1-1-2012

The Effects of Prehabilitation on the Outcome of Anterior Cruciate Ligament Reconstruction

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February 2012

This thesis is submitted to the Royal College of Surgeons in Ireland for the degree of Doctor in Medicine in the Postgraduate School

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To my dearest wife

'In the attitude of silence the soul finds the path in a clearer light, and what is elusive and deceptive resolves itself into crystal clearness. Our life is along and arduous quest after Truth'

-Mahatma Ghandi-

Acknowledgments

I would like to thank:

(1) My supervisors **Prof. O'Byrne, Prof. Moyna, and Mr. Moran** for all their support with this research and the constant reviewing of the protocols and editing of the manuscript.

(2) Mr. Gavin McHugh - Registrar in Trauma & Orthopaedic Surgery. Mr. McHugh assisted in initialising the set-up of the research.

(3) Ms. Alison Quinn & Ms. Eilis Dolan - Head of Physiotherapy, Sports Surgery Clinic Dublin. Their department was principally involved in performing the isokinetic studies and their subsequent analysis.

(4) Professor Steve Eustace - Consultant Radiologist Cappagh National Orthopaedic Hospital. Professor Eustace assisted in analysing the MRI scans.

(5) Dr. Donal O'Gorman - Lecturer in Health and Human Performance, Dublin City University (DCU). Dr O' Gorman has a specialist interest in gene expression and muscle physiology and accommodated the use of the laboratory in DCU.

(6) Christopher O'Hare - Final year DCU student whose dedication and time commitment to this project has been invaluable.

Others: To orthopaedic registrars Dr. Rob-Bruce Brand, Dr. Joshua Ong and Dr. Ray Walls who paved the way for future research in prehabilitation.

To Criona Tobin, Paul O'Connor, and Javier Monedero of DCU who supervised the labwork.

To radiographers Annette White, Mark and Christine in Cappagh Hospital for accommodating all the necessary MRI scans at short notice.

To Prof. David Beard, University of Oxford who lent his D. Phil thesis titled 'Hamstring contraction latency following anterior cruciate ligament rupture, reconstruction and rehabilitation' for referencing purposes.

Presentations and publications relating to this body of work include:

1. Acceptance as an original research article in the American Journal of Sports Medicine for Volume 41, Issue 9 (PMID 23845398 - Epub ahead of print)

2. Review article titled 'Prehabilitation: The Void in the Management of Anterior Cruciate Ligament Injuries – A clinical review. ISRN Rehabilitation, vol.2012, ID 938974, doi:10.5402/2012/938974

3. Cappagh Prize for the Best Research Paper for Oral Presentation, August 2012

4. Oral presentation for the Irish Orthopaedic Association Meeting, June 2012

5. Poster discussion for the International Society of Arthroscopy, Knee Surgery and Orthopaedic Sports Medicine (ISAKOS), Toronto, May 2013

Declaration

I declare that this thesis, which I submit to RCSI for examination in consideration for the award of a higher degree Doctorate of Medicine, is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research programme this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original, and to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

Signed 

Student Number: 99447

1st submission date: 31st January 2012

Revised thesis submission: 18th December 2012

2nd revised thesis submission: 22nd April 2013

3rd revised thesis submission: 21st June 2013

Final approved thesis submission: 26th July 2013

Contents

Abbreviations and Definitions

Akt	also known as Protein Kinase B (PKB), is a serine/threonine-specific protein kinase that plays a key role in multiple cellular processes such as glucose metabolism, transcription and cell migration
Alpha motor neurons (α-MNs)	large lower motor neurons of the brainstem and spinal cord. They innervate extrafusal muscle fibres of skeletal muscle and cause it to contract
AMB	anteromedial bundle
ACL	anterior cruciate ligament
ACLR	anterior cruciate ligament reconstruction
Concentric	contraction in which the muscles shorten while generating force
COV	coefficient of variance. A measure of dispersion of a distribution. In isokinetic testing, this refers to how much a set of repetitions varies from the mean and give a value of the consistency of the results
CSA	cross-sectional area

Eccentric	the muscle elongates while under tension due to an opposing force being greater than the force generated by the muscle.
ELISA	Enzyme-linked immune-sorbent assay is a test that uses antibodies and colour change to identify proteins labelled with luminescence-substrate
Gamma motor neurons (γ-MN)	efferent fibres of the fusimotor system. Provides proprioceptive feedback for the movement, position and extension of muscles
ICC	intraclass correlation coefficient. A measure of the conformity of a data set when it has multiple groups
IGF-1	Insulin-like Growth factor-1. A polypeptide with significant sequence similar to insulin that acts as a potent hypertrophy stimulant in response to Growth Hormone
Isokinetic	muscle contraction at a constant angular velocity but varying resistance
Isometric	static muscle contraction that does not involve change in muscle length

Isotonic	muscle contraction involving change in length of the fibres
MAFbx	muscle atrophy F-box protein. A gene that encodes specific ubiquitin ligases which leads to protein degradation
MGF	Mechano-growth factor. An isoform of IGF formerly named IGF-IEc which may play an important role in satellite cell activation
MHC	Myosin Heavy Chain. A component of the key myofibrillar protein myosin
Modified Cincinnati Knee Rating Score	disease-specific questionnaire for ACL injuries
MuRF-1	Muscle specific ring finger-1. A gene that encodes specific ubiquitin ligases which leads to protein degradation
Peak torque	the highest muscular force output at any moment in a repetition
PLB	posterolateral bundle
RT-PCR	Reverse transcription polymerase chain reaction. A technique used to amplify a defined amplicon from a messengerRNA (mRNA)

SDS-PAGE	Sodium dodecylsulfate polyacrylamide gel electrophoresis. A technique used to separate proteins according to their electrophoretic mobility
Tegner Lysholm Knee Score	disease-specific questionnaire for ACL injuries
Tegner Activity Level Score	scoring system for subject activity level prior to and after injury

Hypothesis

Anterior cruciate ligament reconstruction (ACLR) reduces instability in the knee joint however many patients fail to make a full functional recovery [1], even years following the procedure [2]. One of the major obstacles to full recovery and return to sport activities postoperatively is residual weakness in the quadriceps muscle [3].

We propose that a preoperative exercise programme or "prehabilitation", will lead to significant quadriceps hypertrophy manifest by an increase in peak torque on isokinetic testing and cross-sectional area (CSA) on magnetic resonance imaging (MRI). Postoperatively, patients will undergo less quadriceps atrophy due to improved baseline and will have higher strength and functional levels maintained at 3 months after surgery. Meanwhile on a molecular level, we hypothesise down-regulation of genes involved in muscle atrophy such as MuRF-1 and MAFbx and the up-regulation of the IGF-1 gene involved in muscle hypertrophy. Myosin Heavy Chain (MHC) expression will change into less fatigable isoforms in individuals receiving prehabilitation before ACLR.

Aims

Primary aims

(1) To determine the effects of a preoperative exercise programme on 2 primary outcomes:

a) functional performance test using the single leg hop test

b) isokinetic quadriceps strength

(2) To identify the effects of preoperative exercise programme on:

a) quadriceps MRI CSA

b) disease-specific questionnaires using the Modified Cincinnati Knee Rating System and Tegner-Lysholm Score

c) IGF-1 gene expression

d) atrophy genes MuRF-1 and MAFbx expression

e) MHC isoform expression

Secondary aims

(1) To determine the effects of a preoperative exercise programme on:

a) isokinetic hamstring strength

b) individual muscle fibre MRI CSA

c) subgroups of disease-specific questionnaire

d) cross-section histology of vastus lateralis

Abstract

Introduction Anterior cruciate ligament injury results in quadriceps femoris atrophy. The effects of atrophy can persist after 5 years post anterior cruciate ligament reconstruction (ACLR) and can prolong rehabilitation [4]. Prehabilitation has been defined as preparing an individual to withstand a stressful event through enhancement of functional capacity before surgery [5]. We hypothesise that a preoperative exercise programme would enhance postoperative recovery.

Aims To determine the effects of a 6-week lower limb strengthening and proprioceptive training programme prior to ACLR on lower limb strength and function, muscle cross-sectional area (CSA) and self-reported assessment at baseline, preoperative and 12-week postoperative period. To identify alterations in gene and protein expression involved in muscle hypertrophy and atrophy pathways at the same time points.

Methods 22 volunteers awaiting ACLR were randomly assigned to a control or exercise intervention group. The exercise group completed a supervised 6-week gym and home based exercise programme. Postoperatively, all patients had a standard physiotherapy programme for 12 weeks. Assessments were completed at baseline, preoperatively and 12-week postoperatively. Assessments for primary outcomes include the single leg hop test and isokinetic dynamometry (N=11 for each group). Other assessments such as MRI quadriceps and hamstring CSA, in-line lunge test, Modified Cincinnati Knee Rating System and Tegner-Lysholm Knee Score were also measured (N=11 and 9 in exercise and control group respectively). A percutaneous muscle biopsy of the vastus lateralis muscle was performed at

the same time points under either local or general anaesthetic (during ACLR). IGF-1, MuRF-1 and MAFbx mRNA expression was determined with qRT-PCR. Myosin Heavy Chain (MHC) isoform expression was determined with SDS-PAGE and qRT-PCR.

Results Following 6 weeks of exercise intervention, the single leg hop test improved significantly in the exercise-injured limb compared to baseline and controls (p=0.001, p=0.046 respectively). Quadriceps strength in the injured limb had improved with similar gains in CSA compared to baseline (p=0.001). The vastus medialis CSA was also increased compared to controls (p=0.015). The Modified Cincinnati Knee Rating System was also better compared to baseline.

At 12-week postoperatively, the decline in the single hop test was reduced in the exercise group compared to controls (p=0.001). Similar trends were seen for the quadriceps strength and CSA but were not statistically significant. The vastus medialis CSA had regressed to similar levels as the control group (p=0.008). The Modified Cincinnati Knee Rating System score continued to increase in the exercise group compared to controls (p=0.004).

The expression of IGF-1 gene was significantly increased after the exercise intervention (p=0.028), decreasing back to baseline 12-week postoperatively (p=0.012). MuRF-1 gene expression was decreased after intervention compared to baseline (p=0.05) but increased at 12-week postoperatively (p=0.03). MAFbx levels did not change significantly in either group and within each time point. On mRNA level, there was a shift from MHC-IIx isoform to MHC-IIa after exercise, with significant changes compared to controls preoperatively (p=0.03). Protein testing was only able to reproduce this increment for MHC-IIx isoform expression only.

Conclusion 6-week progressive prehabilitation programme for subjects undergoing ACLR is safe and led to improved knee function, quadriceps strength and CSA, and self-reported assessment pre- and postoperatively. This study supports prehabilitation as an important consideration for patients awaiting ACLR.

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Chapter 1

Introduction

1.1 An introduction on anterior cruciate ligament injury

The anterior cruciate ligament (ACL) is an important stabilising ligament in the knee as it attaches the femur to the tibia in the middle of the knee joint. It prevents excessive forward movement of the tibia in relation to the femur. An ACL injury is the over-stretching or tearing of the ACL in the knee. The injury is characterised by a direct or indirect trauma to the knee which causes joint instability that leads to pain, decreased activity and function, poor knee-related quality of life and an increased risk of osteoarthritis of the knee. ACL reconstruction surgery (ACLR) and rehabilitation are important for the management of ACL injury.

1.1.1 Anatomy of the knee

The ACL is one of the 4 main ligaments in the knee (Figure 1). Embryologically, the ACL is developed by the condensation and differentiation of the blasternal tissue of the interchondral disc which occur from the sixth to the eighth week of intrauterine life [6,7]. The ligament is surrounded by a fold of synovium that originates from the posterior capsule

of the knee joint. Therefore, although the ACL is located intra-articularly, it is developmentally an extra-synovial structure.

Macroscopically, the mature ACL originates from the medial aspect of the lateral femoral condyle in the intercondylar notch and inserts into the interspinous area of the tibia [8] (Figure 2). The ACL is composed of the anteromedial bundle (AMB) which is tight in flexion and limits the anterior translation of the tibia relative to the femur. The posterolateral bundle (PLB) is tight in extension and limits anterior translation of the tibia, but also assists in limiting external rotation [9]. The average ACL length is 38mm with an average width of 11mm [10].

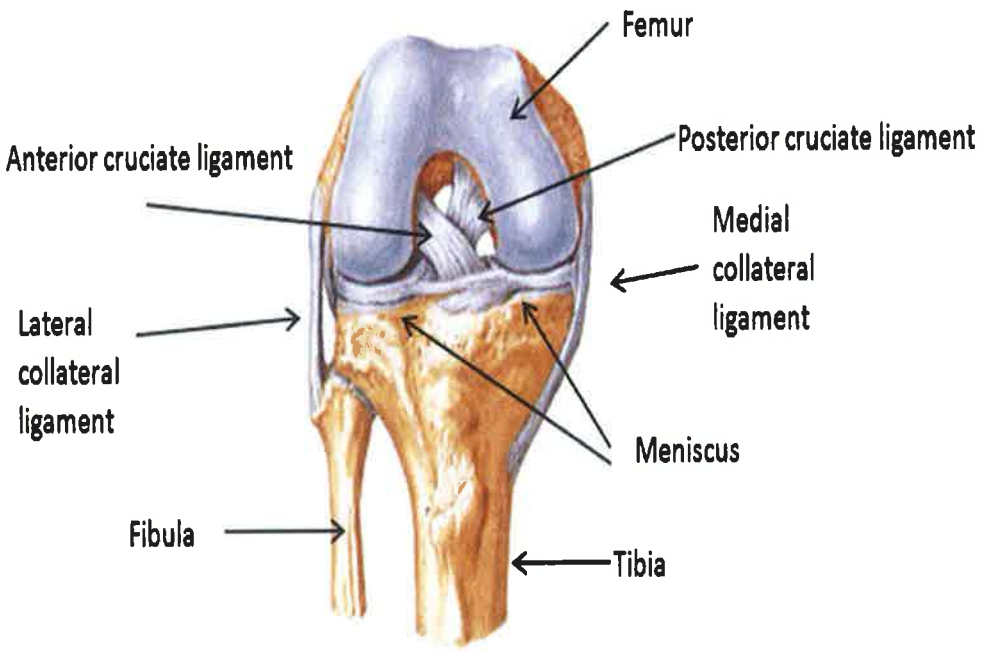


Figure 1: Anatomy of the knee (Adapted from Netters atlas)

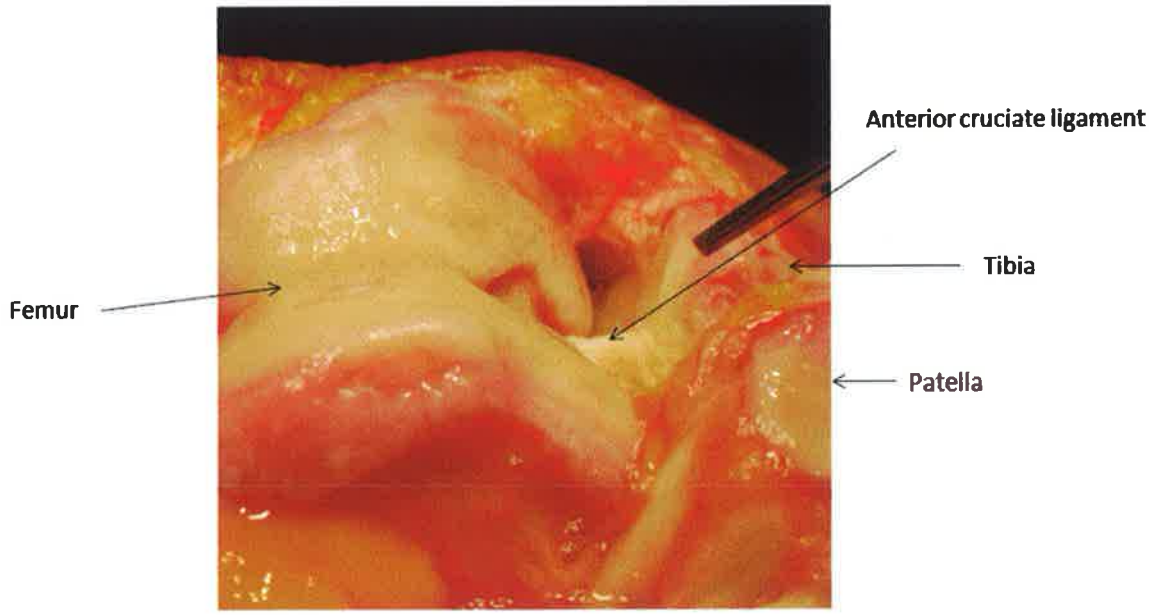


Figure 2: Intra-operative lateral oblique view of the knee in 90° flexion showing the insertion of the ACL into the interspinous area of the tibia

On a microscopic level, the ACL is composed of multiple bundles of mainly collagen units arranged in fascicles. The proximal part of the ligament is the most cellular with abundance of round and ovoid cells. The mid-substance has a high percentage of collagen with low cellularity and elongated spindle shaped fibroblasts. The distal portion is the most solid part of the structure which is rich with chondroblast and ovoid fibroblasts but low in collagen bundles [11,12].

The ACL is innervated by the posterior articular branch of the tibial nerve. The nerve fibres penetrate the posterior joint capsule and accompany the adjacent synovium and vasculature to reach as far as the infrapatellar fat pad [13]. Most of these fibres have a

vasomotor function. However, several small myelinated and unmyelinated fibres are present within the ligament in isolation and are independent of the blood supply. These receptors include Ruffini, Vater-Pacini, and Golgi-like tension receptors, as well as free nerve endings [13,14]. The former three tension receptors are mechanoreceptors that are important in proprioception and provide important signalling pathways during knee postural changes [15]. Direct neural pathways from the ACL to spinal ganglia by the use of tracer technique and axonal transportation have been demonstrated [16]. Together with visual and vestibular input, the input from mechanoreceptors in skin, muscles, tendon, ligaments and joint capsules provide the central nervous system with information about limb position and enable postural control. The relative contribution of each anatomic receptor population is however, still unclear [16].

Sherrington et al, first suggested that 'muscle sense' contributes in determining the position of the joint and knee movement as well [15,16]. The term 'kinaesthesia' refers to the ability to sense the position and movement of our limbs and trunk. The principle muscle receptor in kinaesthesia is the muscle spindle. Muscle spindles are found embedded in extrafusal muscle fibres, composing of 3-12 intrafusal muscle fibres which include both the primary and secondary endings (Group Ia and II sensory fibres respectively) (Figure 3). The different roles of these sensory endings were discovered in the lab of PBC Matthews. When a muscle is stretched, the primary sensory fibres of the muscle spindles respond to changes in muscle length and velocity, transmitting this activity to the spinal cord in the form of changes in the rate of action potentials. Primary endings respond to the size of a muscle and its speed [17]. They are therefore believed to contribute both to the sense of limb position and movement. Secondary endings do not have pronounced velocity sensitivity and signal

only the length changes itself, therefore contributing only to the sense of position [18]. Easily excitable in isolation both by small sharp tendon taps (due to high dynamic sensitivity) and weak electrical stimuli (due to a low electrical threshold because of their larger size), the Group Ia afferents underlie the reflex response to untoward mechanical events such as stumbling [19]. In contrast, the Group II afferents require larger stimuli, whether electrical or mechanical, and can only be activated along with other afferents.

The enhanced role of muscle spindles in signalling changes in limb position was demonstrated using muscle tendon vibration [20]. With high frequency and low amplitude vibration applied to biceps and triceps tendons of the preferred arm, this increased the firing rate of primarily Group Ia muscle spindles. During stimulation, participants were then asked to use the opposite arm to indicate either the position, or speed and direction, of the vibrated arm. As a consequence of the vibration-induced increase in muscle spindle firing rate, the illusory effects in the perception of both elbow joint position and velocity were shown which were consistent with perceived lengthening of the vibrated muscle. Since vibration provides a powerful stimulus for the primary endings of the muscle spindles, this illusion of movement may be attributed to the increase in Group Ia discharge being treated by the sensorium as if it were due to muscle stretch. Various controls established that the effects do depend upon the excitation of muscle receptors rather than of cutaneous or joint receptors. These studies concluded that signals from muscle mechanoreceptors can influence consciousness and do contribute to the subjective awareness of limb position [21].

The stimulation of functionally single fusimotor fibres also has different effects [22]. One fibre type, the 'dynamic fusimotor fibres' has been shown to excite the spindle while

the muscle was held at constant length but also selectively increase the dynamic component of the response to a stretch. Local, non-propagated activity was associated with a low turnover rate of cross-bridges between actin and myosin in sarcomeres and a slow contraction. With the stretch rates typically used on spindles, this represented an effective increase in intrafusal fibre viscosity with little accompanying fibre shortening. This leads to a large increase in the dynamic response of the spindle during stretch, but only weak effects when the muscle was held at constant length. Meanwhile, the second type, the 'static fusimotor fibres' excites the spindle more powerfully when it was held at constant length and reduces the dynamic response during a stretch [22]. Intrafusal activity was actively propagated and contractions were faster. The higher cross-bridge turnover rate meant that a stretch of the contracting intrafusal fibre could be resisted with less viscosity than that for the passive fibre, leading to a fall in the dynamic response. In addition, the substantial intrafusal shortening produce a large increase in static response. The majority of primary endings of spindles appeared to be able to modify in these two distinct ways by fusimotor stimulation, playing a role in the stretch reflex and contributing to the kinaesthetic sense.

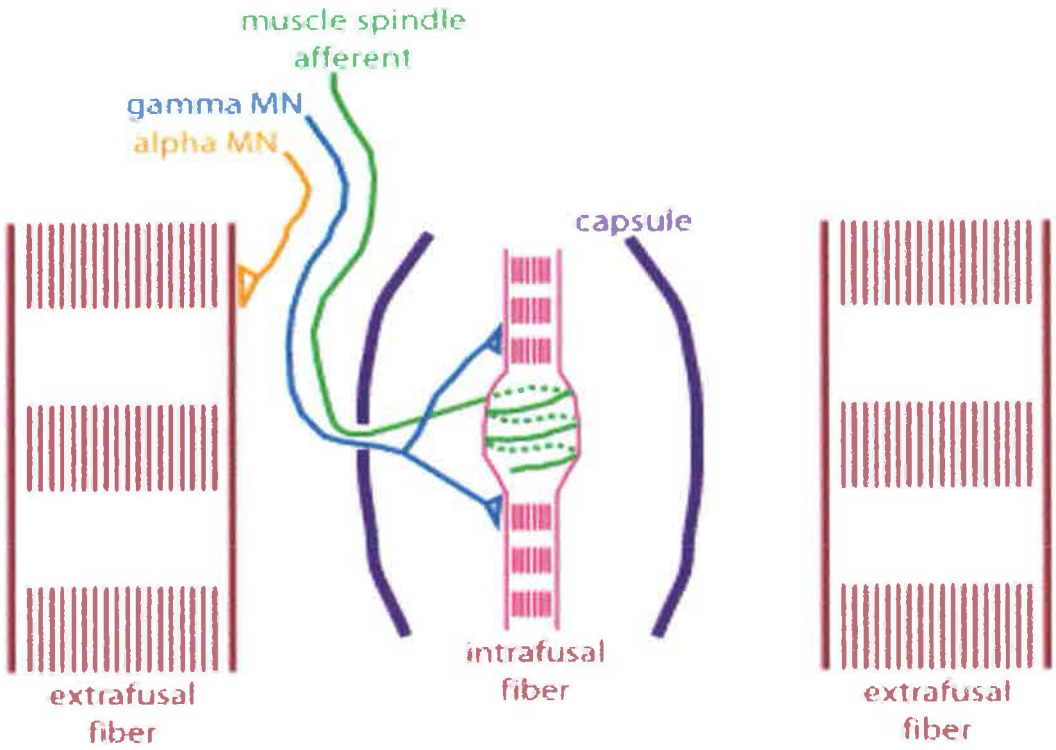


Figure 3. Adapted by courses.washington.edu. The muscle spindle is a stretch receptor with its own motor supply consisting of 3-12 intrafusal muscles. The sensory endings of a primary (group Ia) and secondary (group II) afferent coil around the non-contractile central portions of the intrafusal fibres. The gamma motorneurons activate the intrafusal muscle fibres, which change the resting firing rate and stretch-sensitivity of the afferents.

