baseline Iliopsoas CSA decreased by -2.78% at 14 days, -4.86% at 28 days and -2.78% at 42 days.

2.3.3.3 Quadriceps

Eight studies (Abe *et al.*, 1997; Akima *et al.*, 1997a; Mulder *et al.*, 2006; Maarten D. de Boer *et al.*, 2007; Belavý *et al.*, 2009; Dilani Mendis *et al.*, 2009; Miokovic *et al.*, 2012; Kilroe *et al.*, 2019) reported the changes observed in quadriceps muscles during immobilisation. Six studies (total 63 participants) used BR and two studies (total 30 participants) used ULLI. One study (Abe *et al.*, 1997) reported changes in anterior thigh MT, four reported changes in quadriceps CSA (Akima *et al.*, 1997a; Mulder *et al.*, 2006; Maarten D de Boer *et al.*, 2007; Dilani Mendis *et al.*, 2009) and four reported changes in quadriceps Vol (Akima *et al.*, 1997a; Belavý *et al.*, 2009; Miokovic *et al.*, 2012; Kilroe *et al.*, 2019). Combined quadriceps

muscle Vol decreased by -1.7% at day 3 (D3), -5.0% at D7, -5.71% to -6.5% by D10 to D14, -7.31% at D20, -9.1% by D28, - 12% by D42 and -14.4% by D56. Rates of atrophy in individual quadricep muscles varied, with smaller losses observed in rectus femoris (-3.5% to -4.1% at D14 and -5.1% to -7.4% at D56) compared to vastus muscles (-4.7% to -6.7% at D14 and -5.6 to -15.9% at D56). A full breakdown of quadriceps volume changes is available in Table 2.3. Combined quadriceps CSA changes corelated with volume changes. There was a decrease of -3.9 to -5.9% at D10-14, -7.6% to -10.0% at D20, -7.6% at D28, -10.3% at D42 and -13.6% by D56. For a full breakdown of CSA changes see Table 2.4. Figure 2.2 illustrates the timecourse of changes in quadriceps muscle CSA based on the studies included this review (Akima *et al.*, 1997b; Mulder *et al.*, 2006; Maarten D de Boer *et al.*, 2007; Dilani Mendis *et al.*, 2009). Anterior thigh MT, reported by only one study, decreased by -7.1% by D7, -12.6% by D14 and -12.0% by D20.

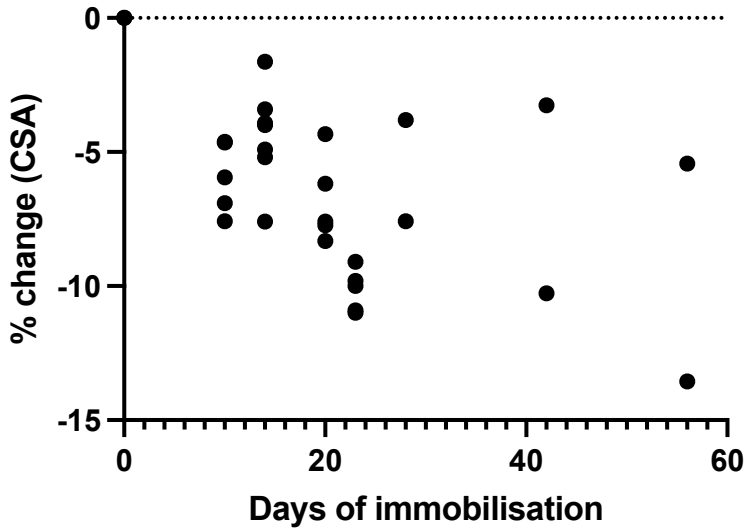

Figure 2.2: Graphical representation of median % change in Quadriceps CSA over time of immobilisation as reported in the four studies in table 2.4

Author	N	Muscle	% change in muscle volume						
			D2	D7	D10 - 14	D20	D28	D42	D56
Akima (1997)	10	Quadriceps (combined)	-	-	-5.71	-7.31	-	-	-
Belavy (2009)	20	Quadriceps (combined)	-	-	-6.5 (+/-3.5)	-	-9.1 (+/-3.4)	-12.0 (+/-3.4)	-14.4 (+/-3.5)
Kilroe (2020)	13	Quadriceps (combined)	-1.7 (+/-0.3)	-5.0 (+/-0.6)	-	-	-	-	-
Akima (1997)	10	Vastus (combined)	-	-	-4.7 to -6.7	-5.5 to -8.0	-	-	-
Belavy (2009)	20	Vastus (combined)			-6.7 (+/-3.7)	-	-9.9 (+/-3.6)	-13.3 (+/-3.5)	-15.9 (+/-3.7)
Miokovic (2012)	9	Vastus (combined)	-	-	-	-	-9.4	-	-5.6
Akima (1997)	10	Rectus Femoris	-	-	-3.5	-3.3	-	-	-
Belavy (2009)	20	Rectus Femoris	-	-	-4.1% (+/-3.5)	-	-2.7 (+/-3.5)	-2.9 (+/-3.4)	-5.1 (+/-3.5)
Miokovic (2012)	9	Rectus Femoris	-	-	-	-	-1.8	-	-7.4

Table 2.3: Percentage change in quadriceps muscle volume in immobilised young healthy volunteers

Author	N	Muscle	% change in muscle CSA				
			D10 - 14	D20 - 23	D28	D42	D56
Akima (1997)	10	Quadriceps (combined)	-5.9	-7.6	-	-	-
Mulder (2008)	10	Quadriceps (combined)	-3.9	-	-7.6	-10.2	-13.6
de Boer (2007)	17	Quadriceps (combined)	-5.2	-10.0	-	-	
Akima (1997)	10	Vastus (combined)	-4.6 to -7.6	-6.2 to -8.3	-	-	-
de Boer (2007)	17	Vastus (combined)	-3.4 to -7.6	-9.1 to -11.0	-	-	
Akima (1997)	10	Rectus Femoris	-4.6	-4.3	-	-	-
Menids (2009)	6	Rectus Femoris	-1.6	-	-3.8	-3.2	-5.4
de Boer (2007)	17	Rectus Femoris	-4.0	-10.9	-	-	-

Table 2.4: Percentage change in quadriceps muscle cross-sectional area (CSA) in immobilised young healthy volunteer

2.3.3.4 Hamstrings

Four studies (Akima *et al.*, 1997a; Belavý *et al.*, 2009; Miokovic *et al.*, 2012; Kilroe *et al.*, 2019) reported the changes observed in knee flexor muscles during immobilisation. Three studies (total 39 participants) used BR and one study (total 13 participants) used ULLI. Two studies reported changes in hamstring CSA (Akima *et al.*, 1997a; Kilroe *et al.*, 2019) whilst all four reported changes in volume (Akima *et al.*, 1997a; Belavý *et al.*, 2009; Miokovic *et al.*, 2012; Kilroe *et al.*, 2019). Combined hamstring Vol decreased by -1.4% at D3, -2.1 % at D7, -6.0 % to -6.21% by D10 to D14, -6.69% at D20, -6.3% to -7.19% by D28, -9.3% by D42 and -11.3% to -16.0% by D56. Full details of hamstring volume changes are shown in Table 2.5. Hamstring CSA decreased by -1.7% to -2.38% at D3, -4.0% to -5.9% at D7, -9.3% at D10 and -9.3% at D20. Figure 2.3 illustrates the timecourse of changes in hamstring muscle Vol based on the studies included this review (Akima *et al.*, 1997a; Belavý *et al.*, 2009; Miokovic *et al.*, 2012; Kilroe *et al.*, 2019).

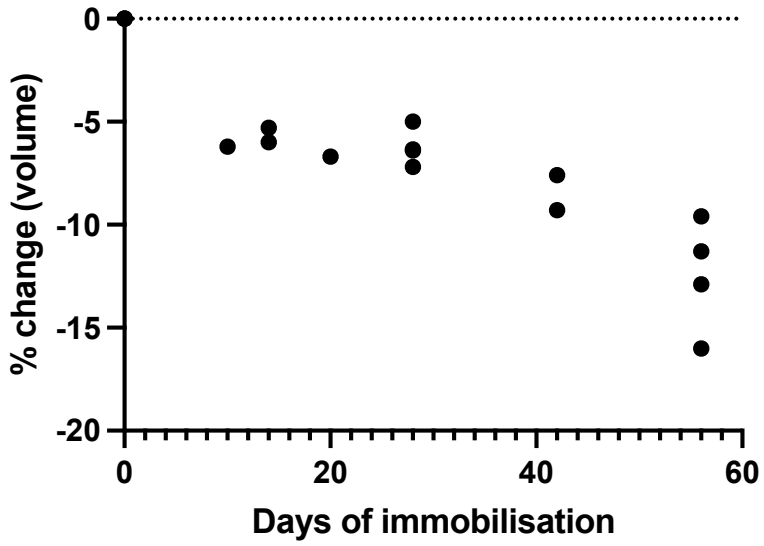

Figure 2.3: Graphical representation of median % change in Hamstring volume over time of immobilisation as reported in the four studies in Table 2.5

Author	N	Muscle		% change in muscle volume				
			D7	D10 - 14	D20	D28	D42	D56
Akima (1997)	10	Hamstrings (combined)	-	-6.2	-6.7	-	-	-
Belavy (2009)	20	Hamstrings (combined)	-	-6.0 (+/-3.3)	-	-6.4 (+/-3.2)	-9.3 (+/-3.2)	-11.3 (+/-3.1)
Kilroe (2020)	13	Hamstrings (combined)	-3.5					
Miokovic (2012)	9	Medial Hamstrings	-	-	-	-7.2	-	-16.0
Miokovic (2012)	9	Lateral Hamstrings	-	-	-	-6.3	-	-12.9

Table 2.5: Percentage change in hamstring muscle volume in immobilised young healthy volunteers

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2.3.3.5 Plantarflexors

Six studies (Abe *et al.*, 1997; Akima *et al.*, 1997a; Rittweger *et al.*, 2005; Seynnes *et al.*, 2008; Belavý *et al.*, 2009; Miokovic *et al.*, 2012) reported the changes observed in plantarflexor muscles during immobilisation. Five studies (total 55 participants) used BR and one study (total eight participants) used ULLI. One study (Abe *et al.*, 1997) reported changes in combined calf MT, and observed decreases of -7.1% at D7, -8.2% at D14 and -6.9% at D20. Four studies reported changes in plantarflexor volume (Akima *et al.*, 1997a; Seynnes *et al.*, 2008; Belavý *et al.*, 2009; Miokovic *et al.*, 2012). Combined triceps surae muscle Vol decreased by -7.8% at D14, -11.2% at D28, -14.4% at D42 and -18.3% by D56.

Rates of atrophy in individual triceps surae muscles varied. In general, smaller losses were observed in the lateral belly of gastrocnemius (LG) (-2.4% to -7.7% at D14 and -14.4% to -16.5% at D56) compared to the medial gastrocnemius (MG) and soleus which showed similar rates of atrophy (-3.0% to -9.4% at D14 and -20.4 to -22.3% at D56). Full results of plantarflexor volume changes are shown in Table 2.6. Figure 2.4 illustrates the timecourse of changes in triceps surae muscle Vol based on the studies included this review (Akima *et al.*, 1997a; Seynnes *et al.*, 2008; Belavý *et al.*, 2009; Miokovic *et al.*, 2012).

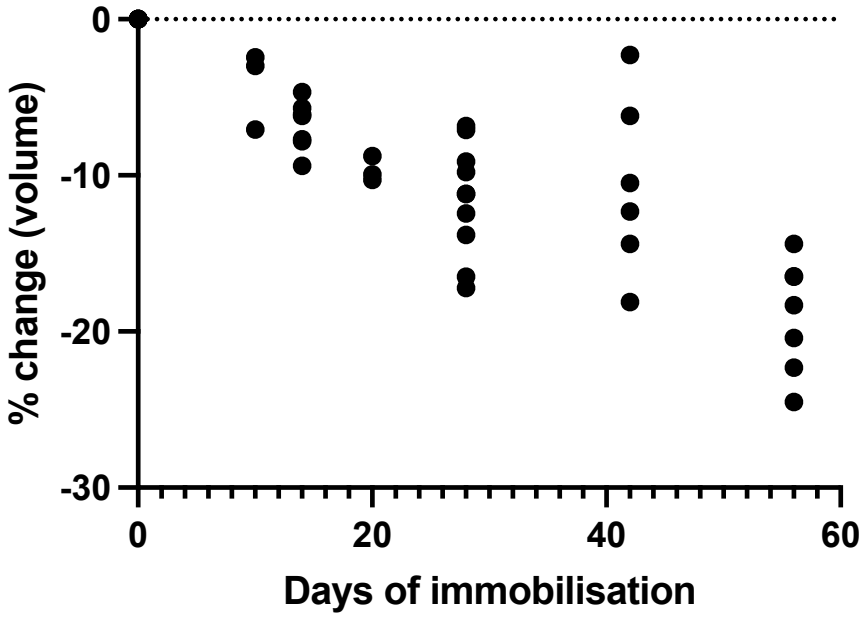

Figure 2.4: Graphical representation of the median % change in Triceps Surae volume during immobilisation as reported in the four studies in Table 2.6

2.3.3.6 Dorsiflexors

Three studies (Akima *et al.*, 1997a; Belavý *et al.*, 2009; Miokovic *et al.*, 2012) (total 39 participants) reported the changes observed in dorsiflexor muscles during BR immobilisation. All three studies reported changes in tibialis anterior (TA) volume, with wide variation in results between studies. Decreases in volume ranged from -0.7% to -9.2% at D14 and 0.8% to -7.7% at D28.

Author	N	Muscle	% change in muscle volume				
			D10 - 14	D20	D28	D42	D56
Belavy (2009)	20	TS (combined)	-7.8 (+/-1.8)	-	-11.2 (+/-1.8)	-14.4 (+/-1.8)	-18.3 (+/-2.0)
Akima (1997)	10	MG	-3.00	-9.93	-	-	-
Belavy (2009)	20	MG	-9.4 (+/-1.5)	-	-13.8 (+/-1.6)	-18.1 (+/-1.1)	-22.3 (+/-1.5)
Miokovic (2012)	9	MG	-	-	-16.50	-	-20.41
Seynnes (2008)	8	MG	-6.09	-	-9.79	-	-
Akima (1997)	10	LG	-2.43	-10.28	-	-	-
Belavy (2009)	20	LG	-7.7 (+/-3.8)	-	-11.2 (+/-2.9)	-10.5 (+/-1.8)	-14.4 (+/-2.8)
Miokovic (2012)	9	LG	-	-	-12.44	-	-16.46
Seynnes (2008)	8	LG	-5.69	-	-7.09	-	-
Akima (1997)	10	Soleus	-7.05	-8.76	-	-	-
Belavy (2009)	20	Soleus	-6.2 (+/-1.8)	-	-9.1 (+/-1.8)	-12.3 (+/-1.8)	-16.5 (+/-1.8)
Miokovic (2012)	9	Soleus	-	-	-17.22	-	-24.51
Seynnes (2008)	8	Soleus	-4.66	-	-6.84	-	-

Table 2.6: Percentage change in Triceps Surae (TS) muscle volumes in immobilised young healthy volunteers

2.3.4 Ankle Fracture patients

Three studies (Vandenborne *et al.*, 1998; Stevens *et al.*, 2004; Psatha *et al.*, 2012a), with a total of 39 patients, reported the changes in lower leg musculature during immobilisation in plaster cast following ankle fracture. All three studies reported change in CSA of triceps surae muscles, with a decrease of -6.0% to -16.0% by D7, -10.6% to -26.4% at D14, -15.5% to -26.5% by D28, -13.5% to -18.9% by D43 and -32.4% by D56. A full breakdown of changes in plantarflexor CSA in ankle fracture patients is available in Table 2.7. Figure 2.5 illustrates the timecourse of changes in plantar flexor CSA in ankle fracture patients based on the studies included this review (Vandenborne *et al.*, 1998; Stevens *et al.*, 2004; Psatha *et al.*, 2012a). One study (Psatha *et al.*, 2012b) reported changes in TA CSA, with a loss of -3.08% by D8, -6.2% at D15, -10.5% at D28, and - 10.3% by D43.

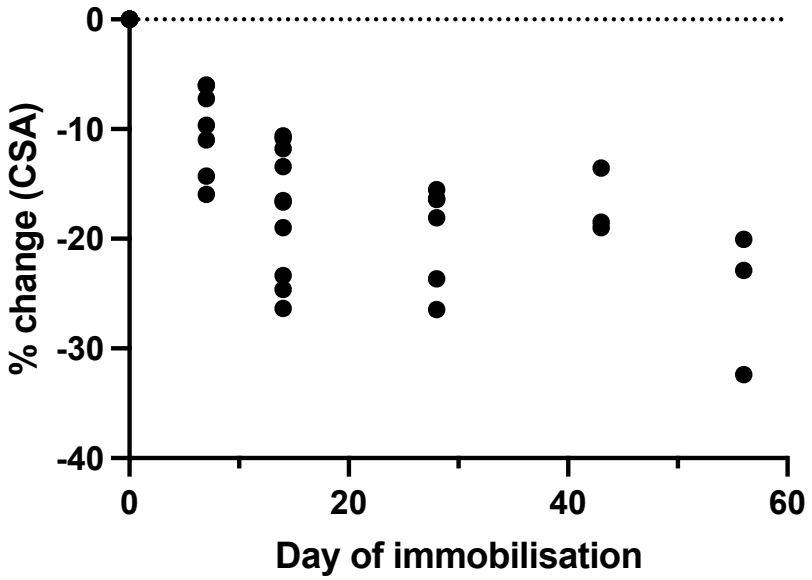

Figure 2.5: Graphical representation of median % change in Triceps Surae CSA in patients following ankle fracture as reported in the three studies in Table 2.7

Author	N	Muscle	% change in muscle CSA				
			D7	D14	D28	D42	D56
Stevens (2004)	20	Posterior lower leg (combined)	-9.66	-18.98	-	-	-
Psatha (2012)	18	Medial Gastrocnemius	-7.2	-16.7	-23.6	-18.5	-
Stevens (2004)	20	Medial Gastrocnemius	-16.0	-26.4	-	-	-
Vandenborne (1998)	1	Medial Gastrocnemius	-	-13.4	-18.1	-	-22.89
Psatha (2012)	18	Lateral Gastrocnemius	-6.0	-11.8	-16.31	-13.6	-
Stevens (2004)	20	Lateral Gastrocnemius	-11.0	-24.6	-	-	-
Vandenborne (1998)	1	Lateral Gastrocnemius	-	-16.5	-26.5	-	-32.4
Psatha (2012)	18	Soleus	-6.0	-10.8	-16.4	-19.0	-
Stevens (2004)	20	Soleus	-14.3	-23.3	-	-	-
Vandenborne (1998)	1	Soleus	-	-10.6	-15.5	-	-20.1

Table 2.7: Percentage change in Triceps Surae muscle CSA in patients immobilised following ankle fracture

2.3.5 ITU patients

Fourteen studies reported changes in lower limb muscle size during ITU admission. Twelve studies reported changes in quadriceps (seven measured CSA (Hirose *et al.*, 2013; Puthucheary, Rawal, McPhail, Connolly, Ratnayake, Chan, Hopkinson, Padhke, Dew, Paul S Sidhu, *et al.*, 2013; Parry and Puthucheary, 2015; Hayes *et al.*, 2018; Nakanishi *et al.*, 2018; Wappel *et al.*, 2018; Borges and Soriano, 2019) and eight measured MT (Parry and Puthucheary, 2015; Turton *et al.*, 2016; Segaran *et al.*, 2017; Toldeo *et al.*, 2017; Hayes *et al.*, 2018; Katari *et al.*, 2018; Pardo *et al.*, 2018)), two reported changes in plantarflexors (1 CSA (Hirose *et al.*, 2013),1 MT (Turton *et al.*, 2016)), two reported changes in dorsiflexors (1 CSA (Hirose *et al.*, 2013), 1 MT (Silva *et al.*, 2019)) and one reported change in hamstring CSA(Hirose *et al.*, 2013) (Table 2.10). Two studies measured combined quadriceps CSA (Hirose *et al.*, 2013; Wappel *et al.*, 2018) and observed a decrease of -13.2% by D7 and -23.9% to -32.5% on D14. The remaining five studies measured rectus femoris (RF) CSA and reported changes of -1.0% to -8.7% at D3, -8.8 to -13.7% at D5, -12.5% to -20.7% by D7, and -17.7% to -29.9% by D14. Figure *2.6* illustrates the timecourse of changes in quadriceps muscle CSA in ITU patients based on the studies included this review (Hirose *et al.*, 2013; Puthucheary, *et al.*, 2013; Parry and Puthucheary, 2015; Hayes *et al.*, 2018; Nakanishi *et al.*, 2018; Wappel *et al.*, 2018; Borges and Soriano, 2019).

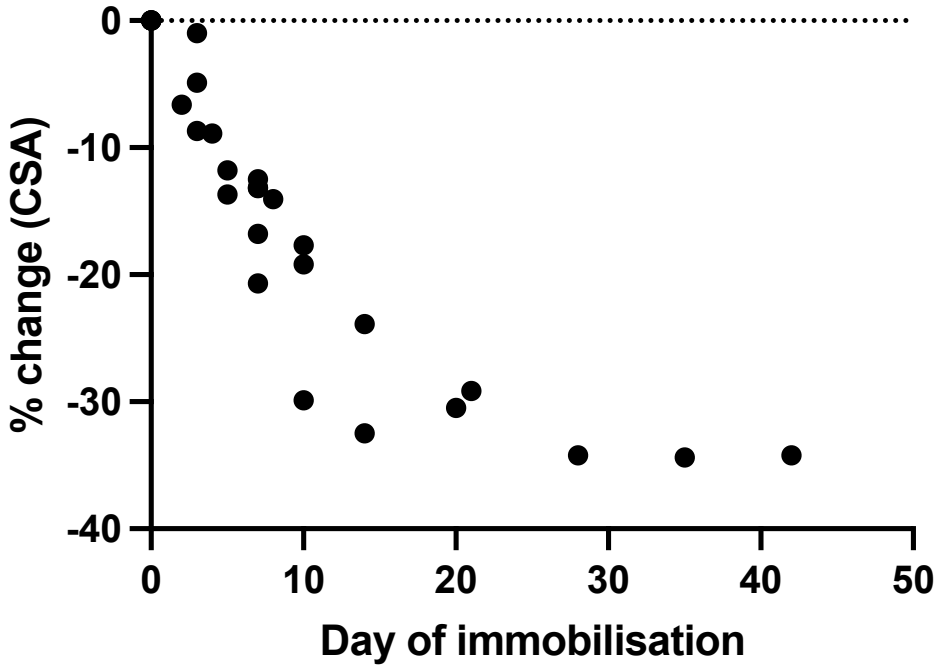

Figure 2.6: Graphical representation of median % change in Quadriceps CSA in immobilised ITU patients, reported in Table 2.9

Author	N	Muscle	% change in muscle thickness				
			D3	D5	D7	D10 -14	D20
Segaran (2017)	17	Quadriceps (combined)	0 to -2.7	0 to -5.0	-6.7 to -16.0	-	-
Toledo (2017)	20	Quadriceps (combined)	-2.59	-	-12.07	-	-
Pardo (2018)	29	Quadriceps (combined)	-0.49	-5.1	-14.8	-20.9	-22.7
Hayes (2018)	25	Quadriceps (combined)	-	-	-	-9.0	--30.3
Parry (2015)	22	RF	-8.7	-16.6	-24.9	-30.4	-
Katari (2018)	100	RF	-8.0	-	-11.0	-	-
Hayes (2018)	25	RF	-	-	-	-25.4	-34.9
Parry (2015)	22	VL	-0.2	-5.7	-6.0	-14.1	-
Turton (2016)	22	VL	-	-8.5	-	-22.9	-
Hayes (2018)	25	VL	-	-	-	-16.4	-32.5
Parry (2015)	22	VI	-1.3	-18.1	-20.0	-29.7	-
Hayes (2018)	25	VI	-	-	-	-16.4	-31

Table 2.8: Summary of % changes in Quadriceps MT in ITU patients

Author	N	Muscle	% change in muscle CSA							
			D3	D5	D7	D10 -14	D21	D28	D35	D42
Hirose (2013)	1	Quadriceps (combined)	-	-	-13.2	-23.9	-29.1	-34.2	-34.4	-34.2
Wapel (2018)	15	Quadriceps (combined)	-	-	-13.2	-32.5	-	-	-	-
Nakanishi (2017)	28	Rectus Femoris	-8.70	-13.7	-20.7	-	-	-	-	-
Parry (2015)	22	Rectus Femoris	-1.0	-11.8	-16.8	-29.9	-	-	-	-
Putuchery (2013)	42	Rectus Femoris	-4.9	-	-12.5	-17.7	-	-	-	-
Borges (2019)	37	Rectus Femoris	-6.61	-8.88	-14.04	-	-	-	-	-
Hayes (2018)	25	Rectus Femoris	-	-	-	-19.2	-30.5	-	-	-

Table 2.9: Summary of % change in quadriceps CSA in ITU patients

Paper	N	Muscle	Measure	% change in measure							
				D3	D5	D7	D10 -14	D21	D28	D35	D42
Hirose (2015)	1	Posterior lower leg	CSA		-	-6.85	-17.11	-22.37	-26.13	-28.01	-28.76
Turton (2016)	22	Gastrocnemius	MT		-3.88	-	-11.63	-	-	-	-
Hirose (2015)	1	Anterior lower leg	CSA	-2.42	-	-15.41	-26.13	-34.74	-39.47	-37.97	-30.76
Silva (2018)	22	Tibialis Anterior	MT		-	-10.05	-20.11				
Silva (2018)	30	Tibialis Anterior	MT		-	-8.67	-13.93				
Hirose (2012)	1	Posterior thigh	CSA			-12.0	-22.7	-28.4	-32.0	-33.8	-35.9

Table 2.10: Summary of % change in other leg muscles of ITU patients

2.3.6 Comparative analysis

2.3.6.1 Healthy vs ITU

Meta-analysis

Meta-analysis for change in rectus femoris CSA after 14 days of immobilisation revealed MD of -0.12 (95%CI -0.49 to 0.24) in healthy participants (I^2 = 0%) (Figure 2.7), whereas in ITU patients the corresponding MD was -1.01 (95%CI -1.32 to -0.69) (I^2=84%) (Figure 2.8). On subgroup analysis this indicates a statistically significant greater loss of muscle in ITU patients (p<0.001|), although it should be noted that changes in ITU patients were subject to considerable heterogeneity.

Analysis of raw data

To allow an illustrative comparison, pooled means were calculated for quadricep muscle CSA at each timepoint for ITU patients and healthy immobilised volunteers. These results show that ITU patients experienced dramatically more muscle loss than healthy immobilised individuals, with -4.6% loss of CSA at D2 (vs -1.6%) and -18.7% at D10-14 (vs -5.5%). Figure 2.9 illustrates this difference, with full results for pooled means shown in Table 2.11.

Study Setting	Papers	Total N	% change in CSA		
			D2	D7	D10 - 14
Healthy	Akima (1997), Mendis (2009), Mulder (2008), Boer (2007), Kilroe (2020)	56	-1.6 (-1.4 to -2.6)	-5.6 (-4.5 to –7.2)	-5.5 (-3.4 to –7.6)
ITU	Parry (2015), Borges (2019), Hayes (2018), Putuchery (2013)	126	-4.6 (-1.0 to -8.7)	-13.9 (-12.5 to -20.7)	-18.7 (-17.7 to -29.9)

Table 2.11: Pooled means of changes in quadriceps CSA in intensive treatment unit (ITU) patients and healthy volunteers after 2 (D2), 7 (D7) and between 10 to 14 (D 10 – 14) days of immobilisation. Range of changes displayed in parentheses.

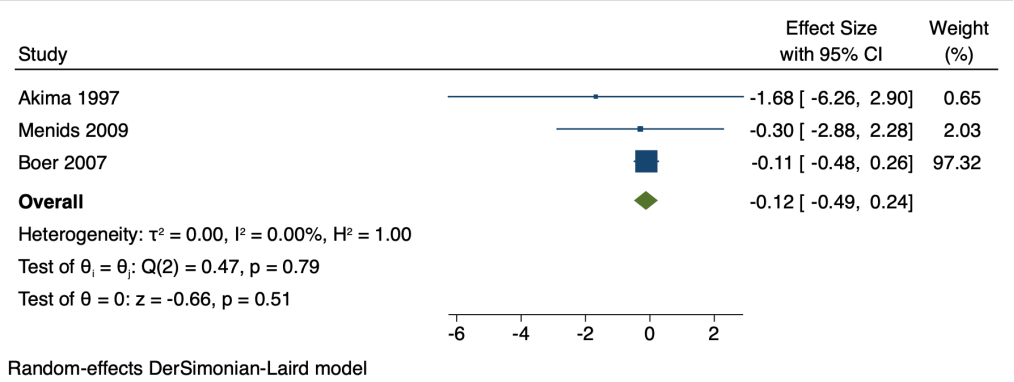

Figure 2.7: forest plot for analysis of change in Quadriceps CSA in healthy volunteers between baseline and day 14

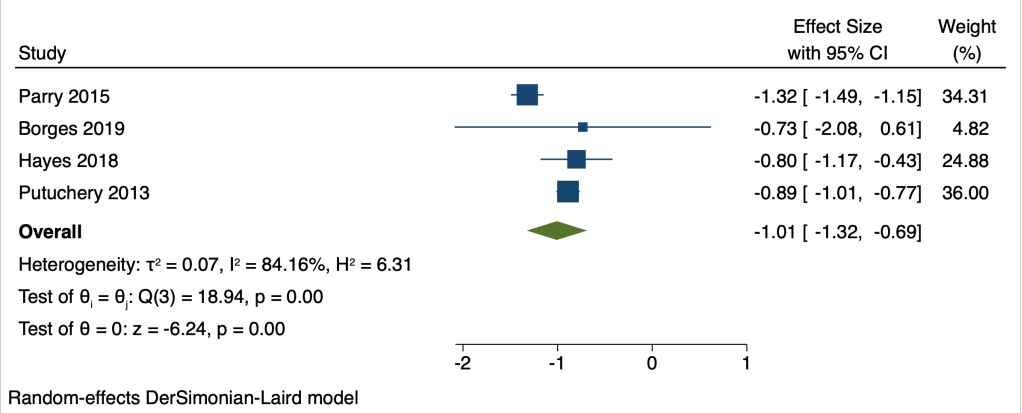

Figure 2.8: forest plot for analysis of change in Quadriceps CSA in ITU patients between baseline and day 14

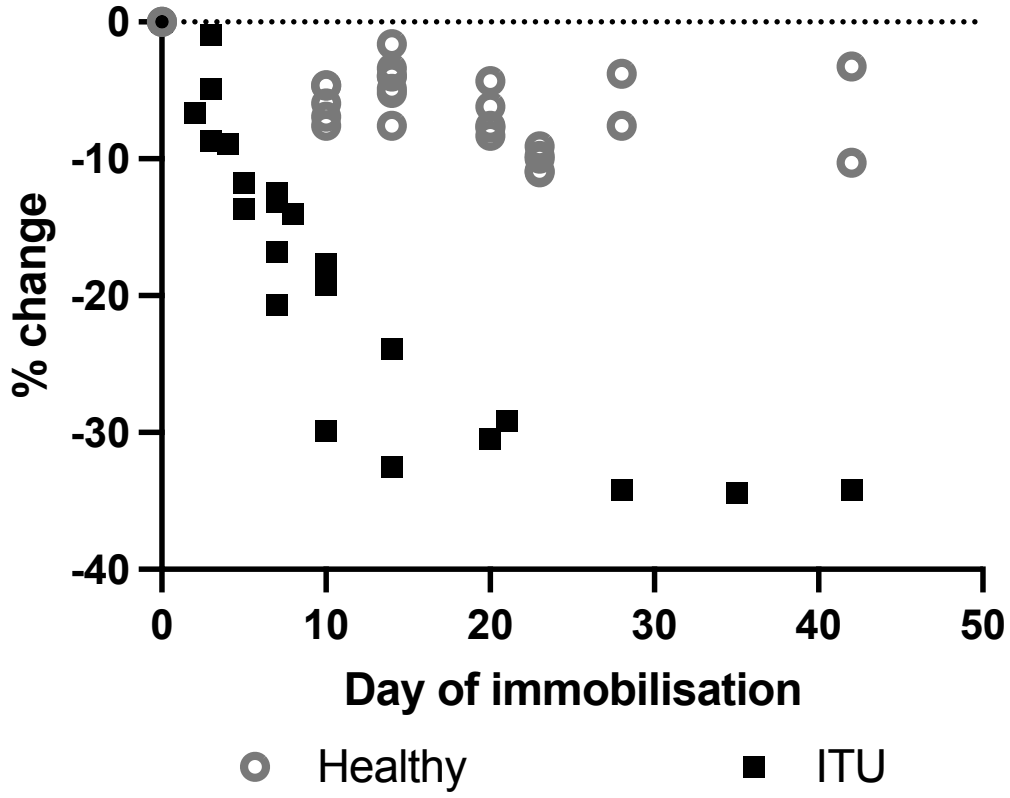

Figure 2.9: Illustrative comparison of median % changes in Quadriceps CSA in ITU patients and healthy volunteers from studies included in this review

2.3.6.2 Healthy vs ankle fracture

There were insufficient original data to allow formal meta-analysis of the difference between muscle loss in immobilised healthy individuals and patients immobilised following ankle fracture. However, the data suggests that muscle loss tends to be greater in patients following ankle fracture than in healthy individuals. Triceps surae muscle Vol decreased by -2.4% to -9,4% by D14 and -6.8% to -17.2% by D28 in healthy participants whereas muscle CSA decreased by -10.6% to -26.4% by D14 and -15.5% to -26.5% by D28 in patients following ankle fracture. Comparison of these changes are illustrated by Figure 2.10.

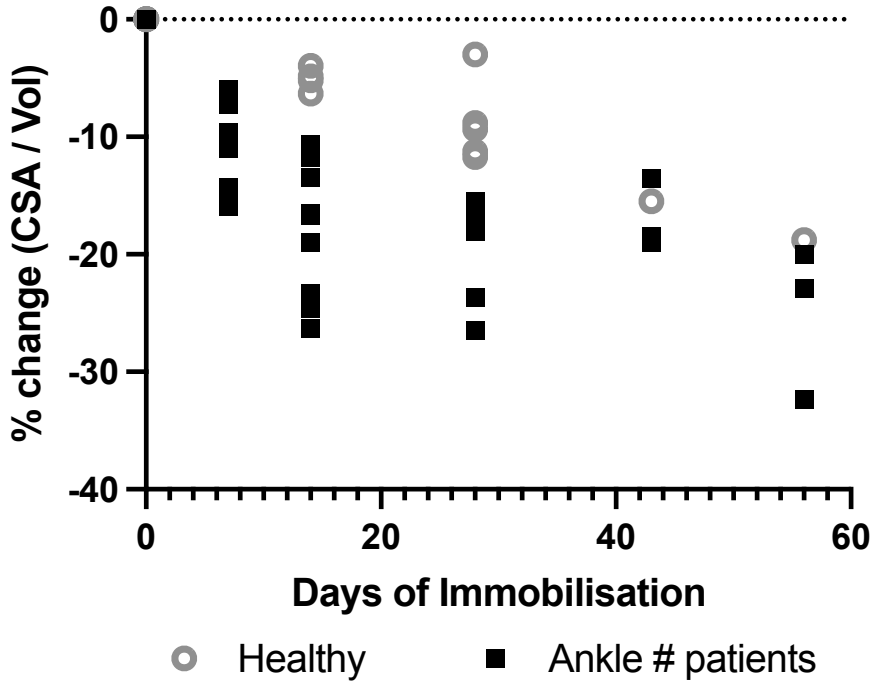

Figure 2.10: Illustrative comparison of median changes in Triceps surae muscles in healthy volunteers and patients following ankle fracture, from studies included in this review

2.3.6.3 Comparison between muscle groups

Formal comparison of the rate of muscle atrophy between muscle groups was not possible as many studies reported percentage change only, with insufficient original data to allow meta-analysis. Pooled means of the change in Vol of each muscle group in healthy participants were therefore calculated to allow illustrative comparison. These results show that tibialis anterior had the lowest rates of atrophy with -1.8% loss at 14 days and -3.2% loss at 28 days, whilst triceps surae showed the greatest losses with -6.96% loss at 14 days and -11.2% loss at 28 days. Figure 2.11 illustrates the difference in rates of muscle atrophy between muscle groups in the lower limb, with full results in Table 2.12.