Structural change within the ACL has a direct effect on the output of muscle spindles through the fusimotor system and its gamma motor neurons. It is these changes that influences the motor activity around the knee and is termed the "ACL reflex" [23]. This neuro-structural system is an essential part of normal everyday activities of the knees which influences the functionality of associated muscle groups.

1.1.2 Biomechanics of a normal ACL

The ACL is integral for joint stability and is the primary restraint to anterior translation of the tibia relative to the femur [24]. The four-bar linkage cruciate mechanism constrains the motion of the femur on the tibia so that there is an effective combination of rolling and sliding movements [25]. This model is simplistic in its explanation of knee motion in the sagittal plane but fails to take into account the rotational components of the femorotibial joint. In native knees the ACL limits anterior neutral-position shift. In contrast, chronic ACL-deficient knees associated with ACL injuries cause anterior translation of the tibia relative to the femur which is four times greater than normal knees [26]. The ACL supports a restraint of approximately 85% to the applied anterior load at 30 degrees of flexion of the knee. This decreases to 80% at 90 degrees flexion.

The AMB and PLB bundles have different mechanisms of action when an anterior draw force is applied to the knee. In vitro studies have found that AMB of the ACL has an in situ tension that increased as the knee flexed from 20 to 90 degrees. In contrast the force in the PLB increased during knee extension [27,28]. PLB dominance in the extended knee provides a basis for developing double-bundle reconstructions.

When the knee joint is near full extension, the ACL functions as an important secondary restraint to internal rotation. Additionally, the ACL provides a minor secondary restraint to external rotation and varus–valgus angulation, especially during weight bearing conditions [29]. This can be tested clinically with the "pivot shift test" which involves

applying a combined internal tibial and valgus torque throughout the range of flexion-extension [30]. Woo et al, studied the tensile properties of the femur–ACL–tibia complex and found that ultimate tensile load to failure and stiffness for young specimens (22–35 years) were 2,160 N and 242 N/mm, respectively. Ultimate tensile load and linear stiffness decrease significantly with age [31].

1.2 ACL injury

1.2.1 Incidence and prevalence of ACL injury

Global statistics on ACL injuries are mainly limited to the Western hemisphere. The USA has an annual incidence of 200,000 ACL injuries [32], which corresponds to a population-based incidence of 60 per 100,000 inhabitants. Over half of these patients will undergo ACLR. Among collegiate athletes, the rate of ACL injuries has increased by 1.3% between 1988 to 2004. This is associated with a subsequent time loss of more than 10 days in 88% of the injured athletes at a cost of USD1 billion for ACLR alone [33]. An Australian study found a similar number of ACLR procedures performed annually as in the United States, with an incidence of 52 per 100,000 [34]. This involved a national annual cost of USD$85 million for surgical expenses alone. The cost was estimated to exceed USD$110 million when non-surgical and indirect expenses were included.

The Scandinavian ACL Registries 2004 to 2007 have also shed light on the annual statistics for ACLR in Scandinavian countries. The annual incidence of primary ACLR was 34,

38 and 32 per 100,000 population in Norway, Denmark and Sweden respectively [35]. However, the authors concluded that the true population incidence for ACL injuries could be approximately 50-100% higher than the reported ACLR incidence.

The 16–29-year age group had an incidence rate of ACL injuries that was twice higher than the rest of the population with a male:female ratio of 2.5:1 [34]. These findings likely reflect the higher rate of athletic participation in this age cohort. There appears to be an increase in the number of females requiring surgery per year and this highlights the need for intervention as an injury prevention strategy [36]. According to the American College of Sports Medicine, women are 2-8 times more likely to sustain an ACL injury than men at the same level of performance [37].

The importance of the ACL has been emphasised in sports requiring stability in running, cutting and kicking. ACL injury accounts for 3-5% of all sporting injuries with football, skiing and gymnastics accounting for most of these injuries [33,24]. Intervention programmes have been shown to transiently reduce the incidence of ACL injuries however, the ideal ACL injury-prevention programme has yet to be identified. Injury-reduction training has been shown to reduce incidence for football [38].

1.2.2 Neuromuscular physiology in ACL injury

Biomechanics in ACL injury

Biomechanical studies of rehabilitated individuals with ACL-deficient knees and ACLR have shown different joint torques in the lower limbs during walking compared with healthy controls. ACL injured patients eventually develop a greater extensor torque at the hip and a reduced extensor torque at the knee during the stance phases of walking and running [39-42]. These observations have been supported by increased electromyographic (EMG) activity in the hamstrings and reduced EMG activity in the quadriceps in ACL-deficient patients during the gait cycle [43-49]. These adaptations are advantageous to individuals with both ACL deficiency and ACLR because they reduce anterior displacement of the tibia relative to the femur and therefore reduce stress on the knee joint while also enabling individuals to perform complex knee movements [39-41,48,50].

The symptoms of knee instability with ACL rupture occur during weight acceptance of the injured limb in the early stance phase of the gait cycle. During this phase of the cycle, the limb accepts full support of the body and attenuates shock via knee flexion which is controlled by an eccentric contraction of the quadriceps. When symptoms of knee instability are felt, patients alter their movement patterns through reduced knee flexion and internal knee extensor moments [40,51]. The alteration in movement pattern results from the hesitancy to fully activate the quadriceps muscles at a range close to full knee extension. Berchuck and Andriacchi [40] termed this alteration as 'quadriceps avoidance' due to the fact that close to full knee extension, quadriceps contraction may cause anterior tibial

translation [52,53] that in turn could result to the symptoms of 'giving way'. The alteration in movement pattern associated with 'quadriceps avoidance', may also represent an increase flexor activity of the hamstrings [39], or the gastrocnemius [54] which has been supported by EMG studies. However, altered movement patterns persist following ACLR [55,56] suggesting mechanical instability is not the sole cause of the gait adaptation.

There is also evidence that ACL-deficient patients walk with a significantly lower internal rotation knee joint moment during the terminal stance phase of the gait cycle [57-59]. Like the adaptation in the early stance phase of gait, this phenomenon is also likely adopted to prevent rotational instability. Termed as the 'pivot-shift avoidance', this gait is also characterised by a significantly decreased internal rotation knee joint moment and by a significantly higher flexion angle during the terminal stance phase of the gait cycle.

Although a number of studies have applied bioinformatics modelling and simulation to understand the muscular adaptations of the gait cycle [56,60,61], only one of these has quantified the effect of muscle compensation on knee instability in ACL-injured patients. Liu and Maitland found that 56% of peak isometric hamstrings force was required to restore anterior tibial translation in the ACL-deficient knee to the values observed in normal gait [62]. This study was performed at the single instant of the gait cycle (heel strike) and only considered the effect of hamstrings muscle action on anterior tibial translation during normal gait speeds.

Patients undergo ACLR with an expectation to resume normal activities and return to sports [63]. Another indication for ACLR is the symptom of knee instability [64]. Current

surgical management strategies include reconstruction with various grafts including the bone-patellar tendon-bone (BPTB) graft and hamstring tendon (HT) graft [65]. Further development in stem cell research pursuing biological repair of the ligament is underway. There is evidence that ACLR with BPTB and HT graft using single bundle technique does not restore the normal kinematics of the knee [66-70]. This has led to interest in examining the use of double bundle ACLR to restore anatomical function in the knee [71,72]. To date, no study has investigated the effects of prehabilitation in combination with surgical reconstruction and accelerated postoperative rehabilitation on ACL injured patients.

Causes of quadriceps weakness in ACL injury

The quadriceps consists of four different muscle groups originating from the proximal femur and converging into the quadriceps tendon 2 cm above the patella. This continues into the patellar tendon and inserts onto the tibial tuberosity. The four muscles are the rectus femoris, vastus medialis, vastus intermedius and vastus lateralis (Figure 4).

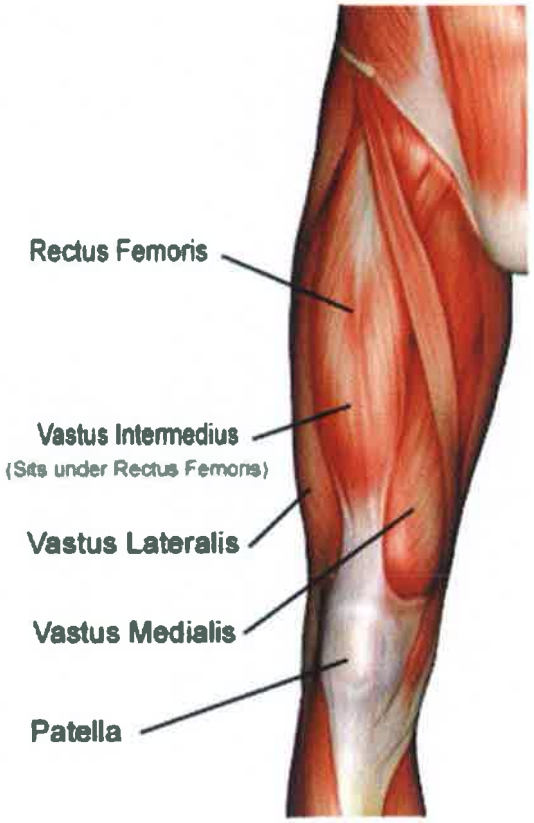


Figure 4: Anatomy of the quadriceps

The quadriceps muscles are the principle extensors of the lower limb. It becomes active during the end of the swing phase of the gait cycle and contracts concentrically during knee extension such as rising from a sitting/squatting position, climbing upstairs and during running and acceleration. It contracts eccentrically during walking down a slope. Decreased levels of physical activity [73-75], weight bearing status [75-77] and immobilisation [78-80] can lead to progressive atrophy. Several factors including decreased protein synthesis [74-76], increased levels of plasma cortisol [77], arthrogenic inhibition [78,79], and altered afferent inflow [80] may be responsible for atrophy after trauma or in periods of prolonged muscular disuse. Though evidence is lacking, this notion alone supports the possible benefits of prehabilitation in preoperative ACL-deficient patients.

Correlative studies of quadriceps muscle hypertrophy and atrophy have employed various measurements such as quadriceps cross-sectional area (CSA) and muscle volume, muscle torque and muscle fibre-typing. Most studies failed to show a relation between quadriceps muscle CSA, morphological measures and muscle strength in ACL-deficient patients [81,82,83,84]. Muscle volume appears to be a better indicator of atrophy than muscle torque because the latter is patient dependent [81]. Measurable atrophy can be observed within 1 to 2 weeks and progresses exponentially [86,87]. The vastus lateralis and vastus medialis (VL and VM respectively) each has approximately 50% type I fibre composition [87]. Both type I and II muscle fibres atrophy significantly, with greater effect on type I fibres [72,74,75]. A concomitant shift from type I to type II (slow to fast fibres) was also found [77, 88-90]. Motor units are also recruited in a consistent fashion from type I to type II.

Disuse muscle atrophy is a known cause of quadriceps weakness. Extensor torque and quadriceps CSA decreased by 25-30% and 14% respectively after 6 weeks of bed rest in healthy volunteers [91]. Berg et al, reported similar results and interestingly found that these changes were completely reversed after 7 weeks of recovery [92]. A reduction of strength likely caused by altered neural function may precede atrophy in periods of decreased activity [93, 94]. Failure of voluntary activation from the central nervous system to the quadriceps muscle as a result of disruption of the ACL has been implicated [84]. Konishi et al, described the abnormal gamma loop function in the injured and uninjured quadriceps muscles of patients with ACL rupture [93]. There is loss of feedback from ACL mechanoreceptors which results in a suppressed state of recruitment of high-threshold

motor units, presumably through the elimination of feedback to gamma motor neurons [94]. This mechanism is also called reflex inhibition or athrogenous muscle inhibition (AMI). This is a protective mechanism preventing future re-injury. Stevens et al, found that the incidence of activation failure in young healthy subjects was approximately 10% [95]. In ACL deficient subjects the incidence of complete inactivation is approximately 30% and can be bilateral [96]. Adjusting contralateral knee strength for activation and considering the activation of the involved quadriceps may allow for a more accurate assessment on the relative contribution of atrophy and activation failure that causes quadriceps weakness. Quadriceps muscle strength is a significant determinant of functional stability in the ACLR knee joint [97]. Considering the emerging evidence linking bilateral quadriceps weakness with unilateral ACL injury, there is a need to further investigate the role of bilateral quadriceps strengthening prior to ACLR.

A reduction in quadriceps moments may also be related to quadriceps weakness despite ACLR [98]. Lewek et al, tested individuals with complete ACL rupture who had >90% or <80% of quadriceps strength using walking and jogging motions [97]. A percentage of these patients underwent ACLR. The ACLR patients had reconstruction using an allograft or hamstring reconstruction. Compared to the uninjured group there was a significant reduction in knee moment at peak knee flexion in both the weak ACLR (quadriceps strength < 80%) and ACL-deficient group during the early stance phase of walking and jogging.

While quadriceps strength is often used as a marker for rehabilitation progress, 'return to play' guidelines following ACLR are not clearly defined and vary considerably [99, 100]. A quadriceps index of 65% and >80% is often used as a criteria for a return to sport

specific training and full competition respectively [99,100]. A full range of movement (ROM) of the knee without evidence of an effusion and self-report of normal activities without symptoms have also been used as a criteria to allow patients to return to full activities [101]. However, another study showed that patients with a quadriceps index of < 80% had walking and jogging pattern similar to patients with ACL deficiency, indicating that premature activities may in fact be detrimental to joint integrity.

Proprioception in ACL injury

Proprioception refers to the specialised variation of the sensory component of joint movement and position. A significant number of mechanoreceptors exist in the fibres of the ACL as discussed previously. Studies have shown decreased proprioception of varying degrees after an ACL injury, as well as a correlation between the measured proprioceptive ability and the patient's subjective extremity function [16].

The first study on proprioception in patients with ACL deficiency showed significantly higher threshold values in 'threshold to detection of passive motion' (TTDPM). This tests the knee that is passively moved either into extension or flexion, and the subject responds as soon as the movement was felt [102]. It has been established that in flexion, the ACL injured knee can employ a net increase in posteriorly directed force acting on the tibia to prevent anterior drawer and pivot shift movement. This may be produced by increased hamstring activity or muscle stiffness [103]. McNair et al, reported that hamstring muscle stiffness in ACL injury correlates with functional stability of the knee suggesting an ACL-

hamstring protective reflex [104]. Therefore, it is advantageous for the ACL injured limb to exhibit both adequate power and rapid reactionary time in the hamstrings to prevent joint instability, in which when the tibia subluxes anteriorly, the hamstrings contract. Therefore, lead researchers in this field proposed that proprioceptive training and emphasis on hamstring strengthening are essential in ACL injured limbs.

Beard et al, reported a delay in the reflex hamstring contraction in ACL-deficient knees compared to the contralateral knee after an impulse generating anterior movement of the tibia relative to the femur [105, 106]. They considered that the reflex response of muscles depended on afferent signals from joint receptors. On the level of baseline muscle stiffness, it has been suggested that the slowing of reflex hamstring activity could be due to loss of proprioceptive input from the ACL and other receptors within the joint. This provided first evidence for the presence of altered stretch reflex excitability in ACL injury.

The muscle spindles as well as the Golgi tendon organs play an essential role in the reflex response of the knee joint [107]. In fact, animal and human studies have proven a direct reflex pathway between the ACL and the hamstrings which is additionally modulated by the central nervous system by gamma motor neurons [108, 109]. The relative contribution of each anatomic receptor population from other parts of the knee joint and the muscles is however, still unclear. Friemert et al, provided evidence that reflexive muscle response consisted of a monosynaptic reflex of the hamstrings (short-latency response) and a later polysynaptic reflex (medium-latency response) [110]. This study observed that the muscle spindles in the distal but also in the proximal part of the muscle are stimulated at the same time during intraoperative ACL stimulation. Melnyk et al, quantitatively assessed

reflex activity in the semitendinosus/semimembranosus after anterior tibial translation in 21 patients with isolated ACL rupture using surface electromyography (EMG) [111]. Patients were divided into non-copers and copers depending whether they had symptoms of 'giving way'. After ACL injury, the medium-latency response was significantly longer while the short-latency response remained unchanged in non-copers indicating that ACL injury is associated with altered stretch reflex excitability. Beard et al, reported that the onset latencies of hamstring reflexes were increased after ACL lesions and improved after a 12-week physiotherapy programme [105, 106]. This study is further discussed in another section, however concluded that sensorimotor training in addition to weight and functional training can help restore sensorimotor control of the knee.

Furthermore, even in sufficient mechanical stability after ACL reconstruction, functional instability characterised by a 'giving-way' pathology has been attributed to a disturbed sensorimotor function [16]. Postoperative functional training has also been shown to significantly decrease the proprioceptive deficit [112]. Apart from altered sensorimotor integration advocated by the leading researchers of proprioceptive training in ACL injury, aberrant muscle recruitment and a decrease in quadriceps activity have also been observed in non-copers, contributing to 'giving way' symptoms. Therefore, a proposed rehabilitation programme pre- and postoperatively should include proprioceptive training amongst others due to non-mechanincal instability relating to altered stretch reflex excitability.

1.2.3 Risk factors for ACL injury

Approximately 2 out of 3 ACL injuries occur following non-contact injuries. Multiple factors, in isolation or combination may contribute to non-contact ACL injury when considerable valgus stress and increased abduction moment during landing occur. This may involve either internal or external rotation [113]. Females are 5 times at a higher risk of sustaining an ACL injury compared to their male athlete counterparts in the same competitive level of sport which support gender and hormonal risk factors [114]. Other anatomical, biomechanical and neuromuscular risk factors also influence the risk of ACL injury.

Anatomical variation such as femoral intercondylar notch width, an increase Q angle (the angle formed by a line from the anterior superior iliac spine to the centre of patella and a second line from the latter to the tibial tubercule) and an increase in posterior tibial slope also increase the risk of ACL injury [115]. The female ACL is smaller in length, cross-sectional area and volume affecting the femoral-notch-impingement theory leading to vulnerability during valgus stress [116]. The female ACL is also less stiff with lower modulus of elasticity and fails at a lower load level [117].

The likelihood of suffering an ACL injury is not evenly distributed across the menstrual cycle. The risk is increased during the preovulatory phase of the menstrual cycle although it is unclear whether oestrodiol has a negative direct effect on the ACL as compared to progesterone or testosterone [118]. There is an elevated risk for ACL injury in females in addition to a 5 and 10-fold increase in high school and collegiate sports

participation respectively over the past 30 years. These factors have led to a significant increase in ACL injuries in females [119].

Cohort studies suggest that a prior history of ACL injury may be a risk factor for a recurrent event on both the ipsilateral and contralateral side [120]. This is probably related to previously injured individuals having smaller ACL, greater posterior slope of the lateral tibia and reduced condylar depth on the medial tibial plateau [121, 122]. Family history also appears to increase predisposition toward ACL injuries [123].

1.2.4 Diagnosis of ACL injury

A focused history and physical examination will allow the diagnosis of an ACL injury. General key features during history taking would include a non-contact mechanism of injury, the presence of an audible 'popping' sound, an early presence of swelling as a result of haemarthrosis, and an inability to continue sport or activities. However an isolated ACL injury occurs in less than 10%, due to the complex rotational force with valgus or varus deformity of the knee. Other associated injuries include meniscus pathology in 60 - 75% [124], subchondral bone injuries or 'bruising' in 80% [125] and complete collateral ligament tear in 5-24% [126].

Physical examination of the ACL injured patient would include a full assessment of all the structures within the knee to outrule any associated injury. The two essential manoeuvres are the Lachmann test and the pivot shift test. The Lachmann test is performed

with the patient supine and relaxed. The examiner places one hand on the lateral aspect of the thigh above the knee. The second hand is positioned on the anteromedial border of the proximal tibia with the thumb placed on the flat bony surface. With the knee flexed at 20-30 degrees, the hand on the tibia attempts to displace the tibia anteriorly in relation to the stabilised femur. The pivot shift test assesses a sudden subluxation of the lateral tibial condyle on the distal femur while the knee undergoes extension to flexion range-of-motion, with a valgus and internal rotation forces applied to the knee. The Lachmann test has specificity and sensitivity of 85% and 94% respectively [127]. However the pivot shift test has a high specificity of 98% with a low sensitivity of 24%.

MRI is the gold standard for diagnosis of an ACL injury. MRI has a specificity of 86%, a sensitivity of 86% and an accuracy of 93% for an ACL tear [128]. With the ease-of-access to MRI and reporting from a musculoskeletal radiologist, the diagnosis of ACL injuries and any associated injury can be appropriately diagnosed and managed.

1.2.5 Management for ACL injury

To operate or not to operate?

There is accumulating evidence to support the use of conservative management strategies for the ACL injured patient [129]. Follow-up studies at 15 years post injury reported good functional and quadriceps/hamstring strength with ACL injuries treated with

rehabilitation. However, many patients were required to modify their activity levels and avoidance of contact sport [130,131].

The KANON study (the Knee, Anterior cruciate ligament, NON-surgical versus surgical treatment) in Sweden [132-135] was a randomised, controlled trial to compare a strategy of structured rehabilitation with early ACL reconstruction within 10 weeks versus structured rehabilitation with delayed ACL reconstruction offered to subjects who continued to have symptoms of knee instability. The KANON study found no difference in muscle power and functional tests between strength training with surgical intervention when compared to conservative management strategies at 2-5 year follow-up. This study also showed no significant difference between the two groups using the Knee Injury and Osteoarthritis Outcome Score (KOOS) as a primary outcome measure. However the development of osteoarthritis in the knee was reported to be increased in non-operative ACL injured patients compared to a delayed progression in patients with ACLR after 15-20 years follow-up. Interestingly, the initial strategy of structured rehabilitation alone instead of structured rehabilitation plus early ACL reconstruction resulted in surgical reconstruction being avoided in 61% of the subjects without compromising the results. This landmark study suggests that the use of rehabilitation plus optional delayed ACL reconstruction for symptoms of instability is favoured, to avoid unnecessary reconstruction without adversely affecting outcomes. However, this study's limitations include self-reporting primary outcome and a non-equivalent control group such as sham-surgery.

A side project of the KANON study [132] reported no significant differences between muscle strength and functional performance in patients with ACL injury treated with

training and surgical reconstruction or training alone. The Limb Symmetrical Index (LSI) was utilised in this study, where a LSI of more than 90% was considered normal [136,137]. Due to these recent trials on the effects of rehabilitation with and without ACL reconstruction, patients with symptomatic unstable knees should be individually assessed regarding symptoms such as knee instability and type and frequency of current and future activities. However, these studies did not investigate the independent and synergistic effects of preoperative prehabilitation in patients who planned and ultimately underwent ACLR.

Non-surgical management

The majority of patients with ACL injury are able to mobilise normally after the initial haemarthrosis dissipates with rest, ice, compression and elevation (RICE). Gentle range-of motion exercises are initiated early to prevent arthofibrosis. Non-steroidal anti-inflammatory medications might be required for analgesia and to reduce swelling. Patients should eventually perform straight-line activities like stair climbing, cycling and jogging. Surgical intervention would be necessary if the patient has any episode of knee instability in their normal activity of daily living, a need to return to pivoting type sports (ie. football, rugby) or occupations requiring knee stability (police, firefighter). These patients are termed as 'non-copers' [138]. The non-operative management may be considered in the elderly patients or less active patients not participating in pivoting type sports (running, cycling). The main goal is to achieve full ROM and strength comparable to the uninjured lower limb.

Open- and closed kinetic chain exercise

Open kinetic chain exercise (OKC) is characterised by the distal segment of an extremity being free to move such as in knee flexion or extension activity [139]. Conversely, a closed kinetic chain (CKC) exercise is described as the distal segment of a limb being fixed, such as in a squat or leg press. This has been preferentially used because of the belief that they are safer for the patellofemoral joint and do not increase additional strain on the ACL, promoting joint laxity. The muscle activation in both was similar pre- and post-rehabilitation. Perry et al, in 2005 concluded that OKC and CKC exercises of the knee extensors following ACL injury do not differ in their effects on knee laxity and function in ACL injury [140]. Tagesson et al, in 2008 stressed the importance of OKC as a conservative non-operative approach to improve quadriceps strength in ACL deficient patients [141]. At 4 months follow-up the OKC group had greater isokinetic quadriceps strength compared to the CKC group.

Home-based physiotherapy

Keays et al, looked into the effectiveness of preoperative home-based physiotherapy [3]. This study was designed to have two matched groups of 12 chronically ACL-deficient patients awaiting reconstruction which were either assigned to receive a home-based exercise and educational programme or no preoperative physiotherapy. Another group of 12 matched uninjured control subjects was also subjected to treatment. After 6 weeks, compared to the non-treated group, the treated ACL-deficient group showed a significant

improvement in quadriceps strength from 85% and 86% to 102% and 103% at 60°/s and 120°/s isokinetic testing speeds respectively. There was no difference in outcome between the non-treated ACL-deficient group versus the treated control group. These patients did not have long-term follow-up and the number of patients that eventually underwent ACLR after this period of rehabilitation remains unknown. Another study conducted by Zatterstrom et al, to evaluate the efficacy of a supervised versus self-monitoring rehabilitation programme in non-operative ACL injuries revealed higher improvement in hamstring and quadriceps strength in the supervised group [142].

Proprioception training

ACL rupture has been shown to impair proprioception and increase the latency of reflex hamstring contraction [143]. Impaired proprioception is a contributing factor predisposing to degenerative joint disease and on-going instability in the ACL deficient knee. The majority of studies in proprioception training have been mainly concentrated on non-operative measures in ACL injured patients. The specific exercises prescribed for balance and proprioception training varied but these protocols challenge balance control.

Beard et al, had a protocol involving wobble board drills, proprioceptive neuromuscular facilitation, dynamic single leg balance, ballistic hamstring catches, mini-trampoline exercises, lateral ski machine training, skipping and bridging with an inflatable ball. Progression of the programme was achieved by decreasing stability of the base and removing visual feedback [143]. This study compared the effects of proprioceptive versus

the traditional exercise regime in ACL deficient patients in a double-blinded, randomised trial. The investigators discussed that whilst most treatments for ACL deficiency are designed to strengthen the quadriceps muscles, some biomechanical studies have suggested that the hamstring muscles can better simulate ACL function [144]. The 12-week programme consisted of two gym sessions interspersed with daily self-supervised home physiotherapy with emphasis on neuromuscular facilitation, rapid hamstring recruitment and dynamic stability. Outcome measures included subjective knee function using the Lysholm and Gillquist scoring scale, proprioceptive index by time to displacement, hamstring reflex contraction latency and acceleration of the tibia. This intervention protocol was well documented, and all patients had ACL injury confirmed by knee arthroscopy prior to recruitment. The traditional regimen arm underwent strengthening exercises with no attempt made to increase the speed of contraction or to improve dynamic stability. Most exercises employed the OKC type and progression was achieved by increasing weight resistance. Meanwhile, the proprioceptive regime was mainly CKC types emphasising facilitation of rapid contraction of the hamstring muscles and to improve dynamic stability through proprioceptive enhancement techniques. Patients subjected to proprioception exercise programme had significantly better functionality with improvement in mean reflex hamstring contraction latency. Limitations to this study included a short follow-up period. Additionally, this study was not designed to specifically examine the effects of this regime preoperatively for planned ACLR candidates.

Angoules et al, prospectively studied knee proprioception following ACL reconstruction and concluded that the former returned to normal by 6 months postoperatively [145]. A neuromuscular training programme termed the perturbation

technique was introduced in 2000, which involved the use of roller and tilt boards for the proprioception programme. The original study by Fitzgerald et al, [146] had 26 ACL-deficient patients, with 12 in the perturbation group over a period of 10 sessions. 92% (11 out of 12) of patients had a successful rehabilitation compared to 50% (7 out of 14) in the standard group ($p<0.05$). Successful rehabilitation is defined as having no episodes of 'giving way' at 6 months follow-up. Subsequent guidelines were proposed for the non-operative management of ACL-deficient patients [129].

The Lund group from Sweden, also looked at neuromuscular training including balance, pylometric, agility drills and sport-specific exercises. Zätterström et al, enrolled 100 patients into 5-8 months period of supervised home-based training with inclusion of postural CKC versus traditional self monitored exercises [147]. The supervised rehabilitation had significant improvement in the Lysholm score at 3 months ($p=0.03$), single leg hop test, and isometric/isokinetic muscle strength in extension at 3 and 12 months. All patients had stability testing and arthroscopy within 10 days of injury. This study had several weaknesses; firstly, half of the patients from the self-monitored group were transferred to the supervised group at 6 weeks due to reduced ROM and therefore an intention-to-treat analysis was required. Secondly, 4 patients in the supervised group requested to have an ACL reconstruction. Lastly, there was a mixture of patient cohorts with ACL injury as well as grade 2-3 medial collateral ligament tears.

Ageberg et al, looked at a subset of the same group of patients as Zätterström (n=63 out of the original 100) and compared them to healthy volunteers (n=60) [148]. Patients were assessed at 6 weeks, 3, 12 and 36 months post-injury with a stabilometry device, and

single leg hop test at the same time frames (except at 6 weeks). The neuromuscular training group had shorter hop distance in both the injured and uninjured limb than the control group at 3 months ($p<0.001$ and $p=0.04$ respectively), but not at 12 or 36 months. When compared to the self-monitored group, the single leg hop test was significantly less than the controls at all time frames. Stabilometry showed differences in both groups compared to controls at all time frames. This study also had an intention-to-treat analysis as 14 patients were transferred from the self-monitoring to the neuromuscular group at 6-weeks follow-up. However, these two Swedish studies only examined the benefits of neuromuscular training in ACL-deficient patients undergoing conservative management.

Surgical management: ACLR

The ultimate goal of ACLR is the restoration of stability, improvement in knee function and delayed progression of knee osteoarthritis [149]. Many patients require an ACLR in order to participate in their preferred level of athletic activities. It is important that patients are provided with an overview of therapeutic options so that they can make an informed decision regarding ACLR. With the plethora of research articles being published on the topic and accessibility of the internet, patients should be given the choice of graft types, timing of surgery and rehabilitation required to return to normal function.

The goal of an ACLR is to restore the stability of the knee with the use of a graft, usually either a bone-patellar tendon bone (BPTB) graft or hamstrings tendon (HT) graft. The graft is passed through prepared bone-tunnels in the femur and tibia intra-operatively, in

the direction of the normal native ACL. Fixation is required on either end of the grafts for the desired knee stability.

Surgical reconstruction is superior to ACL repair as the latter has been shown to have comparable benefits to non-operative measures [150]. In contrast, ACLR improves knee stability and the possibility to return to pre-injury activities when compared to ACL repair with or without augmentation (insertion of tendon graft or synthetic graft) [151].

A recent 2011 Cochrane review reviewing 19 studies with a total of 1597 young to middle-aged patients compared outcomes of ACLR with either BPTB or HT when used in conjunction with modern (extra-cortical) fixation techniques. The meta-analysis showed no differences between both grafts with the single leg hop test, return to activity, Tegner Lysholm scores, subjective measure outcomes and re-rupture rates [152]. Howèver BPTB has a higher risk of developing anterior knee pain. There is also an increasing interest in anatomical double bundle ACLR which improves knee kinematics by reconstructing both the AMB and PLB, but this is technically more demanding and invasive [153]. Short-term follow-up to 2 years showed no difference in patient-related outcomes but did increase rotational stability [154, 155].

Post-ACLR rehabilitation

Many studies have examined the role of physiotherapy in individuals after ACLR. However, there is little consensus regarding the optimal components of a physical therapy programme for specific outcomes such as permitting early range of motion (ROM), immediate weight bearing and returning to pre-injury level of sport and activities.

Continuous Passive Motion (CPM)

There is limited evidence for the use of CPM machine postoperatively following ACLR. A prospective study by Richmond et al, compared 2 groups: one group used CPM for 6 hours per day for 4 days, and the subsequent group for 14 days postoperatively [156]. Both groups had similar baseline characteristics and reconstruction methods were the same. Outcome measures such as swelling, atrophy and ROM were assessed at 2, 7, 14, 28 and 42 days. There were no significant differences between these outcomes at all time points. However the 14-day CPM group had significantly less anterior tibial translation (0.4mm versus 2.4mm , p=0.04) when measured with the KT-1000 arthrometer.

A study in 1993 by McCarthy et al, compared 3 days of CPM with no CPM using pain scales and narcotic usage [157]. All patients underwent BPTB ACLR and were randomised to receiving postoperative physiotherapy on day 1 postoperatively, with the second group using the CPM machine immediately and postoperatively for 16 hours per day for 3 days in addition to routine physiotherapy. Total narcotic dose and usage of patient-controlled

analgesia were significantly increased in the non-CPM group ($p<0.05$). Oral narcotic use on day 2 and 3 postoperative period was increased, but pain scales were similar in all groups at all time periods. However there was no standardisation of narcotic usage per body weight.

Early weight bearing and motion

There has been one study investigating the effects of immediate weight bearing versus delayed weight bearing following ACLR. Tyler et al, randomised 49 patients following BPTB ACLR to either immediate versus delayed 2 weeks weight bearing [158]. Outcomes assessed include ROM, stability, vastus oblique electromyogram, Tegner Lysholm score and anterior knee pain. KT-1000 testing for stability and ROM showed no difference at final follow-up (range 6 – 14 month). ROM showed no statistical difference at final assessment in 7.3 months. Vastus medialis oblique activity was significantly increased in the weight bearing group at 2 weeks ($p=0.002$) but this equalised between both groups at final assessment. Anterior knee pain was significantly increased in the non-weight bearing group at final assessment with 7 out of 20 (35%) non-weight bearing patients and 2 out of 25 patients (8%) reporting pain ($p=0.03$). Anterior knee pain was assessed with the Lysholm scale which evaluated pain in its different subscales such as routine activity, stair climbing and squatting. The Lysholm score was significantly improved from baseline in the weight bearing group ($p=0.03$).

Postoperative bracing

Knee braces were designed to limit ROM to a desired setting and excessive varus and valgus stress. One review analysed 11 studies that evaluated outcomes such as ease of achieving ROM, swelling, wound ooze, knee laxity, pain and protection from injury [159]. Another study showed improved knee extension following a brace locked in extension in the first postoperative week [160]. No study demonstrated a deleterious effect from withholding brace usage. There was no change in postoperative injuries, pain, decreased ROM or increased knee laxity in the control group.

Home-based rehabilitation

Beard et al, assessed a home versus gym exercise programme following 4 to 6 weeks post-ACLR [161]. A computer programme randomised 26 patients and an independent examiner was blinded to patient allocation. Both groups followed a supervised physical therapy twice a week for the first 2 weeks, and then once a week for the following 2-4 weeks. The groups were then randomised to either continue their home-based programme without supervision or to a supervised gym programme for a total of 12 weeks supervision. The home-based group did not complete their compliance forms. There was no difference postoperatively at 3 and 6 month in the Tegner, Lysholm and International Knee Documentation Committee scores, visual analog scale for sports and activities of daily living, instrumented laxity with KT-1000 and isokinetic testing.

A more recent study by Grant et al, randomised 145 patients to either a minimally supervised home-based rehabilitation programme versus a traditional supervised gym protocol [162]. Assessments were blinded and stratified randomisation was performed. Home-based patients attended 4 physiotherapy sessions within the first 3 postoperative months. The gym-based group were enrolled in a twice-weekly session for weeks 2-7, and once weekly from weeks 8-12, with a total of 17 sessions within 3 months postoperatively. The home-based group had a significantly better rate of extension at 96.8% versus 83.3% in the gym-based group ($p=0.02$). The flexion rate was 66.7 % versus 47% in the home and gym-based group respectively ($p=0.03$). There were no differences in instrumented laxity and strength.

These studies do conclude that a minimally supervised physiotherapy programme is a feasible option in ACL rehabilitation.

Quadriceps strengthening: Open kinetic chain (OKC) versus closed kinetic chain (CKC) exercise in post-ACLR

The differences between OKC and CKC were explained in a previous section. The first published study looking at OKC exercise postoperatively was in 1995 by Bynum et al, who investigated the effect of rehabilitation on BPTB ACLR patients followed by a 24-week OKC programme [163]. One hundred patients were prospectively randomised and patients were followed up for a mean of 19 months. Examiners were blinded and patients followed-up at 3 months intervals. 66% returned for the final assessment. There was a significant difference

in the KT-1000 instrumented laxity examination for anterior tibial translation at final assessment. The mean maximum value was 1.6mm and 3.3mm for the CKC and OKC groups respectively (p=0.02). At 9-months follow-up, patella-femoral pain was present in 15% and 38% in the CKC and OKC groups respectively (P=0.46). Subjective patient assessment and the Lysholm and Tegner scores were similar in both groups. 21 patients (42%) in the CKC group perceived that they returned to normal activities earlier than expected versus 10 (21%) in the OKC group (p=0.007). This study determined that CKC exercises were safe and effective whilst produce less strain on the graft in the postoperative phase and reduce patellofemoral pain.

Mikkelsen et al, investigated the addition of OKC at 6 weeks postoperatively following a CKC programme [164]. 44 either had a full CKC programme for 12 weeks or a CKC plus an OKC programme at 6 weeks. At 6 weeks, the OKC group had isokinetic concentric and eccentric quadriceps strengthening between 90 and 40 degrees, with progression to 90 and 10 degrees over the 6 weeks of the OKC programme. The study reported a significant increase in quadriceps strength in the OKC group at 6 months, with no statistics explained. The OKC group had a significantly higher return to pre-injury sporting level at a mean of 31-month follow-up (P<0.05).

From these studies, the evidence has led to a general consensus that CKC is safe immediately post ACLR. However, the addition of OKC in the early phase of rehabilitation led to a significantly greater quadriceps strengthening.

Neuromuscular Electrical Stimulation (NMES)

The use of electrical stimulation as a mode of postoperative rehabilitation has been presented over the last three decades. Arvidsson et al, studied the outcome of NMES following cast immobilisation post-ACLR [165]. 38 patients were randomised and had BPTB ACLR. All patients were splinted for 1 week and then casted for a further 5 weeks at 45 degrees flexion. All patients performed isometric quadriceps within the cast. The patients randomised to NMES received 30-minute session three times daily for 6 weeks postoperatively (40Hz, pulse width 300 microseconds, 20-second duration with 35 second rest). The study assessed computed tomography (CT) preoperatively and approximately 45 days postoperatively, as well as muscle biopsy for histology and muscle enzymes. Hamstring cross-sectional area (CSA) was significantly reduced in male patient subset of the NMES group ($p<0.05$). Within the female cohort, there was a significant reduction in CSA of 31.4% versus 15.6% in the control and NMES group respectively ($p<0.001$). The muscle biopsy exhibited a reduction in muscle fibre area of 5.4% in the NMES female cohort. The control female cohort and all male patients in the study displayed a 30-40% decrease in muscle fibre area. The authors of the study concluded that NMES reduced the rate of muscle atrophy in the female cohort following ACLR.

Snyder-Mackler et al, compared high intensity NMES versus low intensity NMES and high intensity exercise training programme [166]. 110 patients were randomised to 4 groups in a multicentre study using different types of ACLR. Each group had the following: (i) 31 patients had high intensity NMES with 2500Hz triangular alternating current at 75 bursts per second for 15 contractions with 11 seconds on and 120 seconds off 3 times per week (ii) 34

patients had high intensity exercise training (iii) 25 patients had low intensity NMES with a duration of 300 microseconds at 55 pulses per second, 15 seconds on and 15 seconds off for 15 minutes, 4 times per week, 5 days per week (iv) 21 patients had combined high and low intensity NMES. Gait and isometric strength were evaluated at 4 weeks following intervention. High intensity NMES either as a single treatment or in combination with low intensity NMES displayed an increased recovery of the contralateral limb quadriceps strength (70% versus 51% in the low-intensity NMES versus 57% in the high-intensity exercise group) ($p=0.001$). Gait analysis showed a better ROM from flexion to extension with improved normalised gait in the high NMES group. BPTB tendon group scored lower than other groups (allografts or HT) ($p<0.05$).

A 2002 study by Rebai et al, randomised 10 patients to either electrical stimulation with 80Hz versus 20 Hz following ACLR (167). The 20Hz group had a pulse width of 300 microseconds with 15 seconds on and 10 seconds off for 60 minutes. The 80Hz group had a pulse width of 300 microseconds with 15 seconds on and 75 seconds off for 54 minutes. This was performed 5 days per week for a total of 12 weeks. Isokinetic strength and MRI were evaluated at 12 weeks postoperatively. The low intensity NMES had better strength recovery in the contralateral limb, but not with the ipsilateral limb. Hence, there was no significant strength difference between the 2 groups. Muscle volume was equal in both groups. There was less fat accumulation on MRI in the low intensity NMES group (10% versus 20%, $p<0.05$).

Fitzgerald et al, in 2003 randomised 48 patients to a NMES or control group [168]. The difference with this study compared to other NMES study was that it was performed

with the knee in extension. The frequency was 2500Hz with 75 burst per second, twice a week for a total of 12 weeks. Assessments were made at 12 and 16 weeks postoperatively with isokinetic quadriceps strength, knee outcome survey concerning activities of daily living and knee pain score. The NMES group had better quadriceps strength at 12 weeks ($p=0.05$) and faster recovery to commence agility training. The activity scores of daily living were better in the NMES group but pain score was the same in both groups.

Although there are studies that investigated the benefits of NMES, generalised conclusion is difficult due to the variety of parameters used. Although some studies showed improved quadriceps strength, this was not correlated with patient-based outcomes and functional testing.

Proprioception and Balance training

Liu-Ambrose et al, appears to be the only study looking at proprioceptive training post-ACLR [169]. 10 patients were randomised to 12-weeks training of either isotonic strength or proprioceptive training following ACLR. The proprioceptive group had greater percent change in isokinetic peak torque in both quadriceps and hamstring values ($p<0.05$). However functional tests (single leg hop, single leg time hop) and subjective scores were similar between groups.

Accelerated Rehabilitation

One landmark study has led to post-ACLR accelerated rehabilitation as a standard physical therapy in these subjects. A randomised, prospective, and double-blinded trial by Beynnon et al, compared a rehabilitation programme administered over 2 different time intervals [170]. Power analysis was performed to detect a 2.5mm difference in knee laxity between the 2 groups. The accelerated group performed the exercise protocol over 19 weeks, whereas the non-accelerated group performed the same protocol over 32 weeks. Compliance with the exercise protocol was significantly better in the accelerated group (68%) compared to the non-accelerated group (40%). There were no differences in KT-1000 testing, Knee Injury and Osteoarthritis Outcome Score and International Knee Documentation Committee outcome measures, Tegner activity level, single leg hop and biochemical markers of articular cartilage metabolism in synovial fluid. This study concluded that compliance of any exercise programme past 12 weeks would be low and that accelerated rehabilitation of 19 weeks did not result in any deleterious effects when compared to a 32-week programme.

There are several postoperative rehabilitative measures employed in the management of the ACLR patient. While the majority are being used as part of the standard post-ACLR rehabilitation protocol (early weight bearing, accelerated programme, proprioceptive training, open kinetic chain and closed kinetic chain, home physiotherapy), others such as NMES still warrant further research to determine optimal protocol and timing.

1.3 Prehabilitation

1.3.1 Definition

Prehabilitation has been defined as "the process of enhancing functional capacity of the individual to enable them to withstand the stressor of inactivity" [5]. Achieving a higher baseline functional status secondary to preoperative strengthening should in theory attenuate the reduction in strength postoperatively and recovery to pre-stressor baseline function (5).

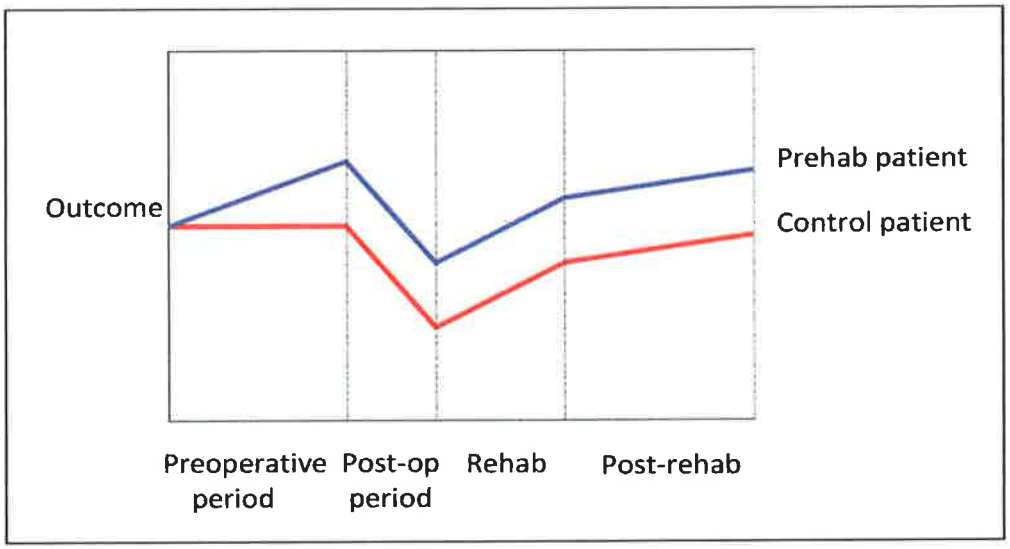


Figure 5. Theoretical model for prehabilitation. (Adapted from Ditmeyer et al)

Initially coined in the 1980s [171], the term 'prehabilitation' is not commonly used in the context of ACL injury rehabilitation. It is however used in relation to patients preparing for other orthopaedic surgery [5] and in prevention of injury in the healthy population [172]. For example, prehabilitation is commonly used prior to total knee replacements [173], spine surgeries [174], cardiac procedures [175] and colectomies [176]. In total knee arthroplasties

(TKA), preoperative function is the greatest overall predictor of postoperative outcome. Therefore, researchers have begun to examine the potential role of prehabilitation as a means of improving patient outcomes postoperatively. For example, a recent, two-arm, parallel, randomised, controlled pilot trial concluded that the intervention elicited clinically meaningful increases in quadriceps strength, walking speed and mental health immediately before TKA but the lasting benefits were not sustained 12 weeks postoperatively [177]. Noyes et al, was the first to propose the use of physiotherapy prior to ACL surgery to increase muscle strength and improve recovery rates [178].

1.3.2 The role of prehabilitation in ACLR

Recent studies have assessed quadriceps function pre- and postoperatively in patients undergoing ACLR. Elmqvist et al, found a 50% reduction in quadriceps strength in the injured leg in the first 14 weeks after injury and reconstruction [179]. After one year, functional performance was still unequal but the injured leg had almost returned to the preoperative non-injured value. Shelbourne et al, measured quadriceps strength and intra-operative patellar tendon width in 540 patients before and after BPTB reconstruction [180]. Smaller patellar tendon width was related to a reduction in quadriceps strength in preoperative patients. Patients with small patellar tendon width (range 20-26mm) had significantly reduced quadriceps strength at 1 and 3 months post-ACLR, compared to the medium and large patellar tendon width at the same time periods. In addition, patients with good preoperative strength (>90% of normal limb) had a higher postoperative strength of 57.5% and 71.6% of uninjured limb at 1 and 3 months respectively. This was compared to

the group with poor preoperative strength (<75% of normal limb). At 2 years follow-up the postoperative quadriceps strength was the same in all groups. Patients in this study were not subjected to a preoperative exercise regime.

Preoperative quadriceps strength is an important predictor on the functional outcome of the knee joint after ACLR [181]. In a study of 73 individuals that underwent ACLR, preoperative quadriceps strength deficits above 20% had persistent and significantly larger strength deficits after surgery. This supports a role for prehabilitation as an intervention to current practices by optimising preoperative knee function.