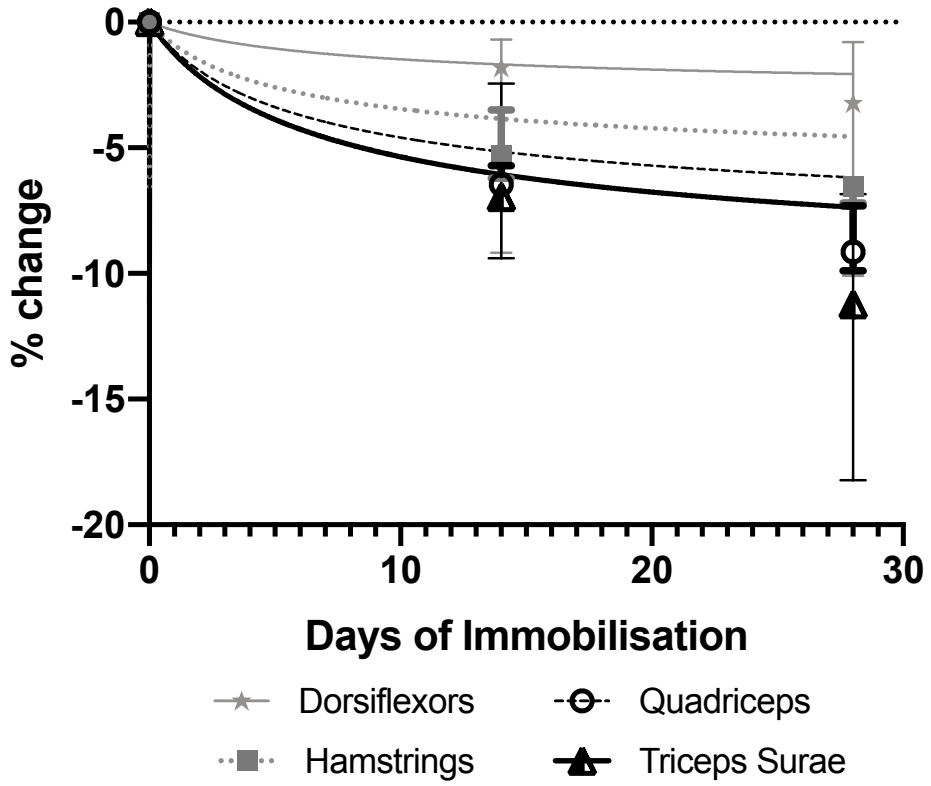

Figure 2.11: Illustrative comparison between the rate of muscle atrophy in different lower limb muscle groups of immobilised healthy volunteers, based on pooled means calculated from studies included in this review

Muscle Group	Papers	Total N	% change in muscle volume	
			D14	D28
Quadriceps	Akima (1997), Belavy (2009), Miokovik (2012), Kilroe (2020)	52	-6.5 (-5.7 to -6.7)	-9.15 (-7.3 to –9.9)
Hamstrings	Akima (1997), Belavy (2009), Miokovik (2012), Kilroe (2020)	52	-5.3 (-3.5 to -6.2)	-6.54 (-6.3 to -7.2)
Triceps Surae	Akima (1997), Belavy (2009), Miokovik (2012), Seynnes (2008)	47	-6.96 (-2.4 to -9.4)	-11.2 (-6.8 to -18.2)
Tibialis Anterior	Akima (1997), Belavy (2009), Miokovik (2012)	39	-1.81 (-0.7 to -9.2)	-3.2 (-0.8 to -10.1)

Table 2.12: Pooled means of changes in muscle volume of different muscle groups in healthy volunteers after 14 (D14) and 28 (D28) days of immobilisation. Range of changes displayed in parentheses.

Similar trends were seen in patients following ankle fracture. In the one paper reporting both outcomes in this patient group (Psatha *et al.*, 2012a) TA CSA reduced by -3.1% by D8 and -6.2% by D15, whereas calf muscle CSA reduced by -6.0% to -7.2% at D8 and -10.8% to -16.7% at D15.

2.3.6.4 Timecourse of disuse muscle atrophy

There was insufficient original data to allow formal meta-analysis of the difference in rates of atrophy over the timecourse of immobilisation. Analysis of pooled means revealed that when averaged over D0 to D14 healthy quadriceps Vol decreased by an average of -0.46%/day, whereas when averaged from D0 to D28 the rate decreases to -0.33%/day. Similarly, for healthy triceps surae the rate of atrophy from D0 to D14 is -0.50%/day whereas for D0 to D28 the rate is -0.40%. In the quadriceps of ITU patients the rate of atrophy when averaged over D0 to D2 is -2.3%/day, from D0 to D7 is -1.99%, and from D0 to D14 -1.34%. These figures suggest that rates of muscle atrophy decrease over time, with more accelerated atrophy in critically unwell patients.

Method of immobilisation

There was insufficient original data to allow formal meta-analysis of the difference in rates of atrophy between BR and ULLI studies. Figure 2.12 shows an illustrative representation of this comparison for change in CSA of quadriceps. This would suggest there is little difference in rates of atrophy between methods of immobilisation, but that ULLI tends to induce a greater degree of muscle atrophy.

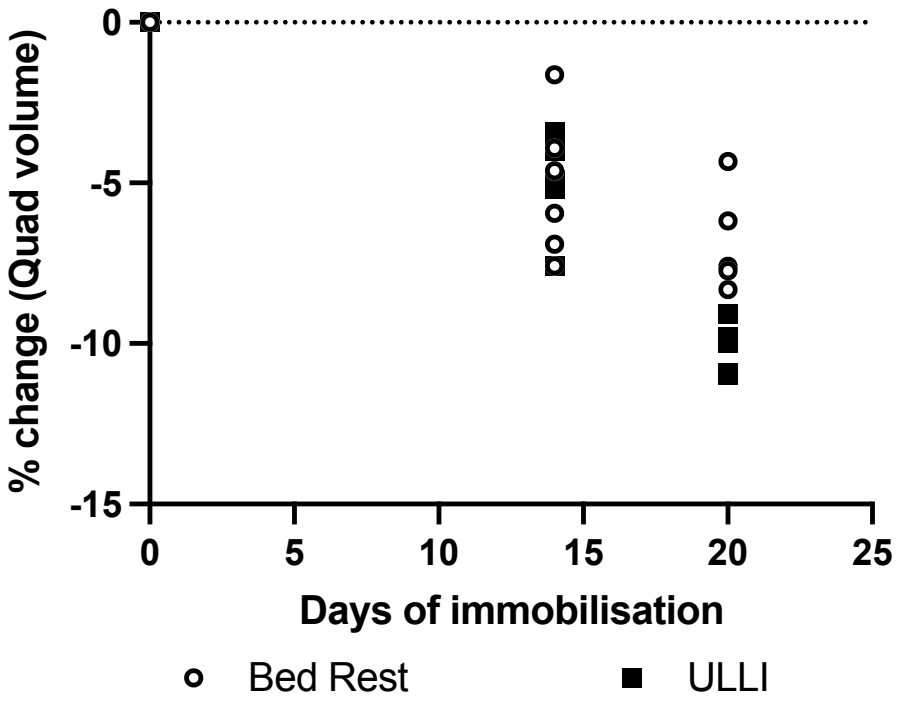

Figure 2.12: Illustrative comparison between median change in quadriceps volume of healthy volunteers immobilised by either bed rest or unilateral lower limb immobilisation (ULLI)

2.3.6.5 Measurement method

Formal comparison of the rate of muscle atrophy as defined by different measurements (MT vs CSA vs Vol) through meta-analysis was not possible due to mathematical constraints as many studies reported percentage change only without original measurements, resulting in insufficient comparable original data. Percentage change of the pooled means of original data were therefore calculated to allow illustrative comparison. In healthy participants quadriceps Vol decreased by -5.6% by D10-14 and by -7.4% by D20-28, whereas CSA decreased by -5.5% and -7.9% respectively. In ITU patients quadriceps CSA decreased by -18.7% by D10-14, whereas MT decreased by -20.4%.

2.4 Discussion

Despite the large amount of research into skeletal muscle atrophy we have found there is limited evidence to characterise the timecourse of DMA in human lower limb muscles in both healthy volunteers and critically unwell patients. The 12 healthy volunteer studies reported combined results from 140 participants, and the 14 ITU studies a total of 422 patients. Studies varied widely in the muscle groups studied, the measurements used and the timepoints reported, resulting in limited comparable data.

Immobilised healthy muscles start to atrophy quickly, with one study reporting a significant decrease in quadriceps volume after just two days (Kilroe *et al.*, 2019). Rates of muscle atrophy are significantly greater in critically unwell patients, with changes in RF CSA being more than 2.5 times greater in ITU patients than in healthy participants. Whilst this is to be expected due to the presence of potent catabolic drivers such as severe systemic inflammation (Costamagna *et al.*, 2015) and starvation (van Gassel, Baggerman and van de Poll, 2020) in ITU patients, rates of atrophy in patients immobilised following ankle fracture also appear to be greater than in healthy volunteers. Whilst patients following ankle fracture are not subject to the same severe systemic inflammation as ITU patients, there is some inevitable localised inflammatory trauma response, which may contribute to the increased rates of DMA. A further possible explanation is in the degree of immobilisation the muscles undergo. Following ankle fracture (or in ULLI for healthy volunteers), legs

are totally immobilised in a cast preventing even the minimal, non-weight bearing contractile activity possible in BR studies.

Rates of atrophy appear to slow as duration of immobilisation progresses. This finding is consistent across all muscle groups and is observed in healthy volunteers, ITU patients and following ankle fracture. This finding is in keeping with observations from other studies (Rudrappa *et al.*, 2016a), where rates of atrophy follow a pattern of exponential decay with the greatest losses in the first 14 days, slowing over time to reach an eventual nadir (Bodine, 2013). This consistent finding suggests that the mechanisms involved in DMA may vary over the duration of immobilisation and that they are at their most potent in the acute early phase (day 0 to day 14).

In ITU patients, results from muscle groups other than quadriceps are limited and no definite variability in rates of atrophy between muscle groups can be identified. However, in healthy volunteers, triceps surae and quadriceps muscle groups appear to be the most susceptible to disuse, and atrophy at around three times the rate of ankle dorsiflexors such as TA, which appear to be the most atrophy resistant. This is in keeping with observations made in other studies (Shackelford *et al.*, 2004; Shah *et al.*, 2006; Bass *et al.*, 2021), and may reflect a trend towards greater atrophy in those muscle which usually contribute the most force during standing and walking (Bodine, 2013).

There is also some evidence of differential rates of atrophy of individual muscles within a muscle group, although it should be noted that this finding is based on a limited number of studies, with not all studies showing consistent findings. For example, in healthy muscles the Vasti of the quadriceps muscle group appear to be more atrophy susceptible than the RF (Akima *et al.*, 1997b; Belavý *et al.*, 2009; Miokovic *et al.*, 2012). These findings mirror those of other studies not included in this review (Schmalz, Blumentritt and Reimers, 2001; Heikkinen *et al.*, 2017) which report variable rates of muscle atrophy within muscle groups following amputation and tendon rupture. If proven, the notion of differential rates of atrophy in individual muscles within a muscle group raises some questions for the proposition of immobility or inactivity being the main driver behind age related muscle loss, as the proportion of each individual muscle within the quadriceps seems to be maintained with advancing age (Trappe, Lindquist and Carrithers, 2001).

Formal comparison of changes in different muscle measurements in the assessment of atrophy was outside the scope of this review. However, from the results included in this review, changes in Vol and CSA in the quadriceps of healthy volunteers, and CSA and MT in the rectus femoris of ITU patients, appear to give broadly similar results. This is in contrast to the findings of other studies which have suggested that MT underestimates atrophy in ITU patients when compared to CSA (Mueller *et al.*, 2016; Paris *et al.*, 2017; Puthucheary *et al.*, 2017).

As multiple studies have demonstrated that acute DMA is accompanied by a corresponding, but even greater loss of muscle function (Wall *et al.*, 2014b), the findings in this review have important clinical implications, especially as in most patients DMA will be accelerated by inflammation and poor nutrition. Further research is required into the mechanisms at work during the acute phase of DMA and potentially strategies to counteract it. Beyond optimising patient care to allow and encourage early mobilisation, techniques such as bed-based resistance vibration exercise and electrical muscle stimulation have shown some promise in the reduction of DMA (Koukourikos, Tsaloglidou and Kourkouta, 2014; Konda *et al.*, 2019).

2.4.1 Limitations

Most included studies were at moderate risk of bias, most commonly due to lack of blinding of the assessors. As the majority of studies did not aim to identify any change in rate of DMA over the period of immobilisation, any unconscious bias in the unblinded assessor is unlikely to have affected our findings. However, several ITU studies were at risk of bias due to high dropout rates as time progressed. As the majority of the dropouts in an ITU population are due to death, this has the potential to accentuate the flattening of the curve with time as those dying sooner are likely to be the most severely unwell and undergoing the most rapid loss of muscle mass.

The major limitation of the current analysis concerns the analysis of raw data from the included studies. Unlike formal meta-analysis, this data does not take account of the fact that individuals within each study are more likely to be similar than those in other studies (patients with sepsis in one study combined with traumatic brain injury patients from another study) and therefore needs to be interpreted with caution.

2.5 Conclusion

In conclusion, further work is required to fully characterise the timecourse of DMA in the human lower limb in both health and disease. However, results from the studies included in this review suggest that DMA occurs rapidly, with the highest rate of muscle loss in the most acute phase, and that these changes are significantly greater in the critically unwell patient. Both findings highlight the importance of early intervention to minimise muscle loss, especially in unwell patients. Further, rates of DMA appear to vary both between muscle groups and between individual muscles within a muscle group, an observation that must be considered during intervention design.

3 Timecourse of disuse atrophy in different leg muscles

Chapter abstract

Introduction

The systematic review and meta-analysis presented in chapter 2 suggests that rates of disuse muscle atrophy (DMA) may vary over the timecourse of immobilisation and between muscles. However, further studies are required to confirm these findings. In this chapter we therefore aim to characterise the timecourse of DMA in various muscles of the leg over the first two weeks of immobilisation. We also validate the utility of ultrasound (US) in assessment of DMA against the gold standard of magnetic resonance imaging (MRI).

Method

Twelve healthy young men had 15 days unilateral lower limb immobilisation using a knee brace and ankle boot, with the other leg acting as a control. Measurements of vastus lateral (VL), medal gastrocnemius (MG) and tibialis anterior (TA) were made using MRI and ultrasound (US) at baseline and every two to three days thereafter (n=8), or at the midpoint and end (n=4) of immobilisation.

Results

Rapid loss of muscle mass occurs in VL and MG (atrophy susceptible (aS)), whilst no significant change in muscle mass was detected in TA (atrophy resistant (aR)). Significant reductions in cross sectional area (CSA) were detected after just five days of immobilisation in both MG (-4.9%) and VL (-5.5%). Although there appeared to be a trend towards a

slowing in the rate of DMA across the two weeks of immobilisation in both VL and MG, no significant difference in the timecourse of DMA was identified over this period. In MG and VL, US CSA was shown to have a strong correlation and concordance to MRI volume (Vol), with good or excellent agreement between measurements. US MT was also shown to have good (MG) or moderate (VL) correlation with MRI Vol, and had good sensitivity and PPV in both muscles. In TA, US CSA showed moderate correlation and concordance with MRI Vol, whilst US MT had only weak correlation.

Discussion

This study provides confirmatory evidence of the findings suggested by the systematic review in chapter 2 and identified atrophy resistant (TA), and atrophy susceptible (MG / VL) muscles. The timecourse of DMA is likely linear over the first two weeks of immobilisation. However, the rate of DMA during this period is rapid and much greater than that observed during longer periods of immobilisation. Whilst the mechanisms underlying the variation in atrophy rate between muscles and over the timecourse of immobilisation are not fully understood, the aRaS pattern revealed by this study provides an ideal model for further investigation. US CSA and MT are reliable measures to assess DMA in MG and VL.

3.1 Introduction

3.1.1 Disuse muscle atrophy

As shown in chapter 2, it appears that there may be an early 'acute phase' of disuse muscle atrophy (aDMA) (Kilroe *et al.*, 2019), with slower relative reductions over longer periods (LeBlanc *et al.*, 2000; Wall *et al.*, 2014a; Rudrappa *et al.*, 2016a), and differential rates of atrophy between muscles during immobilisation (Bass *et al.*, 2021). Some muscle groups appear to be atrophy susceptible (aS) and demonstrate rapid, significant atrophy during periods of disuse, whilst other muscle groups, appear largely atrophy resistant (aR) (John and Nigam, 2019; Bass *et al.*, 2021). However, detailed knowledge surrounding the pattern and degree of loss associated with different periods of immobilisation and in different muscle groups is still lacking. Such knowledge may be crucial in order to design and implement mitigating interventions for physiologically vulnerable patients, such as those with pre-existing low muscle mass, or those about to undergo a known catabolic insult with a resultant period of inactivity such as surgery, where muscle losses during periods of aDMA may have important clinical significance.

3.1.2 Assessment of muscle mass

As discussed in chapter 1, various methods of measuring muscle mass have been described across the literature, including simple anthropometric (Motobe *et al.*, 2004) and whole body measures (Fuller, Laskey and Elia, 1992; Ellis, 2000). However, to investigate the true

timecourse of DMA and the differences between muscles, measurements of individual muscles must be made at multiple time points before, during and after immobilisation. Three main imaging modalities can be used for this purpose: ultrasound (US) (Martino V. Franchi *et al.*, 2018), computed tomography (CT) (Hirose *et al.*, 2013) and magnetic resonance imaging (MRI) (Miokovic *et al.*, 2012).

Due to its excellent soft tissue contrast and multi-planar imaging capability (Kalmar *et al.*, 1988), MRI is considered the gold standard imaging modality for the assessment of muscle (Pons *et al.*, 2018). In muscle it is possible to use MRI to make simple measurements such as cross sectional area (CSA) and volume (Vol), and with advanced techniques fascicle pennation angle (PA), muscle disruption, oedema, myosteatosis and myofibrosis (Fischer *et al.*, 2014). In addition, MRI does not use any ionising radiation, increasing its utility as a research tool. However, MRI is costly (both in terms of equipment purchase and use), sizeable (requiring space and specialised housing unit) and has a relatively long acquisition time (Chianca *et al.*, 2021) (e.g., 30 to 45 mins in this study for lower limb scans only), and as a result is not practical or suitable for use in all settings.

In contrast to MRI, US is quicker to perform, portable and less expensive both in terms of equipment and cost per scan (Nijholt *et al.*, 2017), making it a useful tool in clinical research settings.

US has been shown to produce good intra- and inter-rater reliability in the measurement of both muscle thickness (MT) and CSA (Tillquist *et al.*, 2014; Pardo *et al.*, 2018). In addition, there is also evidence to support the use of US to determine dynamic changes in muscle size in response to exercise training (M. V Franchi *et al.*, 2018). Although these data point to US measurements being able to accurately detect dynamic changes in muscle mass in response to an environmental stimulus, validation studies have mainly been performed with a hypertrophic rather than atrophic stimulus such as immobilisation.

Studies employing US to measure MT and CSA of the quadriceps, gastrocnemius and TA have been conducted to assess the effects of immobilisation (de Boer *et al.*, 2008), and DMA has been observed via US (Segaran *et al.*, 2017). However, very few of these results have been validated against CT or MRI measurements (Scott *et al.*, 2017), and none have looked at this comparison during aDMA. In addition, as US measurements are user dependent and can be altered by a number of factors such as probe pressure and angle (Klimstra *et al.*, 2007), use by inexperienced operators may increase variability in both intra- and inter-rater measurements resulting in relatively large 95% limits of agreement (LOA) for US measurements (Raj, Bird and Shield, 2012). These factors may limit the ability of US measurements to detect muscle mass changes (Raj, Bird and Shield, 2012) and larger sample sizes may be required if US is the imaging modality chosen (May, Locke and Kingsley, 2021).

Considering the current literature in this space, further work is required to validate the utility of US measures in the assessment of DMA, especially in relation to aDMA and across different muscle groups.

3.1.3 Chapter aims

Based on the highlighted knowledge gaps in the existing literature, the primary aim of the work presented in this chapter was to provide high-resolution characterisation of the timecourse of DMA over 2 weeks of immobilisation, including in previously postulated aR and aS muscles. The secondary aim of this work was to assess the relationship between US and MRI-derived measures of muscle architecture, to determine the potential utility of US for quantifying DMA.

3.2 Materials & Methods

3.2.1 Participant recruitment

Twelve healthy, adult males aged 18-30 years were recruited to this study via adverts in the local community. Only male participants were recruited in order to avoid the potential uncontrolled variable of the effect of varying levels of female hormones on muscle physiology (Hansen, 2018). Participants' health was determined according to a 'health history questionnaire' (see Appendix B) to exclude any significant comorbidities or other contraindications to participation. Potential participants were excluded from the study if they had: i) a personal or family history of venous thromboembolism, ii) any history of limb immobilisation, casting or more than five days of hospitalisation or bed rest in the previous 12 months, or iii) current or previous prolonged use of non-steroidal anti-inflammatory medications (due to their potential effects on muscle physiology (Lilja *et al.*, 2018)). Participants who were eligible gave written informed consent to participate in the study before being enrolled. The study was approved by the University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee (ref: FMHS 103-1809) and Nottingham Trent University Human Biological Investigation Research Committee (ref: 586) and was conducted according to the declaration of Helsinki.

3.2.2 Study overview

Once recruited, all participants had 15 continuous days of unilateral lower limb immobilisation (ULLI). This was achieved by placing the dominant

leg in a hinged knee brace (Knee Post op Cool, Össur, Iceland) fixed at 75º knee flexion, and the ankle in an air-boot (Rebound Air Walker, Össur, Iceland) to prevent plantar/dorsi flexion (Figure 3.1).

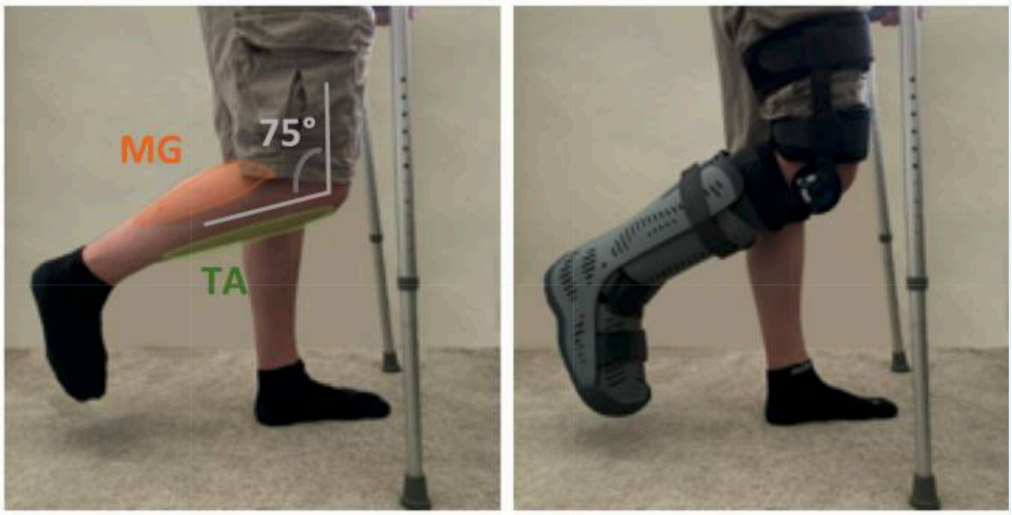

Figure 3.1: Immobilised leg in a knee brace and air-boot.

Both the knee brace and the air boot were adjusted to ensure minimal horizontal and vertical movement, after which 'tamper tags' were fitted to all securing straps to ensure that neither the brace nor boot were removed without the researcher's knowledge. The positioning of the leg in the brace and boot was chosen so that the ankle was fixed in a neutral position to prevent TA lengthening, with the knee held in fixed flexion to prevent MG lengthening. Furthermore, with the immobilised leg fixed in this position, participants were unable to weight bear on the limb, ensuring full immobilisation and disuse. Participants had training in the use of forearm crutches and were encouraged to continue with normal daily activities. Daily contact was maintained with participants throughout. The brace and boot were removed before each scan, allowing for examination of the immobilised leg for any complications,

and for the scans to be performed. The brace and boot were re-fitted by a member of the research team immediately after each scan.

3.2.3 **Assessment of muscle mass**

All participants had baseline (BL) imaging via MRI and US immediately before commencement of immobilisation. MRI and US imaging was repeated every two days during the immobilisation period (unless this fell at a weekend, when the interval for repeat imaging was increased to three days) until day 15. A subset of participants (n=4) only had imaging assessments before, at one time point (TP) (5 days) during, and after immobilisation. Imaging consisted of a whole leg MRI scan and US muscle architecture (MT and CSA) assessment of the VL, MG and TA muscles. These muscles were chosen to be representative of intermediate, aS, and aR muscles, respectively. To account for any potential acute intramuscular oedema after strenuous exercise (Schoenfeld and Contreras, 2014) or fluid pooling due to limb immobilisation, participants rested for at least 30 minutes in a supine position prior to any imaging (Mulder *et al.*, 2015).

3.2.3.1 Ultrasound

Participants lay supine on an examination couch, with knees fully extended and ankles in a relaxed position. Cranio-caudal midpoints of the muscles were then identified from surface landmarks. For VL the mid-point between the greater trochanter of the femur and the palpable joint line of the knee was marked (Figure 3.2).

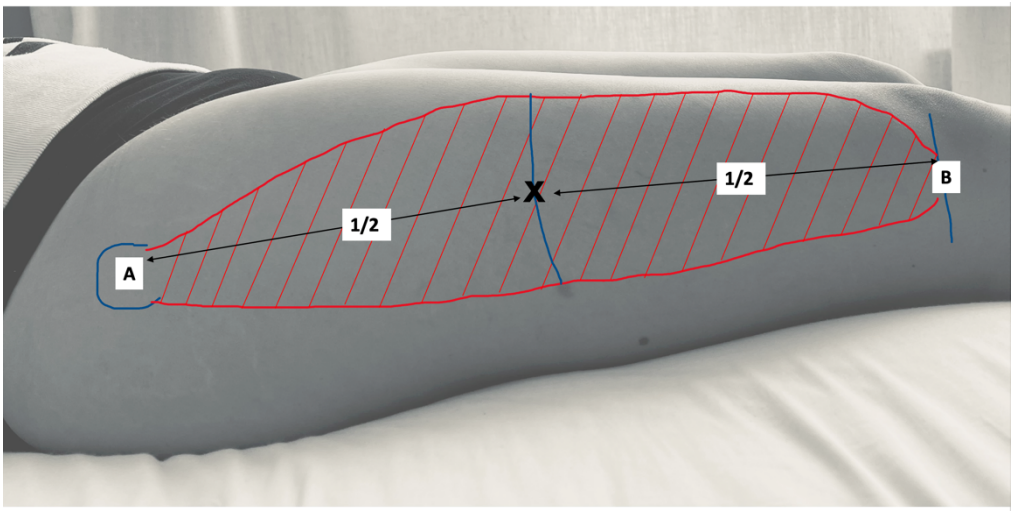

Figure 3.2: Anatomical landmarks used for identification of midpoint of VL

For MG the midpoint of the muscle was estimated at 1/3 of the distance from the posterior knee crease and the medial malleolus of the ankle (Figure 3.3)

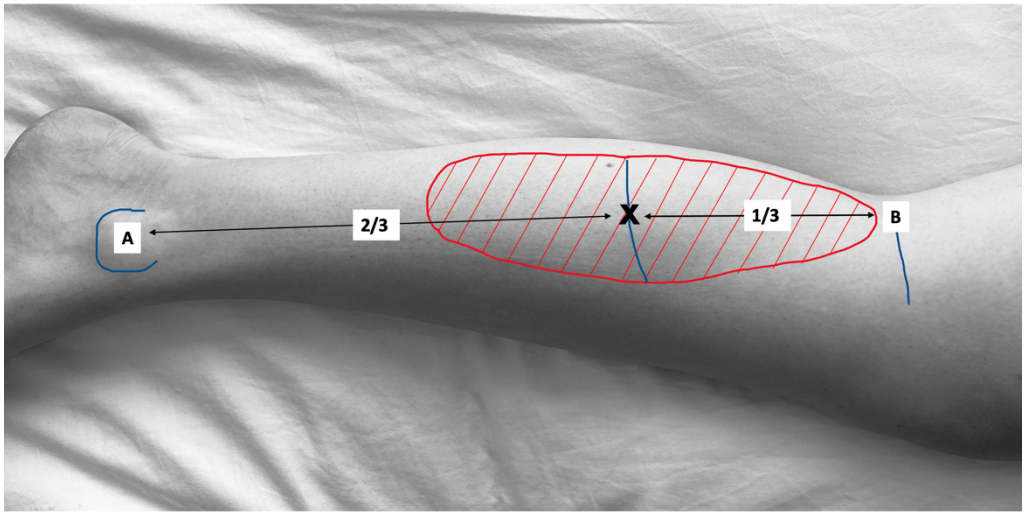

Figure 3.3: Anatomical landmarks used for identification of midpoint of MG

For TA the midpoint of the muscle was estimated as 1/3 of the distance from the palpable joint line of the knee to the lateral malleolus of the ankle (Figure 3.4)

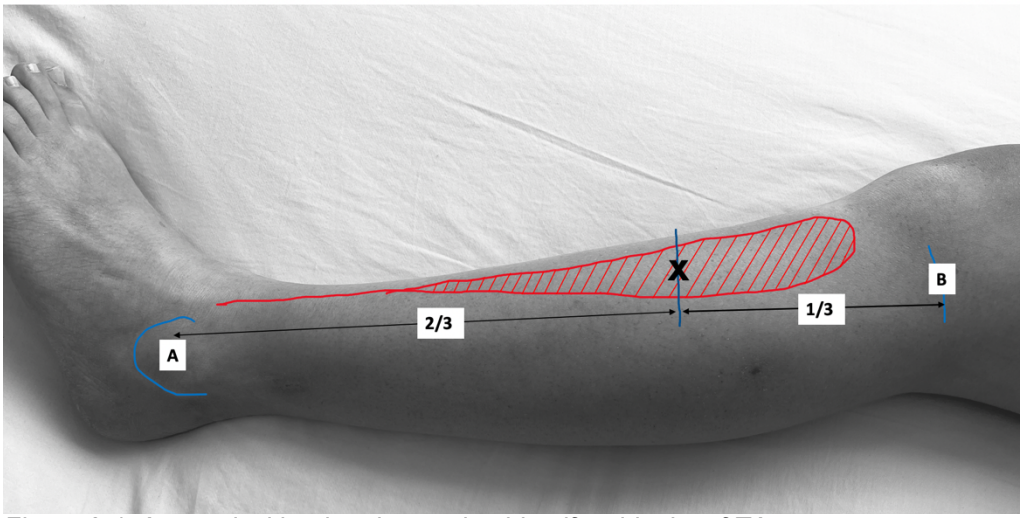

Figure 3.4: Anatomical landmarks used to identify midpoint of TA

At the level of these points, the medial and lateral borders of the muscle were identified using B-mode ultrasonography and the centre point measured and marked. These points were marked using a permanent surgical marker and refreshed at each visit. At the marked centre point of each muscle, three images were captured using B mode ultrasonography (Acuson P500, Siemens, Munich, Germany), with the transducer (VF13-5, Siemens) aligned to the fascicle plane. Care was taken to ensure that there was no compression of the muscle and that the superficial and deep aponeurosis were adequately visualised and transverse in the image. Ultrasounds were performed by an investigator who had undergone training and practiced on fellow investigators until able to produce accurate, repeatable images and been signed off by a supervisor.

CSA and MT were then calculated from these images using ImageJ (NIH, Version 1.53). For VL and MG, MT was determined as the distance between the superficial and deep aponeuroses (Figure 3.5).

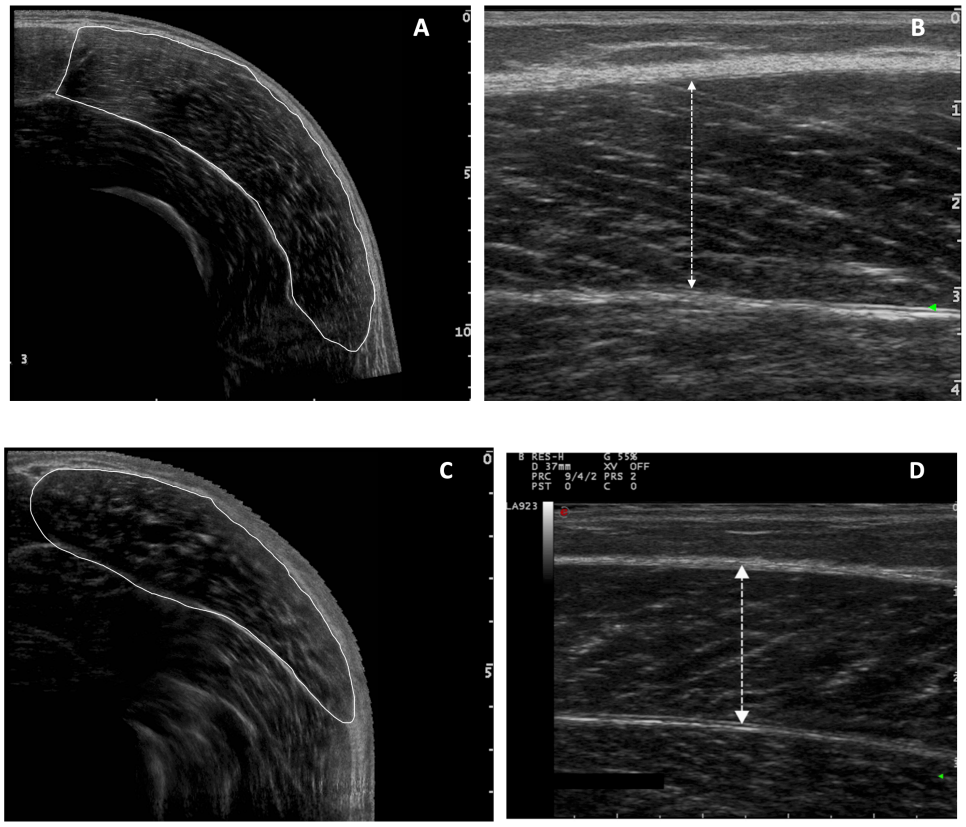

Figure 3.5: Analysis of A) Vastus Lateralis (VL) cross sectional area (CSA), B) VL muscle thickness (MT), C) Medial Gastrocnemius (MG) CSA, D) MG MT. White line in A and C indicates the marked edges of the muscle for calculation of CSA.

Due to the bipennate structure of TA (Varghese and Bianchi, 2014), MT was measured as the distance between the superficial muscle sheath and the most lateral visible point of the central aponeuroses (Figure 3.6).

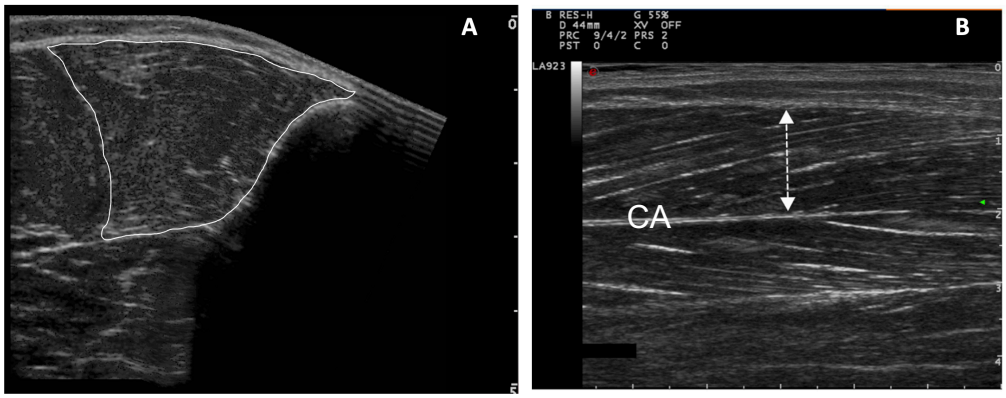

Figure 3.6: Analysis of A) Cross sectional area (CSA) and B) Muscle thickness in Tibialis Anterior. In A) the white line indicates the marked edges of the muscle for calculation of CSA. In B) CA indicates the line of the central aponeurosis

3.2.3.2 Magnetic resonance imaging

Whole leg MRI imaging was performed using a 1.5T MRI system (Avanto, Siemens, Munich, Germany), using a Siemens peripheral angiography coil to optimise signal to noise ratio. A localiser scan was performed to align images and ensure imaging of the whole of both legs (immobilised and non-immobilised). To achieve imaging of the whole leg it was necessary to acquire two sets of axial images which were automatically composited by the scanner software. An imaging matrix of 512 x 235, with a resolution of 835 x 835 μm and slice thickness of 5mm was acquired using a turbo spin echo sequence with an echo time set to the minimum value of 12 ms and a repetition time of 568 ms to optimise the trade-off between imaging time and contrast for a proton density weighted image. MRI images were analysed using 3D Slicer software v4.10 (Open source software, https://www.slicer.org) (Fedorov *et al.*, 2012). The borders of MG and TA were traced for both legs on every slice to allow segmentation by pixel count method, after which semi-automatic filling was performed between slices to calculate muscle Vol (Figure 3.7). Muscle tracing was performed by an independent investigator, unfamiliar with the participants, study protocol or sequencing of the scans in order to avoid bias. Measurements were double checked by a second investigator in random scans, and good agreement between measurements found. Due to human error in the scan acquisition process, several scans did not include the entire VL and Vol could therefore not be reliably calculated for this muscle.

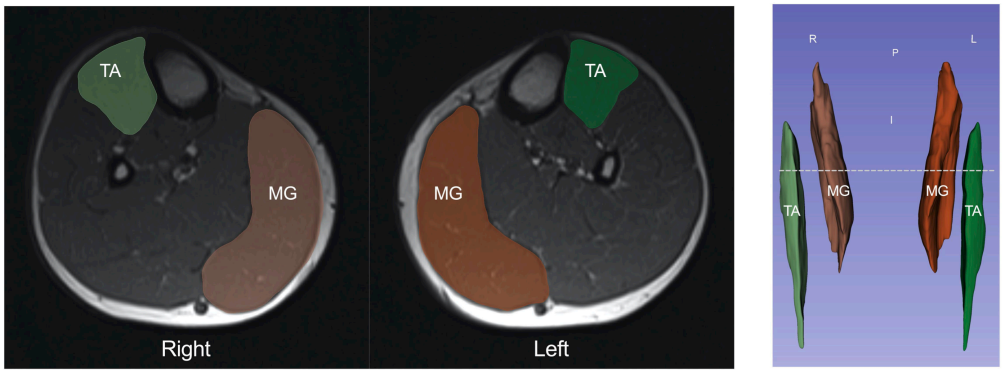

Figure 3.7: Assessment of MRI muscle volume using 3D slicer software, with muscle boundaries traced in every slice followed by semi-automatic volume rendering. TA = Tibialis Anterior, MG = Medial Gastrocnemius

CSA was measured for VL, MG and TA on both legs, again by manually tracing muscle boundaries to allow segmentation by pixel count method (Figure 3.8). CSA measurements were performed at the same points as US measurements determined by bony landmarks (e.g., the joint line of the knee) visible on MRI images. Surface markers (cod liver oil capsules) were also taped over the muscle midpoints marked for US measurement, to allow easy identification of these points on MRI.

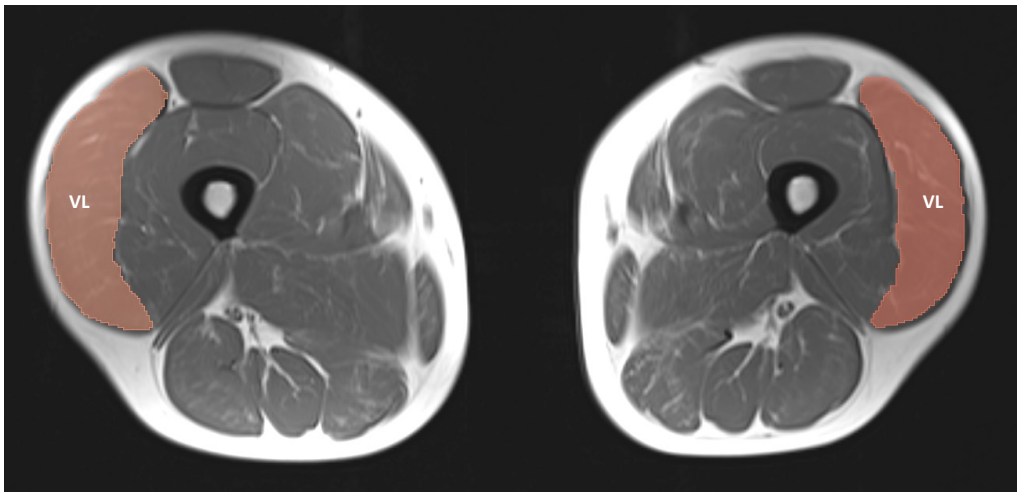

Figure 3.8: Defining muscle cross sectional area for vastus lateralis at the defined point using 3D slicer software for MRI.

3.2.4 Statistical Analysis

Distribution of data was tested using the Kolmogorov-Smirnov test, with normally distributed data expressed as mean (±SD) and non-normally distributed data as median (IQR). To compare changes in the immobilised leg vs. the non-immobilised leg, and to identify differences in the degree of atrophy between muscles, repeated measures two-way ANOVA with multiple comparisons (time vs. immobilisation status) were conducted with Tukey *post hoc* analysis. If there were incomplete data sets for repeated measures comparison mixed-effect model analysis were performed with restricted maximum likelihood (REML) estimators and Dunnet multiple comparison procedure. Statistical significance was accepted as a $p < 0.05$. No formal sample size calculation was performed due to a lack of existing data on which to base estimates.

To assess the agreement between US and MRI measurements of muscle mass losses during immobility, we assessed concordance of change in US CSA from BL to post-immobilisation scans, with MRI CSA over the same time period using Lin's concordance correlation coefficient (CCC), with bootstrapped standard errors to adjust for the longitudinal experimental design (Carrasco and Jover, 2003; Scott *et al.*, 2017). A CCC value of 1.0 indicates perfect concordance, whilst values <0.5 were taken to show poor agreement, 0.5 - 0.7 to show moderate, and >0.7 to show excellent agreement (Lin, 1989; Akoglu, 2018). Bland Altman comparison was performed to assess agreement between US and MRI CSA, with bias and 95% CI reported. Correlation between US CSA / MT,

and MRI Vol / CSA was assessed by calculating Pearson's correlation coefficients (r) with mean absolute differences and 95% limits of agreement. Perfect correlation was indicated by an r of 1.0, whilst r >0.7 was taken to show strong correlation, r 0.5 - 0.7 as moderate and r <0.5 as poor correlation (Akoglu, 2018). For comparison of MRI CSA vs. MRI Vol units of measurement differed, and so it was not possible to assess concordance or perform Bland Altman analysis (Parker *et al.*, 2016). Therefore, to allow an illustration of the degree of agreement between these measurements, the percentage change in each between BL and a set timepoint was compared using mixed-effect model analysis with REML estimators and Sidak's multiple comparison test, with mean difference and 95% CI plotted (Parker *et al.*, 2016). To compare and US MT vs. MRI CSA, the square root of MRI CSA was calculated to allow comparable units and concordance and Bland Altman analysis were then performed as described above. To further assess the utility of US CSA and MT in detecting DMA over short periods of immobilisation, results for all measurements were classified as either showing atrophy or hypertrophy at each time point and for each muscle. Using directional change (i.e., hypertrophy/atrophy) in MRI Vol or CSA (VL) as gold standard reference, sensitivity and specificity of US assessments were calculated as the percentage of 'true' muscle atrophy or hypertrophy respectively. Positive and negative predictive values (PPV/NPV) were calculated as the number of US assessments that detected 'true' muscle atrophy/hypertrophy divided by all US assessments that detected atrophy/hypertrophy, respectively (Scott *et al.*, 2017).

3.3 Results

3.3.1 Participants

A total of 12 young male participants, with no known medical comorbidities were recruited (mean [s.d]) age 22.7 [+/- 3.3], BMI 23.6 [+/- 0.6]) to this study. To characterise the timecourse of aDMA, all participants underwent MRI derived Vol and CSA measurements, as well as US CSA and MT measurements at BL, post immobilisation (END), and every two to three days (two unless research unit weekend closures resulted in three) over a 15-day immobilisation period (this was our "time-course cohort"). Unfortunately, due to participant availability, a subset of 4 participants only had scans at BL, END and at a single TP after 5-7 days of immobilisation (MP).

	All	Time-course cohort
Age (y)	22.7 (±3.28)	22.42 (±3.28)
Height (m)	1.80 (±0.06)	1.85 (±0.05)
Weight (kg)	77.9 (±9.92)	79.8 (±8.08)
BMI (kg/m^2)	23.6 (±0.64)	23.39 (±2.26)

Table 3.1: Participant demographics. Figures are mean[sd].

3.3.2 Timecourse of aDMA

3.3.2.1 Vastus Lateralis

The superior limit of MRI acquisition was manually defined for each scan by an MRI technician. Unfortunately, due to human error, imaging of the superior aspect of VL was incomplete on several MRI scans making it impossible to accurately assess changes in MRI Vol. As a result, MRI-derived CSA was used to define the timecourse of aDMA in VL.

In the measurements completed by all participants, there was no significant difference between limbs at baseline (MD -0.36, 95% CI -2.43 – 1.71, p=0.99) a significant immobilisation x time interaction was detected for VL CSA (p<0.01), with significant decreases in the immobilised (iMOB) limb at MP (-5.50%, MD-1.55, 95CI -2.45 to -0.64, p<0.01) and END (-13.94%, MD-2.28, 95CI -3.31 to -1.25, p<0.01).

Similarly, in the time-course cohort, repeated measures mixed effects analysis revealed a significant immobilisation x time interaction for VL CSA (p<0.01), with no significant difference in BL measurements between the iMOB and Control (CON) limbs (27.04 [±5.72] vs. 25.99 [±5.1], p=0.99). The iMOB limb decreased at all timepoints compared to BL (TP1 -4.5% [p=0.46], TP2 -4.6% [p=0.24], TP3 -10.7% [p<0.01], TP4 -8.9% [p<0.01], TP5 -13.8% [p<0.01], TP6 -14.3% [p<0.01]) (Figure 3.9). There were no statistically significant changes in the CON limb VL CSA over the period of immobilisation (Table 3.3)

Rates of DMA appear to be linear across the period of immobilisation, with significant changes in iMOB VL CSA between both BL and MP (MD -1.55, 95% CI: -2.45 to -0.64, p<0.01) and MP and END (-2.28, 95% CI: -3.31 to -1.25, p<0.01) (Table 3.2). However, secondary analysis of the time-course cohort highlighted significant changes in the iMOB limb from BL to TPs 3 (p=0.01), 4 (p=0.03) and 6 (p=0.02), from TP1 to TPs 3 (p=0.02) and 6 (p=0.049), and from TP2 to TP6 (p=0.03), with no further statistically significant changes observed after TP2 (Figure 3.9).

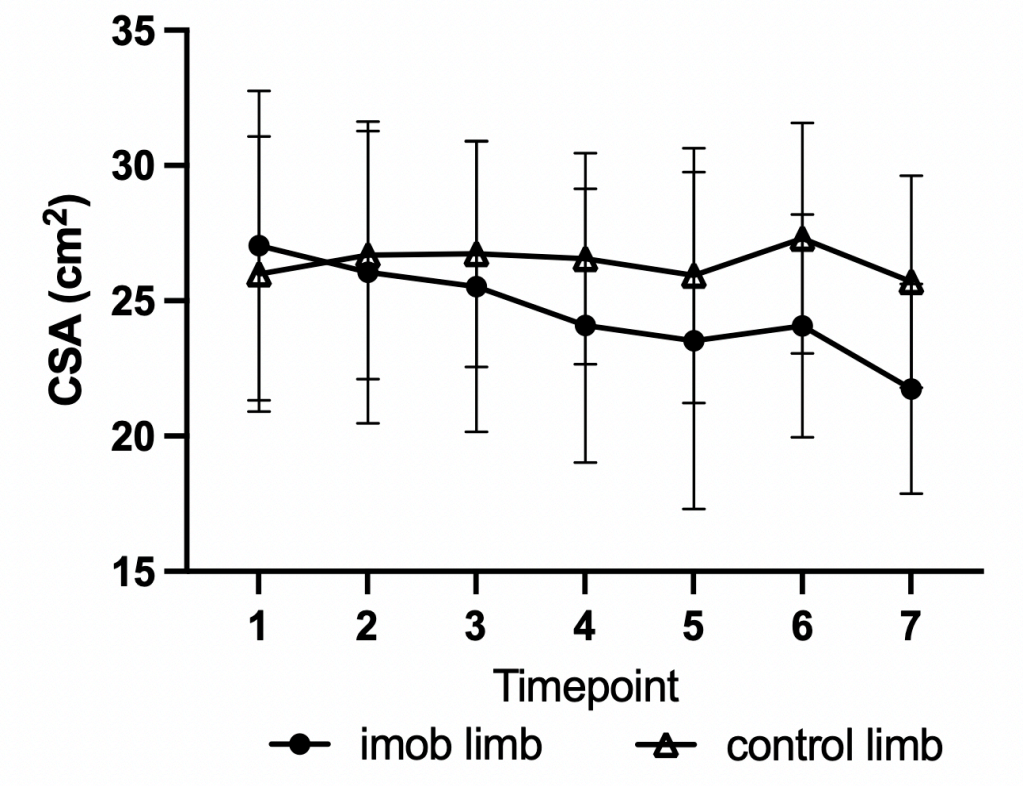

Figure 3.9: Timecourse of changes in MRI measured Vastus Lateralis Cross sectional area (CSA) over 15 days in a control and immobilised (imob) leg. Results shown as mean with sd

	Immobilised				Control		
	Baseline	Midpoint	End		Baseline	Midpoint	End
N	12	12	12		12	12	12
MRI CSA	27.60 (±5.6)	26.05 (±5.2)	23.77 (±5.2)		27.24 (±5.8)	27.77 (±5.1)	27.28 (±5.1)
% change		-5.50	-13.94			1.94	0.14
p		<0.01*	<0.01*			0.89	0.99
US CSA	30.17 (± 4.1)	28.84 (± 4.3)	26.15 (± 4.8)		29.98 (± 4.6)	29.82 (± 3.7)	29.55 (± 4.5)
% change		-4.42	-13.34			-0.54	-1.44
p		0.23	<0.01*			0.99	0.90
US MT	2.72 (± 0.27)	2.62 (± 0.27)	2.53 (± 0.31)		2.68 (± 0.30)	2.78 (± 0.35)	2.68 (± 0.20)
% change		-3.78	-6.98			3.84	0.11
P		0.08	<0.01*			0.32	0.99

Table 3.2: Summary of changes in measurements of Vastus Lateralis in the immobilised and control legs of all participants. Figures are mean (sd)

	Immobilised								Control						
	Baseline	TP1	TP2	TP3	TP4	TP5	TP6		Baseline	TP1	TP2	TP3	TP4	TP5	TP6
N	8	8	7	8	6	5	7		8	7	7	8	5	7	6
MRI CSA	27.04 (±5.72)	26.05 (±5.6)	25.53 (±5.4)	24.09 (±5.0)	23.53 (±6.2)	24.07 (± 4.1)	21.75 (±3.9)		25.99 (±5.1)	26.69 (±4.6)	26.73 (±4.2)	26.56 (±3.9)	25.94 (±4.7)	27.31 (±4.3)	25.70 (±3.9)
% change		-4.45	-4.64	-10.73	-8.90	-13.75	-14.26			1.58	5.23	3.20	4.70	3.67	5.46
p		0.29	0.06	0.01	0.03	0.18	0.02			0.83	0.95	0.98	0.99	0.37	0.99
MT	2.47 (±0.43)	2.46 (±0.52)	2.32 (±0.40)	2.33 (±0.35)	2.29 (±0.35)	2.28 (±0.38)	2.38 (±0.43)		2.55 (±0.16)	2.57 (± 0.29)	2.62 (±0.23)	2.54 (±0.17)	2.55 (±0.28)	2.59 (±0.37)	2.60 (±0.16)
% change		-0.49	-6.22	-4.86	-3.27	-5.21	-7.04			0.67	2.25	-0.19	-1.85	2.69	2.06
p		0.99	0.72	0.39	0.20	0.02*	0.69			0.99	0.77	0.99	0.99	0.99	0.96

Table 3.3: Changes in all measurements of vastus lateralis in the immobilised and control limb of all time-course participants. Figures are mean (sd)

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3.3.2.2 Medial Gastrocnemius

Both MRI Vol and CSA were measured to define the timecourse of aDMA in MG. At baseline, there was no significant difference between the iMOB and CON in either MG Vol (307.62 vs 302.41, MD -5.22, 95% CI: -34.5 to 24.23, p=0.69) or CSA (14.72 vs 13.38, MD -0.89, 95% CI: -2.40 to 0.61, p=0.20). In measurements completed by all participants, a significant immobilisation x time interaction was detected for MG Vol and CSA (p<0.01). In the iMOB limb MG Vol decreased form baseline to MP (-2.09%, MD 6.5, 95% CI: -3.4 to 16.4, p=0.28) and END (-8.16%, MD 22.12, 95% CI: 0.38 to 43.86, p=0.045). Similarly, iMOB limb MG CSA decreased from baseline to MP (-4.99%, MD 0.73, 95% CI: 0.20 to 1.25, p<0.01) and END (- 6.86%, MD 0.99, 95% CI: 0.37 to 1.63, p <0.01). After 15 days, MG CSA increased in the CON limb by +2.74% (p=0.01).

In the timecourse cohort of patients repeated measures mixed effects analysis revealed a significant immobilisation x time interaction for MG Vol and CSA (p<0.01). On post hoc analysis of MG Vol there was no significant difference in BL measurements between the iMOB and CON legs (307.62 [$^{+}/_{-}$ 36.4] vs. 302.41 [$^{+}/_{-}$ 52.2], p=0.69). MG Vol decreased in the iMOB limb at all TP compared to BL (TP1 -0.4% [p=0.99], TP2 -4.7% [p=0.48], TP3 -5.8% [p<0.01], TP4 -6.1% [p=0.04], TP5 -4.3% [p=0.04], TP6 -7.3% [p<0.01]) (Figure 3.10). MG CSA also decreased at all TP in the iMOB limb (TP1 -0.2% [p=0.99], TP2 -6.1% [p=0.04], TP3 -7.9% [p<0.07], TP4 -8.5% [p=0.05], TP5 -9.5% [p<0.01], TP6 -5.4% [p<0.22]).

There were no statistically significant changes in the CON limb MG Vol or CSA over the period of immobilisation.

For both MG Vol and CSA in the iMOB limb there appears to be a period from BL to TP1 where muscle mass remains stable. Muscle mass is lost most rapidly between TP1 and TP4, after which rates slow. In keeping with this notion, changes in CSA between BL and MP in the iMOB limb were statistically significant (MD 0.73, 95% CI: 0.20 to 1.25, p<0.01), but those between MP and END (MD 0.27, 95% CI: -0.62 to 1.17, p=0.89) were not (Figure 3.10). However, it should be noted that there is significant variability in the data and as such these results likely represents a linear loss of muscle and differences in rates over the timecourse of immobilisation may well become less apparent if a larger population was studied.

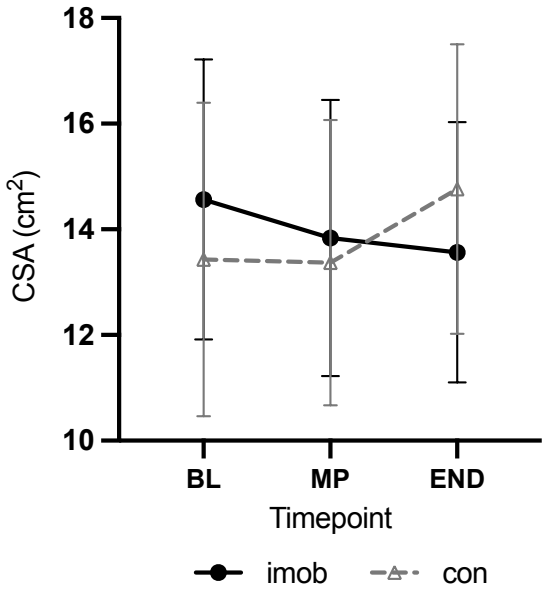

Figure 3.10: Changes in Medial Gastrocnemius cross sectional area (CSA), in control and immobilised (imob) leg. BL = baseline, MP = midpoint (day 5-7), END = day 15. Results are shown as mean with sd

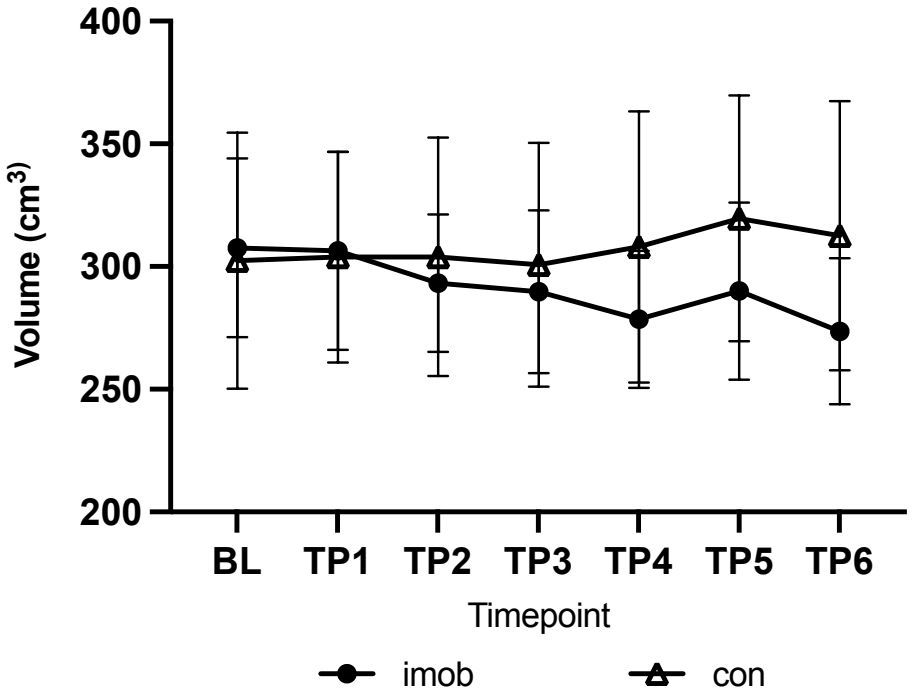

Figure 3.11: Characterisation of the timecourse of changes in Medial Gastrocnemius volume over 15 days in a control and immobilised (imob) leg. BL = baseline, TP = timepoint, gap between timepoints = 2 -3 days. Results are shown as mean with sd

	Immobilised				Control		
	Baseline	Midpoint	End		Baseline	Midpoint	End
N	12	12	12		12	12	12
MRI Vol	291.13 (± 58.5)	285.04 (± 57.2)	267.38 (± 50.7)		280.07 (± 62.7)	287.91 (± 63.6)	286.77 (± 68.6)
% change		-2.09	-8.16			2.8	2.39
p		0.28	<0.05*			0.47	0.73
MRI CSA	14.57 (± 2.7)	13.84 (± 2.6)	13.57 (± 2.5)		13.43 (± 3.0)	13.37 (±2.7)	14.76 (± 2.7)
% change		-4.99	-6.86			-0.43	2.74
p		<0.01*	<0.01*			0.99	0.01*
US CSA	14.86 (± 3.2)	13.85 (±3.0)	12.87 (±3.1)		13.87 (± 3.3)	13.95 (± 3.0)	14.76 (± 4.1)
% change		-6.81	-13.42			0.61	3.49
p		0.06	<0.01*			0.99	0.40
US MT	2.04 (± 0.3)	1.99 (± 0.3)	1.89 (± 0.3)		1.98 (± 0.3)	2.08 (± 0.3)	2.07 (± 0.3)
% change		-2.25	-7.60			4.99	4.32
P		0.57	0.02*			0.06	<0.01*

Table 3.4: Summary of changes in measurement of a medial gastrocnemius in control and immobilised legs of all participants over 15 days. Figures are mean (sd)

	Immobilised							Control						
	Baseline	TP1	TP2	TP3	TP4	TP5	TP6	Baseline	TP1	TP2	TP3	TP4	TP5	TP6
N	8	8	7	8	6	5	7	8	7	7	8	5	7	6
MRI Vol	307.62 (± 36.4)	306.42 (± 40.3)	293.25 (± 28.0)	289.80 (± 33.6)	278.52 (± 27.9)	290.02 (± 36.1)	273.65 (± 29.7)	302.41 (± 52.2)	303.83 (± 2.9)	303.99 (± 48.7)	300.72 (± 49.7)	308.01 (± 55.2)	319.61 (± 50.1)	312.61 (± 54.8)
% change		-0.41	-4.67	-5.80	-6.14	-4.28	-7.26		1.02	2.59	-0.13	1.12	0.39	2.56
p		0.99	0.48	<0.01*	0.04*	0.04*	<0.01*		0.86	0.36	0.85	0.48	0.94	0.35
MRI CSA	14.72 (± 1.9)	14.69 (± 2.0)	13.83 (± 1.9)	13.55 (± 1.9)	13.47 (± 1.9)	13.32 (± 2.0)	13.92 (± 1.5)	13.83 (± 2.9)	14.33 (± 2.8)	13.77 (± 2.5)	14.24 (± 2.9)	14.18 (± 2.6)	14.98 (±2.6)	15.66 (± 2.9)
% change		-0.20	-6.06	-7.97	-8.54	-9.53	-5.46		2.90	0.45	3.13	1.52	5.95	10.32
p		0.99	0.04*	0.07	0.05*	<0.01*	0.22		0.48	0.99	0.61	0.99	0.17	0.15
MT	1.93 (± 0.27)	1.92 (± 0.26)	1.87 (± 0.33)	1.76 (± 0.20)	1.68 (± 0.12)	1.75 (± 0.28)	1.76 (± 0.42)	1.95 (± 0.30)	1.96 (± 0.29)	2.01 (± 0.39)	1.97 (± 0.35)	1.80 (± 0.41)	1.95 (± 0.49)	2.04 (± 0.31)
% change		-0.18	-2.99	-7.79	-8.76	-9.20	-10.38		1.27	2.97	0.24	-8.00	-1.38	2.47
p		0.99	0.57	0.16	0.09	0.44	0.38		0.99	0.95	0.99	0.68	0.99	0.39

Table 3.5: Summary of changes in measurement of Medial Gastrocnemius in the control and immobilised leg of all time-course participants over 15 days of immobilisation. Figures are mean (SD)

3.3.2.3 Tibialis Anterior

Both MRI-derived Vol and CSA were measured to define the timecourse of aDMA in TA. In measurements completed by all participants, no immobilisation x time interaction was detected for either measurement. Furthermore, on secondary analysis, there were no statistically significant changes from BL in the iMOB limb at any time point, with just a -2.94% ±4.35 (p=0.66) change in TA Vol and -1.05% ±8.62 (p=0.90) change in TA CSA after 15 days of immobilisation (Figure 3.12). For a full breakdown of all changes in TA measurements over 15 days of immobilisation see Table 3.6 and Table 3.7.

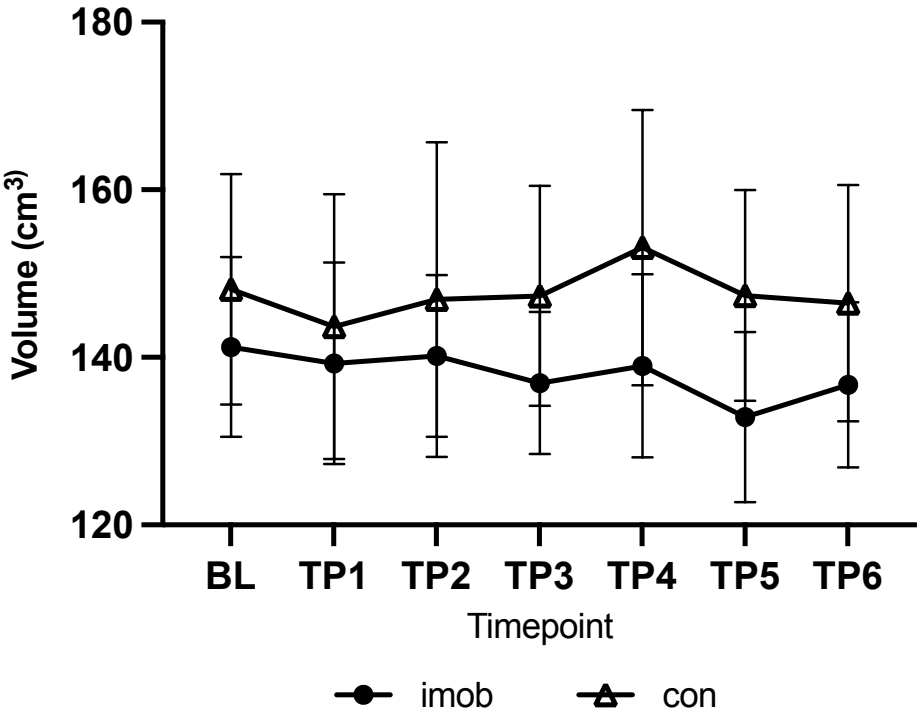

Figure 3.12: Characterisation of the timecourse of changes in Tibialis Anterior volume over 15 days in a control and immobilised (imob) leg. BL = baseline, TP = timepoint, gap between timepoints = 2 -3 days. Results shown as mean with sd.

	Immobilised								Control						
	Baseline	TP1	TP2	TP3	TP4	TP5	TP6		Baseline	TP1	TP2	TP3	TP4	TP5	TP6
N	8	8	7	8	6	5	7		8	7	7	8	5	7	6
MRI Vol	141.24 (± 10.7)	139.30 (± 12.0)	140.18 (± 9.7)	136.95 (± 8.5)	139.00 (± 10.9)	132.89 (± 10.1)	136.73 (± 9.9)		148.14 (± 13.8)	143.69 (± 15.8)	146.90 (± 18.8)	147.34 (± 13.1)	153.10 (± 16.4)	147.39 (± 16.4)	146.47 (± 14.1)
% change		-1.31	-2.33	-2.75	-2.67	-2.48	-1.46			-3.08	-1.87	-0.48	2.19	0.04	1.02
p		0.96	0.99	0.69	0.99	0.53	0.26			0.30	0.99	0.96	0.11	0.99	0.97
MRI CSA	6.48 (± 0.81)	6.48 (± 0.86)	6.67 (± 0.35)	6.71 (± 0.53)	6.77 (± 0.57)	6.41 (± 0.61)	6.16 (± 0.75)		6.99 (± 0.58)	6.98 (± 0.71)	7.23 (± 0.72)	7.29 (± 0.21)	7.43 (± 0.36)	7.28 (± 0.29)	6.92 (± 0.34)
% change		1.28	1.87	4.38	6.22	-3.18	-0.57			1.22	2.85	3.59	6.95	2.48	1.89
p		0.99	0.98	0.87	0.75	0.99	0.85			0.99	0.89	0.67	0.54	0.72	0.99
MT	1.45 (± 0.18)	1.35 (± 0.07)	1.41 (± 0.19)	1.48 (± 0.13)	1.35 (± 0.03)	1.38 (± 0.16)	1.38 (± 0.18)		1.35 (± 0.17)	1.36 (± 0.13)	1.30 (± 0.16)	1.43 (± 0.17)	1.34 (± 0.15)	1.41 (± 0.16)	1.38 (± 0.19)
% change		-7.12	-2.42	1.364	-1.87	-1.28	-2.98			0.66	-2.95	2.48	3.64	7.56	2.13
p		0.83	0.97	0.99	0.74	0.98	0.85			0.99	0.99	0.81	0.99	0.90	0.90

Table 3.6: Summary of changes in measurements of Tibialis Anterior in the control and immobilised leg of timecourse participants over 15 days of immobilisation. Figures are mean (sd)

	Immobilised				Control		
	Baseline	Midpoint	End		Baseline	Midpoint	End
N	12	12	12		12	12	12
MRI Vol	136.13 (± 16.0)	136.45 (± 12.3)	132.13 (± 13.6)		150.11 (± 13.8)	148.2 (± 16.3)	150.1 (± 14.2)
% change		0.23	-2.94			-1.26	-0.01
p		0.99	0.66			0.47	0.47
MRI CSA	6.34 (± 0.76)	6.32 (± 0.61)	6.27 (± 0.62)		6.58 (± 0.82)	6.85 (± 0.78)	6.96 (± 0.81)
% change		-0.19	-1.05			4.17	5.70
p		0.99	0.90			0.17	0.13
US CSA	5.98 (± 0.94)	5.74 (± 0.94)	5.86 (± 1.26)		6.15 (± 0.81)	6.14 (± 0.91)	6.01 (± 0.83)
% change		-3.96	-2.01			-0.14	-2.52
p		0.86	0.96			0.99	0.78
US MT	1.34 (± 0.23)	1.32 (± 0.22)	1.35 (± 0.20)		1.40 (± 0.17)	1.40 (± 0.17)	1.48 (± 0.17)
% change		-1.56	0.63			0.11	5.95
p		0.70	0.96			0.01*	<0.01*

Table 3.7: Summary of changes in Tibialis Anterior measurements of control and immobilised legs of all participants over 15 days. Figures are mean (sd)

3.3.3 Comparison of the timecourse of aDMA between muscles

To allow comparison of changes between muscles of differing size, differences in % change in muscle CSA were analysed. A significant difference in % change by muscle was observed ($p<0.01$) as was a significant time x muscle interaction ($p<0.01$). Secondary analysis revealed a significant difference in % change from BL to TP3 (MD -15.1, 95CI -22.74 to -7.47, p=0.02), TP5 (MD -10.56, 95CI -19.39 to -1.72, p=0.02) between TA and VL, and from BL to TP3 (MD -12.26, 95CI -21.8 to -2.73, p=0.02) and TP4 (MD-12.80, 95CI -23.13 to -2.46, p=0.03) between TA and MG. There was no significant difference between % change in VL between MG CSA at any timepoint (Figure 3.13).