There are currently three studies published on prehabilitation in ACLR. One study presented a 5-week prehabilitation strategy that included progressive muscle strengthening [182]. 100 individuals were included in an exercise programme within 3 months after ACL injury in addition to classifying them as potential copers or non-copers. Baseline and post-test screening examinations included isokinetic quadriceps and hamstrings strength, single leg hop tests and patient-self questionnaires. The exercise therapy programme was divided into 3 subsequent phases in which the initial phase involved resolving knee impairments related to swelling and ROM deficits. Phase 2 was initiated to restore muscle strength and adequate neuromuscular responses employing both single and multiple joint exercises, open and closed kinetic chain exercises, as well as concentric, eccentric and isometric strength exercises. Patients who were not referred to surgery continued rehabilitation in phase 3. 64 out of the 100 patients underwent ACLR. The progressive 5-week preoperative exercise programme led to significantly improved knee function, quadriceps peak torque, torque at 30 degrees of flexion and total work in patients with ACL injury (Figure 6).

However, this was not statistically significant for hamstring muscles strength peak torque, total work and the single leg hop test. This study design did not include a non-intervention group.

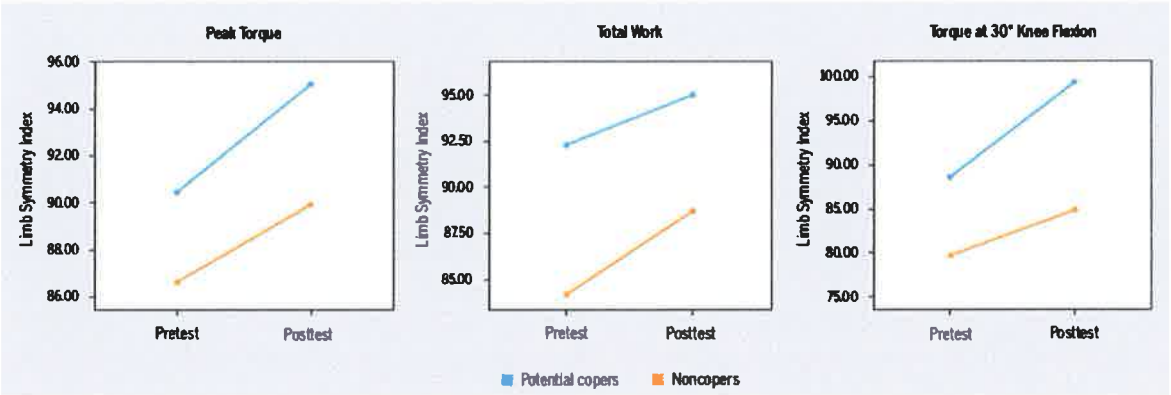


Figure 6. The between-group (pretest and posttest) main effect was significant for 3 quadriceps strength outcomes (p<0.01). (Adapted from Eitzen et al)

A second study by Keays at al, enrolled patients with chronic ACL injuries in a 4-6 weeks prehabilitation programme followed by a minimum of 4-month rehabilitation after surgery [3]. Patients had BPTB ACL reconstruction. There was quadriceps deficit preoperatively at different isokinetic speeds. At 6 months postoperatively there was 22-28% deficit between the injured and uninjured limb. There was no hamstring deficit before and after ALCR. Functional performance improved significantly. Patients wore a knee immobiliser brace locked at zero degree extension for 2 weeks postoperatively. A similar drawback of the study was the failure to include a control group. In addition, although the details for postoperative rehabilitation programme were explained, the preoperative protocol was not provided.

To date one study has examined the effects of preoperative enhancement on proprioception. An American group adopted the perturbation technique prior to ACL reconstruction with the use of rockerboard and rollerboard in 3 different conditions [183]. Subjects who received perturbation training were trained to focus on the somatosensory input from the weight distribution of the foot contact on the board and the afferent information coming from the lower extremity to enhance joint proprioception and muscle response. In this study, Hartigan et al, investigated patients who had poor dynamic knee stability (ACL non-copers) undergoing 10 sessions of perturbation training versus strength training over a period of 3-4 weeks [184]. The perturbation group showed better symmetrical quadriceps strength (97% of the injured leg) and gait (p=0.14) at 6-month postoperatively (Figure 7). Training with the perturbation technique may improve neuromuscular feedback and decrease antagonistic muscle activity, thereby enhancing the quadriceps' ability to stabilise the knee dynamically during gait.

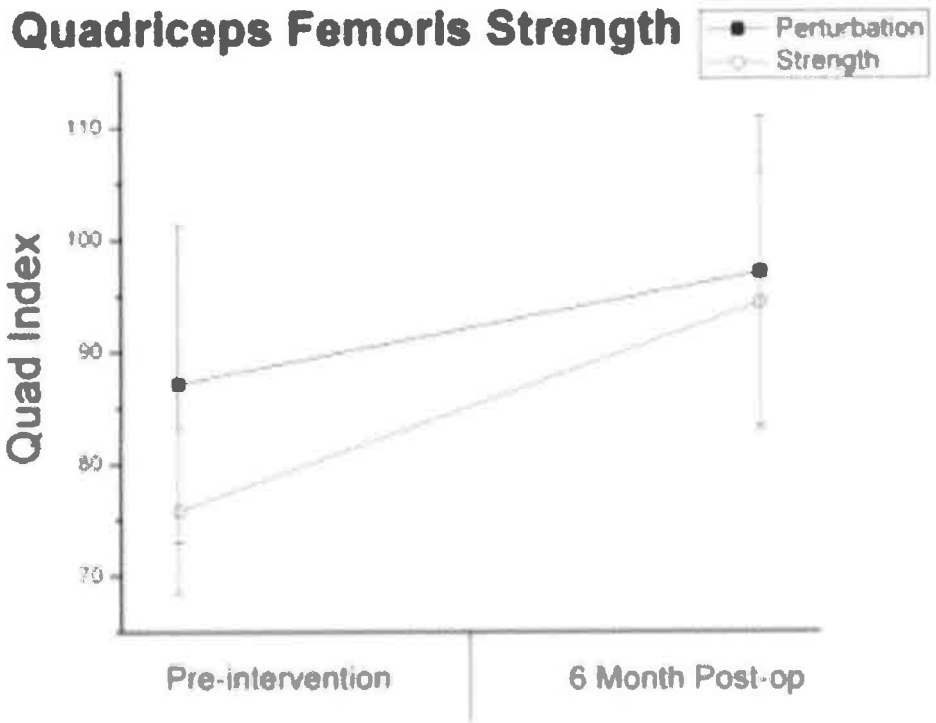


Figure 7. Quadriceps Strength Index prior to intervention and 6 months after ACL reconstruction for each group. Error bars represent standard deviation. (Adapted from Hartigan et al)

These three studies which examined the effects of prehabilitation in ACLR adopted different protocol and outcome measures. Other parameters that include MRI and gene expression of muscular atrophy and hypertrophy are warranted to physiologically enhance our understanding of preoperative neuromuscular training and its effects on long-term functionality.

1.4 Molecular adaptation in ACL rehabilitation

Muscle plasticity is a term for the adaptability of the muscle tissue in various situations such as physical therapy, muscle disuse, stretching, injury and regeneration.

1.4.1 Measurement of muscle hypertrophy: Insulin-like Growth Factor (IGF-1)

IGF-1 has been associated as a factor for promoting hypertrophy in adult mammals. Insulin-like Growth Factor (IGF) is a polypeptide with similar structural properties to insulin and exists in 2 ligand forms: IGF-I and IGF-II. IGF-II is mainly fetal in origin and vital for organ growth. IGF-I is an essential mediator in anabolic growth of skeletal muscle. The liver is the

main IGF-1 autocrine secretory organ in response to growth hormone levels supplying 75% of circulating IGF1. However IGF-I is also released in a paracrine manner in local skeletal muscles. Selectively abolishing the IGF1 production in hepatocytes leads to 75% reduction in IGF1 levels but without growth impairment [185]. An animal study on hypophysectomised rats, which have no further growth hormone secretion, continued to show muscular hypertrophy in response to increased loading indicating that local production of IGF-1 plays the more important role in muscle plasticity [186, 187].

There are three distinct IGF-I isoforms in humans: IGF-IEa, IGF-IEb and IGF-IEc (Figure 8). They are collectively termed mechano-growth factors (MGF) as its expression is amplified from muscular loading. In human skeletal muscle, IGF-IEa is approximately 1000-times more prominent than either IGF-IEb or IGF-IEc [178]. MGF has an E domain which has a role for satellite cell activation [188]. Satellite cells are small mononuclear progenitor cells that fuse to muscle fibres to promote new growth and repair.

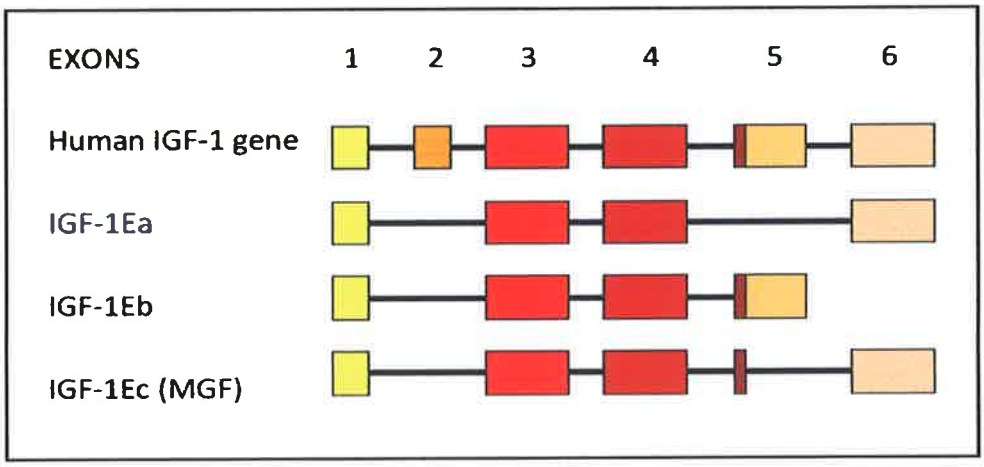


Figure 8: IGF-I isoforms gene (Adapted from Serrano et al)

IGF1 is also expressed in several tissue including muscles induced by stretch or high resistance exercise [181,190]. In humans, increasing the level of circulating IGF1 does not promote muscle protein synthesis [191,192]. In muscle tissue studies, IGF-I has been shown to increase myofibril diameter, suppress proteolysis, promote protein synthesis and induce an increased number of nuclei per length of myofibril [193]. IGF-I also assists myoblast mitosis and differentiation [194]. Hence IGF-I might lead to new myoblasts formation and attaches it to existing myofibrils. One study showed a 163% increase in MGF, a 68% increase in IGF-IEa and 75% increase in IGF-IEb after 5 weeks of resistance training in elderly males [195]. Significant changes in MGF were present in young subjects only. However IGF-IEa in both groups was unchanged at 2.5 hours after resistance training. In contrast, a loss-of function study with an IGF-1 antibody showed a reduction in the number and size of regenerating muscle [196]. Sacheck et al, have shown that IGF-1 inhibits muscle atrophy via the ubiquitin-proteosome pathway [197]. Exercise induces IGF1 production locally in muscle [198], although plasma IGF1 levels are not significantly altered [199]. Based on these findings, IGF1 production is generated by resistance exercise, and can act in a para- and autocrine manner in muscle.

IGF-1 initiates the muscle hypertrophy pathway by binding to its cell membrane receptor (IGF1R). This triggers insulin receptor substrate-1 (IRS-1) which functions as a second messenger to promote the activity of phosphoinositide (PI-3K) which eventually leads to the formation of phosphatidyl-inositol phosphates (PIPs). Protein kinase B, also known as Akt, is a signalling protein kinase which is directed to the plasma membrane and become phosphorylated by phosphoiniositide-dependent kinase (PDK-1). The mammalian target of rapamycin (mTOR) is activated by Akt and also plays a vital role in the hypertrophy

process [200]. The name mTOR is derived due to being selectively inhibited by rapamycin, an immunosuppressant therapy. Bodine et al, demonstrated that rapamycin inhibits muscle hypertrophy without causing atrophy in control muscles [200].

Akt has a dual role as it regulates both muscle hypertrophy and atrophy process. In the presence of increased Akt levels, hypertrophy is mediated by the Akt-mTOR pathway. However when Akt signalling is impaired it mediates the Forkhead box o (FOXO) group, which in turn activates the atrophic genes MAFbx and MuRF-1 (Figure 9).

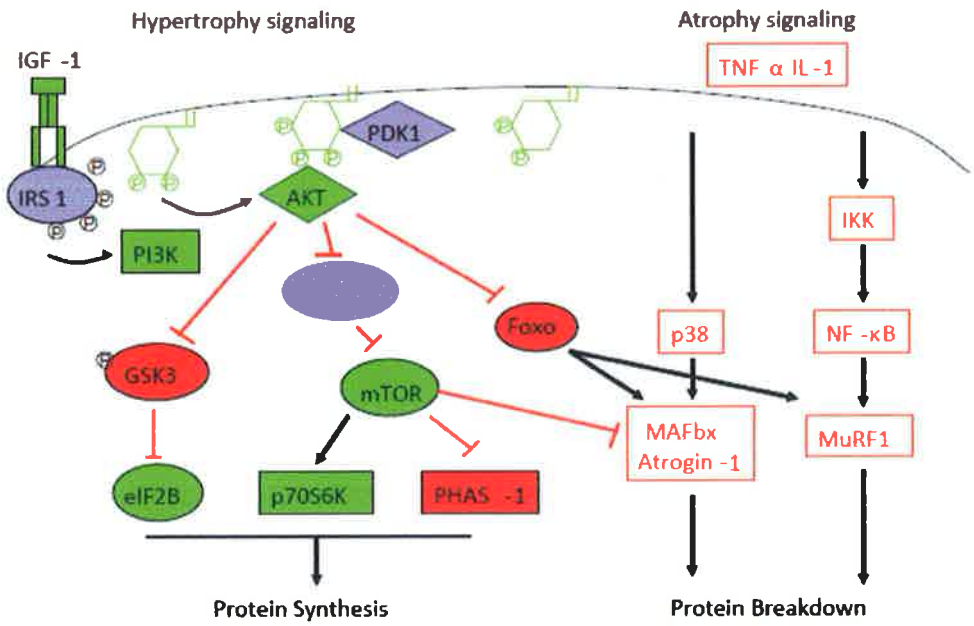


Figure 9: Hypertrophy and atrophy pathways

IGF-1 promotes hypertrophy and prevents atrophy from downstream signalling, by the suppression of the ubiquitin-ligase gene expression. More generally, IGF-1 has been shown to stimulate differentiation of satellite cells and promote the subsequent

incorporation into existing myofibres [201]. It has been reported that serum IGF-1 did not correlate positively with strength in several lower extremity muscles due to local IGF-1 production that is being implicated in muscle hypertrophy [202]. Therefore, local IGF-1 mRNA expression remains the most frequent gene examined to study muscular hypertrophy.

1.4.2 Measurements of muscle atrophy: MuRF-1 and MAFbx

Muscle atrophy is caused by reduced protein production as well as increased proteolysis. The central pathway for proteolysis is termed the ubiquitin-proteasome pathway. Within this process susceptible proteins are 'marked' by multiple copies of ubiquitin, which is a 76-amino acid peptide. This eventually ligates to become a lysine residue and eventually degraded by a large protein complex called 20 S proteasome [203]. This ubiquination process is only made possible by a complex enzymatic process where members of a large group of ubiquitin-ligases participate. Two such proteins have been shown to be upregulated in at least 13 distinct atrophy models: muscle ring finger 1 (MuRF-1) and muscle atrophy F-box protein (MAFbx) [204].

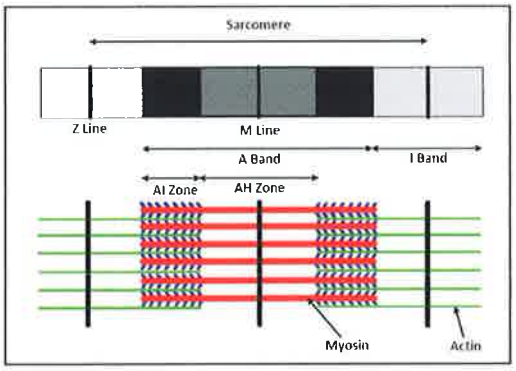
MuRFs are named from their muscular origin and include a RING Finger domain, a B-Box domain and a coiled domain also known as TRIM proteins (Tripartite motif). The RING Finger (Really Interesting Novel Gene) domain is required for ubiquitin ligase activity. MAFbx contains an F-box characteristic of a family of E3 ubiquitin ligases called SCFs. The name SCF is derived because it contains a Skp1 region, a Cullin 1 region and a F-box region [205].

The two genes appear to be overexpressed in many catabolic states. There is a reduction in the levels of both MAFbx and MuRF-1 by 30% following a resistance exercise programme [206]. Mice studies with knock-out genes for the both MAFbx and MuRF-1 have shown 36-56% less muscular atrophy after sciatic nerve denervation versus controls [207]. Although global approach to identify genes required to measure atrophy phenotype in skeletal muscle have been explored [207], MuRF-1 and MAFbx are the most extensively used due to their implications in several types of muscle atrophy, demonstrating the predominant role of the ubiquitin-proteasome pathway during the progression of muscle wasting.

1.4.3 Myosin Heavy Chain (MHC)

Skeletal muscle constitutes approximately 55% of an individual body mass and comprises of multiple bundles of muscle fibres bound together by connective tissue. The muscle fibres are long, cylindrical, multinucleated cells composed of strands of myofibrils. The myofibril in turn is composed of numerous muscle constituents called the sarcomeres which are multiple layers of thin actin and thick myosin filaments bundled in a repeated fashion. This is the functional unit of muscle [208] (Figure 10). The myosin filament is a hexamere composed of 6 polypeptides: 2 heavy chain and 4 light chains. Heavy chains contain 2 elongated globular domains or "heads" which synergistically interact with actin filaments to produce contraction (Figure 11). Actin is a double helix of monomer which is

bound in groups to tropomyosin and individually to troponin T, I and C to form a protein complex [209].



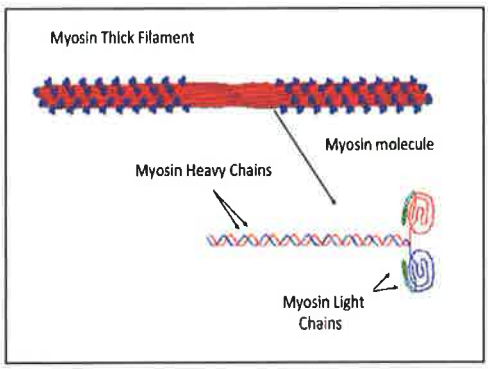


Figure 10: Myofibril structure **Figure 11: Myosin structure**

(Adapted from Andersen et al and Gelfi et al)

The myosin head activates when ATP attaches to ATP binding site. An enzymatic reaction of ATPase hydrolyses ATP to ADP, releasing chemical energy for muscular contraction. The rate-limiting factor for myosin and actin cross-bridging during muscular contraction is the enzyme ATPase. The adult skeletal muscle is comprised of 3 different isoforms of myosin heavy chains: the slow MHC type I and the fast MHC Type IIa and IIx. MHC isoforms are a marker of muscle fibre diversity and alteration due to intrinsic and extrinsic factors [210]. The myofibrils are categorised as Type I, Type IIA or IIB correlated to their content of MHC I, IIa or IIx respectively.

Type I, slow-twitch muscles are specific for a longer duration activity and is a fatigue-resistant phenotype which operate an oxidative metabolism; they have an increased mitochondrial content and a rich capillary bed, so they appear macroscopically red. Type IIa

fibres are also rich in mitochondria and capillaries and hence appear red as well; they stain strongly for succinate dehydrogenase (SDH) but also contain glycolytic enzymes, hence they are classified as fast-twitch oxidative glycolytic fibres and comprised of fast fatigue-resistant units. Type IIx (also called IId) fibres utilised more of glycolytic metabolism. Rat muscle type IIx fibres stain strongly for SDH and are intermediate in velocity of shortening between type IIa and IIb, while in humans the staining of SDH is the weakest as they rely mainly on glycolytic metabolism. Therefore in humans, type IIx are the fastest and most fatigable muscle fibres (Figure 12).

A specific type or muscle mechanical activity induces a switch in the expression of MHC, hence it contributes to the adaptation of muscle to a particular physical activity. Marathon runners and ultra-endurance athletes have been found to express 80-90% of their MHC pool as slow-type MHC Ia, whereas sprinters and weightlifters have predominately IIa/IIx fibres [208].

MHC proteins can be detected with immunohistochemical analysis using anti-myosin antibodies in ELISA or by electrophoresis (SDS-PAGE) on homogenated muscle samples or individual muscle fibres [211]. MHC isoform genes can be quantified using PCR reactions [212].

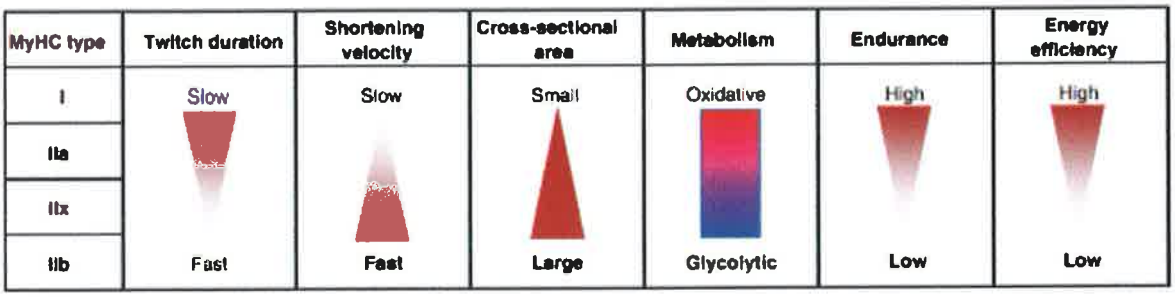


Figure 12. Different isoforms of Myosin Heavy Chain (MHC)

(Adapted from Gundersen et al)

1.5 Summary

There is an increased prevalence of ACL injuries leading to functional, strength and proprioceptive impairment at medium and long-term follow-up. Enhancing the quadriceps strength and function of the knee preoperatively may improve the final outcomes of subjects undergoing ACLR (Figure 13). There are currently three studies published looking at the effects of prehabilitation in ACL subjects. However, these studies utilised different physical exercise therapies and measured distinct outcomes. Some of these studies did not include a non-treated control group, postoperative assessment or molecular effects of prehabilitation. We studied the effects of a preoperative 6-week physical therapy programme which comprised of both eccentric and concentric exercises, open and closed-chain kinetic exercises, lower limb strengthening (with a particular emphasis on quadriceps) and proprioceptive training. This study assessed strength, dynamic function, muscle CSA, self-reported assessment and molecular alterations as effects of prehabilitation in ACLR in a randomised controlled manner.