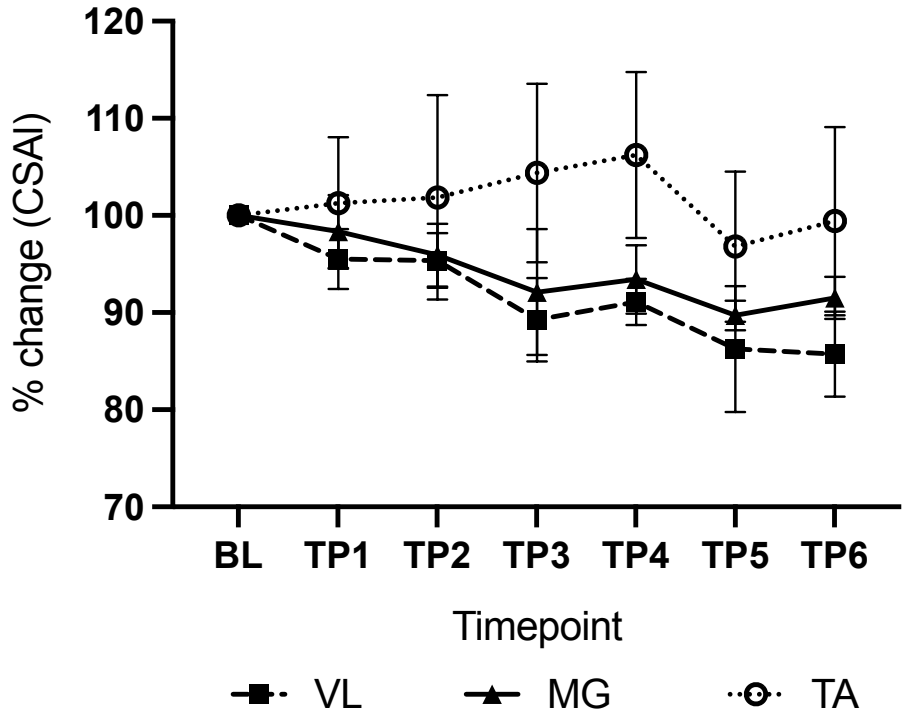

Figure 3.13: Comparison of muscle mass maintenance, assessed as MRI cross-sectional area (CSA) in immobilised vastus lateralis (VL), medial gastrocnemius (MG) and tibialis anterior (TA) over 15-days of immobilisation. 100% represents no change. Results shown as mean with sd. BL = Baseline, TP= timepoint. Gap between TP = 2-3 days

Mixed effects analysis of % change in TA and MG Vol also revealed a significant time x muscle interaction (p=0.04), although secondary analysis only revealed a significant difference in change between TA and MG at TP 6 (MD 8.59, 95CI 0.65 to 16.52, p=0.03) (Figure 3.14).

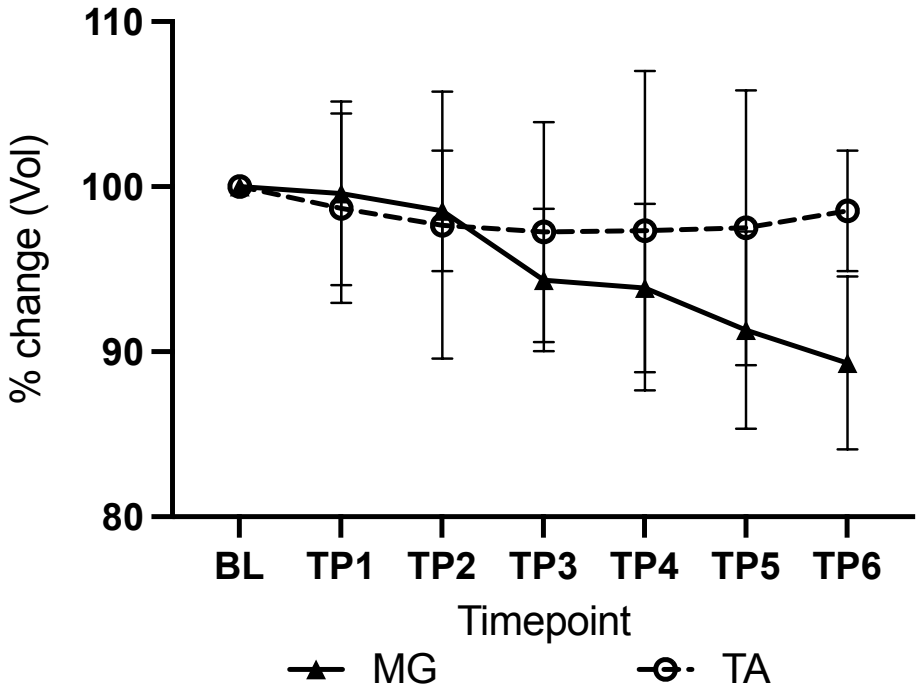

Figure 3.14: Comparison of muscle mass maintenance, assessed as MRI muscle volume, in immobilised medial gastrocnemius (MG) and tibialis anterior (TA) over 15-days of immobilisation. 100% represents no change. Results shown as mean with sd. BL = Baseline, TP= timepoint. Gap between TP = 2-3 days

3.3.4 Agreement between different measurements and modalities

3.3.4.1 Agreement between MRI measurements

For MG, there was excellent correlation between MRI derived Vol and CSA (r=0.83, p<0.01). Furthermore, repeated measures mixed effects analysis did not show any significant difference in % change as measured by Vol or CSA (p=0.70), with mean difference of % change between Vol and CSA in the iMOB limb ranging from -2.20 to 2.61.

In TA, there was no statistically significant correlation between MRI derived Vol and CSA (r=0.49, p=0.65). Whilst no significant difference was observed between % change by Vol or CSA (p=0.23), mean difference in the iMOB limb ranged from -8.89 to 0.71, with a wide 95% CI suggesting poor degree of agreement (Figure 3.15)

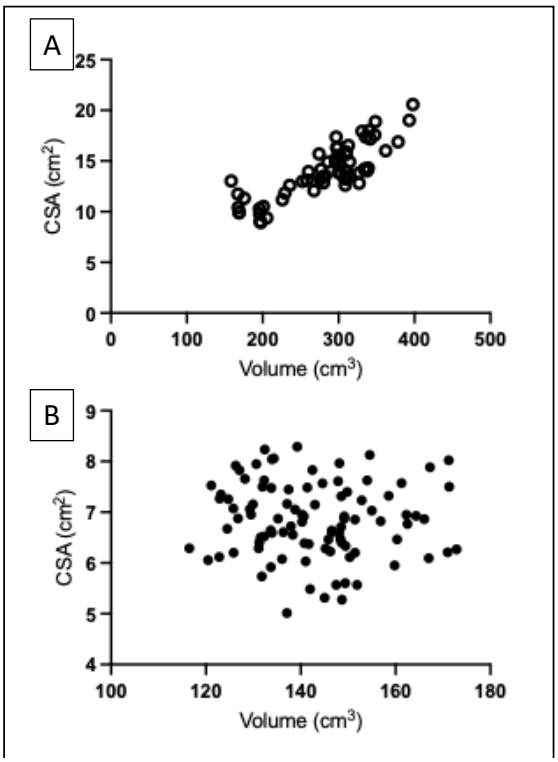

Figure 3.15: Correlation between absolute measurements for MRI-derived volume vs cross-sectional area (CSA), in A) MG (top) and B) TA (bottom).

3.3.4.2 Agreement between MRI and US derived measurements

In VL, there was strong correlation (r=0.77, 95% CI: 0.64-0.85, p<0.01) between MRI and US derived CSA, with strong concordance between these measurements (r_c=0.72, SE 0.58-0.81) via Lin's CCC. Bland-Altman analysis shows a bias of -1.66 (95% CI: -8.49 to 5.16) when comparing US to MRI CSA (Figure 3.16) suggesting good agreement.

Compared to a gold standard (defined as atrophy detected by decrease in VL MRI CSA from BL), in VL US CSA was shown to have a sensitivity of 73.3%, specificity of 40%, PPV of 78.6% and NPV of 33.3% for detecting atrophy. This analysis was based on a comparison of 40 paired measurements.

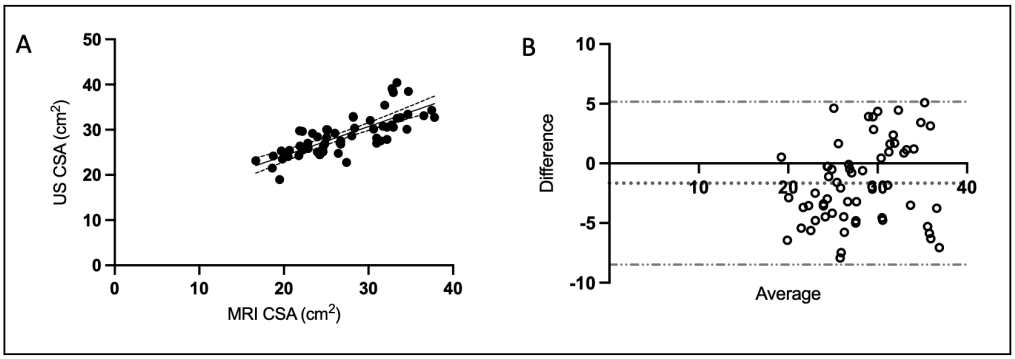

Figure 3.16: A) Correlation and B) Bland-Altman comparison of vastus lateralis (VL) ultrasound-derived (US) cross sectional area (CSA) vs MRI-derived CSA.

Ultrasound derived MT was shown to have moderate correlation with the square root of MRI CSA (SQR MRI CSA) (r=0.54, 95% CI: 0.34-0.70, p<0.01). Bland-Altman analysis showed a bias of 2.50 (95% CI: 1.65 to 3.36) (Figure 3.17).

Compared to the same gold standard as outlined above, in VL US MT was shown to have a sensitivity of 73.1%, specificity of 63.6%, PPV of 82.6% and NPV of 50.0%. This analysis was based on comparison of 111 paired measurements.

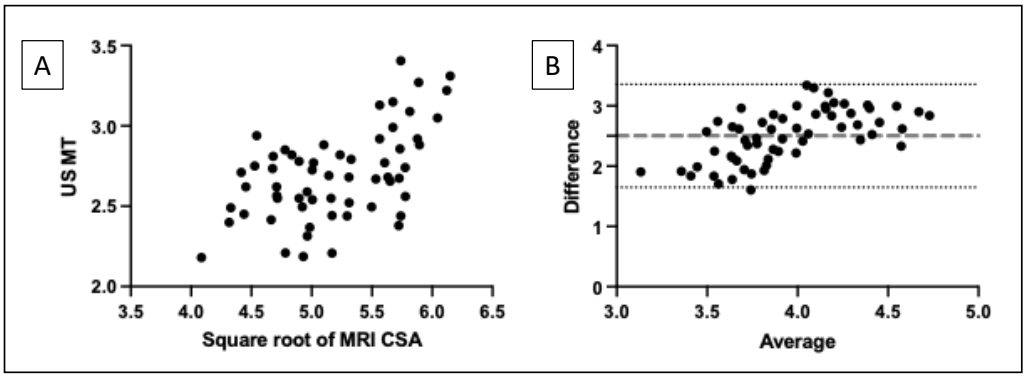

Figure 3.17: Comparison of Ultrasound (US) muscle thickness (MT) vs SQR MRI Cross sectional area (CSA) measurements of Vastus Lateralis (VL). A) Correlation B) Bland Altman comparison

In MG, there was strong correlation (r=0.75, 95% CI: 0.62-0.84, p<0.01) between MRI and US derived CSA, with a suggestion of strong concordance between these measurements via Lin's CCC (r_c=0.73, SE 0.60-0.82). Bland-Altman analysis shows a bias of -0.20 (95% CI: -4.50 to 4.10) when comparing US CSA to MRI CSA (Figure 3.18), suggesting excellent agreement.

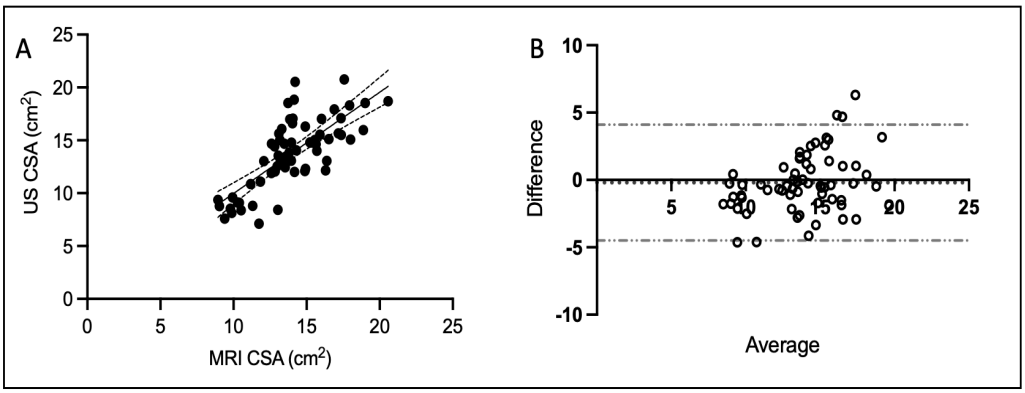

Figure 3.18: A) Correlation and B) Bland-Altman comparison of medial gastrocnemius (MG) ultrasound (US) and MRI derived measurements of cross sectional area (CSA).

Compared to a gold standard (defined as atrophy detected by decrease in MG MRI CSA from BL), in MG US CSA was shown to a sensitivity of 100%, specificity of 52%, PPV of 61% and NPV of 100%. This analysis was based on a comparison of 40 paired measurements.

Ultrasound derived MT was shown to have strong correlation with SQR MRI CSA (r=0.73, 95% CI: 0.6-0.83, p<0.01). Bland-Altman analysis showed a bias of 1.72 (95% CI: 1.25 to 2.21)(Figure 3.19).

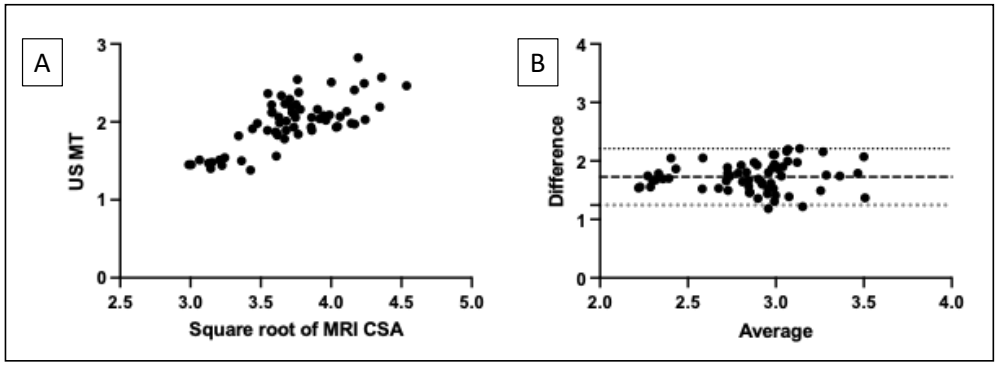

Figure 3.19: Comparison of ultrasound (US) derived muscle thickness (MT) vs SQR MRI derived cross sectional area (CSA) measurements of Medial Gastrocnemius (MG). A) Correlation B) Bland Altman comaprison

Compared to the same gold standard outlined above, US MT was shown to have a sensitivity of 77.3%, specificity of 56.6%, PPV of 68.9% and NPV of 66.7%. This analysis was based on comparison of 119 paired measurements.

In TA, there was moderate correlation (r=0.50, 95% CI: 0.29-0.66, p<0.01) between MRI and US derived CSA with Lin's CCC suggesting moderate concordance between these measurements (r_c=0.43, SE 0.24-

0.59). Bland-Altman analysis showed a bias of 0.43 (95% CI: -1.30 to 2.16) when comparing US CSA to MRI CSA (Figure 3.20), suggesting good agreement.

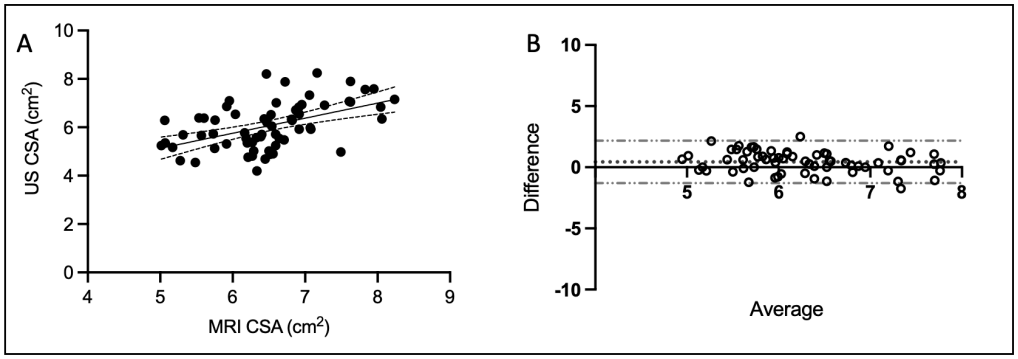

Figure 3.20: A) Correlation and B) Bland-Altman comparison of Tibialis Anterior ultrasound (US) and MRI derived measurements of cross sectional area (CSA).

Compared to a gold standard (defined as atrophy detected by decrease in TA MRI CSA from BL), in TA US CSA was shown to a sensitivity of 65.2%, specificity of 52.6%, PPV of 62.5% and NPV of 55.6%. This analysis was based on a comparison of 40 paired measurements.

Ultrasound derived MT was shown to have moderate correlation with SQR MRI CSA (r=-0.50, 95CI -0.29 to -0.67, p=0.01). Bland-Altman analysis showed a bias of 1.15 (95% CI: 0.81 to 1.50) (Figure 3.21).

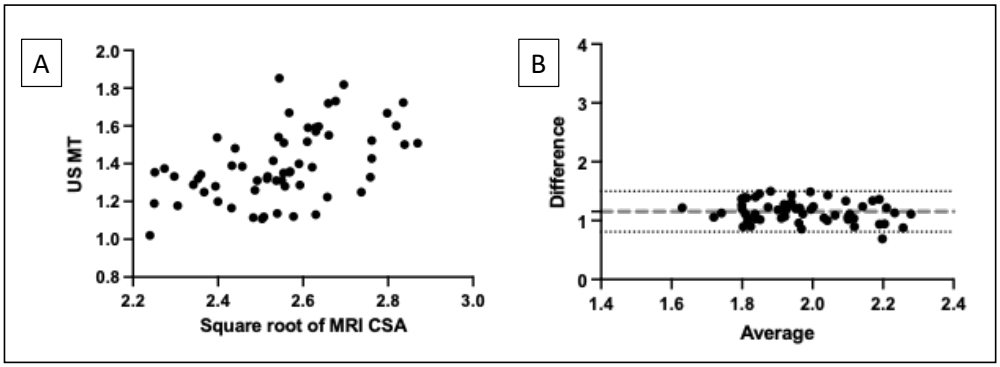

Figure 3.21: Comparison of ultrasound (US) derived muscle thickness (MT) vs MRI derived volume (Vol) measurements of Tibialis Anterior (TA). A) Correlation B) Mixed effects analysis of MD between % change in TA as detected by US MT vs MRI CSA.

Compared to the same gold standard as outlined above, in TA US MT was shown to have a sensitivity of 50.8%, specificity of 53.9%, PPV of 57.1% and NPV of 47.5%. This analysis was based on comparison of 115 paired measurements.

3.4 Discussion

3.4.1 Evidence of aDMA

In this study we found evidence of rapid muscle loss following immobilisation in functionally significant muscles of the leg. In VL, CSA decreased by >5% within the first week of immobilisation, progressing to ~14% after two weeks of immobilisation. Similar figures have been reported by multiple other studies (Backx *et al.*, 2017; Horstman *et al.*, 2019; Kilroe *et al.*, 2019), all of which report a ~5% decrease in combined quadriceps Vol after seven days of immobilisation.

The results of this study showed a greater decrease in VL CSA at 14 days than the studies included in chapter 2 but are in keeping with those

reported elsewhere in the literature. For example Hespel *et al.* reported a 10% decrease in quadriceps CSA after two weeks of immobilisation (Hespel *et al.*, 2001), whilst Thom *et al.,* reported an 11% decrease over the same period (Thom *et al.*, 2001). This variation in VL CSA decrease at 14 days is not easy to explain, although is likely to be at least in part due to the relatively small participant numbers in each of these studies and the resultant overlapping CIs. It is also worth noting that the studies reported in Chapter 2 (Akima *et al.*, 1997a; Mulder *et al.*, 2006; Belavý *et al.*, 2009) are all BR studies, whereas those reporting larger losses are all ULLI studies. Therefore, the greater degree of muscle loss may be explained by the absolute immobilisation experienced with leg casting (BR prohibits weight-bearing but not movement).

In this study MG CSA also decreased by ~5% within the first week, with a statistically significant decrease detected after just five days. After two weeks of immobilisation, MG CSA had decreased by a total of 8%, suggesting a predominance of DMA in the first week of immobilisation. Although these figures are in keeping with those published elsewhere in the literature (Leblanc *et al.*, 1995; Akima *et al.*, 1997a; Christensen *et al.*, 2008; Seynnes *et al.*, 2008), the existent literature base in this space is limited and the results of this well controlled study with an internal comparison add significant further evidence. That our findings provide further evidence of the TA being atrophy resistant (see section below), negates the utility of exploring the timecourse of TA atrophic responses.

3.4.2 Evidence for differential rates of muscle atrophy between muscles

In this study we observed variability in rates of aDMA between different muscles of the leg. As already outlined in the section above, both MG and VL began to atrophy rapidly after immobilisation, with no significant difference in the rates or relative magnitude of aDMA between these muscles. Converse to that seen in MG and VL, we observed no statistically significant change from BL in TA, with changes of just -2.94% and -1.05% in TA Vol and CSA respectively after two weeks immobilisation. As such, rates of DMA were significantly greater in both MG and VL than TA. Differential atrophy rates between TA and MG/VL have been suggested in previously published studies (Belavý *et al.*, 2009; Miokovic *et al.*, 2012). VL has also been compared to other muscles, with differential rates of atrophy between VL (showing aS) and the gracillis and sartorius muscles (showing aR) (Kilroe *et al.*, 2019). However, to our knowledge, this is the first study to characterise the timecourse of these differences demonstrating the temporal aspects of aR in the TA and aS in the MG and VL.

This variation in atrophy susceptibility between muscles is not well understood. However several explanations have been suggested and in reality the mechanism is likely to be multifactorial. The most basic explanation is that rates of atrophy are relative to muscle size at onset of immobilisation (Kilroe *et al.*, 2019). Whilst it is obvious that bigger muscles are likely to lose a greater *total* volume of muscle during

immobilisation, the calculation of a % change negates this as all losses become proportional to the starting size of the muscle. In our study we have demonstrated that % change in muscle mass varies between muscles such that this cannot be a factor underlying differential rates of atrophy between muscles.

A more likely suggestion is that differing size reflects differing daily work and force generating capacity of the muscles, with varying levels of habitual 'training' experienced by different muscles during daily activities. For example, during walking anti-gravity muscles of the leg such as the *Vasti* (e.g. VL) and *Triceps Surae (e.g. MG),* are responsible for far greater force generation than muscles such as foot dorsiflexors (e.g. TA) (Liu *et al.*, 2008). During immobilisation, there is therefore a greater net loss of contractile activity in MG and VL than there is in TA (Anderson and Pandy, 2003), and this results in greater proportional loss of muscles mass in these muscles. This explanation has also been suggested to explain the differential rates of atrophy observed in other studies. For example, Kilroe *et al.* noted that the aR gracillis and sartorius muscles have low gravitational load and activation levels during common habitual activities compared to the aS Vasti of the quadriceps (Kilroe *et al.*, 2019). However, in patients undergoing 'prehabilitation' training programmes before elective surgical procedures, rates of postoperative muscle atrophy have been found to be comparable to those who did no preoperative training (Ditmyer, Topp and Pifer, 2002). Based on this, whilst differing habitual loading of muscles pre immobilisation may

account for some of the variability in atrophy rates observed between muscle groups, it is unlikely to be the sole explanation. Furthermore, differences in rates of DMA within muscle groups, and therefore between muscles with similar habitual activity levels, have been observed. For example, as shown in chapter 2, within the muscles of the calf, MG and Soleus have been shown to atrophy more rapidly than lateral gastrocnemius (LG), and within the quadriceps the *Vasti* atrophy more rapidly than rectus femoris (RF). This difference may be driven by variation in expression of regulatory genes and proteins, although this is yet to be determined. For example, decreases in MPS rates through reduced activation or repression of the IGF-1-Akt-mTOR (Gao *et al.*, 2018) pathway appear to have major modulatory control governing the rate of aDMA (Maarten D. de Boer *et al.*, 2007), but variation in the activation and control of this pathway between aR and aS muscles remains to be established (Gao *et al.*, 2018).

Atrophy susceptibility has also previously been attributed to differences in fibre type composition between muscles (Häggmark, Eriksson and Jansson, 1986). This suggestion is based on the finding that MHC type I muscle fibres are lost preferentially during disuse with a transition in muscle fibre balance from slow (type I) to fast (type II) observed in muscles following DMA (Brocca *et al.*, 2012; Gao *et al.*, 2018). However, despite the different atrophy susceptibility between TA and MG demonstrated in this study, multiple previous studies have shown that TA and MG have a comparable, predominantly type I fibre composition

(Henriksson-Larsén, Lexell and Sjöström, 1983; Bass *et al.*, 2021), indicating that fibre type alone is insufficient to explain variability in DMA between muscles. It should be noted however, that fibre type composition can vary within different domains and motor units of an individual muscle (Piasecki, Ireland, David A. Jones, *et al.*, 2016) and the sampling location within a muscle may therefore impact this finding. Similarly, motor unit organisation may be different even in muscles with similar fibre type composition. At present, these variations are insufficiently characterised to allow a full comparison between muscle, but they may play an important role in DMA susceptibility. Other factors which may be important in defining rates of DMA between muscles include those related to neuromuscular function such as motor unit arrangement and muscle fibre innervation and denervation. In addition, potential fibre losses (hypoplasia) and successful remodelling of motor units may also provide important insight into atrophy susceptibility of muscle, in line with observations seen in aged muscle (Piasecki *et al.*, 2018). It has also been demonstrated that, for the same muscle, rates of atrophy can be varied by immobilising the muscle at different lengths (Booth, 1977), with muscle held at a shorter length losing mass more rapidly than when held under extension (Booth, 1977). Whilst this may initially appear a plausible explanation for the variability in atrophy rates observed in this study, there are several reasons why this is unlikely. Firstly, it must be recognised that evidence for this phenomenon is all based on pre-clinical rodent studies (Dupont Salter, Richmond and Loeb, 2003), with no validation in humans. Furthermore, the aR nature of TA and aS nature of

MG is suggested in results from previous studies where immobilisation was accomplished by BR (Belavý *et al.*, 2009), with no lengthening or shortening restrictions applied to the muscles. Finally, in this study great care was taken to ensure that the muscles were immobilised in a neutral position. If anything, the chosen positioning with the ankle held at 90^{o} and knee flexed at 75^{o}, slightly shortens the TA and lengthens the MG.

Although the underpinning mechanisms of differences in muscle atrophy susceptibility, including the relative commitment of alterations in MPS and MPB, molecular factors, and neuromuscular factors, still need to be determined, the aR nature of TA and aS nature of MG identified by this study provides an ideal experimental model for further investigation of these potential mechanisms.

3.4.3 Timecourse of aDMA

An aim of this study was to establish differential rates of atrophy over the first 15 days of immobilisation in different muscles. Whilst overall rates of atrophy in the aS muscles VL and MG appear largely linear over the study period, there is a suggestion that losses were greatest during the first seven days of immobilisation. However, it is likely that this study is underpowered to fully characterise the true temporality of DMA over the first 14 days of immobilisation.

What can however be concluded from this study is that initial rates of aDMA in VL and MG are rapid, with significant losses in both muscles

after just seven days. Furthermore, our rates of DMA over 14 days are much more rapid than those observed in studies of longer periods of immobilisation (Akima *et al.*, 1997a; Mulder *et al.*, 2006; Maarten D de Boer *et al.*, 2007), providing further evidence of an acute phase of DMA during which losses are at their most rapid.

Whilst the mechanistic basis for the more rapid loss of muscle mass early in aDMA is far from fully elucidated, it is generally accepted that supressed rates of MPS are the primary driver of muscle atrophy during immobilisation (Nunes *et al.*, 2022). Decreases in MPS rates have been detected early during immobilisation, with one study which utilised a ULLI model reporting MPS rates 36% lower in the immobilised leg than the control leg over seven days of immobilisation (Kilroe *et al.*, 2020). Downregulation of genes associated with MPS has also been observed after just 48 hours (Urso *et al.*, 2006) with a significant decrease in expression of anabolic signalling proteins after 14 days (Abadi *et al.*, 2009; Howard *et al.*, 2020). However, studies have also demonstrated that reduction in rates of MPS rate are more severe during days two to seven of immobilisation than during the first two days (Kilroe *et al.*, 2020). Therefore, whilst depression of MPS rates no doubt plays a major role in aDMA, it may not offer a full explanation for the more rapid loss of muscle observed during the earliest stages of immobilisation. In contrast, expression of proteins associated with catabolic processes in skeletal muscle such as MAFbx, MuRF1 (Jones *et al.*, 2004a; Wall *et al.*, 2014a) and Atrogin-1 (Suetta *et al.*, 2012), rise rapidly following immobilisation

and peak in the first 5-14 days (Murton, Constantin and Greenhaff, 2008). For example, MuRF-1 and Atrogin-1 expression (Suetta *et al.*, 2012), and protein ubiquitination (Abadi *et al.*, 2009) have been observed to increase significantly in the first two to four days of immobilisation, but return to normal levels by day 14. Furthermore, the magnitude of the increase in catabolic signalling protein expression varies across muscles (Lecker *et al.*, 2004), with a greater increase observed in MG compared to TA (Gao *et al.*, 2018), mirroring the aRaS pattern observed in these muscles during this study. These findings raise the possibility that an increase in MPB may play an important role in the most rapid loss of muscle mass observed in the early stages of aDMA. However, this suggestion remains controversial (Nunes *et al.*, 2022), and further studies are required to fully clarify the processes involved. The aRaS model identified in this study provides an ideal tool for these further investigations.

3.4.4 Reliability and utility of US in assessing DMA in VL, MG & TA.

In this study we demonstrated that US CSA had strong correlation and concordance with MRI measurements in VL and MG, with good or excellent levels of agreement respectively. Furthermore, US CSA showed high sensitivity and PPV for detection of atrophy in these muscles. In TA, US CSA showed only moderate correlation and concordance to MRI CSA, and only moderate sensitivity and PPV for detection of atrophy.

Reliability of US MT to determine muscle mass varied by muscle. In MG, US MT showed strong correlation with SQR MRI CSA and good sensitivity and PPV for detection of atrophy. In VL and TA, US MT showed only a moderate correlation with MRI SQR CSA. However, VL US MT had high sensitivity and PPV for detection of atrophy. In TA, sensitivity and PPV for detection of atrophy was low, although it must be considered that no significant atrophy was seen in this muscle even via the gold-standard of MRI.

MG US CSA and MT are accurate and reliable estimates of muscle mass and can be used for the assessment of atrophy in both research and clinical settings. In VL, US CSA provides an accurate and reliable estimate of muscle mass and can be used for the assessment of atrophy. The reliability of VL US MT is less certain, and as such clinical and/or research findings based on this measurement alone should be treated with caution. US assessment of TA is more challenging and therefore whilst TA CSA showed only a moderate correlation to MRI measurements, techniques for measurement of TA (both CSA and MT) need further development and validation.

Several possible explanations exist for the poor correlation of TA US with MRI measurements observed in this study. The fascial plane between muscles in the anterior compartment of the lower leg become difficult to discern on both MRI and US imaging below the midpoint of the TA muscle belly. Furthermore, due to angulation caused by the bony prominence of

the tibia along the medial aspect of the TA, smooth panoramic ultrasound images are technically difficult to produce. In addition, due to the bi-pennate fascicle arrangement in the TA, there is a central aponeurosis which was used in this study to measure muscle thickness. This central aponeurosis varies in depth across the width of the muscle, and whilst every attempt was made to record measurements at the most lateral point (where the central aponeurosis is deepest), it is possible that variability in this was a source of error. In summary, TA is a technically difficult muscle to quantify using ultrasound and poor reliability of TA US may reflect these difficulties.

There is limited existing evidence published regarding the reliability of US measurements of MT and CSA in VL, MG and TA in the context of DMA. Of these, the most evidence has been published for the use of US in determination of VL MT (Giles *et al.*, 2015; M. V. Franchi *et al.*, 2018) and CSA (Stokes *et al.*, 2021), with both showing good correlation to MRI measurements. Furthermore, good correlation between VL changes by US and MRI in terms of both atrophy (Ahtiainen *et al.*, 2010; Stokes *et al.*, 2021) and hypertrophy (M. V. Franchi *et al.*, 2018) have been shown. One previous study which reports the validity of US CSA assessment of the whole quadriceps and gastrocnemius muscle groups during DMA found close agreement with MRI CSA measurements for the quadriceps groups, but poor concordance in gastrocnemius (Scott *et al.*, 2017). However, the same study also found that there was good correlation between change in US CSA and MRI CSA during immobilisation in both

muscle groups. The results of this study provide further evidence for the utility of US CSA in VL to determine muscle mass and provide new evidence surrounding its reliability in MG and TA.

3.4.5 Limitations

Whilst the number of participants involved in this study is not small for a study of its type, the number of participants may still impact the statistical power of this study to detect changes in rate of aDMA across a period of immobilisation and fully characterise the timecourse of aDMA. This was further affected by missing data points, which resulted from restrictions in facility opening hours and individual participant availability despite the best efforts of the research team. Furthermore, as participants in this study were all healthy, young men, it is accepted that these findings may not be representative of all populations, and further studies need to be performed to examine these findings in populations of older and female populations.

3.5 Conclusion

Rapid muscle loss is experienced in response to very short periods of immobilisation. Rates of aDMA vary between muscles, with TA being aR whilst MG and VL are aS. In aS muscles, rates of muscle loss appear largely linear across the first two weeks of immobilisation. Ultrasound CSA is a reliable tool for the assessment of dynamic changes in muscle mass in response to disuse in the aS muscles of MG and VL.

4 Functional effects of acute disuse muscle atrophy

Chapter abstract

Introduction

The true clinical importance of muscle atrophy lies in its effects on the various functions of skeletal muscle. Whilst it is well established that in the quadriceps, muscle strength and power is lost more rapidly than muscle mass, there is a lack of published research in the functional outcomes of immobilisation in muscles of the lower leg, especially over shorter periods of immobilisation. Furthermore, it remains to be established whether loss of muscle function mirrors the pattern of decrease in muscle mass observed in atrophy resistant/atrophy susceptible (aR/aS) muscles as identified in chapter 3.