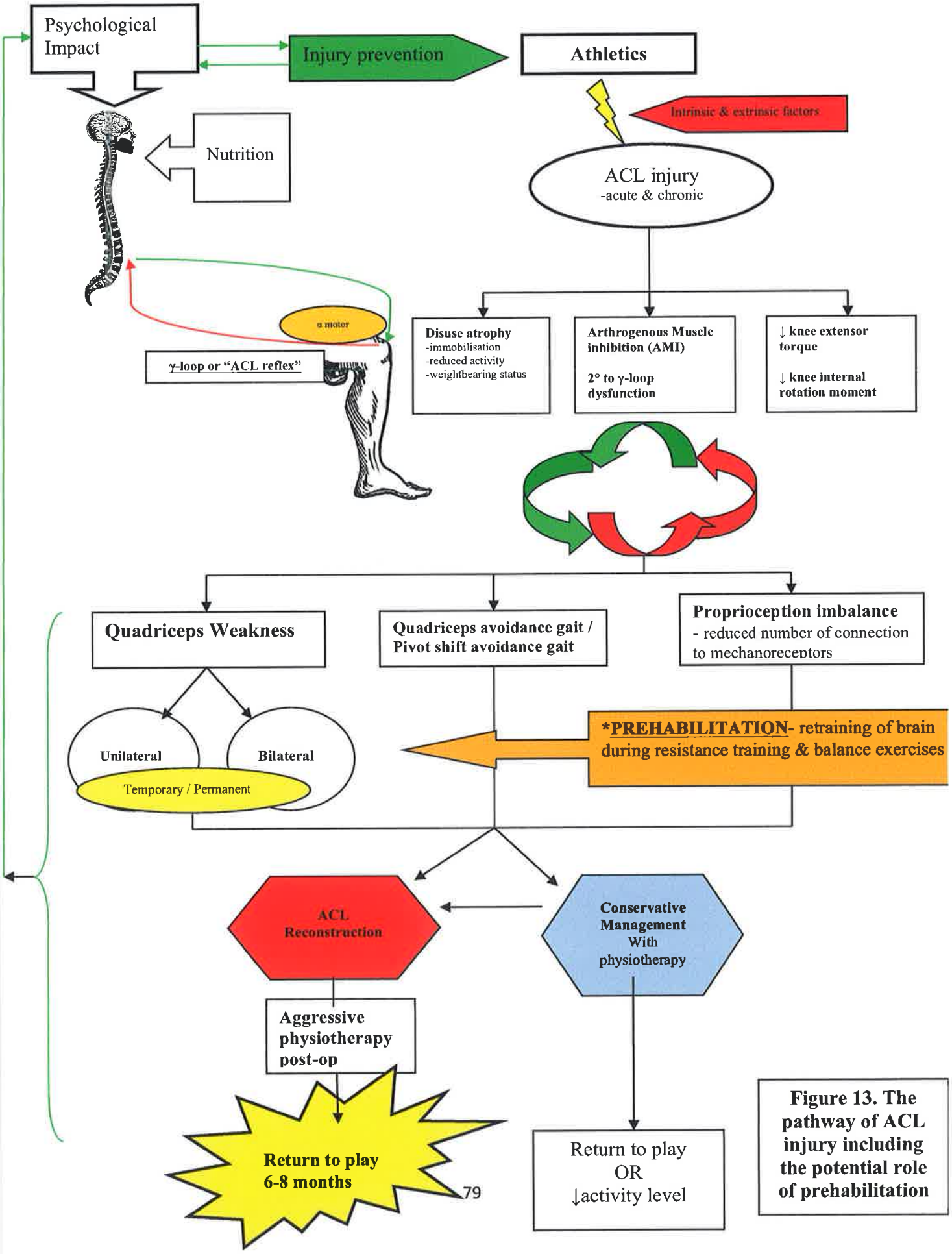


Figure 13. The pathway of ACL injury including the potential role of prehabilitation

Chapter 2

Materials and Methods

2.1 Subjects

Patients diagnosed with rupture of the anterior cruciate ligament were recruited from the Sports Surgery Clinic and Cappagh National Orthopaedic Hospital between December 2010 and December 2012. The inclusion criteria were males between the ages of 18 to 45 years old with an isolated ACL tear in the knee. Patients were excluded from the study if they were female, had any associated fracture, meniscal repair, associated collateral ligament injury requiring repair/reconstruction, had comorbidities contraindicating high physical exertion, and living outside Greater Dublin area for practical reasons with exercise supervision and gym usage. Patients were allowed to participate if they had a partial menisectomy. The study was designed and conducted in accordance with the Declaration of Helsinki. Ethics was approved by the Cappagh National Orthopaedic Research Committee and the Sports Surgery Clinic Research and Ethics Committee.

The waiting list from one orthopaedic consultant was used to recruit patients. Patients were provided with a complete protocol of the study and informed consent was obtained. Consent was also obtained from the consultant orthopaedic surgeon in charge of their care. Twenty-two patients were enrolled into the study, with 11 subjects in each of the prehabilitation exercise group and control group. During the enrolment process, opaque

envelopes were used to randomly assign patients to each group. Patient selection and allocation are depicted in the CONSORT flow diagram as shown in the next chapter.

2.2 Study design

The study involved a supervised 4-6 weeks gym and home based preoperative exercise programme (Appendix B). Both groups undertook a standard postoperative inpatient physiotherapy programme.

Muscle strength, quadriceps cross-sectional area (CSA), and physical performance were assessed at baseline (4-6 weeks preoperatively), prior to the ACLR and 12-week postoperatively. Pain and function were also assessed using the Modified Cincinnati score and Tegner Lysholm score at all 3 time points. A percutaneous muscle biopsy of the vastus lateralis muscle was performed at baseline, prior to ACLR and 12-week postoperatively. Peak torque, average peak torque, coefficient of variance and the hamstring to quadriceps ratio were assessed using isokinetic dynamometry.

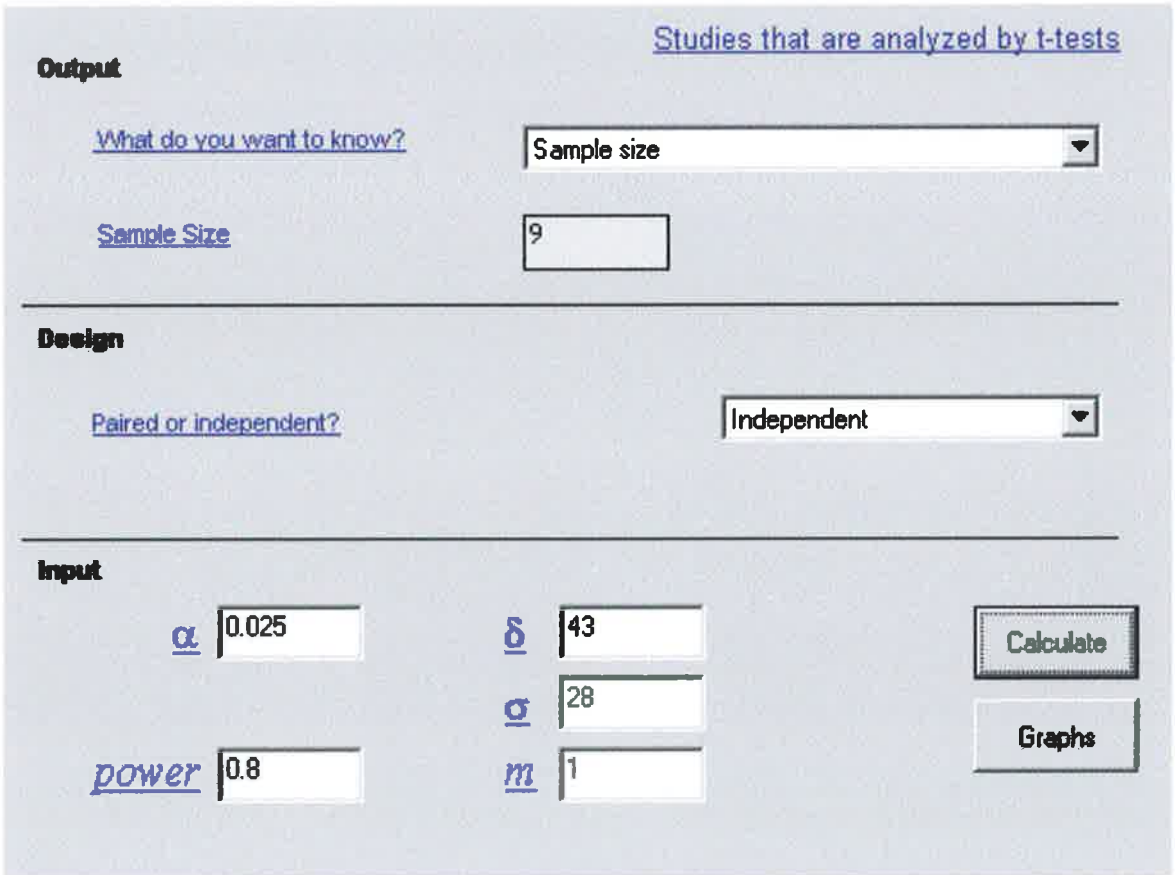
2.3 Power Analysis

Based on the 2 primary outcome measurements of the first five patients, we proposed that the potential improvements in quadriceps peak torque and the single leg hop

test following the exercise intervention were 22% and 30% respectively. The power was set at 0.8 and the significance level p=0.025.

Peak torque 1st five patients (T3)	Mean = 196.9 N	sd = 28.13
Proposed improvement =	22% Mean x 0.22 = 43.318	
Single leg hop test 1st five patient (T3)	Mean = 132.4	sd= 29.167
Proposed improvement =	30% Mean x 0.3 = 39.72	

A. Quadriceps peak torque



B. Single leg hop test

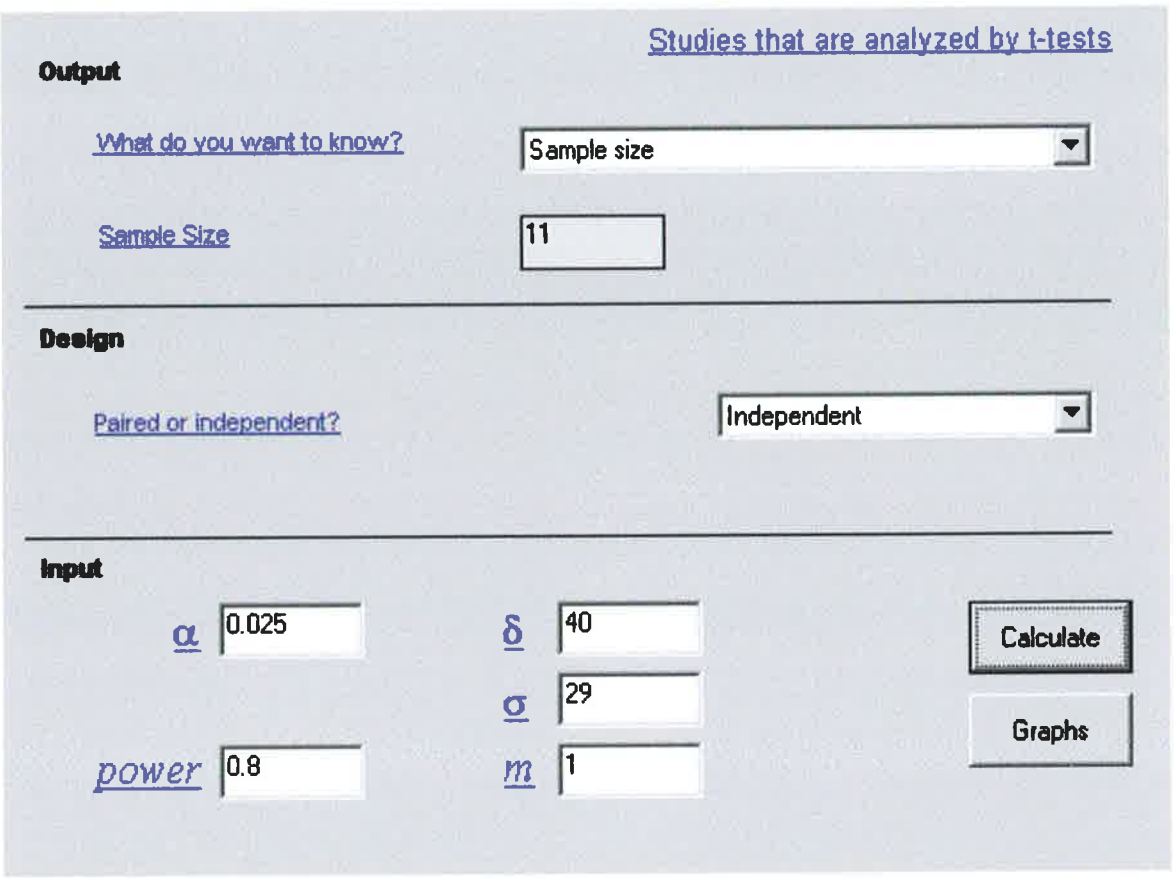


Figure 14. An automated calculator for sample size calculation for A, quadriceps peak torque and B, the single leg hop test using the Vanderbilt Power Size and Calculator Software programme version 3 (2009)

An automated programme using the Vanderbilt Power Size and Calculator Software version 3 (2009) was then used to calculate the sample size (n) [213]. With p value=0.025 and power=0.8, the sample size was estimated to be 9 and 11 for each treatment group for the quadriceps peak torque and single leg hop test respectively (Figure 14). The projected sample size of 22 fulfilled the power for these two primary outcomes.

2.4 Exercise Allocation

All patients had standard preoperative care that included a meeting with the physiotherapist at the initial visit where they were given written and verbal instructions on simple exercises to perform. The control group was not discouraged to do exercise or normal activities of daily living prior to the ACLR. The exercise group was enrolled in a 6-week exercise programme preceding surgery. This exercise programme consists of resistance training and balancing programme as shown in Appendix B. The programme consists of four supervised exercise periods per week: 2 gym sessions interspersed with 2 home sessions. The starting weights in the gym were determined by the best single maximal effort. There were 3 sets of 12 repetitions with a weight increase of 10-15% per week. During the last session of the gym exercise, the weights were reverted to the previous week values to favour the muscular response to endurance and gaining mass [214]. Home exercise consisted of the same programme as the gym but with the use of a *Thera-Band®* instead of weights. Patients used a Don Joy knee brace locked at 20° extension to reduce the risk of further straining the ACL pre- or postoperatively during open chain kinetic (OKC) exercise [215]. The brace also functions as a confidence factor during OKC exercise.

2.5 Strength Assessment

The average peak torque, average work per repetition, coefficient of variance and deficits of the quadriceps and hamstrings were assessed using isokinetic dynamometry

(Cybex Humac Norm®) (Figure 15). Since its introduction in the 1970s, researchers have argued that isokinetic contractions do not mimic the normal everyday activities of the thigh musculature. The Cybex dynamometer assesses isokinetic quadriceps strength with good reliability, when compared to either isotonic or isometric testing [216,217]. The dynamometer remains an integral part of research and as a rehabilitative tool after any knee surgery.

Figure 15: The Cybex isokinetic dynamometry machine with an anti-shear device

An isokinetic contraction occurs when a limb moves at a constant velocity with a joint as the axis of rotation. The velocity of the movement is kept constant with the dynamometer which in turn measures the resistance. This resistance is directly proportional to the muscular force produced. Isokinetic testing has several advantages. Firstly, it measures forces in dynamic motion unlike isometric contraction where the conditions are static; the muscle length and joint angles remain constant during muscular contraction.

Secondly the testing is considered safe as the resistance generated by the machine depends on the patient's effort. It is an objective measure of muscle performance that is validated and commonly used for the knee joint. The test-retest reliability is often assessed using Spearman or Pearson correlation coefficients. The correlation coefficient only describe how strongly associated units measured in the same group resemble each other. Intraclass coefficient (ICC) is considered a better measurement and a value over 0.8 is considered a responsive test. Larsonn et al, have shown an ICC of 0.93 for peak torque during repetitive concentric knee extensions [218].

The isokinetic sessions were supervised by the same researcher. Any measurement on the device was noted for future test purposes. Once patients were seated on the isokinetic machine, they were secured with upper crossing torso and thigh stabilisation straps. The seat was adjusted to align the patient's knee axis of rotation to the dynamometer shaft. The shin attachment was secured with the Johnson anti-shear device as shown in Figure 15. The anti-shear device is used to prevent excessive anterior translation of the tibia in relation to the femur. Subjects were verbally encouraged to give maximal effort.

Subjects performed 3 sets of 10 concentric isokinetic repetitions with a rest period of 10 seconds between each set. The first set was the process where patients performed submaximal efforts for most of the repetitions, except for the fourth and fifth trial where maximum effort was achieved to fulfil familiarisation. The next 2 sets were recorded at maximal effort and were performed at 90°/sec. This speed was chosen as this was deemed

the normal velocity of the knee joint and was also the testing speed at this unit. Due to the ACL injury and the risk of shearing, a mean arc of 20° – 110° was used.

The coefficient of variance (COV) shows the average of peak torque values for each repetition within a set. It was used as a guide to reliability of the data. Factors such as pain, apprehension, poor instruction and lack of effort or concentration can increase the COV. The test was repeated if the COV was more than 15%.

2.6 Outcome Questionnaires (Appendix C)

The Modified Cincinnati knee rating system, Tegner-Lysholm and Tegner Activity score were completed at baseline, preoperatively and at 12-week postoperatively. The questionnaires were checked upon completion and any enquiry was explained to the patient [219].

Modified Cincinnati Knee Rating System

The Modified Cincinnati Knee Rating Score is a self-monitored questionnaire that assesses symptoms, functional limitations with athletic and daily activities, perception of patient's knee condition, and current activity level in the ACL injured or ACLR patient. It uses a battery of questions in eight sections with a total score of 100. The questionnaire has been

validated psychometrically and has been shown to detect a clinically relevant difference over time after ACL injury [220,221].

Tegner-Lysholm Knee Scoring Scale

The Tegner-Lysholm scale is another well-validated functional score designed for knee ligament injuries as well other knee pathologies [222,223]. The scale is similar to the Cincinnati score with its eight sections of questions totalling 100 points. However it has been noted that patients tend to score higher on the Tegner-Lysholm questionnaire as compared to the Cincinnati score [223].

Tegner Activity Score

The Tegner Activity score determines the level of athletic and occupational activity in patients before and after their injuries. The scoring scale ranges from 1 to 9 depending on activity levels.

2.7 Physical Performance Tests (Appendix D)

Single Leg Hop Test

The single hop test is considered the gold standard to assess the functional status of the thigh musculature [224]. Although it has a low sensitivity rate, it has a high specificity and low false-positivity to confirm any abnormal limb symmetry [225]. Patients commenced the test with their non-injured leg. They initially were given a trial jump, followed by three further jumps on the same leg. The best single leg hop value was recorded. Patients were instructed to wear a knee brace with free range-of-motion to improve their confidence when jumping on their injured leg.

In-Line Lunge Test

The in-line lunge test is one of the aspects of the Functional Movement Screen [226] and this test for lower limb stability. The tibia length of the patient was measured so it was referenced to the floor as the distance to be covered during the lunge by the lagging lower limb. The patient placed their heel at the beginning of the tape measure. The dowel was placed behind the back touching the head, thoracic spine and sacrum. The hand opposite to the front foot grasped the dowel at the cervical spine while the contralateral hand held at the sacral level. The patient then stepped out on the tape measure on the floor placing the heel of the opposite foot at the indicated mark. The patient then lowered the lagging knee to be able to touch the surface behind the heel of the front foot and returned to the starting

position. The test was performed three times bilaterally in a slow controlled manner. The score range was from one to three.

2.8 Muscle Cross-Sectional Area (CSA)

Magnetic resonance imaging (MRI) was performed at baseline, preoperatively and at 12-week postoperatively to assess changes in quadriceps CSA. Imaging was performed using a Philips Gyroscan Intera 1.5 Tesla MRI scanner (Phillips Healthcare, Netherlands).

Patients were placed supine with a surface body coil around both thighs. A field of view of 30 cm was used (256 x 256 pixel matrix). An initial coronal scouting scan was used to establish the level of the knee joint. Subsequent axial cuts were taken 15 cm proximal to the joint line. T2 weighted axial cuts were taken. A slice thickness of 4 mm and slice gap of 0.4 mm were used with an echo time of 100 ms and a relaxation time of 3000 ms. An experienced radiologist manually outlined the entire quadriceps as a region of interest and the area was calculated using a commercialised validated built-in software package (EasyVision Philips Medical Systems ®). Analysis was performed on a single fixed slice at the 12^{th} MRI slide. This equates to the fixed 15cm from the joint line for the first slide in addition to 12-four millimetre axial cuts. Intra-observer reliability of cross-sectional measurement has previously been shown to have a co-efficient of variation of 0.78 % [227]. Each slice was analysed 3 times by a consultant radiologist and the mean value was used for final analysis.

The use of MRI in the assessment of quadriceps femoris cross-sectional area (CSA) is well validated. Mitsiopoulis et al, compared thigh-adipose tissue free skeletal muscle on MRI axial cuts with cadaveric dissections on the same specimens and found correlation coefficients of 0.99 [228]. With regard to repeatability, Mitsiopoulos found an intraobserver error for skeletal muscle CSA of 2%. MRI quadriceps CSA is also responsive to a short term strengthening programme. Ploutz et al, found a mean increase in CSA of 5% in healthy subjects following a 9-week resistance training programme [229].

2.8 Muscle Biopsy Procedure

The baseline muscle biopsy was performed under local anaesthetic using an aseptic technique. A site was marked on the lateral aspect of the thigh midway between the anterior superior iliac spine and the knee joint, just anterior to the iliotibial band. The site is the standard location for access to the vastus lateralis muscle and is away from longitudinal incision used in ACLR. During the second muscle biopsy which was performed intraoperatively, the site was compressed by the tourniquet applied to the thigh to prevent a haematoma.

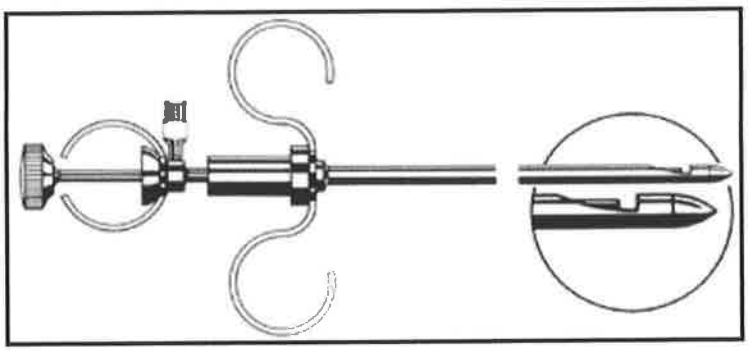


Figure 16: Bergstrom muscle biopsy needle

The patient was asked to contract their thigh muscles to confirm the location over the vastus lateralis muscle. A small area of skin was shaved if necessary. 5 ml of local anaesthetic (1 % lignocaine) was infiltrated into the skin and subcutaneous tissue as far as the fascia but not into the muscle itself. The skin was painted with an iodine antiseptic solution and a sterile drape placed over the thigh. A 5 millimetre skin incision was made through the skin and the deep fascia with a number 11 blade scalpel.

The obturator was removed from the biopsy needle (Figure 16). The needle was inserted with window closed (inner needle advanced to its furthest forward position). The needle was advanced, moving easily through skin until resistance was felt from the muscle sheath and then pushed through the sheath.

Suction was applied to the needle with the aid of an assistant using a large syringe and connection tubing. A sample of tissue was guillotined by opening the outer needle window (retracting inner needle) and then closing the window with forward thrust of the inner needle. Careful timing was required between the surgeon and the assistant in that as soon as the barrel was plunged in, the suction was released to avoid sucking the sample up into the tubing. This procedure was repeated approximately 3 times without removing the needle from the thigh. The needle was then removed and the obturator used to evacuate the specimen (usually around 100 mg).

Pressure was applied for approximately 5 minutes and once haemostasis was achieved, several steristrips were applied to close the incision. A waterproof dressing was

placed over the wound. An elastic stockinette was placed over the thigh for further compression for a few hours if required. The patient was instructed not to remove the dressing for 5 - 7 days. The second biopsy was performed immediately prior to surgery under general anaesthetic, obviating the need for a local anaesthetic. This further minimized patient discomfort and stress. The third muscle biopsy was performed at 12-week postoperatively using the protocol performed for the baseline sampling.

2.9 Preparation of muscle sample and analysis

The muscle sample was divided into a small (~ 20 mg) and a large, (~ 80 mg) portion. The smaller portion was mounted in Tissue-Tek® OCT™ Gel and frozen in isopentane cooled to the temperature of liquid nitrogen. The larger portion was frozen directly in liquid nitrogen. Both were then stored at –80°C.

Histopathology in Haematoxylin and Eosin Staining

Frozen muscle samples from 5 patients in each treatment group were fixed in 4% formaldehyde. Cross-sectional specimens cut using a knife pre-cooled to -20°C were mounted into slides and dried at room temperature for 1 hour. Slides were incubated with haemotoxylin solution (Mayer®) for 5 minutes and subsequently washed using warm running tap water for 5 minutes. Acetic acid was added to dilute Eosin solution (Merck®) to 0.5%. The slides were subsequently incubated with Eosin for 10 minutes. Slides were then

wash in a glass chamber three times for 1 minute with distilled water. The slides were dehydrated using 70% ethanol for 1 minute and subsequently with Xylene for 30 seconds. The slides were mounted with 1-2 drops of a Xylene-based mounting media (Depex®) and covered with cover slides avoiding bubbles. The slides were pressed under heavy weight for 10 minutes at room temperature and stored. The microscopy images were acquired at 200X magnification with the Leica DM RBE microscope®.

Muscle preparation for SDS-Page

Approximately 25 mg of frozen muscle was placed in eppendorf tubes. The top of each eppendorf tube was pierced with a needle and the samples were lipophalised (freezed dried) overnight. Connective tissue or clotted blood was dissected out from each sample under light microscopy.

Protein assay (microplate)

A BioRad DC ® (Detergent Compatible) protein assay was used as described by Lowry [230]. The protein content of each sample was determined to allow standardisation of samples. The same amount of protein per well must be loaded for SDS-PAGE to ensure that differences in protein content are not due to variation in initial volume loaded. A standard was first prepared using bovine serum albumin (BSA) mixed with homogenate buffer at the following concentrations; 10, 7.5, 5.0, 2.5, 1.0 and 0 mg/ml. 5 ul of each standard was added

to a 96-well microplate in duplicate. The samples were thawed on a water bath at 56°C and 5 µl of each sample were added to wells in duplicate. 25 µl of reagent A and 200 µl of reagent B of this commercialised assay were finally added to each well and the plate loaded onto the plate reader. The absorbance of each sample was measured at 595 nm as per manufacturer's instructions.

The protein concentrations were automatically determined from a standard curve and the mean of the duplicate values used to determine the volume of each sample required. 360 µg of each sample was made up in final volumes of 300 µl to give a final protein concentration of 1.2 µg/µl. This included 75 µl of Laemmli buffer and the required amount of homogenate buffer to create the total volume.

Gel setup

The glass plates were cleaned with 95% ethanol. The running gel was prepared with 58.2 ml of ultra pure H_2O, 30ml of 1.5 M Tris buffer (pH 8.8), 30ml of 30% Acrylamide, 1200 µl of 10% sodium dodecyl sulfate (SDS), 600 µl of 10% ammonium presulfate (APS) and 60 µl of *N,N,N',N'* tetramethylethylenediamine (TEMED) as described by Talmadge and Roy [231]. The plates were assembled and the running gel loaded and allowed to set for 1 hour. Air bubbles were removed with 1.2 ml of Isopropanol.

The stacking gel was prepared with 12.4 ml ultra pure H_2O, 5ml of 0.5 M Tris buffer (pH 6.8), 2.6 ml of 30% Acrylamide, 200 µl of 10% SDS, 200 µl 10% APS and 20 µl TEMED. Gel combs were placed in the plates and the stacking gel loaded to form lanes for the

electrophoresis. After one hour, lane dividers were carefully removed and the lanes straightened. The plates were placed in the gel rig and filled with running buffer. Running buffer contained 15.15g Trisma, 72.07g Glycine and 5g SDS per 5 litres of ultra pure H_2O. The samples were then carefully thawed again in a water bath incubator at 56°C for 20 minutes and loaded carefully into the wells with a pipette. The plates were transferred to the running rig and the rig was filled with running buffer.

The electrodes were connected and the gel run at 35 milliamps (mA) in the cold room at 4°C. To optimise electrophoretic separation of the isoform bands, conditions for the gel runs were varied. Running time ranged from 16 – 60 hours. At 16 hours, the tracking dye had just reached the end of the gel whilst at 60 hours it was no longer visible. The amount of protein loaded per well was also varied from 1-20 µg .

Gel constituents were also changed from 6 – 8 % Acrylamide and Glycerol was added in line with the technique described by Talmadge and Roy (118). The gels were stained directly in Coomasie blue (Sigma Aldrich laboratories) for 1 hour and rinsed in ultra-pure H_2O until protein bands were visible. They were then scanned with a conventional scanner and photographed. Images were processed with EZQuant-Gel 2.17 software and graphical intensity analysis of the bands calculated. Bands were identified using known migration patterns from previous studies and verified with commercialised rabbit muscle as positive control. In addition the rainbow marker allowed identification of the approximate molecular weight of the bands (220 – 240 kD).

RNA extraction using TRI-reagent ®

RNA was isolated using TRI-reagent® (Sigma-Aldrich) according to the manufacturer's instructions. 25-30mg of frozen muscle sample was added directly to 500 ul of TRI-reagent. To further break down cellular material, the homogenate was repeatedly drawn up with a 1 ml syringe and 19 and/or 21 G needles. It was then centrifuged at 12000 revolutions per minute (rpm) for 10 minutes at 4°C. The supernatant was transferred to a new tube and allowed to stand at room temperature for 5 minutes. 150ul of chloroform was added and the mixture shaken vigorously using a Vortex for 15 seconds and allowed to stand at room temperature for 10 minutes. It was then centrifuged at 12000 rpm for 15 minutes at 4°C. The colourless upper aqueous phase which contained the RNA was transferred into a new eppendorf, carefully leaving the cloudy middle phase containing DNA. 250ul of isoproponalol was added to the samples. The mixture was shaken vigorously using a Vortex for 15 seconds and left at room temperature for 10 minutes. This was followed by another centrifuge at 12000 rpm for 10 minutes at 4°C. The supernatant was removed carefully, isolating the RNA pellet which was subsequently washed using 500 ul of 75% ethanol and vortexed for 15 seconds. It was then centrifuged at 12000 rpm for 5 minutes at 4°C. The supernatant was again removed carefully and the pellet was allowed to dry for 10 minutes by placing the eppendorf sideways on tissue paper. The pellet was than dissolved in 30ul of diethylypyrocarbonate (DEPC)-treated water. Samples were stored at -80°C.

Before reverse transcription, the RNA was quantified and quality assessed using a spectrophotometer. The ration of the absorbance at 260 and 280nm (A260/280) is used to assess the purity of RNA from DNA and protein contamination. A 260/280 ratio of more than 1.9 was achieved.

Reverse Transcription

For quantification of messenger RNA (mRNA), equal quantities (500 ng) of RNA were reverse transcribed into complementary DNA (cDNA) using the Quantitect Reverse Transcription kit (Qiagen, Valencia, CA). The purified RNA samples were briefly incubated in genomic DNA (gDNA) Wipeout Buffer at 42 °C for 2 minutes to effectively remove contaminating genomic DNA (Table 1). The RNA was reverse-transcribed using a master mix of reverse transcriptase, buffer and primer mix (Table 2). The entire reaction took place at 42 °C and was then inactivated at 95 °C . Following this, the cDNA samples now formed were transferred to ice for 5 minutes and then stored at -20 °C until analysed. This experiment was performed under strict RNA-ase free conditions (Figure 17).

Table 1. Genomic DNA elimination reaction components

Component	Volume/Reaction	Final concentration
gDNA Wipeout Buffer, 7x	2ul	1x
Template RNA	5 ul (500 ng)	
RNAase-free water	7 ul	
Total volume	14 ul	

Table 2. Reverse-transcription reaction components

Component	Volume/Reaction	Final concentration
RT Master Mix		
Quantiscript Reverse-Transcriptase*	1 ul	1x
Quantiscript RT Buffer, 5x^	4 ul	
RT Primer Mix	1 ul	
Entire gDNA-free template RNA	14 ul	
	20 ul	

* Also contain RNAase inhibitor

^Includes $Mg^{2+}$and dNTPs

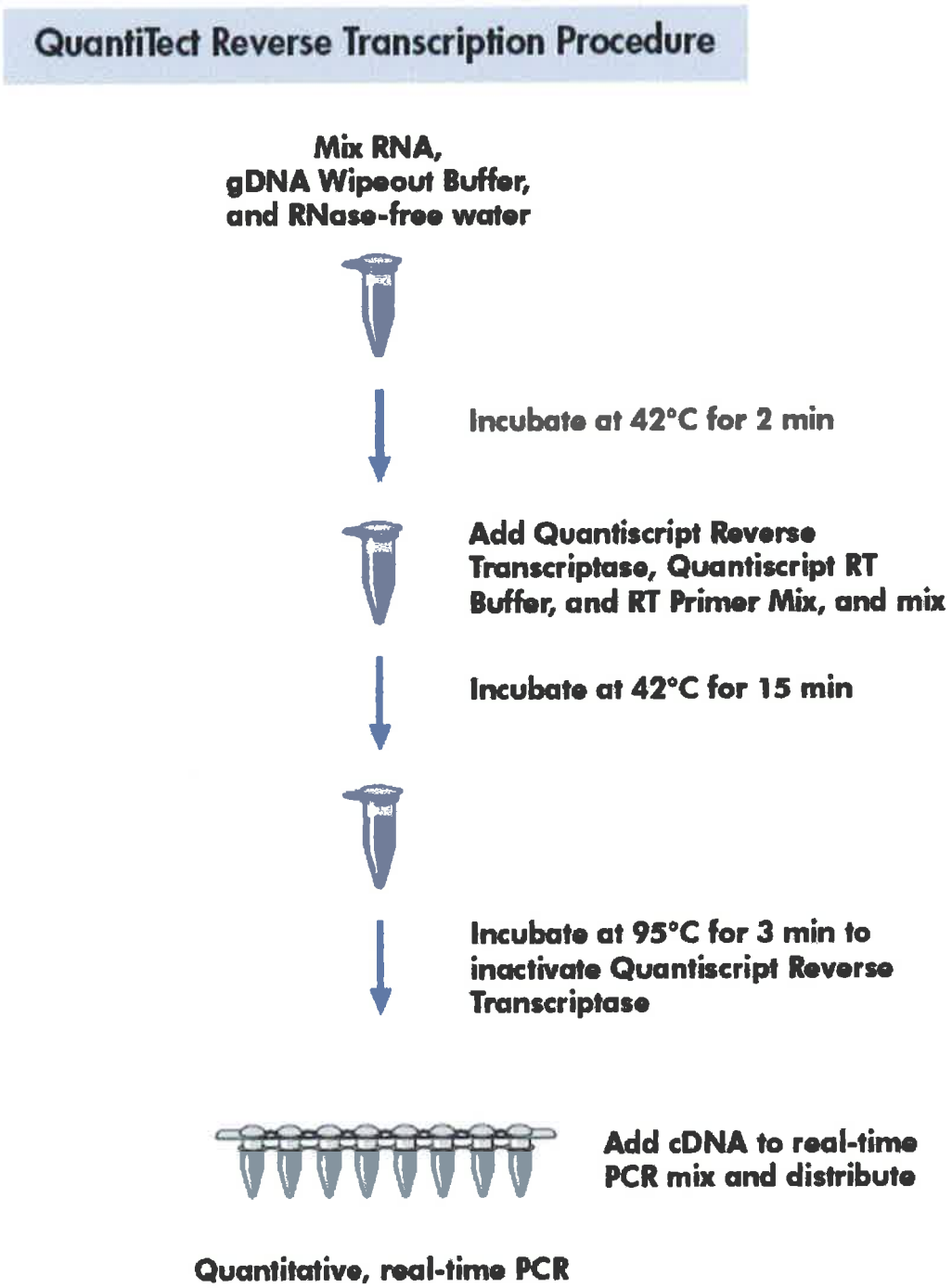


Figure 17: PCR-QuantiTect RT Procedure

The resulting cDNA was a template for quantitative real-time PCR. Oligonucleotide primers were synthesized (MWG, Biotech, Ebersberg) and quantitative PCR was performed in 20 ul reaction containing 2 ul template cDNA, SYBR Green MasterMix ® (Roche, Basel, Switzerland) and 10 pmol of each forward and reverse primer (Table 3). Amplification for mRNA was performed on the Roche LC 480 Lightcycler® in triplicate samples, including non-template controls. Conditions were 45 repeat cycles of 95°C for 15 seconds and 58-65 °C for 60 seconds depending on the melting temperature of the primers. Relative expression of genes relative to GAPDH was determined using the $2^{-\Delta\Delta Ct}$ method.

Table 3. Primers

Primers	Sequence (5' -> 3')	Tm (°C)	GC-Content
GAPDH (F)	CATGAGAAGTATGACAACAGCCT	58.9	43.5%
GAPDH (R)	AGTCCTTCCACGATACCAAAGT	58.4	45.5%
IGF-1 (F)	GATGGGGTCTCGCACTGTCCC	65.7	66.7%
IGF-2 (R)	GAGCCGAGATCATGCCACTG	61.4	60%
MURF-1 (F)	CCTGAGAGCCATTGACTTTGG	59.8	52.4%
MURF-2 (R)	CTTCCCTTCTGTGGACTCTTCCT	62.4	52.2%
MAFbx (F)	GCAGCTGAACAACATTCAGATCAC	61.0	45.8%
MAFbx (R)	CAGCCTCTGCATGATGTTCAGT	60.3	50%
MHC I (F)	TCGCCGAGTCCCAGGTCAAC	63.5	65%
MHC I (R)	TGGGGCTTTGCTGGCACCTC	63.5	65%
MHC IIa (F)	GATCGAGGAGCTGCGGGCCACT	67.7	68.2%
MHC IIa (R)	GGTGGGCGCTGGTGTCCTGCT	67.6	71.4%
MHC IIx (F)	CGCAAACATGAGAGAAAAGTGAAGGAAC	63.7	42.9%
MHC IIx (R)	CCGGAATTTGGAGAGGTTGACGTT	62.7	50%

2.10 Statistical Analysis

Statistical analysis were performed using SPSS version 20.0 (Chicago,Illinois). A one-way analysis of variance (ANOVA) was used to evaluate potential group differences in their baseline characteristics (age, height, weight, time until surgery, Lachmann test, anterior drawer test and pivot shift test). A mixed- design ANOVA for repeated measures was used to analyse the different groups over time (6-week preoperatively, preoperative and 12-week postoperative time points). Where the data violated the assumption of sphericity, the Greenhouse-Geisser correction was used. The level of significance was set to 5%, with Bonferonni adjustment made for multiple comparisons. A linear mixed model analysis was also performed on all data requiring longitudinal (repeated) measures to validate the *p*-values generated from the mixed-design ANOVA. The results were expressed as mean value ± standard deviation (SD). Pearson linear correlation coefficient was calculated with 95% confidence interval to analyse correlation of different parameters.

Chapter 3

Results

A total of 439 patients were assessed for eligibility from November 2010 until December 2012, as shown in Figure 17. From a pool of 58 eligible patients, 44 were contactable and 14 were non-respondents. A total of 14 patients were initially allocated to the exercise group and 11 to the control group. Three of the participants in the exercise group did not complete the preoperative physiotherapy programme due to time constraint and their data were not used in the final analysis. The measurements for 22 patients were analysed for the single leg hop test and quadriceps peak torque. Other measurements include correlative analyses were performed in 20 patients (N=11 and 9 in exercise and control groups respectively). All patients in the exercise group completed > 90% of the prescribed exercise regime (Figure 18).

As this was a longitudinal study with three time points, a normalised ratio was used to account individual's differences. This standardisation was measured as a ratio of the test's value of the assessed limb at a time point over the value of the injured limb at baseline. Values of the injured limb at baseline were normalised to '1'. For example, the uninjured limb cross-sectional area (CSA) value at baseline would be above '1' if it were greater than the injured limb CSA value at baseline. Therefore, graphs that indicate the data as ratio in the y-axis are normalised ratio as opposed to absolute values.

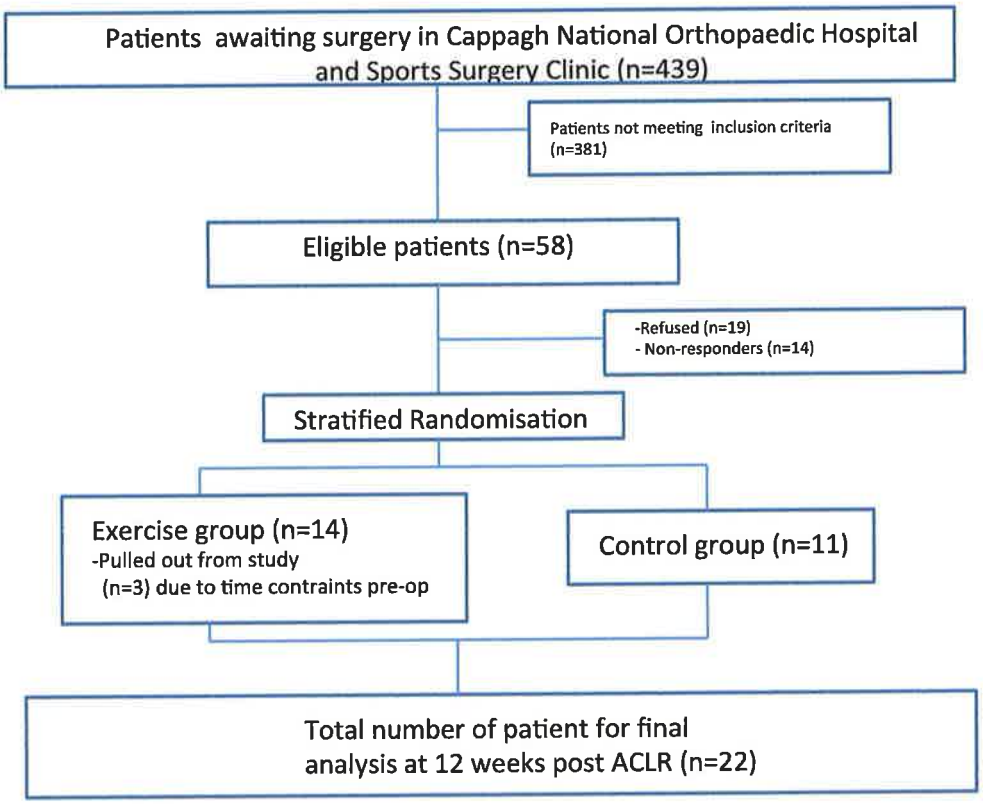


Figure 18: CONSORT flow diagram of the study

3.1 Baseline characteristics

There were no significant differences in the age, height, weight, body mass index (BMI), surgery waiting-time and Tegner activity level between the groups at baseline (Table 4). Patients had similar mean Tegner activity level score pre- and post-injury in both groups. All patients had a positive anterior drawer and Lachmann test. It was not always possible to perform the pivot-shift test in the outpatient setting due to pain. However, all patients had a positive pivot test during preoperative assessment after a general anaesthetic.

Table 4: Baseline characteristics (N=22)

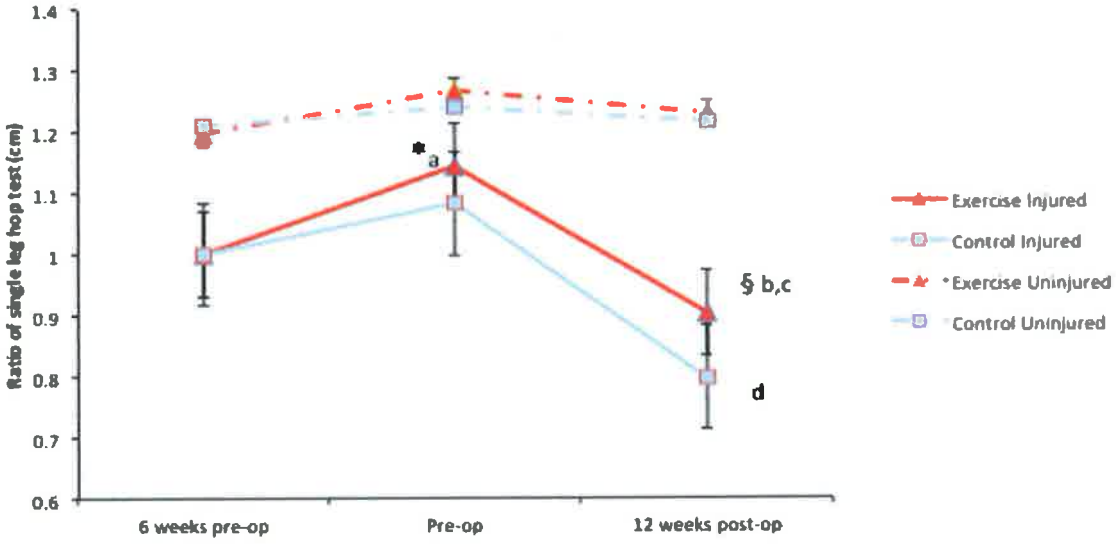
Parameter	Experimental Condition		P-value
	Exercise	Control	
Age	27.55 ± 7.85	31.36 ± 7.72	0.26
Height	180.6 ± 7.4	177.1 ± 7.60	0.29
Weight	78.91 ± 9.52	85.09 ± 16.01	0.28
BMI	24.21 ± 2.71	26.94 ± 3.41	0.052
Weeks till surgery (weeks)	4.50 ± 0.9	4.27 ± 0.6	0.66
Tegner Activity Before Injury	8.27 ± 1.00	8.27 ± 1.27	1.00
Tegner Activity After Injury	3.91 ± 1.13	4.36 ± 1.70	0.47
Injured Limb Right: Left	7 : 4	8 : 3	0.66
Dominant Limb Right: Left	9 : 2	10 : 1	0.56
Anterior drawer test	+	+	
Lachmann test	+	+	
Pivot shift test	+	+	

3. 2 Functional tests

Single leg hop test

Performance of the single leg hop test assessing dynamic knee function is illustrated in Figure 19. After exercise intervention, there was an improved trend in the single leg hop test for both exercise and control groups in both limbs. However, the improvement in the performance of the injured limb was statistically significant in the exercise group preoperatively compared to baseline within the same group (p=0.001). The mean (mean,SD) preoperatively was higher for the injured limbs in the exercise group (183.1, 15.55) compared to controls (156.5, 38.49) and this was statistically significant (p=0.046). In percentage, the increase in performance was 13.5% in the exercise group compared to 9% in the control group post-exercise therapy.

The single leg hop test scores of the injured limbs were reduced at 12-week postoperatively for both groups. However, the exercise group had a higher score for their injured limbs compared to controls. This was statistically significant (p=0.001).

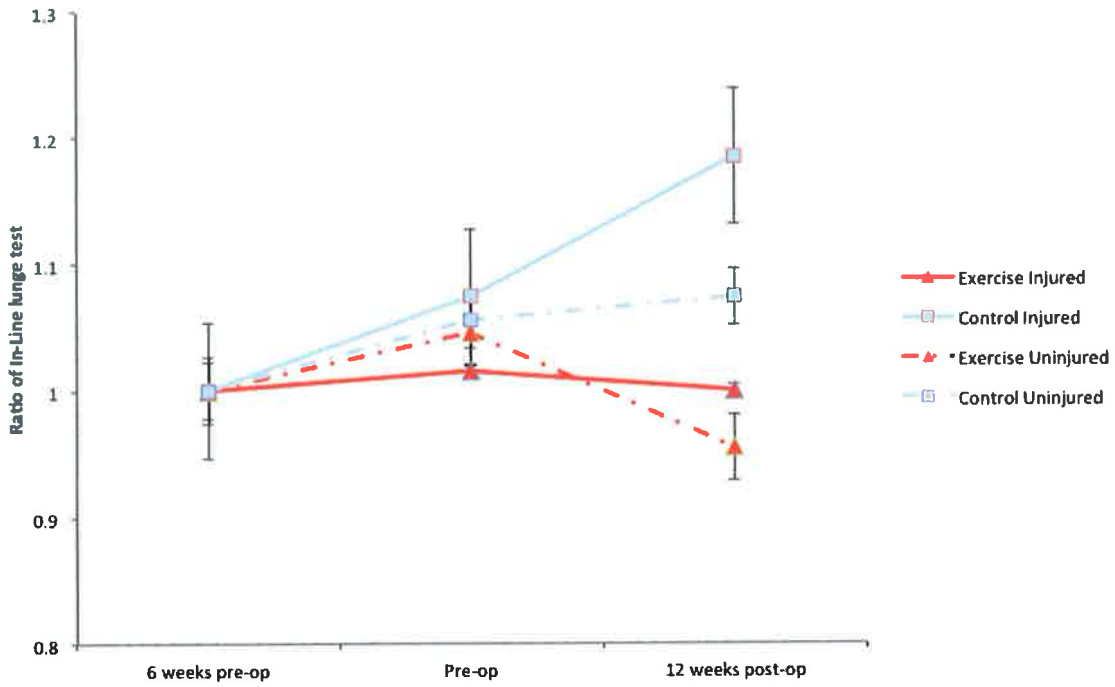


*p= 0.046 v Control Injured
§p= 0.001 v Control Injured
[a] p= 0.001 v 6 wk pre-op
[b] p= 0.01 v pre-op
[c] p= 0.047 v 6 wk pre-op
[d] p<0.001 v 6 wk pre-op & pre-op

Figure 19: Ratio of single leg hop test (N=11 for each group). Three tests were performed on both injured and uninjured limbs in which the best value was recorded at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op*). A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05. The graph represents a ratio in which the respective injured limb's value at baseline is the common denominator.