Method

Fifteen healthy young men were recruited to have unilateral lower limb immobilisation (ULLI) as described in chapter 2. Ten participants were immobilised for 15 days, whilst the remaining five had five days of ULLI. Maximum voluntary contraction (MVC), knee extension one repetition maximum (1-RM), leg extension power, jump performance (height, power, take-off speed), force control, balance (centre of pressure (COP) distance moved and ellipse area), timed up and go, and CPET (anaerobic threshold (AT) and VO2peak) were assessed at baseline and end of immobilisation.

Results

After 15 days ULLI knee extension and plantarflexion muscle strength decreased by 20-30%. No significant change was identified in dorsiflexion MVC. Leg extension power decreased by 25%, whilst jump power decreased by 9.3%, and jump height decreased by 18.1%. There was a significant worsening in balance performance, with COP distance moved increasing by 14.8% and COP ellipse area increasing by 29% in the immobilised leg. Timed up and go increased by 8.2%, AT decreased by 14.8% and VO2peak decreased by 11.5%. Although changes in function after five days of immobilisation mirrored those observed after 15 days, no results achieved statistical significance at this timepoint.

Discussion

There was a decrease in all aspects of mechanical muscle function secondary to immobilisation. These changes occur more rapidly than loss of muscle mass. Whilst the pattern of muscle function loss reflects the aRaS pattern identified in chapter 3, loss of muscle mass alone is not sufficient to explain these changes. Decrease in muscle function is likely due to complex, multifaceted muscle remodelling, which occurs in the early stages of immobilisation. The aRaS model provides an ideal method for further investigation to clarify the processes underlying these changes. Furthermore, the rapid and severe loss of muscle function identified in this chapter highlights the need for early targeted interventions and treatments in individuals with reduced mobility (e.g. after surgery).

4.1 Introduction

4.1.1 Skeletal muscle function

The primary focus of the preceding chapters has been to assess the rate of change in muscle mass. However, the true clinical importance of muscle atrophy lies in its effects on the various functions of skeletal muscle.

Whilst skeletal muscle also has important metabolic functions (as outlined in chapter 1) the primary function of skeletal muscle is to act as the engine driving movement of the skeleton through the process of excitation contraction coupling., The importance of this mechanical function cannot be underestimated, as it allows both the maintenance of posture and mobility (Goodpaster *et al.*, 2006b), through which participation in social/occupational activities, and independence with activities of daily life are achieved (Frontera and Ochala, 2015). The maintenance of this mechanical muscle function is therefore essential for continuing vitality and maintenance of independence into older age (Di Girolamo *et al.*, 2021a), and will therefore be the focus of this chapter.

4.1.2 Assessment of mechanical function

The complexity and variety of mechanical tasks undertaken by skeletal muscles requires a variety of different characteristics. For example, some tasks need short periods of high force generation, others require repeated low force movements, whilst others still require sustained

moderate force. These different characteristics of mechanical function can be assessed under three general categories: strength, power and fatiguability.

4.1.2.1 Strength

Strength describes the peak torque that can be developed against a load and is a measure of the ability of a muscle to generate force with a single maximal effort Decreases in strength have been shown to correlate with loss of lean muscle mass in older adults, but at a more rapid rate (Cooper *et al.*, 2013). It is therefore possible that changes in muscle strength may be detected before any decrease in muscle mass during the aDMA process. Furthermore, a greater proportional change in strength compared to mass may point to alterations in 'muscle quality' (Brzycki, 1993) and the involvement of other factors beyond mass, such as neuromuscular drive, in determining muscle strength.

The two main measures of muscle strength in a research setting are 1 Repetition Maximum (1-RM) (Grgic *et al.*, 2020) and Maximum Voluntary Contraction (MVC) (Perrin, 1994). 1-RM is an isotonic measurement of muscle strength and is defined as the heaviest weight that can be lifted once across a complete range of motion. 1-RM has the advantages of being relatively simple and inexpensive to perform, and allows measurement of the strength of a single joint movement (Amarante do Nascimento *et al.*, 2013). However, whilst easy to apply to movement of large joints such as the knee and elbow via leg extension and bicep curl

respectively, 1-RM is difficult to measure for all movements at smaller joints such as foot dorsiflexion. Furthermore, given the isotonic nature of the measurement and the use of relatively high loads, if not performed properly there is a risk of muscle injury and post-test muscle pain (Fung, Choo and Seng, 2013). The necessity for high loads can be overcome by the use of 5- or 10-RM assessments (Reynolds, Gordon and Robergs, 2006), with compendium tables available to covert these assessments to 1-RM values. These forms of assessment may be preferable and more practicable for older adults (Falck *et al.*, 2017) and certain patient populations (McNair, Colvin and Reid, 2011), but do still require participants to be able to undertake a full range of motion for the chosen exercise.

MVC is an isometric measurement of muscle strength during which participants apply the maximum pressure possible against a static fixed load which is most commonly a force transducer plate or strap. The force generated is detected by a digital dynamometer giving a standardised result for maximum force generated. Due to the static nature of this test, there is a lower risk of muscle injury, and it is easier to test strength across small joints. MVC may not however be as representative of functional capabilities as strength across a range of movement is not assessed.

4.1.2.2 Power

Power is a product of velocity and force of contraction, reflecting the ability of a muscle to exert a maximal force in as short a time as possible (Beaudart *et al.*, 2019). Due to the integration of these two aspects, power depends not only on generation of force, but also coordination of movement. It has been observed that in age related muscle changes, power is lost faster than strength (Skelton *et al.*, 1994) and is a better predictor of mortality (Metter *et al.*, 2004). Multiple techniques have been used for the assessment of power, including jump analysis, dynamometry, stair climbing and “all-out” cycle tests (Gray and Paulson, 2014).

Vertical jump analysis is a commonly used method for the assessment of leg extension power (LEP), most often used in sports performance settings (Darmiento, Galpin and Brown, 2012). In its most basic form, the vertical jump test is a measure of the height gained when a participant jumps, with greater heights gained correlating to greater leg extension power. However, combining data on the distance jumped and the participant’s weight can give a more detailed estimate of leg extension power (Klavora, 2000). In recent times, inertial sensors worn on the participant have enabled measures of maximum jump height, take-off force, take-off speed and maximum power (Andrenacci *et al.*, 2021), all of which reflect the functional ability of lower limb muscles.

A further method of assessing LEP is the Nottingham Power Rig (Bassey and Short, 1990). For this assessment participants are seated in an adjustable chair, with their feet against a foot pedal so that their hips, knees, and ankle joints are angled at a similar position to when rising from a chair. Participants then extend their legs as quickly as possible against the footpad, which in turn accelerates a flywheel to give a measure of the average LEP in the push (Bassey and Short, 1990). As participants are seated for this measurement of LEP, it is particularly well suited to older participants, those with poor balance, and those with back problems (Blackwell *et al.*, 2009) who may not be able to perform jump-based power assessments.

4.1.2.3 Fatigability

Fatigability describes the exercise induced reduction in the ability of a muscle to exert power as a result of contractile activity (Mitchell *et al.*, 2012). Muscle fatigue is distinct from muscle exhaustion which is defined as the inability to sustain a muscle contraction, however the two are sometimes used interchangeably (Vøllestad, 1997). Fatigability has many contributors, including contraction type and task intensity and can vary greatly between muscle groups (Avin and Law, 2011). As generation of muscle contraction is controlled by a complex system of events, fatigue may arise at a variety of levels including central nervous system activation of motoneuron signal generation, neuromuscular junction (NMJ) activity, muscle nutrient delivery and intrinsic muscle processes such as excitation contraction coupling (Vøllestad, 1997).

Given this complexity, various different techniques for assessment of muscle fatigue have been described in the literature. Furthermore, given the multiple systems governing muscle fatigue, measurements must take these into account and attempt to control for the many different possible confounding factors.

The simplest method of assessing muscle fatigue is using direct assessment of maximal force or power generation, and the time taken for this to reduce. For example, maintaining a muscle contraction at a set proportion of MVC (e.g. 80%) for as long as possible, with the time to fatigue assessed as the time until torque declines by a set parameter (e.g. >5% for >2 seconds) (Mulder *et al.*, 2015). One benefit of using this method is that it encompasses 'overall' fatigue irrespective of the mechanism of reduction in force generation (Vøllestad, 1997).

4.1.2.4 Force steadiness

Whilst measurements of MVC, 1-RM, power output and even fatiguability may be used to define a muscle's ultimate functional capacity, most everyday tasks require controlled sub-maximal contractions rather than explosive maximal force (Blomkvist *et al.*, 2018). The ability of muscles to maintain these steady, submaximal contractions may therefore define an individual's experience of their muscle function. For example, can they control the force need to rise from a chair, pour water from a kettle or maintain a normal gait?

During muscle contraction, the desired force output naturally fluctuates around an average point, rather than being at a constant level (Pethick and Piasecki, 2022). These fluctuations in force output are governed by inherent variabilities in motor unit firing rates and have been demonstrated to increase with age (Ely *et al.*, 2022). Force steadiness is a determining variable in balance and dexterity (Ely *et al.*, 2022) and an independent predictor of functional performance in every-day tasks, such as standing from sitting, walking and stair climbing (Blomkvist *et al.*, 2018). Furthermore, force steadiness has been demonstrated to decrease in muscles as they fatigue (Hunter and Enoka, 2003; Contessa, Adam and De Luca, 2009). This may be experienced palpably as muscles near the point of exhaustion. Measurement of change in force steadiness may therefore give a predictor of changes in functional performance, as well as insight to the modulation of motor unit control in response to immobilisation.

4.1.2.5 Measurements of physical performance

Global physical performance goes far beyond simple, isolated aspects of muscle function such as maximum force generation. Physical performance reflects not only the neuro-muscular components of co-ordination and the maintenance of muscle contraction, but also includes the cardiovascular systems' capability for oxygen delivery and the muscles' oxygen utilisation capacity to meet the energy demands of physical activity (Tieland, Trouwborst and Clark, 2018).

With these factors in mind, multiple measurements of overall physical performance have been described in the literature. The short physical performance battery test (SPPB) assesses balance, gait speed and sit-to-stand capacity (Treacy and Hassett, 2018). These tests focus on lower extremity function since this has been shown to correlate with mobility, disability and patient outcomes including hospitalisation, institutionalisation and mortality (Beaudart *et al.*, 2016). The SPPBT takes approximately 10 minutes to complete with the test scored to a maximum of 12 points. Participants scoring ≤ 8 points are described as having “poor” physical performance (Alfonso J. Cruz-Jentoft *et al.*, 2010). It is the most widely used battery of tests for physical function and has been validated in multiple large studies (Beaudart *et al.*, 2019). Although a useful tool in monitoring the physical function of frail or ‘at risk’ older adults, it is less effective in higher functioning adults (Treacy and Hassett, 2018).

The timed up and go (TUG) test, which assesses sit to stand capacity and gait speed in a combined assessment, has also been shown to be a good predictor of overall physical performance, and changes in TUG have been shown to be associated with adverse health outcomes in both young and older individuals (Kear, Guck and McGaha, 2017). In addition, TUG has been shown to correlate with a much more complex assessment of physical function (cardiopulmonary exercise testing (CPET), described below), and as such potentially predict surgical risk (Boereboom *et al.*, 2021).

4.1.2.6 Balance

One of the most important measures of physical performance for independent day-to-day life is standing balance (Pu *et al.*, 2015). Balance impairments have been shown to be associated with falls, development of future disabilities and hospitalisation rates in older adults (Bergquist *et al.*, 2019). Maintenance of standing balance relies on multiple factors including vision, proprioception, central processing time, nerve conduction velocities, tissue stiffness, and muscle strength and power (Pu *et al.*, 2015). Standing balance ability has been shown to correlate to muscle strength and power in both young and older individuals (Muehlbauer *et al.*, 2012; Hammami *et al.*, 2016; Gouveia *et al.*, 2020). Furthermore, decreases in balance have been observed after immobilisation (Caplan *et al.*, 2015), although this is not well characterised, especially over shorter periods of immobilisation. Whilst balance is multifactorial, any changes observed after short periods of immobilisation in otherwise fit and healthy young individuals, are likely to reflect changes in muscle function.

Many different techniques have been described in the literature for the assessment of standing balance (Bergquist *et al.*, 2019). One of the most commonly used in a clinical setting is the Borg Balance Scale (Muir *et al.*, 2008), elements of which are included in the SPPB. Whilst this allows a crude assessment of standing balance based on parallel, semi-tandem and tandem standing times, use of electronic force pressure platforms have allowed more detailed measurements such as assessment of the

centre of pressure (COP), which allow early detection of balance degradation (Quijoux *et al.*, 2021). The COP represents the centre point of pressure in the foot-ground contact surface and is the point where plantar ground reaction force is applied (Chen *et al.*, 2021). The distance by which the COP moves during standing can be used as a measure of postural sway and balance steadiness (Chen *et al.*, 2021). Furthermore, an ellipse that encloses 95% of all COP observations can be calculated (Schubert and Kirchner, 2014) with the size of the ellipse demonstrating the degree of postural sway. Beyond providing a more detailed assessment of balance, these advanced techniques avoid the 'ceiling effect' often present in higher functioning individuals, allowing better participant characterisation and determination of change.

4.1.2.7 Measurements of muscle oxidative capacity

Cardiopulmonary exercise testing (CPET) provides assessment of the entire integrated response of the body to exercise, and therefore results reflect respiratory, cardiovascular, neuropsychological, and skeletal muscle system function (Albouaini *et al.*, 2007). However, changes to CPET outcomes after a short period of immobilisation (especially if only one limb is immobilised whilst the participant is encouraged to maintain their normal activity levels), may be presumed to be driven by changes in skeletal muscle oxidative capacity as theoretically respiratory, cardiovascular, and neuropsychological system function should remain unchanged. Indeed, based on studies of skeletal muscle, mitochondrial respiration, muscle oxidative capacity has been shown to be reduced

after two weeks of single limb immobilisation (Gram *et al.*, 2014). Although CPET analysis can yield a wealth of information, two measurements are of particular interest with regards to changes in muscle function following immobilisation: the ventilatory anaerobic threshold (AT) and VO_2max.

After a prolonged period of strenuous exercise, anaerobic metabolism occurs because oxygen supply cannot keep up with the increasing metabolic requirements of exercising muscles. As this occurs, there is a significant increase in lactate production in the muscles and CO_2 excretion increases more rapidly than O_2 consumption (Wright *et al.*, 1999). The VO_2 at which this change occurs is known as the AT and is an estimate of an individual's exercise capacity. As discussed in Chapter 3, there is evidence for a shift in fibre type distribution after immobilisation, with greater atrophy of MHCI (slow/aerobic) fibres. As a result, after immobilisation MHCII (fast/anaerobic) fibres (Fitts *et al.*, 2010; Brocca *et al.*, 2012) make up a greater proportion of the overall muscle fibre type and this may be expected to result in a reduction in AT (and therefore exercise capacity).

VO_2max is defined as the maximal rate at which oxygen can be consumed on a whole-body level. Whilst VO_2max during whole-body exercise may be presumed to be limited by oxygen delivery to muscle, VO_2max has been shown to be proportional to maximum muscle oxidative capacity (Van Der Zwaard *et al.*, 2016), suggesting that this is

the main determining factor. The explanation for this is that as activity level increases, skeletal muscle consumes an ever larger fraction of the total body oxygen uptake, and change in VO_2max therefore provides an estimate of change in muscle oxygen consumption (Liu and Marcinek, 2017). As VO_2max represents the highest possible rate at which the body can consume oxygen, well defined criteria for identification of VO_2max have been developed (see section 4.2.3.6). Many participants, including those recovering from a physiological insult or who are unaccustomed to exercise, may fail to match these criteria and instead reach VO_2peak. Whilst not a reliable measure of change in absolute maximal whole-body oxygen consumption due to the subjective nature of termination of this assessment, changes in VO_2peak may represent an estimate of muscle fatiguability and endurance and have been shown to strongly correlate with changes in VO_2max (James *et al.*, 2007).

4.1.3 Changes in muscle function with immobilisation

The loss of muscle function with disuse is well established. In terms of muscle strength, knee extension (KE) MVC has been shown to reduce by 8% after just five days immobilisation (Mulder *et al.*, 2015) and 25% after six weeks (Berg, Larsson and Tesch, 1997). Similarly, plantarflexor MVC decreased by 26% after five weeks bed rest (LeBlanc *et al.*, 1988) in healthy individuals, and approximately 50% after eight weeks cast immobilisation in ankle fracture patients (Shaffer *et al.*, 2000).

KE power has also been shown to decrease with immobilisation, with a 43% reduction reported after 20 days of bed rest (Funato *et al.*, 1997). Contractile work capacity (a measurement of maximum power output) has also been shown to decrease for both quadriceps and plantarflexors after two weeks of bed rest (Winnard *et al.*, 2019). Jump height has also been shown to decrease significantly after just five days of immobilisation, although no change in jump power or peak force was observed (Mulder *et al.*, 2015).

Balance is also known to decrease after immobilisation, but these changes tend to occur over more prolonged periods (Winnard *et al.*, 2019; Saumur *et al.*, 2020). For example, a systematic review examining changes in balance secondary to bed rest has shown that the median length of immobilisation in studies that did report a change in balance measurements was 45 days, whilst for studies which reported no change it was 20 days (Saumur *et al.*, 2020).

Changes in muscle fatiguability after immobilisation are less clear. Whilst several studies report increased fatiguability after immobilisation (Winnard *et al.*, 2019) other studies have reported this to be unchanged (Bosutti *et al.*, 2020), with increased fatigue resistance (Shaffer *et al.*, 2000; Mulder *et al.*, 2015) also reported.

4.1.4 Correlation between changes in muscle mass and function

The loss of muscle strength with disuse is commonly reported to exceed the loss of muscle size. For example, following eight weeks of bed rest, quadriceps CSA declined by 14% compared to a 17% decline in strength (Mulder *et al.*, 2006). A systematic review of the effects of simulated microgravity has shown that moderate decreases in power, MVC and 1-RM are the first changes to be detected at approximately seven days, with statistically significant changes in Vol, MVC and fatiguability present at ~14 days (Winnard *et al.*, 2019).

4.1.5 Differential changes in function depending on muscle group

As discussed in more detail in chapter 3, there is good evidence that during a period of disuse, some muscles (and muscle groups) are atrophy resistant (aR), whilst others are atrophy susceptible (aS). However, the degree to which this pattern translates into changes in muscle function is not well established. For example, whilst some studies have demonstrated plantar flexor MVC to decrease by approximately 25% after five weeks of bed rest (Gogia *et al.*, 1988; LeBlanc *et al.*, 1988), other studies have found no significant change in plantarflexor function over shorter periods of immobilisation (Mulder *et al.*, 2015). Similarly, whilst some studies report a 28% decrease in dorsiflexor torque after six weeks (Geboers *et al.*, 2000), others failed to identify similar losses, with a reduction of just 8% identified after five weeks of bed rest (Gogia *et al.*, 1988).

One study which compared changes in both dorsi and plantarflexor strength in the same participants found that after five weeks of immobilisation, plantarflexor strength decreased by 26% whilst no significant change was detected in dorsiflexors (LeBlanc *et al.*, 1988). A systematic review of a large number of bed rest studies also identified that dorsiflexor strength appears to decrease less than KE and plantarflexor strength (Winnard *et al.*, 2019). However, this review also demonstrates that there is a lack of published research in the functional outcomes of immobilisation in both plantarflexors and dorsiflexors, especially over shorter periods of immobilisation (Winnard *et al.*, 2019). Further research is therefore required to assess for differential rates of change in function between muscle groups during the acute phase of DMA.

4.1.6 Study aims

The primary aim of this study is to characterise the changes in leg muscle function over 15 days of immobilisation. Secondary aims include comparison of rates of change in muscle function in different muscle groups and correlation of rate of change in muscle function with changes in muscle mass. A further aim is to compare the rate of change in muscle function over 5 and 15 days of immobilisation in order to assess whether the rate of functional loss varies over the timecourse of immobilisation.

4.2 Method

4.2.1 Participant recruitment

Fifteen healthy, adult males aged 18-30 years were recruited to participate in this study via adverts placed in the local community (e.g., posters, social media advertising etc). Interested individuals were provided with a written information sheet and invited to attend the university if they were still interested in participation. After explanation of the study (including all potential risks) by a member of the research team, participants were requested to provide full written consent. Following this, all participants attended a screening session, lasting approximately 30 minutes and completed by a medically qualified doctor, to ensure they were safe to have immobilisation and testing of muscle function, including CPET. This screening session involved completion of a full medical history, auscultation of the heart sounds and lung fields, recording a 12-lead ECG, non-invasive blood pressure (NIBP), and measurement of height (cm) and weight (kg) for calculation of BMI. Participants were excluded from the study if they had; i) a personal or family history of venous thromboembolism, ii) any history of limb immobilisation, casting or more than 5 days of hospitalisation or bed rest in the previous 12 months, iii) prolonged use of non-steroidal anti-inflammatory medications or iv) any absolute or relative contra-indications to CPET testing as defined by the ATS/ ACCP guidelines for CPET (Figure 4.1) (American Thoracic Society and American College of Chest Physicians, 2003).

TABLE 8. ABSOLUTE AND RELATIVE CONTRAINDICATIONS FOR CARDIOPULMONARY EXERCISE TESTING

Absolute	Relative
Acute myocardial infarction (3–5 days)	Left main coronary stenosis or its equivalent
Unstable angina	Moderate stenotic valvular heart disease
Uncontrolled arrhythmias causing symptoms or hemodynamic compromise	Severe untreated arterial hypertension at rest (> 200 mm Hg systolic, > 120 mm Hg diastolic)
Syncope	Tachyarrhythmias or bradyarrhythmias
Active endocarditis	High-degree atrioventricular block
Acute myocarditis or pericarditis	Hypertrophic cardiomyopathy
Symptomatic severe aortic stenosis	Significant pulmonary hypertension
Uncontrolled heart failure	Advanced or complicated pregnancy
Acute pulmonary embolus or pulmonary infarction	Electrolyte abnormalities
Thrombosis of lower extremities	Orthopedic impairment that compromises exercise performance
Suspected dissecting aneurysm	
Uncontrolled asthma	
Pulmonary edema	
Room air desaturation at rest ≤ 85%*	
Respiratory failure	
Acute noncardiopulmonary disorder that may affect exercise performance or be aggravated by exercise (i.e. infection, renal failure, thyrotoxicosis)	
Mental impairment leading to inability to cooperate	

Figure 4.1: Absolute and Relative contra-indications for CPET - ATS / ACCP guidelines on exercise testing - (American Thoracic and American College of Chest 2003).

The study was approved by the University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee (ref: FMHS 103-1809) and conducted according to the declaration of Helsinki.

4.2.2 Study overview

4.2.2.1 Duration of Immobilisation

Once recruited, participants were allocated into two groups, dependant on participant preference. One group (n=10) had 15 continuous days (15D) of unilateral lower limb immobilisation (ULLI) (as described in chapter 3), with tests of muscle function and assessment of muscle mass performed before and after immobilisation. These are the same participants as described in chapter 3. The second group (n=5) were immobilised for five days (5D) with tests of muscle function only performed before and after immobilisation. Participant characteristics for both groups are summarised in Table 4.1. Due to the difficulties in participant recruitment and the impactful nature of 15 days ULLI, in order

to recruit a sufficient number of participants it was decided to allow participants to decide on their own allocation rather than randomising.

	15 days	5 days
N	10	5
Age	22.4 (± 3.1)	28.8 (± 5.7)
Height	1.8 (±0.1)	1.8 (± 0.1)
Weight	76.5 (±10.2)	81.5 (± 4.1)
BMI	23.2 (±1.9)	25.7 (± 1.0)

Table 4.1: Participant Characteristics

4.2.2.2 Leg immobilisation

The process of ULLI is described in full in chapter 3. In brief, ULLI was achieved by placing the dominant leg in a hinged knee brace and the foot in an air-boot, with both adjusted to ensure minimal horizontal and vertical movement. Tamper tags were fitted to all securing straps to ensure that neither the brace nor boot were removed without the researcher's knowledge. With the immobilised leg fixed in this position participants were unable to weight bear on the limb or perform dorsi/plantar flexion, ensuring full immobilisation and disuse. Participants were trained in the use of forearm crutches and were encouraged to continue with normal daily activities. Regular contact was maintained with all participants and with the provision available that, if participants reported pain or discomfort, the knee brace and boot would be removed by a member of the research team and the limb inspected by a medical practitioner to ensure no evidence of DVT, pressure sores or any other problems. However this was not required for any participants.

4.2.3 Assessment of muscle function

4.2.3.1 Strength

1 repetition maximum

Participants were seated on a cable-based knee extension weight machine, with the seat adjusted so that the pivot point of the machine aligned with the middle of the knee joint (Figure 4.2). Participants first attempted five repetitions of knee extension with resistance set at 50% of anticipated 1-RM (based on stature and prior physical activity) to ensure correct technique was adopted and that participants were comfortable with the exercise. After a 1-minute rest and based on participant rating of the five previous repetitions (using a 0-10 modified Borg scale (Borg, 1982) resistance was increased to ~70% of the anticipated 1-RM and participants again attempted five repetitions at the new weight. If this was achieved and following another minute rest, a third set was performed at 85–90% of the predicted 1-RM and the participant asked to complete five repetitions. If only one repetition could be completed, this was deemed their 1-RM. If the participant could complete between two and four repetitions, a predicted 1-RM was calculated using the prediction coefficients shown in Table 4.2 (Brzycki, 1993).

Repetitions	Leg press coefficient
1	1.00
2	1.0475
3	1.13
4	1.1575

Table 4.2: 1 repetition maximum (1-RM) prediction coefficients (Brzycki, 1993)

Figure 4.2: Image of setup used for knee extension 1 repetition maximum (1-RM) testing

Maximum voluntary contraction

For the assessment of MVC participants were seated on a custom build chair with their hip joints at 110° and their knees and ankles at 90°. For assessment of knee extension, the lower leg was secured to a force dynamometer with non-compliant straps placed just above the medial malleolus (Figure 4.3 A).

For assessment of plantar and dorsiflexion, the foot was placed on a custom built dynamometer (purpose built calibrated strain gauge, RS125 Components Ltd, Corby, UK) which consisted of a plate at a 45° angle to the floor attached to a force transducer with a strap placed over the ball of the foot (Figure 4.3 B)

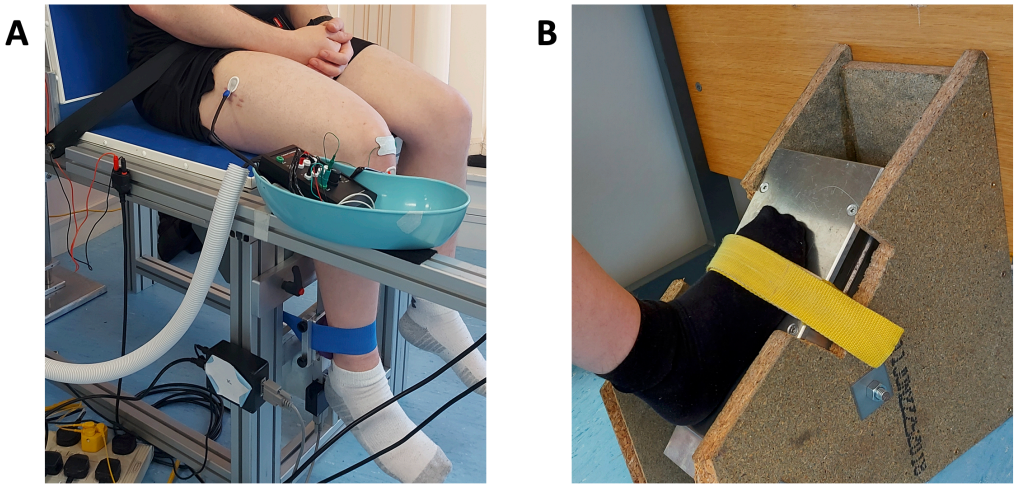

Figure 4.3: Image of equipment used to assess maximum voluntary contraction (MVC) for A) knee extension and B) Ankle dorsiflexion and plantarflexion

Before assessment of MVC in each muscle group, as a warm-up, three moderate intensity contractions were carried out with visual on-screen feedback. Three maximal attempts were then performed, with the highest value taken as MVC. For dorsiflexion MVC, the participant was instructed to lift their foot against the strap as hard as they could. For plantar flexion MVC, the foot was in the same position and participants were instructed to push the ball of their foot down into the plate while keeping their foot flat and prevent any ankle and knee lift. For knee extension, using a waist belt to prevent hip lifting and facilitate isolation of the knee extensors, participants were instructed to attempt knee extension against

the ankle strap. Verbal encouragement was performed for each exercise and on-screen visual feedback for these tasks was provided using Spike2 software (Cambridge Electronic Design, Cambridge, UK).

4.2.3.2 Power

Overall lower limb power was assessed using a countermovement jump, with movements recorded by a BTS G-sensor (BTS Bioengineering S.p.A., Milan, Italy) placed on the participant's waist at the S1 vertebral level and secured with a belt. The G-sensor was connected via Bluetooth to G-Studio (version 3.3.22.0, BTS Bioengineering).

A countermovement jump involves the participant standing with feet shoulder width apart, hands on hips, bending to a 90° knee angle before springing up to land in an upright position. This movement profile was demonstrated to participants before they attempted it. The participant carried out three jumps with a gap of 30 seconds between each. The jump with the greatest maximal power was recorded for analysis along with the respective take-off speed and jump height measurements.

Unilateral lower limb power was assessed using the Nottingham Power Rig (Bassey and Short, 1990). This device allows measurement of lower limb power using a leg press plate attached to a flywheel, which in turn is connected to a device which calculates movement power. The seat was adjusted so the participant's knee joint was at 90° with the foot resting on the plate and the seat measurement recorded for the follow-

up visit. The flywheel brake was applied, and the participant placed their foot on the plate. Following a countdown from three, the participant was instructed to push as hard as possible as the brake was simultaneously released. As the seat does not have a full back rest, this prevents any input from postural changes during the power measurement. (Figure 4.4). The digital box provides a power measurement which was recorded. For each leg, participants carried out two attempts. If these attempts were within 10% of each other, then that concluded this measurement. If the attempts were greater than 10% apart, a final attempt was made. The highest measurement was taken from the two or three attempts.

Figure 4.4: Image of Nottingham power rig, as used to test leg extension power

4.2.3.3 Force control

Following MVC assessment, participants were instructed to hold a 12-second contraction at 10%, 25% and 40% of MVC. This was visualised by the participant as a horizontal on-screen cursor on Spike2 software. Participants were instructed to control their force output as close to the line as possible. As with MVC, this was performed for isometric dorsiflexion, plantar flexion and knee extension. Four contractions at 10 and 25% were recorded for each muscle along with two at 40%, with a 10 second rest between each. These contraction intensities were chosen to give a range of low to mid-level contractions which represent the intensity of activities of daily living, for example walking on a flat surface, standing from a chair or climbing stairs. The degree of variation of force applied was measured using Spike2 software, to give a coefficient of variation (COV). An increase in COV represents a worsening of force control, whilst a decrease in COV represents an improvement in force control.

4.2.3.4 Balance

Using a 1-metre pressure force plate (Footscan advanced, Materialise, Belgium), balance was assessed for both legs together and then each leg individually. For the bilateral assessment participants stood naturally in the centre of the force plate, with COP, distance moved and ellipse area recorded for 30s. For the unilateral leg balances, participants were instructed to raise their opposite foot by at least five inches from the floor (Figure 4.5). A countdown from five was given before the balance

recording started with participants told to raise their foot on two, to prevent balancing longer than necessary. The unilateral balances were also for 30s with COP, distance moved and ellipse area measured.

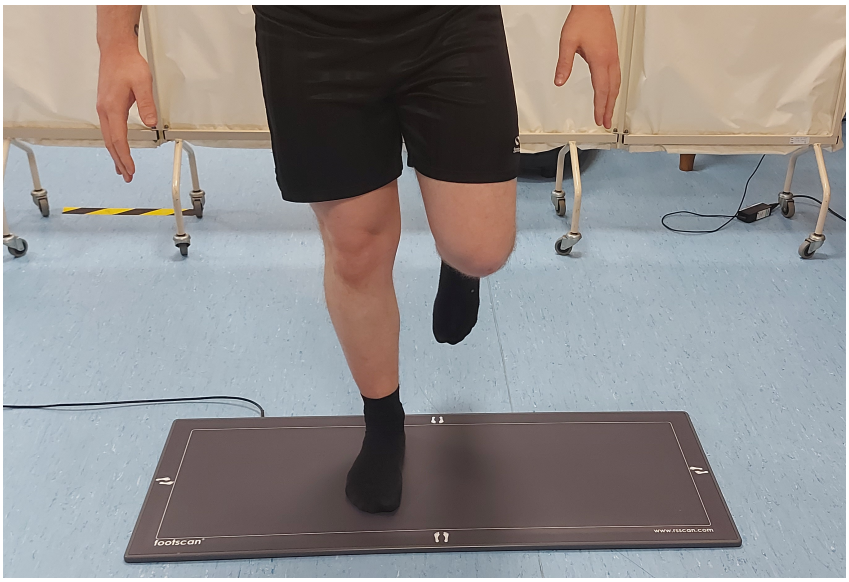

Figure 4.5: Image of the pressure force plate used to measure distance moved and ellipse area of centre of pressure to assess balance

4.2.3.5 Physical Performance

Timed up and go

The Timed Up and Go test (Podsiadlo and Richardson, 1991) was used to asses general mobility and physical function. Participants began seated in a chair without arms, with an obstacle placed three metres from of the chair. Participants were instructed to stand, without the use of their hands, walk as fast as possible without running around the obstacle and back to the chair, and then to sit back down on the chair without the use of the hands. The process was demonstrated to the participant before they carried out two attempts. Movement was measured using a BTS G-sensor (BTS Bioengineering, location) placed on the L2 vertebrae, with the quickest time recorded from G-Studio (BTS) for analysis.

4.2.3.6 Cardio-pulmonary exercise testing

Cardiopulmonary exercise testing was carried out using a metabolic cart (ZAN 680, nSpire Health, Colorado, USA) which is subject to annual servicing and validation. Flow rate is calibrated against a known volume using a three litre diamond cut syringe prior to each CPET with the inline gas analyser calibrated against two precision gas mixtures (Weisman *et al.*, 2003). Ambient room conditions at time of testing were recorded via an environmental module including room temperature (degrees Celsius), humidity (%) and atmospheric pressure (mbar) to ensure consistent conditions for pre- and post-intervention testing.

Before each test a thorough explanation of the CPET protocol, including safety and termination criteria was explained to participants. Next, a 12-lead ECG (CardioCollect12S, DelMar Reynolds, USA) and pulse oximeter (iMEC8 Mindray, Shenzen, China) were attached to the participant to record continuously throughout the CPET. A non-invasive blood pressure cuff (SunTech Medical, Morrisville, USA) was placed on the patient's left arm and set to record every two minutes.

After monitoring equipment was attached, participants were fitted with an appropriately sized silicone face mask secured with elasticated head straps (V2 mask, Hans Rudolph, Shawnee, USA). A flow sensor was attached to the front of the mask for the quantification of volume and sampling of inspired and expired gases during rest and exercise. The mask was then checked for air leaks.

Participants were helped onto a cycle-ergometer (Lode Corival, Lode, Groningen, Netherlands) with the seat height adjusted for comfort and the settings recorded to ensure consistency and reproducibility at re-test. The ramp protocol selection was based on participants estimated VO_2peak information based on a subjective physical activity questionnaire (Dukes Activity Questionnaire (DASI) (Hlatky *et al.*, 1989)) and the clinical opinion of an experienced CPET operator (American Thoracic Society and American College of Chest Physicians, 2003).