In-line lunge test

Performance in the in-line lunge test to assess static knee function is illustrated in Figure 20. There were no statistically significant differences between the injured or uninjured limbs at any time point for both groups.



p= nil significant

Figure 20. Ratio of in-line lunge (N=11 and 9 for exercise and control groups respectively). Three tests were performed on both injured and uninjured limbs in which the best value was recorded at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op*). A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05. The graph

represents a ratio in which the respective injured limb's value is the common denominator.

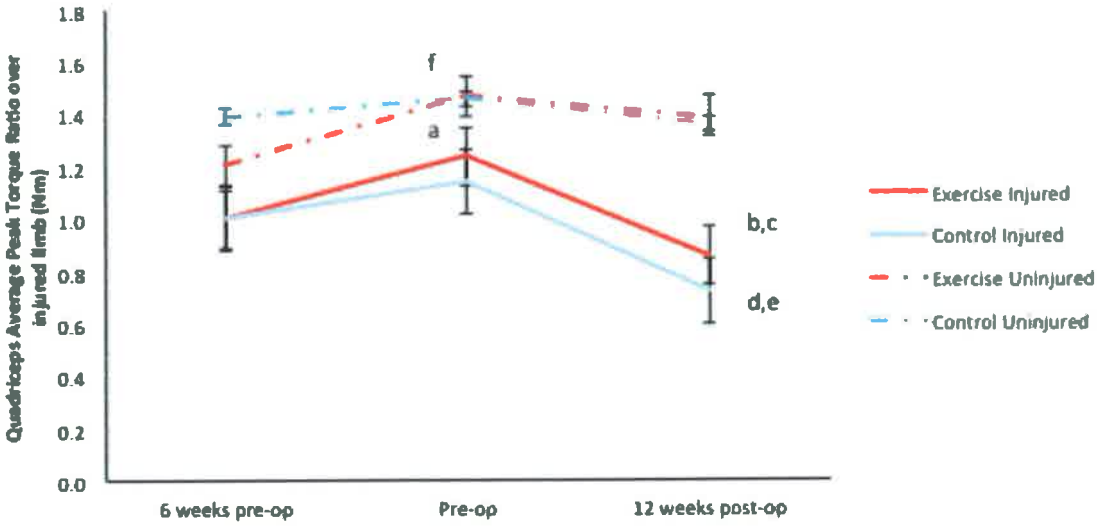
3. 3 Isokinetic Dynamometry

Quadriceps Peak Torque

Quadriceps peak torque assessing quadriceps strength is illustrated in Figure 21. Quadriceps peak torque increased significantly in both the injured and the uninjured limbs after preoperative exercise programme compared to baseline ($p=0.001$, $p=0.006$ respectively). Compared to baseline and preoperative values, there was a significant decrease in quadriceps peak torque of the injured limb in the exercise group at 12-week postoperatively ($p=0.033$, $p<0.001$ compared to baseline and preoperative time points respectively). This was similarly seen in the control group ($p=0.001$, $p<0.001$ compared to baseline and preoperative time points respectively).

Though there were no statistically significant differences between the exercise and control group for the injured limbs at any time point, there seems to be an improved trend in the exercise group at preoperative (mean, SD) (151.1, 30.21 vs 138.7, 43.92 for the exercise and control groups respectively) and 12-week postoperative time points (102.1, 22.18 vs 89.27, 34.70 for exercise and control groups respectively). In percentage, the increase in average quadriceps peak torque post-exercise was 20% versus 11% from baseline in the exercise group compared to control group. Meanwhile the decrease in

average quadriceps peak torque was 19% in the exercise group and 31% in the control group at 12-week postoperatively.



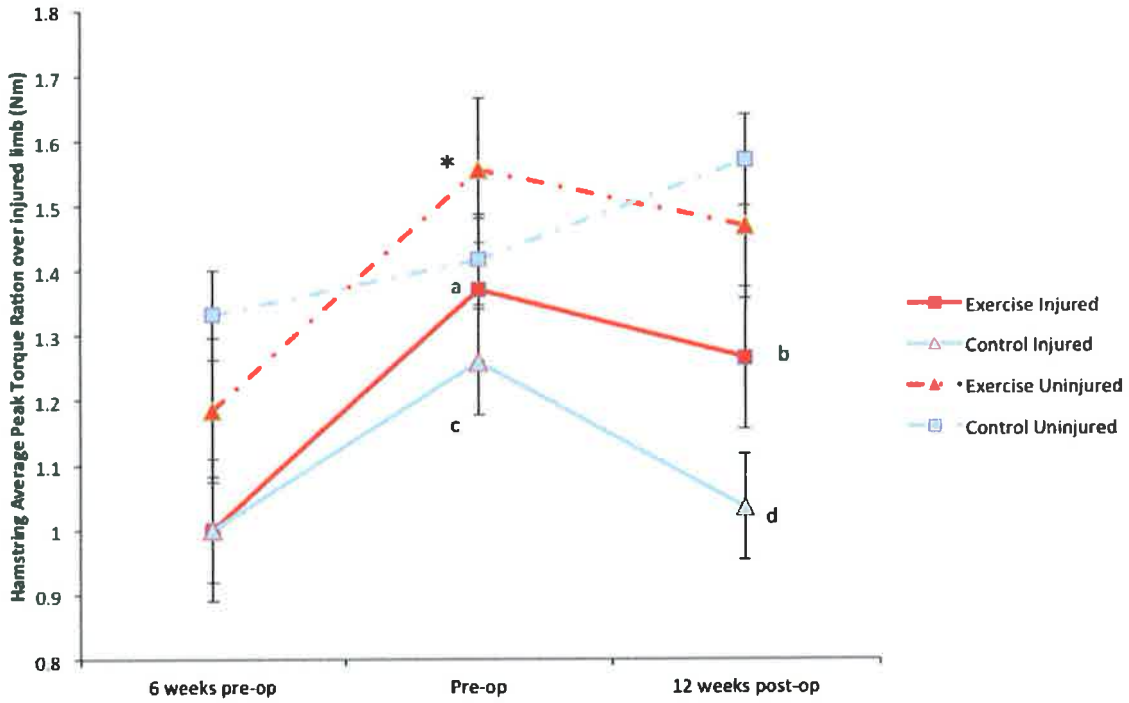
[a] p= 0.001 v 6 wk pre-op
[b] p<0.001 v pre-op
[c] p=0.033 v 6 wk pre-op
[d] p<0.001 v pre-op
[e] p=0.001 v 6wk pre-op
[f] p=0.006 v 6wk pre-op

Figure 21: Quadriceps peak torque ratio (Nm) (N=11 for each group). Three tests were performed on both injured and uninjured limbs in which the best value was recorded at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op*). A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05. The graph represents a ratio in which the respective injured limb's value at baseline is the common denominator.

Hamstring Peak Torque

Hamstring peak torque assessing hamstring strength is illustrated in Figure 22. Compared to baseline, preoperative hamstring peak torque increased significantly in the injured limb in both the exercise ($p= 0.034$) and control group ($p<0.001$). Though this was not statistically significant, the increase in peak torque was 33% in the exercise group compared to 22% increase in the control group preoperatively. For the uninjured limb, the exercise group had statistically significant higher scores compared to the control group preoperatively ($p=0.02$).

There was a significant decrease in hamstring peak torque in the injured limb of both the exercise ($p=0.05$) and control group ($p=0.018$) at 12-week postoperatively compared to preoperative values. However, there was an improved trend postoperatively for the exercise group although this was not statistically significant compared to the control group ($p=0.45$). In percentage, the hamstring power remained 21% higher than baseline in the exercise group compared to 3% in the control group.



[a] p= 0.034 v 6wk pre-op
[b] p= 0.05 v pre-op
[c] p< 0.001 v 6wk pre-op
[d] p= 0.018 v 6wk pre-op
*p= 0.02 v Control Uninjured

Figure 22. Hamstring average peak torque ratio (Nm) (N=11 and 9 for exercise and control groups respectively). Three tests were performed on both injured and uninjured limbs in which the best value was recorded at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op*). A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05. The graph represents a ratio in which the respective injured limb's value is the common denominator.

3.4 MRI Cross-Sectional Area (CSA)

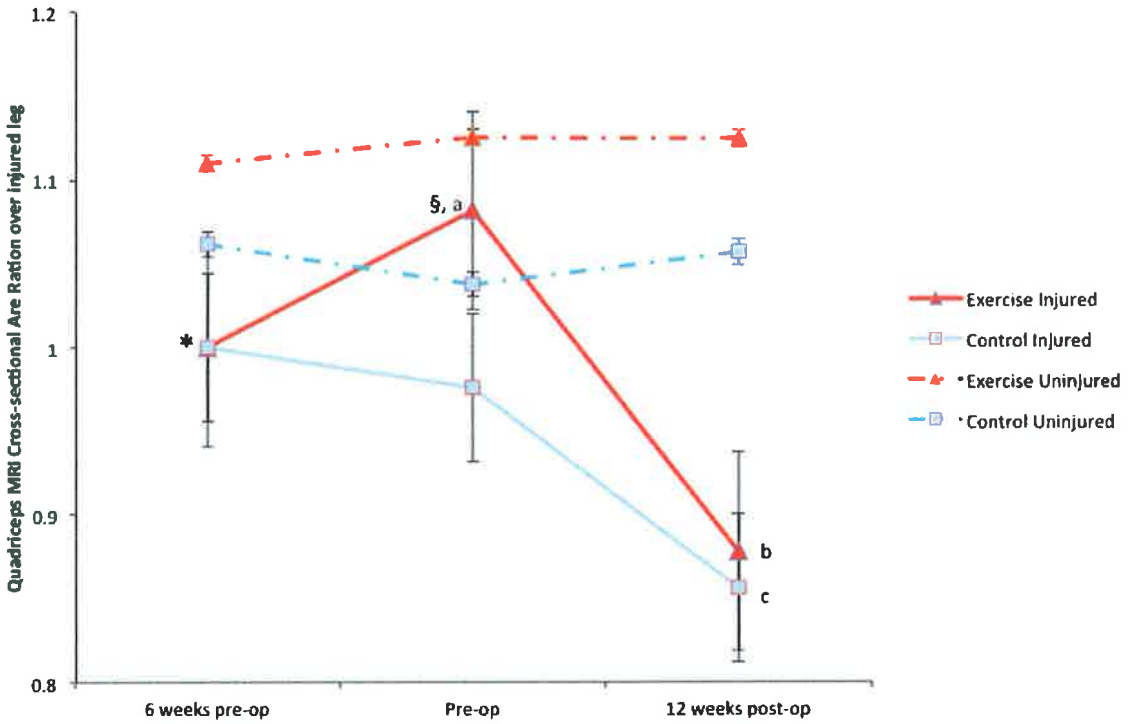
Muscle Groups CSA

Quadriceps – Injured Limb

The Quadriceps CSA at baseline was significantly different between the two groups at baseline (p=0.046) (Figure 23). Compared to baseline, there was a significant increase (p=0.001) in quadriceps CSA in the exercise group preoperatively while there was no change in the control group for the same time point. The difference between the two groups preoperatively was statistically significant (p=0.0024). This effect was not maintained in both groups as the CSA decreased significantly at 12-week postoperatively from preoperative values (p<0.001 for both).

Quadriceps – Uninjured Limb

Compared to baseline, there was no change in quadriceps CSA in the uninjured limbs at preoperative and postoperative time points for both groups.

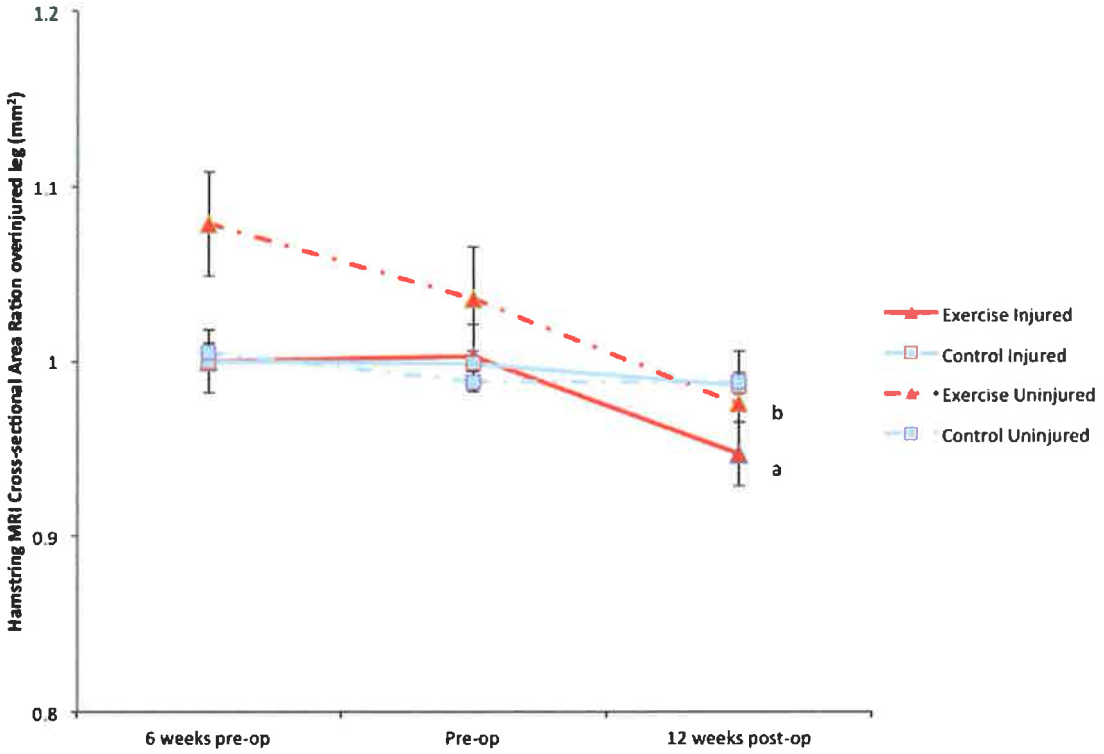


*p= 0.046 v Control Injured
§p= 0.0024
a p= 0.001 v 6wk pre-op
b p< 0.001 v 6wk pre-op
c p< 0.001 v 6wk pre-op & pre-op

Figure 23: Quadriceps MRI CSA as ratio (mm^2) (N=11 and 9 for exercise and control groups respectively). Three measurements were performed on both injured and uninjured limbs in which the mean value was recorded at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op*). A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05. The graph represents a ratio in which the respective injured limb's value is the common denominator.

Hamstrings

There was no change in hamstring CSA in both the exercise or control group for the injured limbs prior to surgery (Figure 24). There was a 6% decrease in CSA for the uninjured limbs for the exercise group from baseline however this was not statistically significant ($p=0.32$). Compared to preoperative time point, hamstring CSA decreased significantly at 12-week postoperatively in both the injured and uninjured limb of the exercise group ($p=0.024$, $p<0.001$ respectively).



[a] p= 0.024 vs 6wk pre-op Exercise Injured
[b] p< 0.001 vs 6wk pre-op Exercise Uninjured

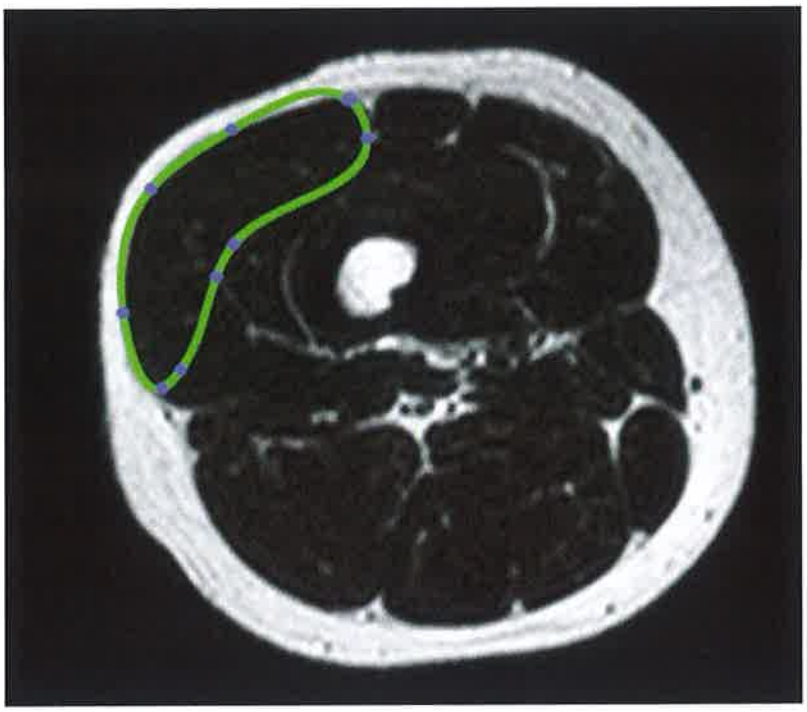
Figure 24: Hamstring MRI CSA as ratio (mm^2) (N=11 and 9 for exercise and control groups respectively). Three measurements were performed on both injured and uninjured limbs in which the mean value was recorded at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op*). A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05. The graph represents a ratio in which the respective injured limb's value is the common denominator.

CSA of Individual muscles

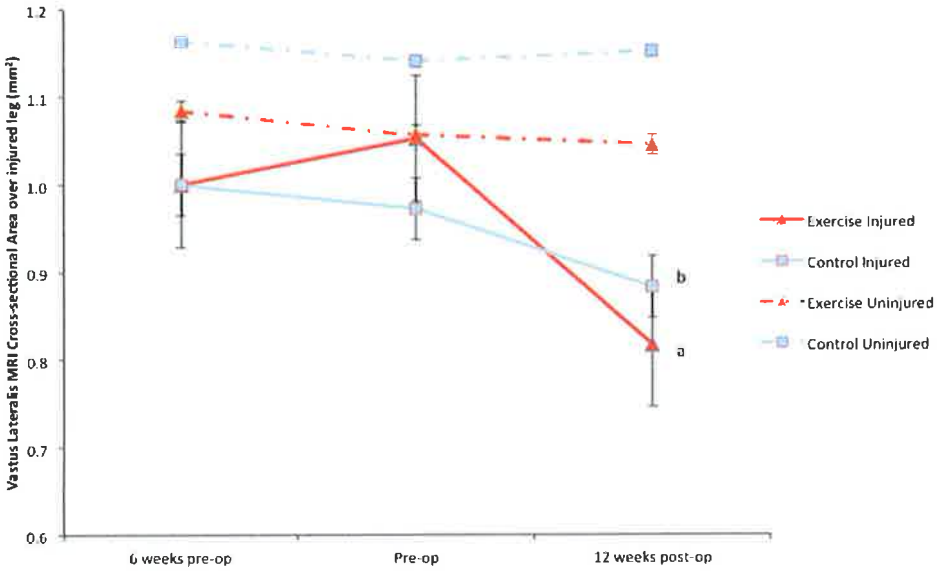
Vastus lateralis, vastus medialis and vastus intemedius

The effect of prehabilitation on vastus lateralis is shown in Figure 25. The vastus lateralis CSA of the exercise group had a 5% increase preoperatively compared to baseline while the control group had a 4% decrease for the same time point. The difference between exercise and control group was not statistically significant. This effect was not maintained at 12-week postoperatively with a 19.3% reduction in CSA from baseline ($p<0.001$). The control group had a 12.7% reduction at 12-week postoperative time point from preoperative values ($p=0.004$).

A.



B.



[a] p< 0.001 v 6wk pre-op & pre-op
[b] p= 0.004 v 6wk pre-op

Figure 25: A. MRI showing CSA measurement. B. Vastus lateralis MRI CSA ratio (mm^2) (N=11 and 9 for exercise and control groups respectively). Three measurements were performed on both injured and uninjured limbs in which the mean value was recorded at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op).* A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05. The graph represents a ratio in which the respective injured limb's value is the common denominator.

The effect of prehabilitation on vastus medialis CSA is shown in Figure 26. After exercise prior to surgery, the vastus medialis CSA was significantly increased in the exercise group compared to both baseline and the control group (p=0.03, p=0.015 respectively). However, this effect was not maintained at 12-week postoperatively with significant reduction in CSA compared to baseline for both exercise and treatment groups (p<0.001, p=0.002). The mean CSA (mean, SD) in the exercise group (18.4, 3.8) was significantly lower than the control group (22.6,4.3) at 12-week postoperatively (p=0.034).

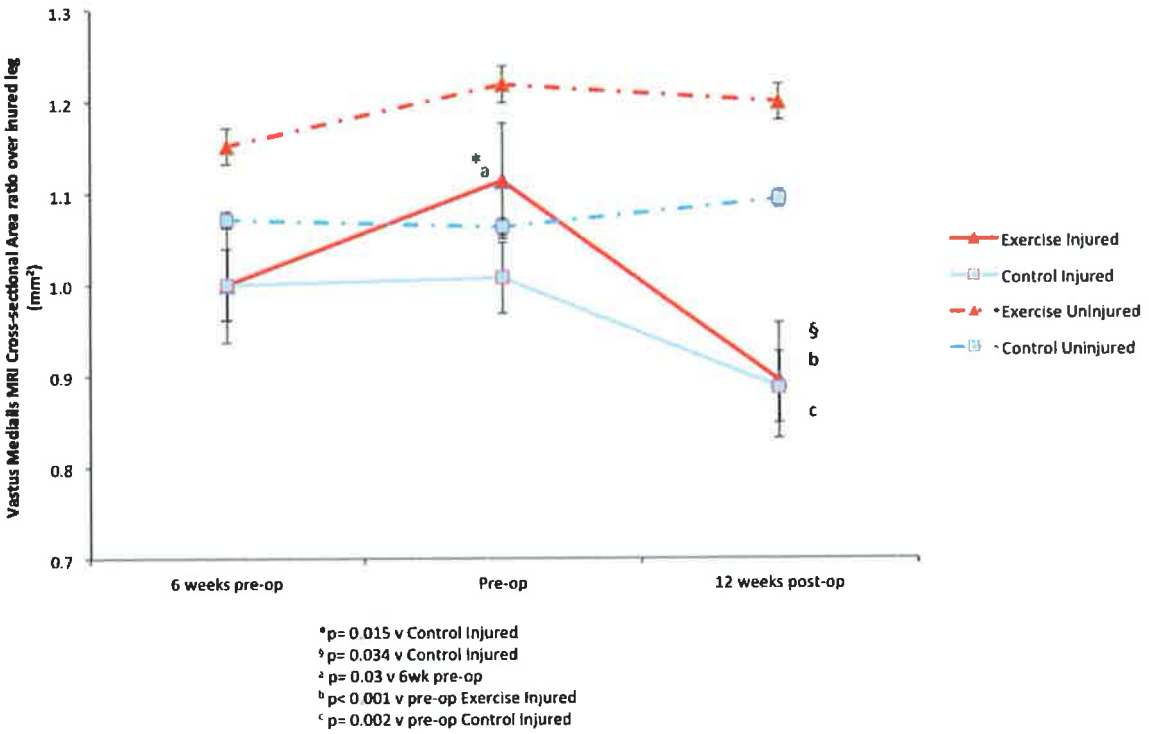


Figure 26: Vastus medialis MRI CSA ratio (mm^2) (N=11 and 9 for exercise and control groups respectively). Three measurements were performed on both injured and uninjured limbs in which the mean value was recorded at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op).* A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05. The graph represents a ratio in which the respective injured limb's value is the common denominator.