After a two minute warm up of unloaded cycling, resistance was increased in a ramp-wise manner as per the Bruce Ramp protocol(Bruce, 1971) with a range of 5-30 Watts per minute aiming to achieve VO_2peak in 8-12 minutes. Participants were encouraged to maintain a cadence of 50-60 revolutions per minute (RPM), whilst the investigator ensured participants were exercising at 85% or more of age predicted maximal heart rate (max HR = 208–(0.7*Age) (Tanaka, Monahan and Seals, 2001)) and an exercise to a respiratory exchange ratio (RER; VCO_2/VO_2) above 1.1. Tests were ended when participants indicated they had reached exhaustion, or they were unable to maintain a cadence above 50 RPM. After tests were ended, cycling resistance was removed for a five minute recovery period of low load cycling (load 10W) where the participant was instructed to cycle at 30 RPM. Immediately after test cessation, subjective effort level was measured using a modified (10-point) Borg scale rating of perceived exertion (Noble, 1982) and the participant was asked what caused them to stop.

All participants were monitored for the signs and symptoms which should result in immediate termination of the test, as defined by the American Thoracic Society (ATS) guidelines (American Thoracic Society and American College of Chest Physicians, 2003). For a full list of the termination criteria see Appendix C. However, no participants experienced any of these features and no CPET was terminated before completion.

CPET Analysis

All CPET data were analysed independently by two experienced CPET assessors, blinded to subject and time point with any disagreement resolved in consultation with a third, experienced CPET assessor. Prior to analyses, CPET data were reviewed to ensure reliability, including that resting values were comparable to normal limits and that there was no evidence of an air leak, pseudo-threshold, or acute hyperventilation (Ozcelik, Ward and Whipp, 1999; Luks, Glenny and Robertson, 2013). Individual breath by breath data were interpreted as five breath rolling averages, with absolute values (ml/min) from each CPET divided by body weight (kg) to derive AT and VO_2peak in relative units (ml/kg/min). AT was determined using a dual methods approach considering both the V-slope and Ventilatory Equivalents method (Wasserman *et al.*, 1994; ERS Task Force *et al.*, 2007). Absolute VO_2peak (L/min) was determined as a 15 data point rolling average including the highest value (at the midpoint) attained during the last 20 seconds of the test (Phillips *et al.*,

2017). Tests were considered to have achieved VO_2max if three or more of the following criteria were achieved: a plateau in the oxygen uptake curve (sustained flattening of VO_2 curve despite rising VCO_2), a respiratory exchange ratio (RER) of >1.15, HR over 85% age-predicted maximum, and a rating of perceived exertion (RPE) on a modified 0-10 Borg scale(Borg, 1982) ≥9 immediately following the test (Blackwell *et al.*, 2017).

4.2.4 Assessment of change in muscle mass

The method used for MRI analysis of muscle mass is described in more detail in chapter 3.2.3.2. In brief, whole leg MRI imaging was performed using a 1.5T MRI system (Avanto, Siemens, Munich, Germany), with a Siemens peripheral angiography coil to optimise the signal to noise ration of the resulting images. A localiser scan was performed to align images and ensure imaging of the whole of both legs. An imaging matrix of 512 x 235, with a resolution of 835 x 835 μm and slice thickness of 5mm was acquired using a turbo spin echo sequence with an echo time set to the minimum value of 12ms and a repetition time of 568ms to optimise the trade off between imaging time and contrast for a proton density weighted image.

MRI images were analysed using 3D Slicer (v4.10) software (Open source software, https://www.slicer.org)(Fedorov *et al.*, 2012). The borders of MG and TA were traced for both legs on every slice to allow

segmentation by pixel count method, after which semi-automatic filling was performed between slices to calculate muscle volumes (Figure 4.6).

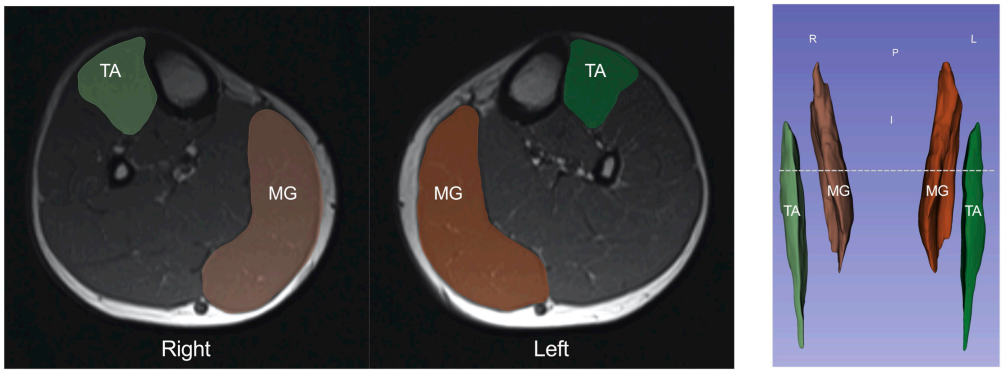

Figure 4.6: Assessment of MRI muscle volume using 3D slicer software, with muscle boundaries traced in every slice followed by semi-automatic volume rendering

CSA was measured for VL, MG and TA on both legs, again by manually tracing muscle boundaries to allow segmentation by pixel count method. CSA measurements were performed at the muscle midpoints after measuring from bony landmarks (e.g., the joint line of the knee) visible on MRI images. Data was unavailable to analyse changes in muscle mass for three participants due to breakdown of the MRI scanner at the required timepoint.

4.2.5 Statistical Analysis

Distribution of data was tested using the Kolmogorov-Smirnov test, with normally distributed data expressed as mean (±SD) and non-normally distributed data as median (IQR). To compare changes in the immobilised leg vs. the non-immobilised leg repeated measures two-way ANOVA with multiple comparisons (time vs. immobilisation status) were conducted with Tukey *post hoc* analysis. If there were incomplete data

sets for repeated measures, comparison mixed-effect model analysis was performed with restricted maximum likelihood (REML) estimators and Dunnet multiple comparison procedure. For whole body data with only pre- and post-immobilisation measures, paired t-tests were performed. Statistical significance was accepted as an alpha <0.05.

Correlation between changes in muscle mass and muscle function was assessed by calculating Pearson's correlation coefficients (r) with mean absolute differences and 95% limits of agreement. Perfect correlation was indicated by $r = 1.0$, whilst $r > 0.7$ was taken to show strong correlation, r 0.5 - 0.7 as moderate correlation and $r < 0.5$ as poor correlation (Akoglu, 2018).

4.3 Results

4.3.1 Muscle Strength

4.3.1.1 Knee Extension

There was no significant difference at baseline between the immobilised (iMOB) or control (CON) leg in either the 15D (531.99N [±91.2] vs 498.29N [±129.2], p=0.06) or 5D group (523.84N [±136.6] vs 470.28N [±134.4], p=0.08) for knee extension MVC. There was no significant time x immobilisation interaction (p=0.06) or significant differences detected in either the iMOB (-11.5%, p=0.16) or CON (2.17%, p=0.91) leg after five days. A significant time x immobilisation interaction was detected in the 15D cohort (p<0.01). Knee extensor MVC decreased by 32% in the iMOB leg (364N, MD=174.1, 95% CI: 110.8 to 237.5, p=<0.01), with no significant change detected in the CON limb (-6%, p=0.93) (Figure 4.7).

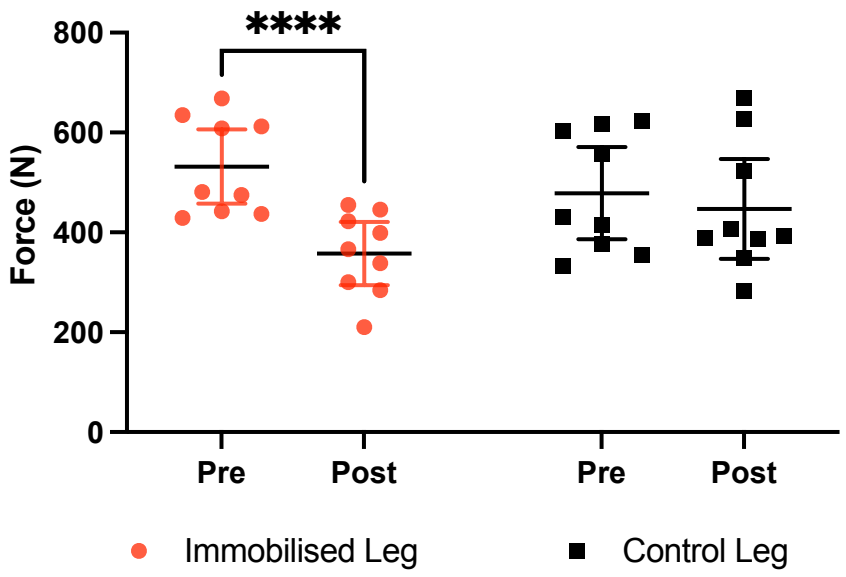

*Figure 4.7: Knee extension maximum voluntary contractile (MVC) force in Newtons (N) in a control leg (no intervention) and in the contralateral leg after immobilisation for 15 days. Data shown are individual values with mean and SD. N=10, Analysis via 2way ANOVA. ****=p<0.001 vs. pre-immobilisation in the same limb.*

There was no significant difference at baseline between the iMOB or CON leg in either the 15D (62.3kg [±13.1] vs. 56.9 [±9.9], p=0.06) or 5D cohort (67.7kg [±11.6] vs. 60.9kg [±16.2], p=0.08) for knee extension 1-RM. A significant time x immobilisation interaction was detected in both the 5D (p=0.02) and 15D cohorts (p<0.01). However no significant difference was detected in either the iMOB (-3.5%, p=0.82) or CON (11.23%, p=0.13) leg 1-RM after five days. In the 15D cohort, knee extension 1-RM decreased by 20.0% in the iMOB leg after 15 days (49.8kg, MD=12.4, 95% CI 4.6 to 20.7, p=<0.01), with no significant change detected in the CON limb (-2.9%, p=0.89) (Figure 4.8).

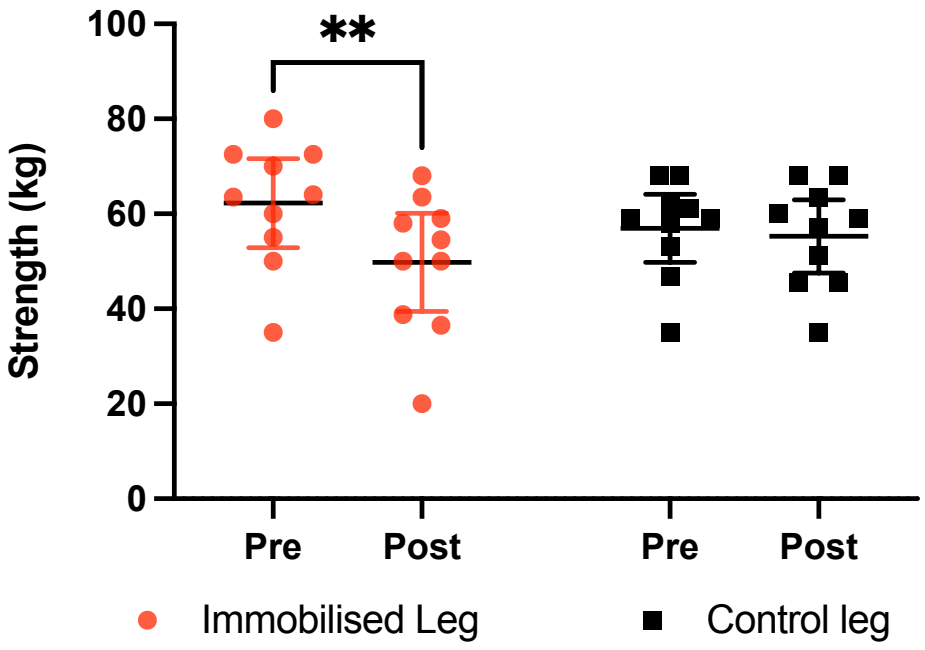

*Figure 4.8: Knee extension strength (assessed a one-repetition maximum (1-RM)) in kilograms (kg) in a control leg (no intervention) and in the contralateral leg after immobilisation for 15 days. Data shown are individual values with mean and SD. N=10, Analysis via 2way ANOVA. **=p<0.01 vs. pre-immobilisation in the same limb*

4.3.1.2 Plantarflexion

At baseline, plantarflexor MVC was significantly greater in the IMOB leg than the CON leg in the 15D cohort (372.2N [±212.5] vs. 354N [±219.0], p=0.04) but not in the 5D cohort (382.0N [±208.3] vs. 423.8 [±178.9], p=0.13). A significant time x immobilisation interaction was detected in both the 5D (p=0.02) and 15D cohorts (p<0.01). However no significant difference was detected in either the iMOB (-11.3%, p=0.58) or CON (-20.2%, p=0.08) leg MVC after five days. In the 15D cohort, plantarflexor MVC decreased by 18.7% in the iMOB leg after 15 days (302.7N [±212.3], MD=12.4, 95% CI: 5.1 to 19.7, p=<0.01), with no significant change detected in the CON limb (-8.0%, p=0.71) (Figure 4.9).

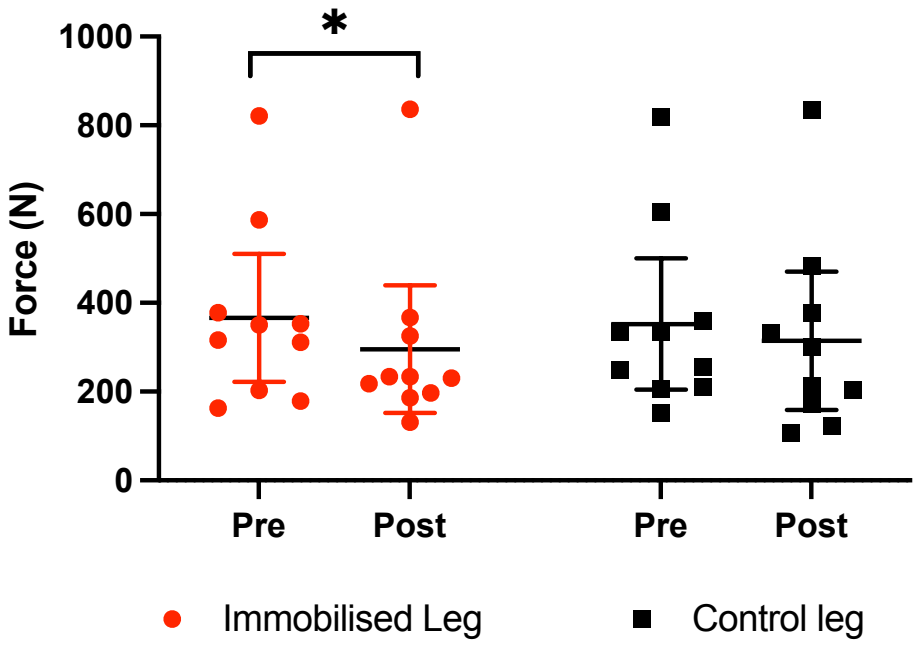

*Figure 4.9: Plantar flexion maximum voluntary contractile (MVC) force in Newtons (N) in a control leg (no intervention) and in the contralateral leg after immobilisation for 15 days. Data shown are individual values with mean and SD. N=10, Analysis via 2way ANOVA. *=p<0.001 vs. pre-immobilisation in the same limb*

4.3.1.3 Dorsiflexion

There was no significant difference at baseline between the iMOB or CON leg in either the 15D (197.6N [±44.8] vs. 191.8N [±70.4], p=0.97) or 5D group (162.1N [±57.5] vs. 184.6N [±56.8], p=0.81). There were no significant time x immobilisation interactions detected in either the 5D (p=0.96) or 15D (p=0.43) cohorts (Figure 4.10).

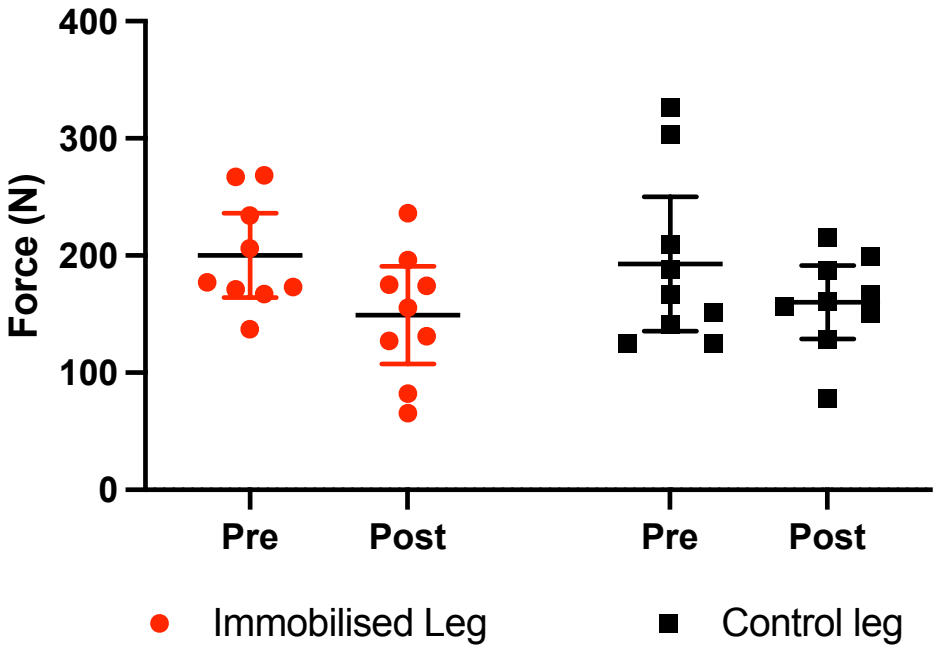

Figure 4.10: Dorsiflexion maximum voluntary contractile (MVC) force in Newtons (N) in a control leg (no intervention) and in the contralateral leg after immobilisation for 15 days. Data shown are individual values with mean and SD. N=10, Analysis via 2way ANOVA.

Table 4.3 below outlines the changes observed in strength measurements after 5 and 15 days immobilisation for all movements.

	Baseline	Day 5 % Change	Day 15	Day 15 % Change
VL MVC iMOB (N)	531.9 *(± 91.2)*	-11.5%	364.0 *(±79.9)*	-31.6%
VL MVC CON (N)	*498.3 (±129.2)*	*2.2%*	*470.4 (±143.6)*	*-5.6%*
MG MVC iMOB (N)	372.2 (±212.5)	-11.3%	302.7 (±212.3)	-18.7%
MG MVC CON (N)	*354.3 (±219)*	*-20.2%*	*325.9 (±227.8)*	*-8.0%*
TA MVC iMOB (N)	197.6 (±44.8)	-13.1%	152.3 (±51.9)	-22.9%
TA MVC CON (N)	*191.8 (±70.4)*	*-12.4%*	*161.8 (±38.7)*	*-15.6%*
KE 1RM iMOB (kg)	62.3 (±13.1)	-3.5%	49.8 (±14.5)	-20.0%
KE 1RM CON (kg)	*56.9 (±9.9)*	*11.2%*	*55.3 (±10.8)*	*-3.0%*

Table 4.3: Summary of changes in muscle strength in a control leg (CON) and immobilised leg (iMOB) after 5 and 15 days (for 5 days n=5, for 15 days n=10). Absolute values with (SD). VL = Vastus lateralis, MG = medial gastrocnemius, TA = tibialis anterior, KE = knee extensions MVC = maximum voluntary contraction, 1RM = 1 repetition maximum, Analysis via 2way ANOVA

4.3.2 Muscle Power

4.3.2.1 Nottingham Power Rig

There was no significant difference at baseline between the iMOB or CON leg in either the 15D (280.2kW [±103.7] vs. 267.2 [±99.1], p=0.42) or 5D cohort (324.0kW [±149.7] vs. 321.4kW [±141.2], p=0.99). No significant time x immobilisation interaction was detected in the 5D cohort (p=0.426), but leg extension power decreased in both the iMOB (-24.2%, 245.5kW [±158.9]) and CON limbs (-15.0%, 273.3 [±204.1]. A significant time x immobilisation interaction was detected in the 15D cohort (p=0.01). Leg extension power decreased significantly after 15 days in the iMOB leg (-25.1%, MD = 80.6, 95% CI: 15.32 to 146.4, p=0.02), but not in the CON limb (-0.03%, p=0.91) (Figure 4.11).

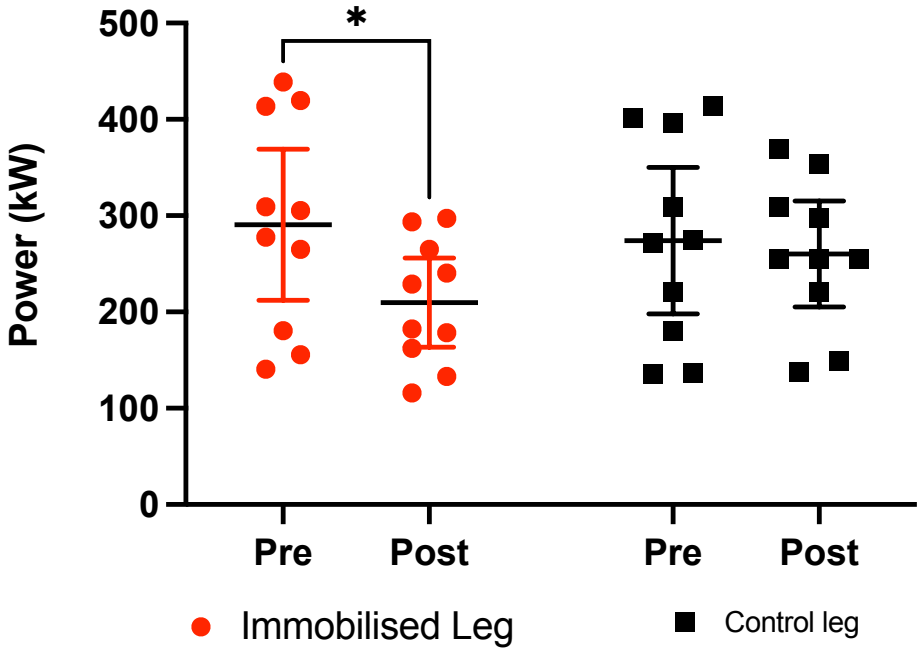

*Figure 4.11: Leg extension power in Kilowatts (kW) in a control leg (no intervention) and in the contralateral leg after immobilisation for 15 days. Data shown are individual values with mean and SD. N=10, Analysis via 2way ANOVA. *=p<0.05 vs. pre-immobilisation in the same limb.*

4.3.2.2 Jump power

There was no significant change in maximum concentric jump power (3.32kW [±0.54] vs. 2.85kW [±0.55], -14.3%, p=0.11), jump height (22.96cm [±5.22] vs. 18.38cm [±6.65], -20%, p=0.17) or take-off speed (2.29m/s [±0.34] vs. 2.03m/s [±0.39], -11.3%, p=0.19) although all measurements decreased over the period of immobilisation in the 5D cohort. However, after 15 days of immobilisation maximum concentric jump power (4.31kW [±0.97] vs. 3.91kW [±1.18], -9.3%, p=0.04), jump height (29.6cm [±8.5] vs. (24.3cm [±9.0], -18.1%, p<0.01) and take-off speed (2.7m/s [±0.3] vs 2.4m/s [±0.4], -9.4%, p<0.01) all decreased significantly (Table 4.4).

	Baseline	Day 5 % Change	Day 15	Day 15 % Change
Notts Rig iMOB (kW)	280.2 (± 103.7)	-24.2%	209.7 (±64.8)	-25.1%
Notts Rig CON (kW)	*267.2* *(±99.1)*	*-14.9%*	*260.3* *(±76.9)*	*-0.03%*
Jump Power (kW)	4.31 (± 1.0)	-14.3%	3.91 (±1.2)	-9.3%
Jump Height (cm)	29.6 (±8.5)	-20.0%	24.3 (±9.0)	-18.1%
Take off speed (m/s)	2.66 (±0.33)	-11.3%	2.41 (± 0.41)	-9.4%

Table 4.4: Summary of changes in leg muscle power as assessed by leg extension and jump analysis after 5 and 15 days immobilisation (for 5 days n=5, for 15 days n=10). For leg extension power was assessed in a control leg (CON)) and in the contralateral leg (iMOB)) with analysis via 2way ANOVA. Jump analysis was via paired t-test.

4.3.3 Force Steadiness

There was no significant time x immobilisation interaction detected for any measurement of force steadiness in any muscle, and no firm conclusions can therefore be drawn from any trends observed. None of these changes reached statistical significance (Table 4.5).

	iMOB Day 5 % Change	CON Day 5 % Change	iMOB Day 15 % Change	CON Day 15 % Change
KE 10% MVC	-7.4%	-10.2%	12.5%	0.5%
KE 25% MVC	-6.7%	-12.3%	10.6%	-1.3%
KE 40% MVC	-19.4%	17.3%	20.5%	3.2%
Plantarflex. 10% MVC	10.4%	50.8%	18.1%	-9.2%
Plantarflex. 25% MVC	53.9%	21.9%	23.0%	1.25%
Plantarflex. 40% MVC	23.8%	18.9%	-3.1%	-12.5%
Dorsiflex. 10% MVC	39.7%	15.6%	37.6%	16.4%
Dorsiflex. 25% MVC	31.9%	11.9%	24.4%	18.9%
Dorsiflex. 40% MVC	27.6%	8.3%	27.8%	-0.6%

Table 4.5: Summary of changes in force steadiness, measured as coefficient of variation (COV) from a target force line in different leg muscles at different intensities in a control leg (CON) and in the contralateral leg (iMOB) after 5 and 15 days immobilisation (for 5 days n=5, for 15 days n=10). Analysis via 2way ANOVA.

4.3.4 Balance

The distance by which the COP moved whilst standing on both legs increased significantly after 15 days of single leg immobilisation (50.9mm [±29.8] vs 83.9mm [±49.9], +64%, p<0.01). There was a significant time x immobilisation interaction detected between the iMOB and CON legs after 15 days (p=0.02). COP distance increased significantly in the iMOB leg (493.6mm [± 356.6]. vs. 566.7 [±180.8], +14.8%, p<0.01), with no significant change in the CON leg (491.4mm [± 314.0] vs. 438.0 [±100.3], -10.8%, p<0.96) (Figure 4.12).

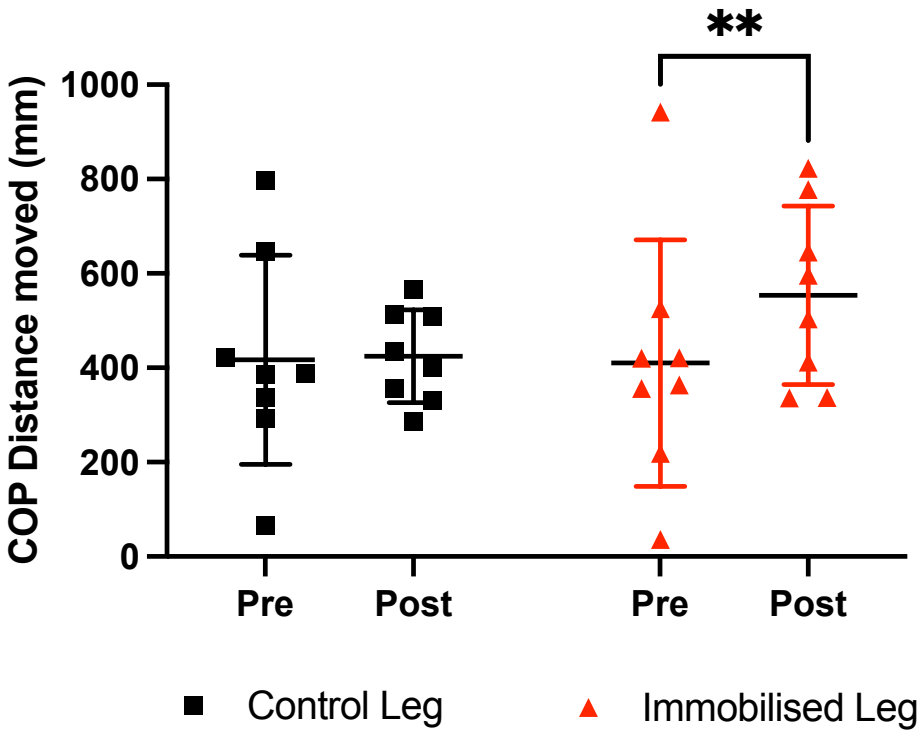

*Figure 4.12: Comparison of change in distance moved by COP in a control leg (no intervention) and in the contralateral leg after immobilisation for 15 days. Data shown as individual values with mean and SD, N=10, Analysis via 2way ANOVA. *=p<0.05 vs. pre-immobilisation in the same limb.*

COP ellipse area numerically increased after 15 days of immobilisation when standing on both legs and on the iMOB or CON leg. However, none of these changes reached statistical significance, and there was no time x immobilisation interaction detected when comparing the CON and iMOB legs. No significant changes were detected in any measures of balance after five days (Table 4.6).

	Baseline (mm)	Day 5 % Change	Day 15 (mm)	Day 15 % Change
Distance Moved - Both	50.9 (± 29.8)	+ 109%	83.9 (± 49.9)	+ 64.8%
Distance Moved – iMOB	493.6 (± 356.6)	+ 59.9%	566.7 (± 180.9)	+ 14.8 %
Distance Moved - CON	491.4 (± 314.0)	- 36.4%	438.0 (± 100.3)	- 10.9%
Ellipse - Both	4.7 (± 3.8)	+13.3%	8.9 (± 5.4)	+ 89.1%
Ellipse - iMOB	61.3 (± 33.1)	+ 93.9%	79.1 (± 61.5)	+ 29%
Ellipse CON	49.0 (± 27.7)	+ 3.1%	52.9 (± 17.7)	+ 7.9%

Table 4.6: Summary of changes in centre of pressure distance moved and ellipse area in a control leg (CON) (no intervention) and in the contralateral leg after immobilisation (iMOB) after 5 and 15 days of immobilisation. (for 5 days n=5, for 15 days n=10). Analysis via 2way ANOVA.

4.3.5 Timed Up and Go

Timed up and go time increased after 5 days (7.36s [±0.5] vs. 8.12s [±1.1], +10.3%, p=0.12) and 15 days of immobilisation, reaching significance in the 15D cohort only (6.96s [±1.6] vs 7.54s [±1.8], +8.2%, p=0.02) (Figure 4.13).

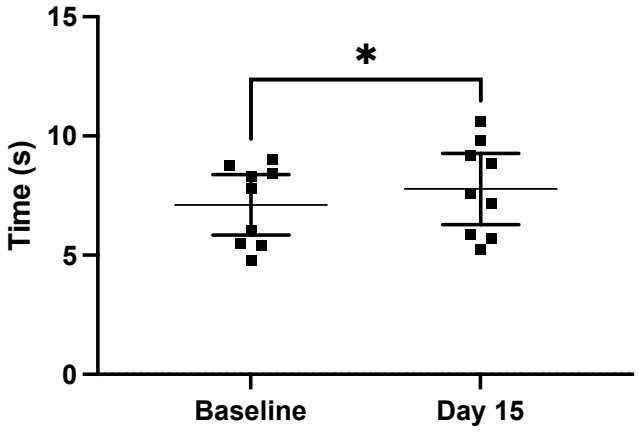

*Figure 4.13: Change in 'Timed Up and Go' time after 15 days of immobilisation. Data shown as individual values with mean and SD. * demonstrates significance. N=10, Analysis via paired t-test*

4.3.6 Cardiopulmonary Exercise Test

CPET was only performed in the 15D cohort. After 15 days of single leg immobilisation, AT reduced by 14.8% (25.3 ml/kg/min [±6.2] vs. 21.6 ml/kg/min [±5.8], p<0.01) and VO_2peak by 11.5% (42.4 ml/kg/min [±8.2] vs. 37.55 [±8.2], p<0.01). The reason given for termination of the test was fatigue in the iMOB leg in 90% of participants, and overall maximal effort in the remaining 10% of participants (Figure 4.14).

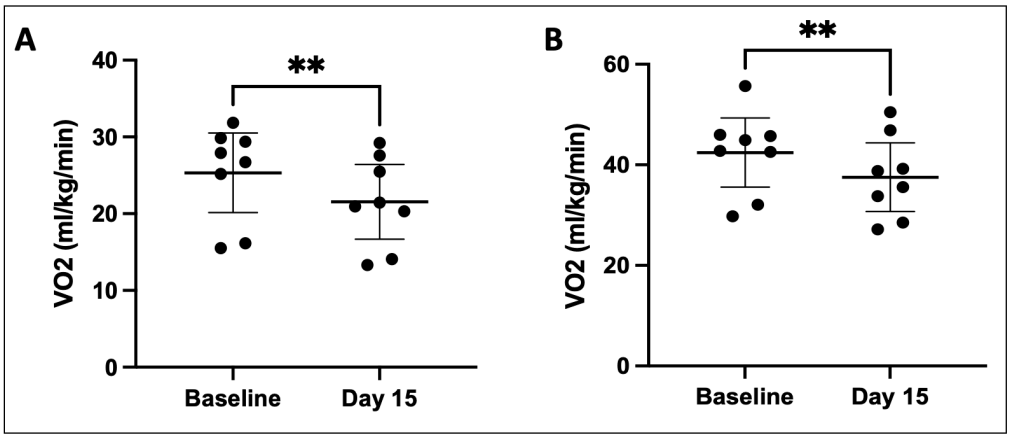

*Figure 4.14: Change in A) VO_2 at Anaerobic threshold and B) VO_2peak after 15 days of immobilisation in n = 10 participants. Data shown are individual values with mean and SD. Analysis via paired t-est. **=p<0.01 vs, baseline*

4.3.7 Correlation between changes in function and muscle mass

There was strong correlation between change in VL CSA and change in VL MVC (r=0.76, p=0.01) (Figure 4.15). There was no statistically significant correlation between change in any other measure of muscle function and change in muscle mass across the different muscles (VL, MG and TA) (for full results see Appendix E).

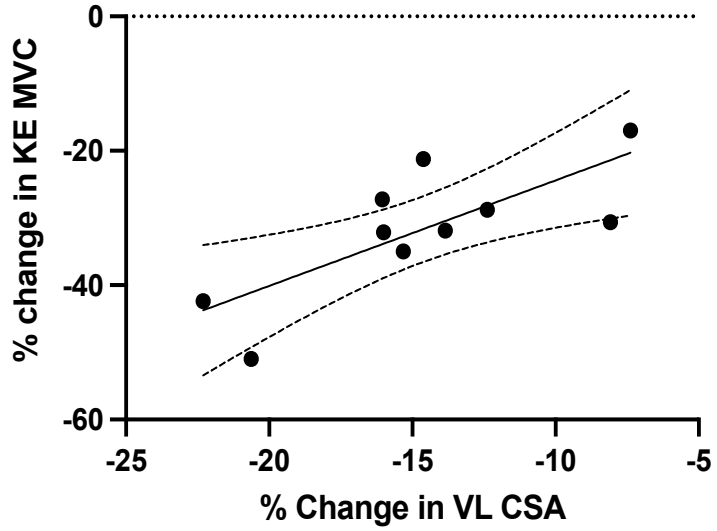

Figure 4.15: Correlation between % change in vastus lateralis (VL) cross sectional area (CSA) and Knee extension maximum voluntary contraction (MVC) after 15 days immobilisation. N=10 for both cohorts. Analysis via calculation of Pearson's correlation coefficient for paired data

4.4 Discussion

4.4.1 Changes in muscle function after immobilisation

In this study we have demonstrated a significant decrease in multiple aspects of physical function, including skeletal muscle strength, power, aerobic capacity and fatigue resistance after 15 days of limb immobilisation.

Measurements of muscle strength and power decreased by 20 to 30% over the period of immobilisation, equating to a 1.3 - 2% loss of muscle strength and power for each day of acute immobilisation. These findings are in keeping with the results of other studies which have shown dramatic losses of muscle force generation after just a short period of immobilisation. For example, knee extensor MVC has previously been demonstrated to reduce by approximately 9% after three (Demangel *et al.*, 2017) to five days (Wall *et al.*, 2014b; Mulder *et al.*, 2015), 14% after 10 days (Monti *et al.*, 2021), and 22 - 27% after 14 days (Jones *et al.*, 2004b; Glover *et al.*, 2008; Wall *et al.*, 2014b). Considering the participant characteristics and the immobilisation employed in these studies it appears that losses of muscle strength occur at a similar rate across gender and method of mobilisation.

To put these losses into context, a 30% reduction in leg strength and power is comparable to the difference between a healthy individual in their twenties and one in their sixties (Rejc *et al.*, 2018).

Whilst the rate of reduction in muscle power observed in this study was similar to that reported in other studies (Rejc *et al.*, 2015, 2018) the pattern of greater losses of power than of strength observed in aging (Skelton *et al.*, 1994), and suggested by some authors as being present with immobilisation (Monti *et al.*, 2021), was not observed. This may be due the movement profile of the exercises chosen to test power in this study, both of which (Nottingham Power Rig and vertical jump test) involved whole leg extension. As only knee and ankle joints were fixed during the immobilisation employed in this study, hip extensors were not immobilised and therefore likely maintained a higher level of mass and function. As hip extensors play a key role in explosive whole leg extension, the lack of their immobilisation may have attenuated the overall reduction in power observed. However, in a study involving two weeks of bed rest (where hip extensors are immobilised equally to other muscle groups), leg extension power and knee extension strength were found to reduce at equal rates in both young and older individuals (Rejc *et al.*, 2018). Combined with the finding of this study, these results suggest that the proposed pattern of greater losses of power than of strength may not occur in the acute phase of DMA.

Importantly, in this study we have demonstrated an association between changes in isolated aspects of muscle function and changes in markers of ‘global’ physical function.

For example, we saw a 10% increase (representing worse mobility) in TUG times and a 15-30% reduction in balance in the immobilised leg after 15 days. Interestingly, balance on both legs was also worse after the immobilisation period, suggesting that the control limb is not able to compensate for defects in the affected limb. These changes were small and there was likely a 'ceiling effect' with the participants ability partially masking the changes. However, given that rates of muscle function losses with immobilisations have been shown to be similar between young and elderly individuals (Urso, Clarkson and Price, 2006), with a tendency towards a greater rate of functional losses with increasing age (Rejc *et al.*, 2018), these results highlight the serious impacts of periods of immobilisation in older individuals.

Any loss of muscle power can have a severe detrimental impact on functional capacity in older adults (Rejc *et al.*, 2018), and a 20-30% loss of muscle strength and power in some may be enough to translate into a loss of independence. Studies have shown that muscle function is recovered more slowly and less completely in older people undergoing retraining (Rejc *et al.*, 2018), and that repeated periods of immobilisation and associated losses of muscle function accelerate the trajectory of sarcopenia and the point at which older adults reach a dependency threshold (Oikawa, Holloway and Phillips, 2019). Given the clear impact of just a short period of immobilisation on muscle function, treatment strategies must be implemented early during any period of immobilisation in older people in order to try and mitigate any loss of muscle function.

4.4.2 Relationship between change in muscle mass and function

In this study we demonstrated a strong correlation between changes in VL CSA and knee extensor MVC, but no correlation between change in mass and function for the MG and TA. Whilst this may be expected in TA as neither change in mass or function reached significance, the reason for lack of correlation in MG is less clear. Irrespective of muscle group, relative changes in muscle strength and power were more than double the observed change in muscle mass. This pattern of functional losses exceeding atrophy is well established in the literature, with reductions in muscle function 2-3 times greater than reductions in muscle mass repeatedly observed (Wall *et al.*, 2014b; Rejc *et al.*, 2018; Monti *et al.*, 2021). However, whilst this trend is well established, a complete explanation for the mechanisms underlying it is not (Monti *et al.*, 2021).

Clearly, the consistent finding of greater losses of muscle function than mass suggests that reduced muscle mass provides only a partial mechanistic explanation for loss of muscle function. Indeed, muscle fibre specific tension (maximum force generation per muscle fibre CSA) reduces after both short (Lamboley *et al.*, 2016) and prolonged (Larsson *et al.*, 1996) periods of immobilisation, suggesting a change in muscle contractile qualities.

One potential explanation is that muscle unloading results in a reduction in MPS, which disproportionately affects contractile proteins compared to

other sarcoplasmic proteins (Fitts, Riley and Widrick, 2000). This results in a significant decrease in myofibrillar protein content per unit of muscle cell volume (Larsson *et al.*, 1996; Campbell *et al.*, 2013), and hence a greater decrease in contractile force than fibre CSA.

Muscle stiffness, tone and elasticity has also been found to decrease rapidly with immobilisation resulting in a decrease in muscle tension force (Demangel *et al.*, 2017). Similar changes have been demonstrated in tendons (Reeves *et al.*, 2005), with decreased stiffness and decreased elastic modulus (i.e. less force required to stretch tendon) (Reeves *et al.*, 2005; Maarten D de Boer *et al.*, 2007) after immobilisation. These changes occur rapidly and have been demonstrated after just 14 days (Couppé *et al.*, 2012), so are likely contributing to the reductions in muscle function observed in this study. Changes in tendon stiffness may be due to decreased tendon collagen turnover and cross linking, decreased tendon glycosaminoglycan and water content, and increase in nonuniform orientation of collagen fibrils (Maganaris and Narici, 2005). Combined these changes in muscle and tendon stiffness may impair transmission of force (Monti *et al.*, 2021).

There is also evidence that immobilisation induces changes in sarcoplasmic reticulum (SR) calcium handling. For example, after immobilisation there is a rapid reduction in the overall SR calcium content (Lamboley *et al.*, 2016; Monti *et al.*, 2021), maximal SR calcium capacity (Lamboley *et al.*, 2016) and responsiveness of SR calcium release

channels (Monti *et al.*, 2021). This decrease in overall calcium stores and release of calcium in response to neuromuscular junction (NMJ) depolarisation may result in a reduction in muscle contractility and therefore its ability to generate force.

Furthermore, growing evidence suggests that neuromuscular (mal)adaptations may underly much of the disproportionate loss of muscle function compared to mass. Whilst we found that immobilisation did not significantly impact force steadiness, indicating a preservation of basic motor control following disuse, there is evidence that immobilisation results in rapid adaptation to motor units (MU) (Inns *et al.*, 2022), the NMJ (Monti *et al.*, 2021) and muscle fibre denervation (Arentson-Lantz *et al.*, 2016a; Demangel *et al.*, 2017; Monti *et al.*, 2021). Given that a significant proportion of early gains in muscle strength in response to exercise training are due to improved neural recruitment (Moritani and deVries, 1979), it is logical that the converse would also be true and that neuromuscular adaptations underly a significant proportion of the loss of muscle strength with immobilisation. In support of this suggestion, a reduction in overall electromyography (EMG) activity has been demonstrated after two weeks of immobilisation (Deschenes *et al.*, 2002). Furthermore, recent findings by our group demonstrate rapid MU dysregulation secondary to immobilisation, with a reduction in MU firing rate suggesting impaired neural input to muscle (Inns *et al.*, 2022). In addition, after two weeks of immobilisation MU potential (MUP) size decreased significantly whilst MU complexity increased significantly.

MUP size is proportional to the total number and size of muscle fibres contributing to it, and given that function decreases more quickly than CSA this suggests partial denervation of MU fibres (Inns *et al.*, 2022). Increase in MUP complexity is suggestive of increased electrophysiological dispersion between fibres of the MU, which may represent increased fibre diameter variability, reinnervation or axonal sprouting (Piasecki, Garnés-Camarena and Stashuk, 2021), and is a similar pattern to that seen in myopathies (Inns *et al.*, 2022).

Further evidence of partial fibre denervation in response to immobilisation is provided by studies showing an increase in neural cell adhesion molecule (NCAM) positive muscle fibres after immobilisation (Demangel *et al.*, 2017). NCAM expression is associated with the axonal sprouting response observed in denervated muscle and therefore suggestive of fibre denervation (Walsh *et al.*, 2000). These changes have been identified after just three days of immobilisation, and as such suggest that this denervation and change in MU recruitment patterns may occur very early in the context of disuse (Demangel *et al.*, 2017).

In summary, the greater loss of muscle function compared to muscle mass reflects a decreased muscle fibre specific force generation (Larsson *et al.*, 1996; Lamboley *et al.*, 2016) due to complex, multi-faceted muscle remodelling which occurs rapidly in the early stages of immobilisation. This remodelling may include a reduction in muscle cell myofibrillar content (Larsson *et al.*, 1996; Fitts, Riley and Widrick, 2000;

Campbell *et al.*, 2013), decreased muscle (Demangel *et al.*, 2017) and tendon (Reeves *et al.*, 2005; Maarten D de Boer *et al.*, 2007; Couppé *et al.*, 2012) stiffness, changes in SR calcium handling (Lamboley *et al.*, 2016; Demangel *et al.*, 2017; Monti *et al.*, 2021), MU remodelling (Inns *et al.*, 2022), decreased neural input (Deschenes *et al.*, 2002; Inns *et al.*, 2022), NMJ instability (Monti *et al.*, 2021), and rapid partial fibre denervation (Arentson-Lantz *et al.*, 2016b; Demangel *et al.*, 2017; Monti *et al.*, 2021).

4.4.3 Changes in muscle oxidative capacity

After 15 days of single leg immobilisation there was a significant reduction in both AT (-14.8%) and VO_2peak (-11.5%), each of which are accepted measures of cardiorespiratory fitness (Ghosh, 2004; Umapathi and Nguyen, 2022). As participants were instructed to continue their normal daily activity levels during this study, and CPETs were repeated just two weeks apart, it is unlikely that respiratory or cardiovascular system alterations played a major role in the changes observed, and the reductions in AT and VO_2peak likely represent a reduction in skeletal muscle oxidative capacity. This conclusion is supported by other immobilisation studies which have demonstrated that skeletal muscle oxidative capacity, based on measures of mitochondrial respiration, is reduced significantly after two weeks of immobilisation (Gram *et al.*, 2014).

As discussed in chapter 3, there is evidence of a shift in fibre type composition after immobilisation, with greater atrophy of MHC I (aerobic) fibres compared to MHC II. For example, after just three days of immobilisation it has been shown that there is a significant increase in the proportion of type I fibres exhibiting a hybrid fibre status (with MHC IIX) indicating that type I fibres rapidly lose their specific slow type, aerobic capabilities (Demangel *et al.*, 2017). A similar decrease in the proportion of type IIa fibres has also been demonstrated following a period of immobilisation (Arentson-Lantz *et al.*, 2016a), with an overall effect that type IIx (fast / anaerobic) fibres (Fitts *et al.*, 2010; Brocca *et al.*, 2012) make up a greater proportion of total muscle fibres.

Further muscle level adaptations in response to immobilisation which may explain this decrease in muscle oxidative capacity have also been identified. Muscle capillary density has been shown to be reduced after just 14 days of immobilisation, with capillary density correlating to post immobilisation peak aerobic capacity (Arentson-Lantz *et al.*, 2016b). Furthermore, as mentioned above, muscle mitochondrial oxidative capacity has also been shown to reduce significantly after short periods of immobilisation with these reductions demonstrated to be due to a decrease in mitochondrial number, rather than intrinsic mitochondrial function (Gram *et al.*, 2014).

4.4.4 Differential changes in muscle function by muscle group

In this study we identified evidence of differential decreases in muscle function between muscle groups of the lower leg. Whilst plantarflexor MVC decreased significantly, no significant changes were observed in dorsiflexor MVC. To the author's knowledge, this is the first time this trend has been demonstrated over short periods of immobilisation. Although few other studies have been published which a provide direct comparison between functional changes in different muscle groups of the lower leg, those which have demonstrate a similar trend. For example, LeBlanc et al., found that after five weeks of bed rest, plantarflexor strength decreased by 26% whilst no significant change was detected in dorsiflexors (LeBlanc *et al.*, 1988). A systematic review of a large number of bedrest studies also identified that dorsiflexor strength decreases less than plantarflexor strength (Winnard *et al.*, 2019).

As shown in chapters 2 and 3, there is good evidence that plantar flexor muscles (such as the MG) are atrophy susceptible, whilst dorsiflexor muscles (such as TA) are atrophy resistant. However, as already discussed, decreases in muscle mass appear to only provide a partial explanation for decreases in muscle function, and the greater loss of function in plantarflexors is therefore likely due to greater susceptibility to all of the muscle remodelling processes described above (section 4.4.2).

The results of this study, which show that when exposed to the same period and degree of immobilisation different muscles exhibit varied but co-ordinated susceptibility (or resistance) to both atrophy and functional decline, may suggest a common control pathway mediating the response of muscle to immobilisation, both in terms of atrophy and the remodelling processes responsible for decreased function.

4.4.5 The timecourse of changes in muscle function

Whilst no changes in muscle function achieved statistical significance after five days of immobilisation, this was likely a result of the study being underpowered due to the small sample in the 5D cohort (n=5). Assessing the numerical values obtained in this study, we found that after five days of immobilisation MVC, across all muscles assessed, decreased by 11 - 13%, compared to 18 - 31% after 15 days. Conversely, average numerical decreases in power, balance and TUG after five days were not less than the losses observed in the 15D cohort. Collectively this suggests that functional changes occur rapidly during the first 14 days of immobilisation. This suggestion is supported by a systematic review exploring the effects of bed rest on muscle mass and function in older individuals, which showed that muscle function is lost at approximately 3% per day over the first two weeks of immobilisation, but with a sharp initial reduction after which rates of loss stabilise between day 5 and day 14 (Figure 4.16) (Di Girolamo *et al.*, 2021c).

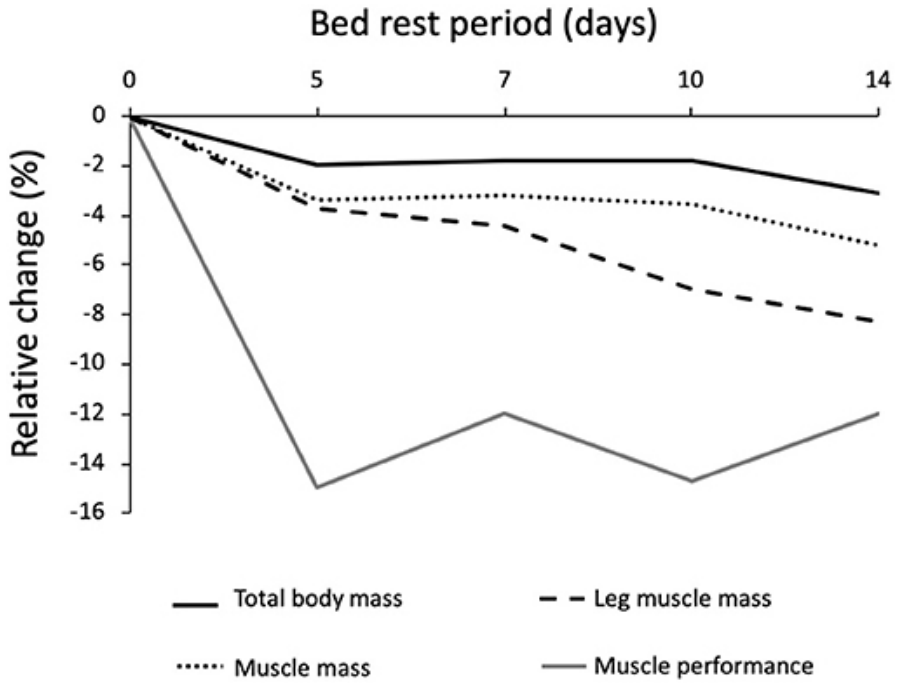

Figure 4.16: Graph from a systematic review by Di Girolamo et al., (2021) showing combined results from multiple studies to illustrate the timecourse of changes in leg muscle mass and performance over the first two weeks of immobilisation in older adults (Di Girolamo *et al.*, 2021c).

However, not all studies report this pattern of decline. For example, a study with repeated measurements made in the same group of immobilised individuals showed a linear rate of muscle strength loss (approx. 1% per day) over three weeks of immobilisation, with a loss of 14.7% at day 14 and 21% at day 24 (Maarten D de Boer *et al.*, 2007). It must however be noted that this study did not perform any measurements before day 14, and variability in rate of strength loss before this time point may have been present. Based on the available evidence, it can be concluded that rates of decline in muscle function do appear to be more rapid during the first month of immobilisation, with stabilisation of losses over longer periods. For example, in studies of prolonged immobilisation knee extensor MVC reduced by 25% after six weeks (Berg, Larsson and Tesch, 1997) and 17% after eight weeks (Mulder *et al.*, 2006), whilst

plantar flexor MVC decreased by 26% after five weeks (LeBlanc *et al.*, 1988), and muscle power decreased by 30% after 12 weeks of bed rest (Rittweger *et al.*, 2007). If the initial decrease of a 1% loss of function per day over two weeks identified by de Boer et al had continued on a linear trajectory, rate of loss by 12 weeks would be 84%, far greater than that observed by Rittweger et al.

Whilst the exact timecourse of changes in muscle function over the first two weeks of immobilisation remain uncertain, multiple studies have demonstrated that functional declines in this period are detectable after just short periods (Demangel *et al.*, 2017), (Di Girolamo *et al.*, 2021b) and proceed rapidly. This finding is very significant in the clinical setting and highlights the need for early, targeted interventions and treatments in individuals with reduced mobility to prevent detrimental losses of muscle mass and function.

4.4.6 Limitations

Due to the impact of 15 days ULLI on normal life, this study was challenging to recruit to. As a result a strategic decision was made to allow participants to choose whether to be allocated to the 15D or 5D cohort. This resulted in an imbalance in participant characteristics between the 2 groups, with the 5D cohort having a slightly higher age and BMI. However, these differences are small with both groups consisting of healthy young males. More importantly, the 5D cohort is underpowered due to the small sample size recruited, and as such no

firm conclusions can be made from the data associated with this timepoint. It does however provide interesting numerical insight with which to design future studies in this research space. Another potential limitation is the lack of immobilisation of the hip extensors with regard to the impact this may have on whole leg extension based power assessments, and these results should be considered with this in mind. Despite this limitation, our method of immobilisation does mimic the situation for people who are casted/using crutches after a single lower limb injury and also, unlike studies of bed rest for example, allows bilateral comparisons between and immobilised and non-immobilised limb; and the impact of muscle disuse to be studied independent of reductions in global physical activity.

4.5 Conclusions

In conclusion, rapid losses of leg muscle strength and power, aerobic capacity and endurance occur after just two weeks of immobilisation. Muscle function is lost at a much greater rate than mass, likely due to complex, multi-faceted muscle remodelling, involving a reduction in muscle cell myofibrillar content, motor unit remodelling, decreased neural input, fibre denervation, decreased capillary density and fibre type transition. Given that loss of muscle function occurs rapidly, early and to different extents across muscle groups, targeted interventions are needed for individuals subject to a period of disuse, especially those with reduced physiological resilience, to prevent harmful losses of muscle mass and function.

5 Postoperative electrical muscle stimulation attenuates loss of muscle mass and function following major abdominal surgery: A split body randomised control trial

Chapter abstract

Introduction

Significant loss of muscle mass and function occurs after major abdominal surgery. Whilst the cause of this muscle loss is multifactorial (including inflammation and malnutrition), immobility is likely to play a major role. Increasing physical activity in postoperative patients is difficult. Neuromuscular electrical stimulation (NMES) is a way of increasing muscle contractile activity, without the need for patients to increase activity. NMES has been shown to reduce muscle atrophy in some patient groups, but evidence in postoperative patients is limited. This study assesses the efficacy of NMES for attenuating muscle atrophy and functional declines following major abdominal surgery in older adults.

Methods

Fifteen patients undergoing open colorectal resection completed a split body randomised control trial. Patient's' lower limbs were randomised to control (CON) or NMES (STIM). The STIM limb underwent 15 minutes of quadriceps NMES twice daily on postoperative day (POD) one to four. Ultrasound measurements of Vastus Lateralis cross sectional area (CSA) and muscle thickness (MT) were made preoperatively and on POD five, as was dynamometry to determine knee extensor strength (KES). All outcomes were statistically analysed using linear mixed models.

Results

NMES significantly reduced the loss of CSA (-2.52% vs -9.16%, $p<0.001$), MT (-2.76% vs -8.145, $p=0.001$) and KES (-10.35% vs -19.69%, $p=0.03$) compared to CON. No adverse events occurred, and patients reported that NMES caused minimal or no discomfort and felt that ~90-minutes of NMES daily would be tolerable.

Discussion

NMES reduces losses of muscle mass and function following major abdominal surgery and as such, may be a promising tool to postoperative recovery. This is important in preventing long-term postoperative dependency, especially in the increasingly frail older patients undergoing major abdominal surgery. Further studies should establish the efficacy of whole leg, bilateral NMES for improving patient-centred outcomes.

5.1 Introduction

Substantial losses of skeletal muscle mass and function occur after major gastrointestinal (GI) surgery, due to the physiological insult of surgery (Farhan *et al.*, 2016), physical inactivity (van Wijk *et al.*, 2021) and inadequate protein nutrition (van Wijk *et al.*, 2021) in the postoperative period (Carli, 2015), with the greatest losses occurring in the first postoperative week (Aoyama *et al.*, 2016). In patients who had a colorectal resection, a 6.5% reduction in quadriceps cross sectional area (CSA) was reported after six days (Vinge *et al.*, 1996), with similar losses reported following oesophagectomy (4.8%) (Shimoda *et al.*, 2019). Even greater losses were noted in patients admitted to an intensive treatment unit (ITU), with a median reduction of rectus femoris CSA of 12.5% over the first seven days, rising to 17.7% by day 10 (Puthucheary, Rawal, McPhail, Connolly, Ratnayake, Chan, Hopkinson, Padhke, Dew, Paul S. Sidhu, *et al.*, 2013). Higher rates of postoperative muscle loss have been shown to be associated with increased risk of postoperative complications (van Wijk *et al.*, 2021), greater disease recurrence following cancer surgery (Kobayashi *et al.*, 2016), and worse survival (Shimoda *et al.*, 2019; van Wijk *et al.*, 2021).

The majority of major abdominal surgery is performed in patients over 60 years of age (*National Bowel Cancer Audit*, 2020; British Geriatrics Society, 2020). Older patients regain muscle function more slowly and less completely following major abdominal surgery (Watters *et al.*,

1993a), and surgery related muscle loss is associated with declines in the muscle function important for independence (Watters *et al.*, 1993b), a slower return to normal activities, and reduced quality of life (Dronkers, Witteman and van Meeteren, 2016). Furthermore, the cumulative effect of repeated short bouts of muscle disuse in older age, such as those associated with surgery, may be a key factor in the development of sarcopenia (Wall, Dirks and van Loon, 2013), frailty, and loss of independence (Dronkers, Witteman and van Meeteren, 2016); all of which incur burden to individuals, families, and society (Clark and Manini, 2010), and are associated with numerous negative health outcomes (Deschenes, 2004).

Immobilisation, inflammation and starvation are recognised catabolic drivers, all of which are present in patients following major GI surgery (Carli, 2015). Quantifying the relative contribution of each to the development of postoperative muscle atrophy is challenging, however results from healthy volunteer studies of immobilisation show that disuse is likely a major contributor, with immobilisation alone shown to elicit a 3.5% loss of quadriceps CSA after five days (Wall *et al.*, 2014b), representing over half of the 6.5% loss seen in postoperative colorectal resection patients over a similar time frame (Vinge *et al.*, 1996).

Although increased contractile activity would seem the obvious answer to mitigate physiological declines associated with muscle disuse, it is clear that after major abdominal surgery patients are unable to perform

the level of physical activity required (Noro *et al.*, 2003). For example, following oesophagectomy, patients were sedentary for 96% of the first five days, taking just 86 (46-210) steps on POD 1 and only 474 (302-805) steps by POD 5 (Hussey *et al.*, 2019). More strikingly, even in patients treated with an enhanced recovery after surgery (ERAS) protocol following colorectal resection, the median number of steps on POD 5 was less than 1500, and in patients not following ERAS steps were fewer than 500 (Henriksen *et al.*, 2002a). Even in a patient cohort where over 80% had laparoscopic resection, patients receiving standard care mobilised less than 500 steps per day on POD 1-3, while patients receiving intense, twice-daily mobilisation support only achieved a maximum of 1000 steps per day (Fiore *et al.*, 2017). The most frequently cited factors preventing further mobilisation were haemodynamic instability (Hussey *et al.*, 2019), fatigue (Henriksen *et al.*, 2002b), pain (Noro *et al.*, 2003), and attachment to drains, feeding apparatus and pumps (Low *et al.*, 2019). As a result, optimising muscle maintenance is not easily addressed. Studies in healthy older adults undergoing bed rest have shown that 2,000 steps per day is not enough to maintain skeletal muscle mass (Arentson-Lantz *et al.*, 2019b), therefore it is clear that postoperative patients are unable to perform enough physical activity to prevent muscle atrophy.

NMES is a technique of eliciting muscle contractions using electrical impulses without the requirement for voluntary contraction. Electrical impulses are transmitted transcutaneously and generate muscle fibre

action potentials that would normally be transmitted via motor neurons to cause voluntary muscle contraction. In ITU patients, NMES has been shown to cause an increase in mTOR phosphorylation, suggestive of its ability to activate the cell-signalling pathways associated with muscle protein synthesis (MPS) (Dirks *et al.*, 2015). Similarly, in patients who had major abdominal surgery, NMES applied during recovery reduced markers of muscle protein breakdown (MPB) (Strasser *et al.*, 2009). Taken together, these findings suggest that NMES may be a pragmatic substitute for exercise in the postoperative period.

NMES has been shown to reduce muscle atrophy in immobilised healthy volunteers (Dirks *et al.*, 2014), and in patients following sporting (Gibson, Smith and Rennie, 1988) and spinal cord injuries (Erika Scremin *et al.*, 1999). In patients with chronic heart failure, NMES elicits comparable improvements in measures of fitness and strength to conventional exercise training based cardiac rehabilitation (Deley *et al.*, 2005; Dobsák *et al.*, 2006; Karavidas *et al.*, 2010). There are however very few studies reporting the use of NMES following major abdominal surgery. Vinge *et al.* did report that NMES significantly reduced losses of skeletal muscle mass following colorectal resection, with associated improvements in MPS (Vinge *et al.*, 1996), but no assessment of muscle function was performed. In addition, whilst this study yielded promising results, it is a single study from over 20 years ago, before ERAS was widely introduced. Therefore, there remains a need for further studies to assess the ability

of NMES to attenuate loss of muscle mass and function in patients following major GI resection in current clinical settings.

The primary objective of this study was to assess the ability of NMES to attenuate Vastus Lateralis (VL) muscle mass losses following major colorectal resection in older adults. Secondary objectives included determining the impact of NMES on muscle function (knee extensor strength (KES)) and muscle architecture and characterising postoperative physical activity levels and typical dietary intake in this patient cohort. We also assessed the tolerability of NMES in this patient cohort to determine its viability as a treatment modality.

5.2 Methods

This split body randomised control trial was approved by the NHS Research Ethics Committee (20/EM/069, IRAS ID: 274048) and was registered with clinicaltrials.gov (NCT04199936). Adult patients scheduled to have major open colorectal resection, and who met the study eligibility criteria based on their routine preoperative assessment, were approached by the research team and provided with information regarding the study. Patients were eligible for inclusion if they: i) were having open major colonic resection; ii) had sufficient mobility to complete normal ERAS; and iii) were able to give informed consent. Patients were excluded if they had: a) any pre-existing neuromuscular disease, b) a pacemaker, implantable cardiac defibrillator, nerve simulator device, or bilateral metal orthopaedic implants c) inability to give informed consent; d) disability preventing normal mobilisation after surgery; e) peripheral vascular disease (PVD), chronic kidney disease (CKD), chronic congestive cardiac failure (CCF); or f) intubation for more than 24 hours postoperatively. Patients with PVD, CKD and CCF were excluded due to the potential of these conditions to interfere with normal muscle physiology (Adams and Vaziri, 2006; Zizola and Schulze, 2013). Patients who returned to theatre during the study period were excluded from final analysis. After eligibility was confirmed in person on the day of the patient's operation, written informed consent was obtained and baseline measurements were performed. All baseline measurements were repeated on POD 5 after four days of unilateral NMES.

5.2.1 Randomisation and blinding

After baseline measurements were complete, patients' lower limbs were randomised using random permuted block sizes, to act as control (CON) or undergo NMES (STIM). Due to the nature of the intervention, patients and the primary researcher were not blinded, however all ultrasound measurements (the primary outcome for this study) were double checked by a second, blinded assessor.

5.2.2 Muscle mass assessment

Changes in muscle mass were assessed as the primary outcome. To determine muscle CSA, the craniocaudal midpoint of VL was identified as halfway between the greater trochanter of the femur and the midpoint of the patella. Medial and lateral boundaries of VL were identified at this level using B-mode ultrasound (Esaote, LA523/923 probes) and the intersection of the craniocaudal and medial-lateral midpoints was identified and marked using permanent ink. Marks were refreshed on each POD. Muscle CSA was measured at this point using video panoramic ultrasound as previously described (Sarto *et al.*, 2021). Muscle architecture measures including MT, pennation angle (PA) and fascicle length (FL) were also made as described in chapter 3. Ultrasound images were interpreted in ImageJ (NIHR, USA), with a mean value of three measures for each assessment at each timepoint used.

5.2.3 Muscle function assessment

To determine KES, patients were seated on the edge of the bed, with legs fully supported up to the knee joint and both knees flexed to 90 degrees. The bed was adjusted so that patients' feet were hanging freely. A portable dynamometer (Lafayette Manual Muscle Tester, IN 47903, USA) offering static resistance was placed against the lower shin and patients were instructed to extend their leg against the device with maximum effort.

5.2.4 Muscle stimulation protocol

NMES was performed on the assigned leg twice each day on POD 1-4 for 15 minutes per session with over three hours between sessions. NMES was delivered using an NHS approved, CE marked device (Premier Combo Plus, Med-Fit Ltd, UK) using two large (7.5x13cm) electrodes placed proximally and distally over the lateral quadriceps. NMES was delivered at a frequency of 30Hz and delivered in a one second on, one second off pattern. Amplitude settings were determined prior to baseline measurements and set at the minimum level required to produce both visible contractile activity in the muscle and involuntary movement at the knee joint with patients seated, knees flexed at 90 degrees and feet hanging freely. Mean (SD) amplitude was 36.5 ($\pm$6.8) mAmp.

The choice of this NMES protocol was based on a review of previously published studies and clinical guidelines, and designed to reach a balance between optimum muscle activation whilst minimising patient discomfort (Alon, 2013; Hart and White, 2017).

After the final NMES session, patients rated the level of discomfort elicited by NMES on a text-based Likert Scale and reported the maximum duration of NMES per day they considered tolerable.

5.2.5 Physical activity levels and dietary intake

Physical activity levels were recorded using a self report questionnaire (Appendix D). Distance walked was measured by a member of the research team after patients identified landmarks to which they had mobilised. Step counts were derived using a conversion factor of 1.439 steps per meter (Burnfield and Powers, 2006). Dietary intake on each POD was recorded using a questionnaire completed by the patient alone, or with a researcher as required, and cross referenced against hospital documentation (Appendix D). Details of food options were provided by the hospitals catering supplier with nutrient composition calculated using specialist software (Nutritics Ltd, Dublin, Ireland). Energy intake values were compared to 25-30 kcal/kg/d and protein to 1.5g/kg/d, based on the ESPEN guidelines regarding clinical nutrition in surgery (Weimann *et al.*, 2017).

5.2.6 Statistical analysis

Formal sample size calculation was not possible due to the lack of existing data, and the study was therefore performed as a pilot study Distribution of data was tested using the Kolmogorov-Smirnov test, with normally distributed data expressed as mean (±SD) and non-normally distributed data as median (IQR). If only one measurement was compared, paired t-tests or Wilcoxon signed rank was used as appropriate. To account for the structure of the data we analysed outcomes using linear mixed models. Times (pre and post) were included with random intercepts and slopes, and each leg was nested within individuals. Outcomes are reported as the interaction between time and leg. Where possible, an unstructured variance-covariance structure was used for the random effects. Results are presented as mean differences (MD) with 95% confidence intervals (CI) and p-values. We regarded $p<0.05$ as statistically significant. All analyses were conducted using Stata Version 16.1.

5.3 Results

Eighteen patients were recruited and randomised to take part in the study, with 15 completing the study (Figure 5.1). No patients withdrew because of NMES. There were no adverse events reported. Patient demographics for those who completed the study are summarised in Table 5.1.

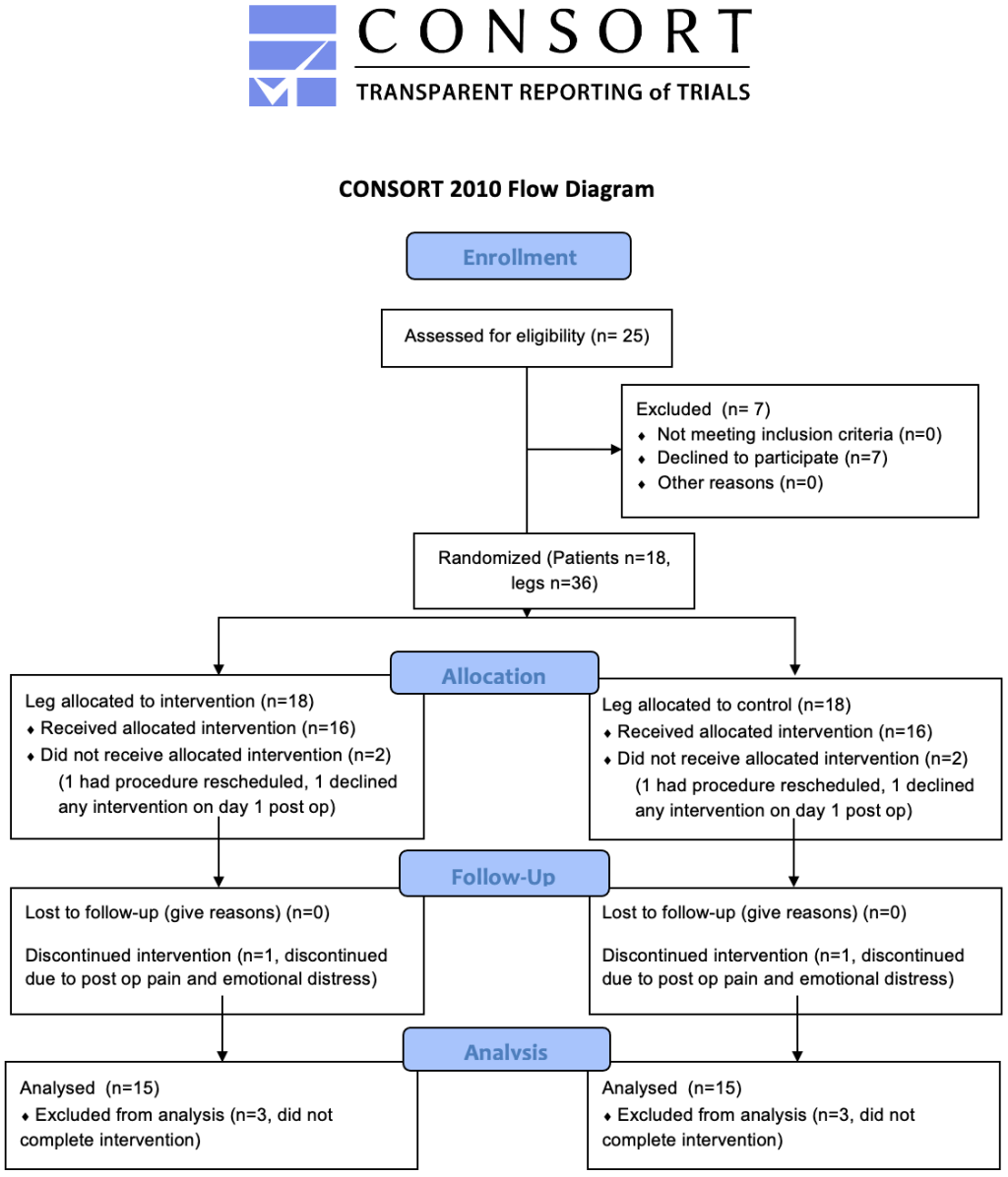

Figure 5.1: CONSORT diagram outlining participant recruitment, assessment and follow for this study

N	15
Male : Female	4:1
Age (years)	66 (±5.9)
BMI (kg/m^2)	28 (±4.0)

Table 5.1: Demographics of participants included in study

All patients had open major colonic resection; 13 for rectal cancer, one for hepatic flexure cancer in the presence of ulcerative colitis, and one for stricturing diverticular disease. Of the three patients who withdrew from the study, two had no baseline data collected, and the third received no NMES. It was therefore decided to exclude all of these participants from analysis.

5.3.1 Muscle mass, function and architecture

The was no significant difference between VL CSA of the CON (17.18cm^2 (± 2.85)) and STIM (16.48cm^2 (± 2.56)) legs preoperatively (p=0.16). By POD 5 VL CSA in the CON limb had decreased by 1.60cm^2 (± 0.54) to 15.58cm^2 (± 2.43), representing a loss of 9.16% (± 2.0). VL CSA in the STIM leg decreased to 16.06cm^2 (± 2.48) by POD 5, a change of -0.42cm^2 (± 0.2), representing a loss of 2.52% (± 1.07). CSA loss in the CON leg was significantly greater than in the STIM leg (p<0.001) (Figure 5.2).

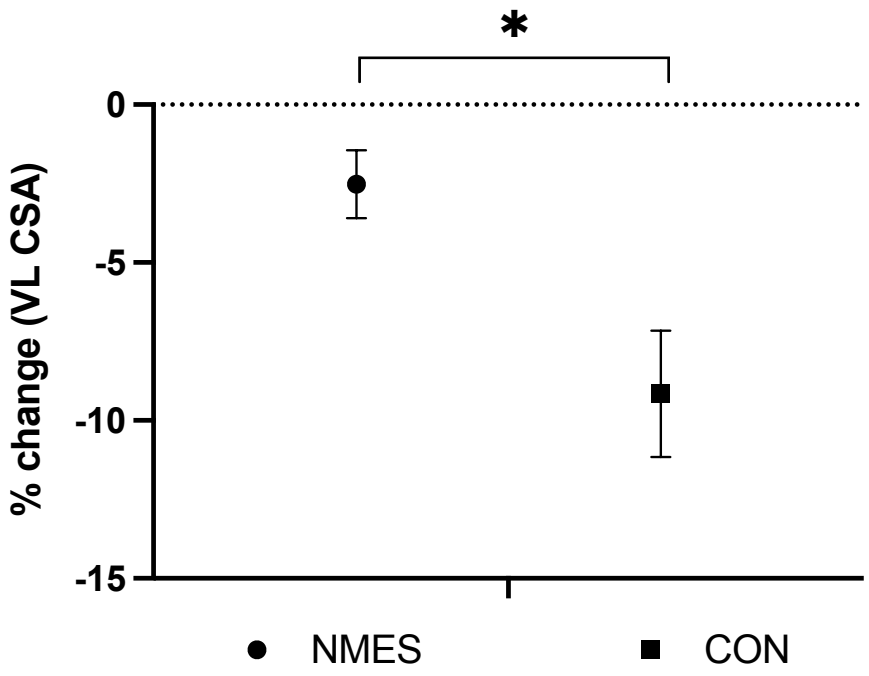

*Figure 5.2: Percentage change in vastus lateralis (VL) muscle cross sectional area (CSA) from baseline to postoperative day 5 (POD 5) with and without neuromuscular electrical stimulation (NMES). *=p<0.05. MD 1.18 (95% CI 0.75 to 1.61; p<0.001).*

The was no significant difference between VL MT of the CON (2.08 cm (± 0.30)) and STIM (2.06 cm (± 0.25)) legs preoperatively (p=0.74). By POD 5 VL MT in the CON limb had decreased by 0.17cm (± 0.13) to 1.91cm (± 0.26) (8.14% (± 5.93) loss). VL MT in the STIM leg decreased by 0.05cm (± 0.06) to 2.01cm (± 0.28) by POD5 (2.76% (± 3.56) loss). The losses in the CON leg were significantly greater than in the STIM leg (p<0.001) (Figure 5.3). There was no difference between legs pre or postoperatively and no difference in change between legs for FL or PA (Table 5.2).

	NMES D0	NMES POD5	Change	% Change		CON D0	CON leg POD5	Change	% Change		Comparison of losses
Fascicle length (cm)	7.77 (± 1.8)	8.32 (± 1.9)	0.55 (± 1.0)	7.69 (± 15.0)		7.61 (± 1.9)	8.58 (± 1.5)	0.97 (± 1.8)	16.51 (± 25.4)		MD -0.42 (95% CI -2.24 to 1.4; p=0.66)
Pennation angle (degrees)	14.58 (± 3.8)	13.43 (± 3.2)	-1.15 (± 3.5)	-4.26 (± 27.1)		15.48 (± 4.7)	13.28 (± 2.7)	-2.21 (± 3.8)	-8.84 (± 26.1)		MD 1.05 (95% CI -1.9 to 4; p=0.44)

Table 5.2: Vastus lateralis muscle architecture from day of surgery to postoperative day 5 (POD 5) with (STIM) and without (CON) neuromuscular electrical stimulation (NMES).

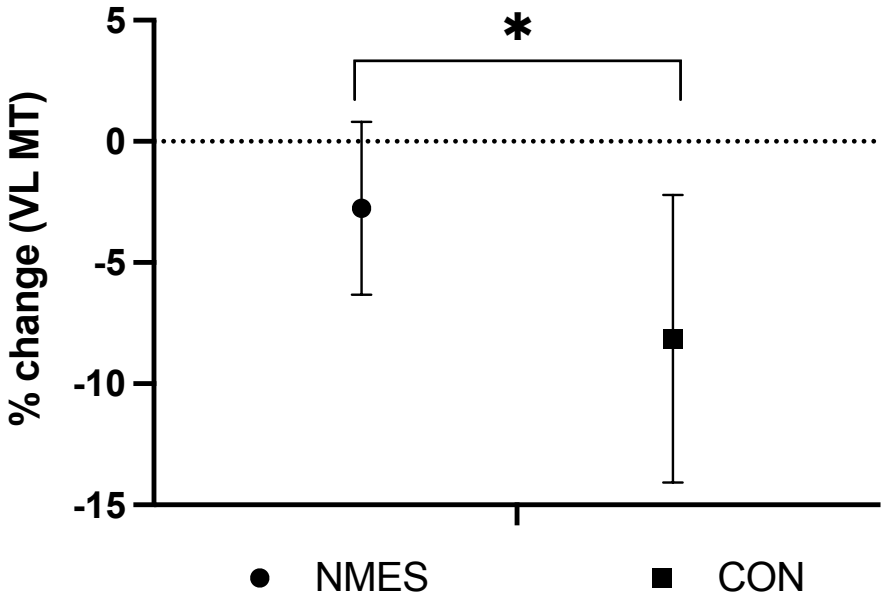

*Figure 5.3: Percentage change in vastus lateralis (VL) muscle thickness (MT) from baseline to postoperative day 5 (POD 5) with and without neuromuscular electrical stimulation (NMES). *=p<0.05. MD 0.12 (95% CI 0.04 to 0.2; p=0.001).*

There was no significant difference in KES between the CON (44.44lbs (± 8.15)) and STIM (44.7lbs (± 7.26)) legs preoperatively (p=0.93). By POD 5, KES in the CON limb had decreased by -9.30lbs (± 6.72) to 35.13lbs (± 6.56) (-19.69% (± 12.91)). STIM leg KES decreased by -4.82lbs (± 4.65) to 39.88lbs (± 6.71) (-10.35% (± 8.98) by POD 5. The decrease in the CON leg was significantly greater than in the STIM leg (p=0.03) (Figure 5.4).

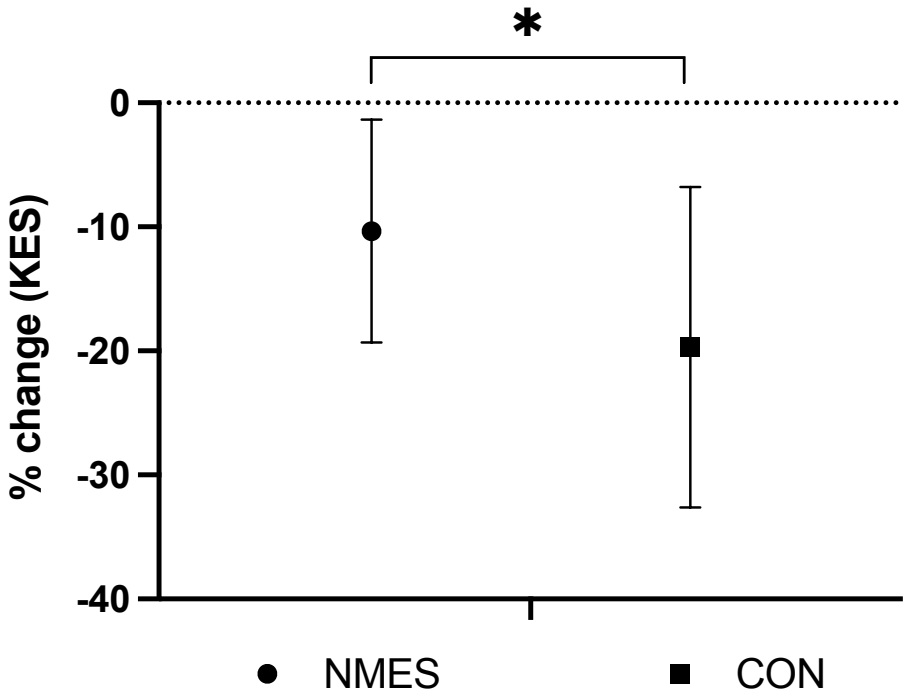

*Figure 5.4: Percentage change in knee extensor strength (KES) from baseline to postoperative day 5 (POD5) with and without neuromuscular electrical stimulation (NMES). *=p<0.05. MD 4.48 (95% CI 0.00 to 8.97; p=0.03).*

5.3.2 Postoperative physical activity

Although patients spent the majority of POD 1-4 in bed, time spent in bed was significantly less on POD 3 (18.7 hours (± 4.2)) and POD 4 (18.25 hours (± 4.9)) compared to POD 1 (23 hours (19.8 (±3.5)), both p<0.01). Numerically, time spent mobilising (15.45 (± 9.3) to 26.36 (± 21.1) minutes), p=0.06) and distance mobilised increased (30.0m (± 23.6) to 93.6m (± 166.6), p=0.11), but these changes did not reach statistical significance.

5.3.3 Postoperative dietary intake

Full dietary intake information was collected for 11 patients. Four patients developed postoperative ileus, beginning on POD 2 for one patient and POD 3 in the others. Mean daily energy intake was 536.6 (±527.8) kcal across POD 1-4, and 490.0 (±470.7), 505.0 (±251.8), 541.0 (±488.1), 610.5 (±900.5) kcal on each POD respectively. Despite a numerical increase day-on-day, there was no significant difference in energy intake between the days. Mean daily protein intake was 15.6 (±16.4) g across POD 1-4, and 13.9 (±14.1), 14.9 (±10.2), 15.0 (±15.6), 18.5 (±25.6) g on POD 1-4 respectively. No significant difference in protein intake was found between the days. This equated to a mean daily energy intake over POD 1-4 being just 25.3 (±24.9) % of the ESPEN recommendation, reducing to 12.8 (±2.6) % in patients with ileus. Mean daily protein intake was only 12.3 (±12.9) % of the ESPEN recommendations, and only 5.7 (±0.9) % for those with ileus. Overall, only one patient consumed the recommended energy intake (on POD 4). No patients managed to achieve over 50% of the recommended protein intake on POD 1-3, with just one patient achieving this (78%) on POD 4.

Collectively for all patients, the percentage of energy consumed as protein was 11.7 (±2.0) on POD 1, 12.3 (±4.9) on POD 2, 11.1 (±4.9) on POD 3, and 11.9 (±3.9) % on POD 4, indicative of a low protein diet across this period. Interestingly, compared to the day-on-day increase in energy intake across the post-operative period, the percentage of energy consumed as protein did not increase.

5.3.4 Patient preferences

Overall, 26.7% of patients stated that NMES caused no discomfort, whilst the remaining 73.3% reported it to cause slight discomfort. The median maximum time patients felt NMES would be tolerable for was 88.75 minutes per day, with answers ranging from 45 to 240 minutes.

5.4 Discussion

In this study, NMES significantly attenuated the loss of VL CSA, MT and KES following open colorectal resection in older adults. To our knowledge, this is the first time the effects of NMES on muscle function following major abdominal surgery have been reported, and the first time the effects on muscle mass have been reported in the current clinical environment (i.e., since the implementation of ERAS).

Although there is no comparable study of NMES after abdominal surgery in the current era, it is known that NMES reduces losses of muscle mass (Silva *et al.*, 2019) and function (Liu *et al.*, 2020) in ITU patients, and loss of muscle function following cardiac surgery (Sumin *et al.*, 2020). Our results, along with those of previous studies, suggest that NMES may represent a useful therapy to enhance current ERAS regimes in order to decrease muscle loss after surgery.

There is variability in reports of the efficacy of NMES on muscle 'health', with some meta-analyses reporting inconclusive evidence for its benefit in patients following, for example, orthopaedic surgery (Conley *et al.*,

2021) and ITU admission (Edwards *et al.*, 2014). This may be due to the significant heterogeneity in the administration of NMES observed across, and indeed sometimes within studies (Arpin *et al.*, 2019; Conley *et al.*, 2021). Variations include the length of time NMES is applied, the amplitude, frequency and/or pulse width of the stimulation within in a session, and the frequency of sessions. Stimulation site may also affect NMES effectiveness, with recent work showing that peripheral nerve stimulation recruits from a wider pool of motor units that conventional NMES (Inns *et al.*, 2021).

Dose-response studies are required to establish the optimum NMES delivery protocol to prevent losses of muscle mass and function in healthy immobilised, critically unwell, and postoperative patients, each of whom will likely have differing physiological responses to, and tolerance of, NMES. Encouragingly, in this study our NMES protocol was well tolerated, causing only mild or no discomfort, and all patients felt they would be able to tolerate longer periods. This is an important consideration if NMES is to be developed into an accepted clinical therapy.

The reductions in VL CSA and MT observed in our control limb are consistent with those previously described following major abdominal surgery. Although numerically the losses in this study appear to be larger than those previously reported, this may be due to differing disuse atrophy susceptibility of individual muscles (Bass *et al.*, 2021), with

previous studies often reporting whole body (Shimoda *et al.*, 2019), psoas (Otsuji *et al.*, 2017) or grouped quadriceps (Vinge *et al.*, 1996) changes. Muscle function decreases in the CON are also in keeping with previous studies showing loss of muscle strength (Henriksen *et al.*, 2002a) and a delay in return to normal walking activities (Fiore *et al.*, 2017) following major abdominal surgery. The greater loss of muscle function compared to muscle mass as seen in this study has been previously described with disuse (Suetta, 2017), and may be due to a decline in muscle quality as well as quantity (Goodpaster *et al.*, 2006b; Suetta, 2017) (see chapter 4). In the latest definition of sarcopenia from the European Working Group on Sarcopenia in Older People (EWGSOP) (Cruz-Jentoft *et al.*, 2019), muscle quality is described as “micro- and macroscopic aspects of muscle architecture and composition”, and although we did not see any changes in muscle architecture parameters (FL and PA) in either leg, other aspects of muscle quality such as myosteatosis (Correa-de-Araujo *et al.*, 2020) and neuromuscular connectivity (Guo *et al.*, 2021) may have contributed to the attenuation of functional declines observed with NMES. The underlying mechanisms of NMES-induced preservation of muscle mass and function in postoperative patients needs further exploration.

Despite this study being conducted on a background of ERAS, patients in our study had low levels of postoperative mobility and walked, on average, less than 100m by POD 4. As there is insufficient evidence to support specific values (Gustafsson *et al.*, 2019), ERAS guidelines do

not set any daily recommended activity level targets, leading to wide variability in practice. For example, ERAS patient information from one UK NHS trust advises a target of 250 steps on POD 1 increasing to 1250 steps on POD 4 (Kings College Hospital NHS trust, 2021); a target not met by the patients in this study. Our results are similar to a number of other studies which have also shown poor overall mobility following major abdominal surgery (Henriksen *et al.*, 2002a; Noro *et al.*, 2003; Haines, Skinner and Berney, 2013; Fiore *et al.*, 2017; Hussey *et al.*, 2019). This observation of low levels of post-operative mobility further supports the potential benefit of NMES to compensate for challenges in full adherence to ERAS.

Patients in our study also had poor postoperative nutritional intake and in particular consumed low levels of protein. The importance of the interaction between nutrition and muscle contractions in maintaining MPS rates is well established (Breen *et al.*, 2013), with adequate protein intake required to potentiate the effects of contractile activity on MPS (Breen and Phillips, 2012). It is therefore possible that combining NMES with optimised postoperative nutrition may increase its benefits in preventing loss of muscle mass and function. ERAS guidelines encourage recommencing early dietary intake in patients post major abdominal surgery, but with the aim of accelerated GI recovery, decreased hospital length of stay and decreased rate of complications and mortality (Carmichael *et al.*, 2017), rather than any beneficial effects on preservation of muscle mass and function. Although early dietary

intake is encouraged in ERAS (Carmichael *et al.*, 2017; Weimann *et al.*, 2017), patients often consume low protein food. In our study 65.9% of meals consumed were soup, jelly or ice cream over POD 1-4. It has been shown that it is possible to increase patients' calorific and nutritional intake through supplementation of normal food with high quality protein powder (Munk *et al.*, 2014). Development of protein enhanced forms of the foods most often consumed by postoperative patients should therefore be investigated. Further studies of NMES in postoperative patients should also seek to optimise patients' nutritional intake in order to maximise the potential benefits of NMES as a therapeutic intervention.

5.4.1 Limitations

Whilst the ability of NMES to significantly decrease the loss of muscle mass and function in postoperative patients demonstrated in this study is encouraging, it is recognised that the current results have several limitations. Although the VL is widely recognised as functionally important for locomotion and other activities of daily living, to bring the most meaningful benefit to patients it may be more beneficial to stimulate all the major muscle groups involved in locomotion. However, the effectiveness of NMES in other muscle groups, which may have different fibre composition and motor unit structure, will need to be investigated. The assessment of KES at POD5 may also have been affected by the patient's abdominal incision causing pain and reducing the ability to use core muscles to assist in force generation. Steps were taken to minimise this by ensuring that patients legs were fully supported to the knee joint,

the patients used their arms braced against the bed for support, and that patients had sufficient analgesia before performing the test. Whilst this may have had some influence on the total reduction in KES at POD5, as both legs were observed under the same circumstances this is unlikely to have any effect on the differences observed between legs. However, in future studies (not affected by COVID restrictions) it would be beneficial to test patients muscle function in a controlled lab environment rather than at the bedside. Furthermore, whilst we demonstrated the effectiveness of NMES in reducing losses of KES, the ability of postoperative NMES to preserve whole leg muscle function, including those associated with independence maintenance and activities of daily living (e.g., standing, walking and balance), remains to be determined.

5.5 Conclusion

In conclusion, this study has demonstrated the ability of NMES to attenuate loss of mass and strength in the functionally important quadriceps muscle. Further studies are now required to optimise NMES and establish the practicality and utility of bilateral whole leg NMES in patients following major abdominal surgery.

6 Discussion

Skeletal muscle is often overlooked as a vital organ, but maintenance of muscle mass and function is essential for continued vitality and independence into older age, as well as being linked to whole body health and overall survival. It is, of course, well known that reduced muscle activity leads to a reduction in muscle mass and strength. However, the timecourse of these changes and the degree to which atrophy varies between muscles are still not fully characterised. Development of effective, clinically suitable, therapeutic interventions to attenuate the loss of muscle due to immobilisation is also required.

In the systematic review (chapter 2) it was identified that despite the large amount of research into DMA, limited evidence was available to characterise its timecourse, especially over the first two weeks of immobilisation. However, rates of DMA appeared to slow over the period of immobilisation, with this finding being consistent across healthy, critically unwell, and ankle fracture patients. Rates of disuse appeared to be greatest during the first two weeks, but there was insufficient data to characterise the timecourse of DMA over this period. Furthermore, it appeared that rates of atrophy vary by muscle, although limited evidence was available for many muscles. Further work was therefore required to characterise the timecourse of DMA over the first two weeks of immobilisation and identify any evidence of differential rates of atrophy between muscles.

In order to investigate these findings further, in the next two chapters young, healthy men underwent unilateral lower limb immobilisation (ULLI), using a knee brace fixed at 75^{O} flexion, and ankle air boot. The advantage of this experimental design is that it allows the participant's other leg to act as their own control, perfectly matched for demographic factors as well as hormonal, dietary and activity levels over the period of immobilisation. Furthermore, ULLI also allows participants to mobilise (using crutches) and continue to be involved in normal daily activities. This not only reduces the inconvenience that taking part in an immobilisation study may usually involve, but also mitigates the adverse health consequences associated with whole body immobilisation.

Our first experimental study (chapter 3) characterised the changes in muscle mass of the vastus lateralis (VL), medial gastrocnemius (MG) and tibialis anterior (TA) over the first two weeks of immobilisation. It was demonstrated that loss of muscle mass occurs very rapidly, with a 4% reduction in VL cross sectional area (CSA) after just 3 days, and changes reaching statistical significance after five days in both VL (-5.5%) and MG (-4.9%). Although there was no statistically significant variation in the timecourse of DMA identified over the first 15 days of immobilisation, there was a trend towards more rapid loss of muscle mass during the first week. Furthermore, no significant change in muscle mass was identified in TA, suggesting that this muscle is atrophy resistant (aR) in response to immobilisation. The mechanisms controlling DMA are not fully understood, and the presence of both aR and atrophy susceptible (aS)

muscles in the lower leg provides an ideal model for further investigation of these processes.

Whilst our study was ongoing, a paper was published by Kilroe at al, which also demonstrated very rapid loss of muscle mass, with a statistically significant change in total thigh muscle volume (Vol) identified after just two days of immobilisation, and a 6.7% reduction in quadriceps Vol after seven days of immobilisation. Furthermore, this study identified greater losses of muscle mass in the quadriceps muscle group than in the hamstrings, suggesting variation in atrophy sensitivity between muscles (Kilroe *et al.*, 2019). These findings complement those of our study and confirm the rapid timecourse of DMA during the first two weeks of immobilisation, as well as the presence of aS and aR muscles.

During this study we also validated the use of ultrasound (US) measured CSA and muscle thickness (MT) against the gold standard of MRI derived Vol/CSA. US CSA had strong correlation and concordance with MRI measures in both VL and MG, with good to excellent levels of agreement, and high sensitivity and PPV for detection of atrophy. These findings suggest that US CSA can be used to accurately identify and assess the rate of DMA in the aS muscles of MG and VL. This is an important finding for clinical studies, where access to MRI scanners may be impractical (e.g. after surgery) or potentially unsafe (e.g. critically unwell patients), and the effectiveness of potential therapeutic interventions in these settings can therefore be monitored using US CSA.

In chapter 4, the functional effects associated with aDMA were investigated in healthy immobilised individuals. There was a significant decrease in all aspects of mechanical muscle function after two weeks of immobilisation. Muscle function is lost to a greater extent than muscle mass over the same period of immobilisation with a 20-30% reduction in MVC observed after two weeks. This chapter significantly increased the available evidence for characterisation of changes in muscle function in muscles of the lower leg in response to immobilisation, and for the first time we demonstrated that differential rates of decline in muscle function mirror the aRaS picture identified in the response of muscle mass to immobilisation. A decline in muscle oxidative capacity was also identified after two weeks of immobilisation. This, coupled with the more rapid loss of function compared to mass, suggests that functional changes secondary to immobilisation are likely due to complex, multifaceted and as yet not fully characterised muscle remodelling. The aRaS model provides an ideal method for further investigation to clarify the processes underlying these changes. Furthermore, the rapid and severe loss of muscle function identified in this chapter highlights the need for early targeted interventions and treatments in individuals with reduced mobility (e.g. after surgery).

As demonstrated in the systematic review in chapter 2, rates of muscle atrophy are much more rapid when muscle disuse is accompanied by another catabolic driver, such as inflammation or malnutrition. In patients

recovering after major abdominal surgery, immobility, inflammation and poor nutrition are all likely to be present and decreases in muscle mass and function in this patient group can therefore be expected to be severe.

The split body randomised control trial (RCT) reported in chapter 5, was conducted to characterise the changes in muscle mass and function during the first five days after major abdominal surgery, and test the efficacy of neuromuscular electrical stimulation (NMES) in attenuating the loss of muscle mass and function. As with ULLI, the split body design of this RCT allowed the participant's other leg to act as their own control, and so there was perfect matching for demographic, operative and postoperative factors between the control and intervention limb. This study demonstrated that rates of DMA are approximately twice as fast in postoperative patients as in healthy immobilised individuals with a 9.16% reduction in VL CSA and 19.7% decrease in knee extensor by postoperative day (POD) 5. However, twice daily NMES reduced the rate of aDMA and functional decline in postoperative patient's VL by 50%. This is a significant finding and is the first time the effectiveness of NMES in reducing loss of muscle function and mass in patients after major abdominal surgery has been reported in the modern clinical setting. The effectiveness of NMES in this patient group is very encouraging, and further studies must be completed in order to develop and test the effectiveness of bilateral, whole leg NMES in patients after major abdominal surgery.

There is variability in reports of the efficacy of NMES on muscle health in the literature, with some meta-analyses reporting inconclusive evidence for its benefit in patients following surgery (Conley *et al.*, 2021) and ITU admission (Edwards *et al.*, 2014). However, these findings are likely due to significant heterogeneity in the administration of NMES across, and sometimes within, studies (Arpin *et al.*, 2019; Conley *et al.*, 2021). As would be the case during the development of any drug based therapeutic intervention, dose-response studies are required to establish the optimum NMES delivery protocol.

Conclusion

This thesis has characterised the timecourse of acute DMA in muscles of the leg over the initial two weeks of immobilisation. It has revealed rapid significant reductions in muscle mass and function, and variation in rates of atrophy between aR and aS muscles of the leg. This aR/aS model can be utilised in future ULLI studies to investigate and identify the mechanisms controlling muscle atrophy and loss of muscle function. Furthermore, we have demonstrated the effectiveness of NMES in reducing loss of muscle mass and function in patients after major abdominal surgery. Further studies should be conducted to investigate the optimum delivery of NMES, and to develop and test the effectiveness of bilateral, whole leg NMES in postoperative patients.

7 Appendices

Appendix A

Example search strategy

#	Database	Search term	Results
1	Medline	LEG/	61949
2	Medline	exp "HAMSTRING MUSCLES"/	351
3	Medline	exp "MUSCLE, SKELETAL"/	255196
4	Medline	exp MUSCLES/	646327
5	Medline	*ATROPHY/ OR *"MUSCULAR ATROPHY"/	9757
6	Medline	exp ATROPHY/ OR exp "MUSCULAR ATROPHY"/	42154
7	Medline	exp TIME/ OR exp "TIME FACTORS"/	1349631
8	Medline	(2 OR 3 OR 4)	646327
9	Medline	(1 AND 8)	9954
10	Medline	(5 OR 6)	42154
11	Medline	(9 AND 10)	248
12	Medline	(7 AND 11)	21
13	Medline	exp IMMOBILIZATION/	25990
14	Medline	exp "MUSCULAR ATROPHY"/	12810
15	Medline	exp *"MUSCULAR ATROPHY"/	8595
16	Medline	(14 OR 15)	12810
17	Medline	(1 AND 13 AND 16)	17

18	Medline	(7 AND 17)	1
19	Medline	11 [Human age groups Adult OR Aged] [Humans]	175
20	Medline	12 [Human age groups Adult OR Aged] [Humans]	17
21	Medline	17 [Human age groups Adult OR Aged] [Humans]	9
22	Medline	18 [Human age groups Adult OR Aged] [Humans]	1
23	Medline	((((leg OR thigh OR calf OR lower limb) AND muscle) AND atrophy) AND (immobil* OR inactiv* OR reduced activity OR bed rest)).ti,ab	162
24	Medline	((((leg OR thigh OR calf OR lower limb) AND muscle) AND atrophy) AND (immobil* OR inactiv* OR reduced activity OR bed rest)).ti,ab,af	251
25	Medline	exp "LOWER EXTREMITY"/ OR exp LEG/ OR exp THIGH/	155966
26	Medline	(13 AND 14 AND 25)	35
27	Medline	23 [Human age groups Adult OR Aged] [Humans]	77
28	Medline	24 [Human age groups Adult OR Aged] [Humans]	136
29	Medline	26 [Human age groups Adult OR Aged] [Humans]	19
30	Medline	exp "BED REST"/	3801

31	Medline	(1 AND 14 AND 30)	16
32	Medline	(7 AND 31)	4
33	Medline	31 [Human age groups Adult OR Aged] [Humans]	12
34	Medline	32 [Human age groups Adult OR Aged] [Humans]	3

Appendix B

Health history questionnaire

Name or Number .....

Please complete this brief questionnaire to confirm fitness to participate:

1. **At present,** do you have any health problem for which you are:

(a) on medication, prescribed or otherwise Yes ☐ No ☐

(b) attending your general practitioner Yes ☐ No ☐

(c) on a hospital waiting list Yes ☐ No ☐

2. **In the past two years,** have you had any illness which require you to:

(a) consult your GP Yes ☐ No ☐

(b) attend a hospital outpatient department Yes ☐ No ☐

(c) be admitted to hospital Yes ☐ No ☐

3. **Have you ever** had any of the following?

(a) Convulsions/epilepsy Yes ☐ No ☐

(b) Asthma Yes ☐ No ☐

(c) Eczema Yes ☐ No ☐

(d) Diabetes Yes ☐ No ☐

(e) A blood disorder Yes ☐ No ☐

(f) Head injury Yes ☐
No ☐

(g) Digestive problems Yes ☐
No ☐

(h) Heart problems Yes ☐
No ☐

(i) Problems with bones or joints Yes ☐
No ☐

(j) Disturbance of balance / coordination Yes ☐
No ☐

(k) Numbness in hands or feet Yes ☐
No ☐

(l) Disturbance of vision Yes ☐
No ☐

(m) Ear / hearing problems Yes ☐
No ☐

(n) Thyroid problems Yes ☐
No ☐

(o) Kidney or liver problems Yes ☐
No ☐

(p) Allergy to nuts, alcohol etc. Yes ☐
No ☐

(q) Any problems affecting your nose e.g. recurrent nose bleeds Yes ☐
No ☐

(r) Any nasal fracture or deviated nasal septum Yes ☐
No ☐

4. **Has any,** otherwise healthy, member of your family under the age of 50 died suddenly during or soon after exercise? Yes ☐
No ☐

5. Are there any reasons why blood sampling may be difficult? Yes ☐
No ☐

6. Have you had a blood sample taken previously? Yes ☐ No ☐

7. Have you had a cold, flu or any flu like symptoms in the last Month? Yes ☐ No ☐

Women only (delete if not applicable)

(xiii) *8. Are you pregnant, trying to become pregnant or breastfeeding?*

Yes ☐ *No* ☐

If YES to any question, please describe briefly if you wish (e.g. to confirm problem was/is short-lived, insignificant or well controlled.)

.....
.....
.....

Appendix C

Termination criteria for CPET

Signs and symptoms indicating the need for immediate termination of CPET as defined by the ATS guidelines (American Thoracic Society and American College of Chest Physicians, 2003):

- Chest pain or tightness
- Faintness
- Sudden pallor
- Loss of co-ordination
- Confusion
- Dizziness
- Signs of respiratory failure
- Palpitations
- Ischaemic changes (>2mm ST depression with symptoms or >3mm without symptoms) on ECG
- Arrhythmias on ECG (prolonged runs of multiple ectopic heart beats or heart block)
- Blood pressure drops by greater than 20mmHg from previous reading
- Systolic blood pressure >250mmHg or diastolic >120mmHg
- Sudden onset bradycardia with a drop in heart rate of >20bpm from previous reading
- Desaturation with a SpO2 <80%

Appendix D

Postoperative Dietary Intake Questionnaire

Participant ID:_____

Date: _____ / _____ / _____

Postoperative Day: 1 / 2 / 3 / 4 / 5 (please circle as appropriate)

Please record all food and drink that you have had in the last 24 hours

(Record specifics of the food and approximate quantities – e.g. half a bowl of soup)

Breakfast

Lunch

Dinner

Snacks / other food

Drinks

To be completed by researcher:

Record any prescribed nutritional support not described above (e.g. Fortisip, NG feed etc)

Postoperative level of activity questionnaire

Participant ID:_____

Date: _____ / _____ / _____

Postoperative Day: 1 / 2 / 3 / 4 / 5 (please circle as appropriate)

In the last 24 hours, how many hours did you spend…	
Asleep	hours
Awake in bed	hours
Sitting out in a chair	hours
Standing	hours
Walking	hours

If you were able to walk in the last 24 hours, how far did you walk:

Please provide distance with units (e.g. 10 meters). If you are not sure of the distance you can describe it (eg – 1x whole length of the ward)

In the last 24 hours, what proportion of the following activities were you able to complete without assistance:

	(circle as appropriate)
Getting washed	**None / some / all**
Getting dressed	**None / some / all**
Eating meals	**None / some / all**

Appendix E

Summary of correlation between % change in muscle mass and measurements of muscle function after 15 days immobilisation.

Measurement	r	p
Compared to % change VL CSA		
Power (Notts rig)	0.14	0.74
Jump power	0.72	0.07
COP (distance moved)	-0.59	0.17
1RM	0.49	0.22
Compared to % change MG CSA		
MVC	-0.21	0.62
Jump height	0.30	0.63
Jump power	0.34	0.45
COP (distance moved)	-0.33	0.47
Compared to % change in TA CSA		
MVC	0.64	0.07
Jump height	0.85	0.07
Jump power	0.63	0.13
COP (distance moved)	0.75	0.06

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