The effect of prehabilitation on vastus intermedius CSA is shown in Figure 27. The vastus intermedius showed significant differences in which the exercise group had a decreased CSA mean (mean, SD) (16.6, 2.9) compared to the control group (20.7, 4.4) at baseline (p=0.022). CSA was increased significantly in the exercise group compared to control group (p=0.005). At 12-week postoperatively, there were a 4% and 17% reduction of CSA for exercise and control group respectively (p=0.01, p=0.048).

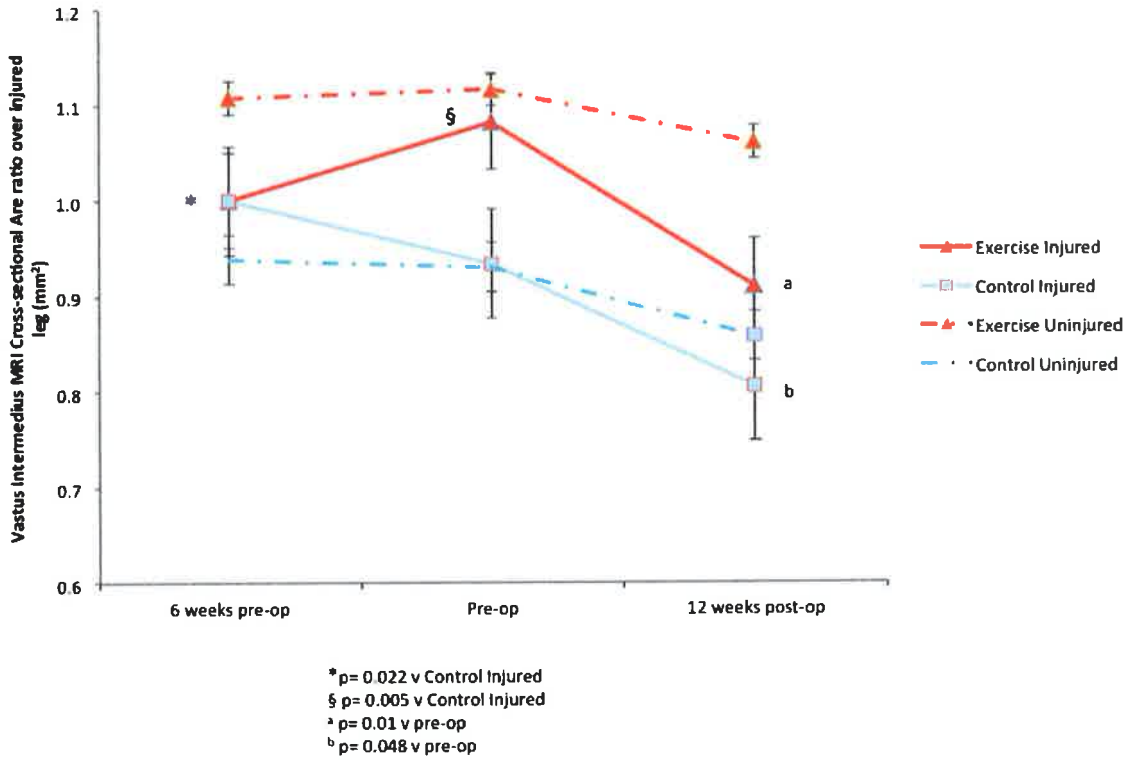
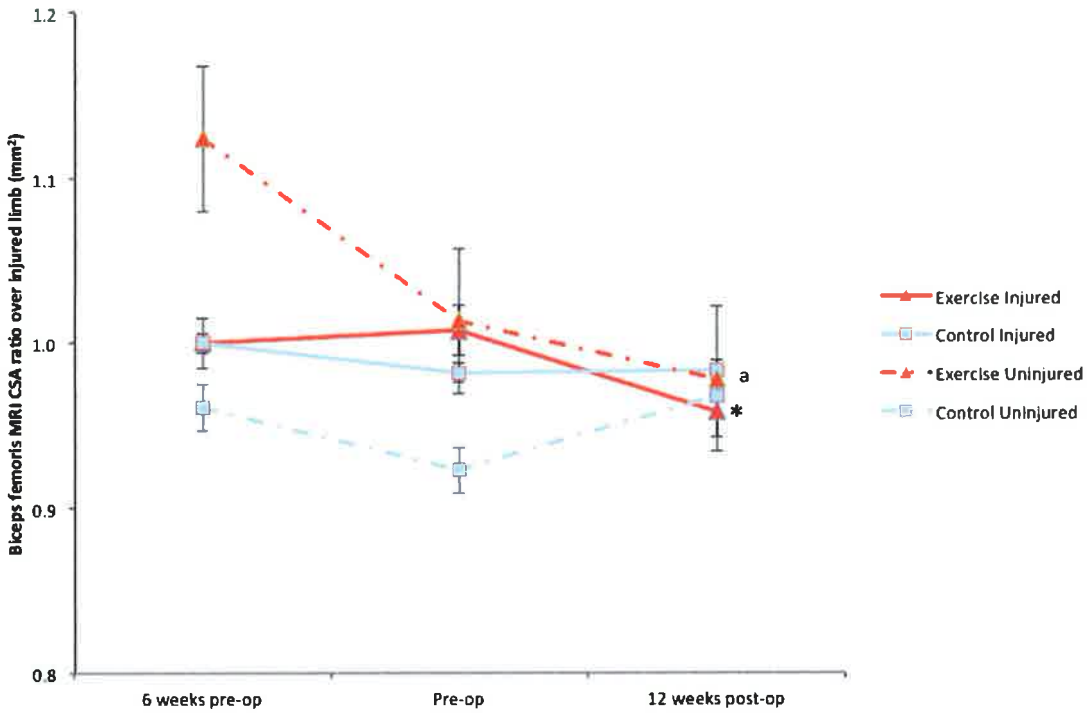


Figure 27: Vastus intermedius MRI CSA ratio (mm^2) (N=11 and 9 for exercise and control groups respectively). Three measurements were performed on both injured and uninjured limbs in which the mean value was recorded at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12- week postoperatively (*12 weeks post-op*). A three-way ANOVA test was

used to calculate differences between the groups with a significant p-value cut-off of 0.05. The graph represents a ratio in which the respective injured limb's value is the common denominator.

Biceps Femoris, Semitendinosus and Semimembranosus

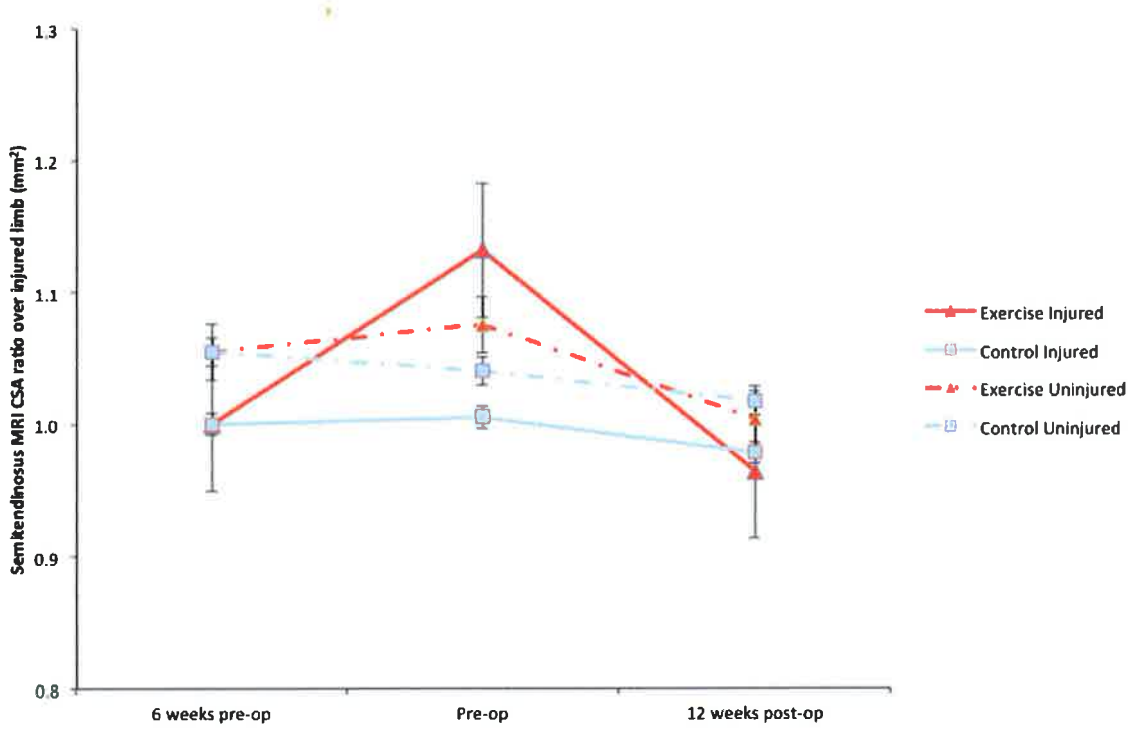
The biceps femoris CSA showed a 4.2% and 1.2% reduction in exercise and control groups respectively at 12-week postoperatively which was statistically significant (p=0.039) (Figure 28). While there was no difference in the uninjured limbs for the control group, the CSA was appreciably more reduced in the exercise-uninjured limb from baseline (p=0.023).



*p= 0.039 v Control Injured
[a] p= 0.023 v 6wk pre-op Exercise Injured

Figure 28: Biceps femoris MRI CSA ratio (mm^2) (N=11 and 9 for exercise and control groups respectively). Three measurements were performed on both injured and uninjured limbs in which the mean value was recorded at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op*). A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05. The graph represents a ratio in which the respective injured limb's value is the common denominator.

Figure 29 showed CSA changes for semitendinosus. Although the injured semitendinosus CSA showed an increased trend preoperatively compared to baseline, there were no differences between the CSA in both groups for both the injured and uninjured limbs.



p= nil significant

Figure 29: Semitendinosus MRI CSA ratio (mm^2) (N=11 and 9 for exercise and control groups respectively). Three measurements were performed on both injured and uninjured limbs in which the mean value was recorded at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op).* A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05. The graph represents a ratio in which the respective injured limb's value is the common denominator.

The changes of semimembranosus CSA are illustrated in Figure 30. At 12-week postoperative time point, both exercise and control groups had a 4% and 12% increase in CSA compared to baseline, although this was only statistically significant for the control group (p=0.003). The increased CSA was also seen in the uninjured limb of control groups compared to the exercise group. The decrease CSA in the exercise-uninjured limb was statistically different from the increased trend in CSA in the control-uninjured limb (p=0.05).

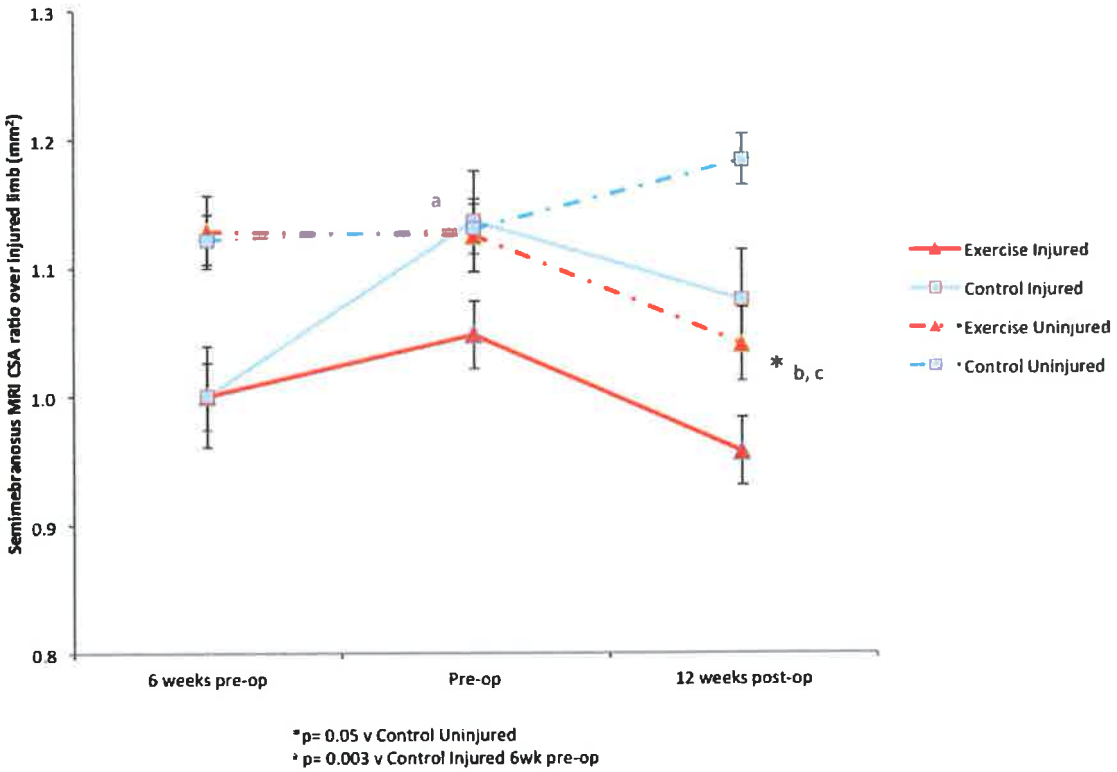


Figure 30: Semimembranosus MRI CSA ratio (mm^2) (N=11 and 9 for exercise and control groups respectively). Three measurements were performed on both injured and uninjured limbs in which the mean value was recorded at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12- week postoperatively (*12 weeks post-op).* A three-way ANOVA test was

used to calculate differences between the groups with a significant p-value cut-off of 0.05. The graph represents a ratio in which the respective injured limb's value is the common denominator.

3. 5 Patient-self assessment questionnaires

Modified Cincinnati Knee Rating System

The total Modified Cincinnati Knee Rating System scores are shown in Figure 31. The total scores showed an increase trend for both the exercise and control groups at 12-week postoperative time point. However, these were not statistically significant for control groups at all time points. Meanwhile, the scores were increased significantly at preoperative and 12-week postoperative time points in the exercise group (p=0.004, p=0.001 respectively). There was a significant increase in the scores of the exercise group at 12-week postoperative time point compared to the control group (p=0.004). Similar trends were observed for subgroups of the Modified Cincinnati Knee Rating System Score such as pain (Figure 32), swelling (Figure 33), giving way (Figure 34), walking (Figure 35), running (Figure 37) and jumping (Figure 38) albeit with different statistical significant p-values between the exercise and control groups and the time points within the same group. The stairs sub-score showed no differences between the exercise and control groups (Figure 36). A breakdown of the scores is included in Table 5.

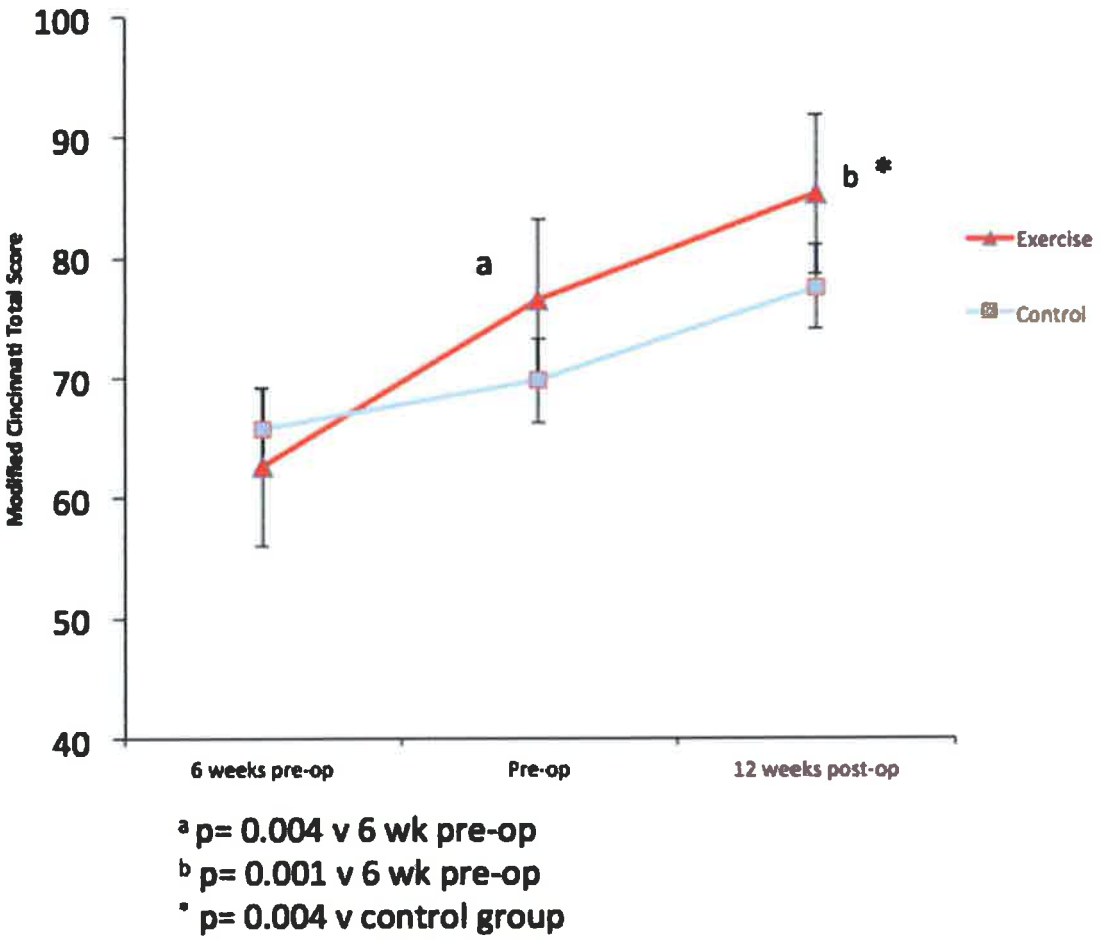
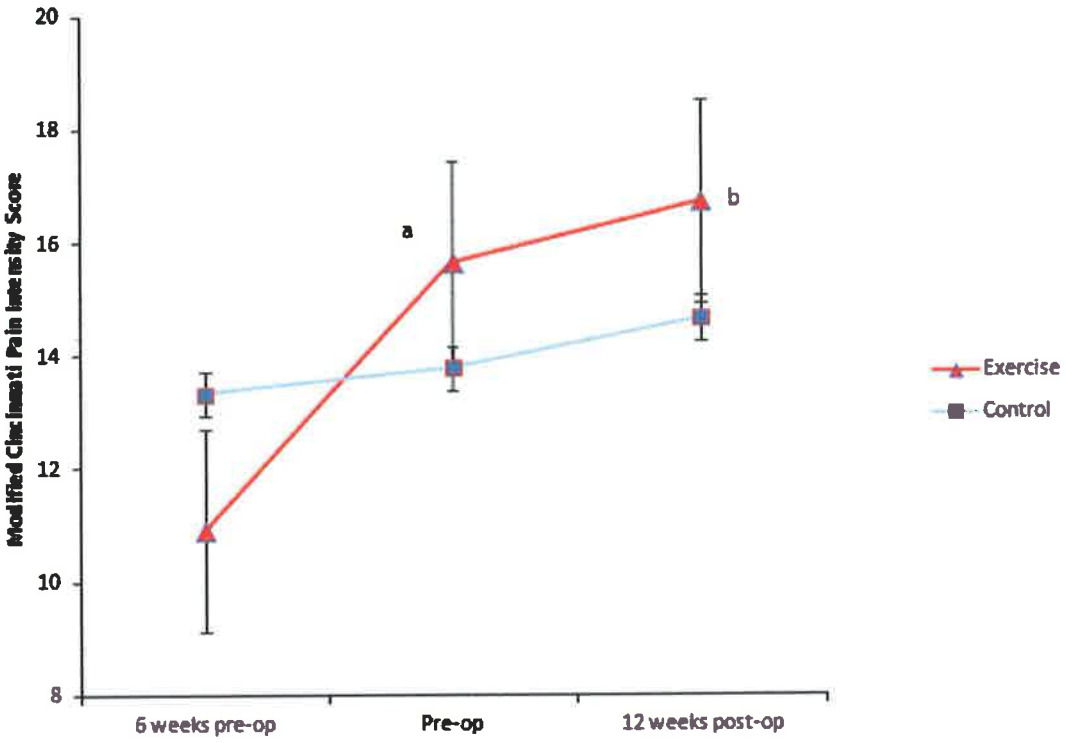


Figure 31: Total Modified Cincinnati Knee Rating System Score (N=11 and 9 for exercise and control groups respectively). Total scores were calculated at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op).* A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05.



[a] p= 0.002 v 6 wk pre-op
[b] p= 0.004 v 6 wk pre-op

Figure 32. Pain sub-scale of the Modified Cincinnati Knee Rating system score (N=11 and 9 for exercise and control groups respectively). Total scores were calculated at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op).* A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05.

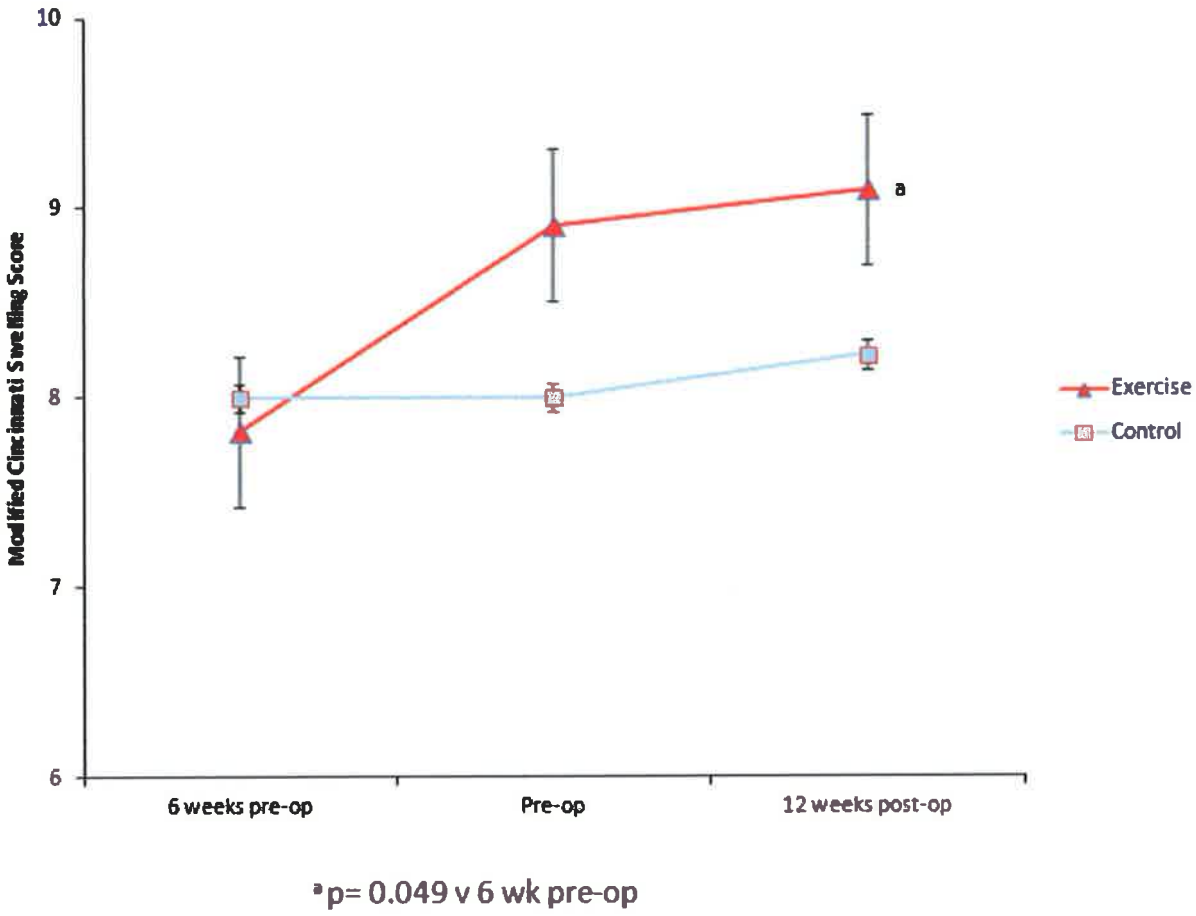
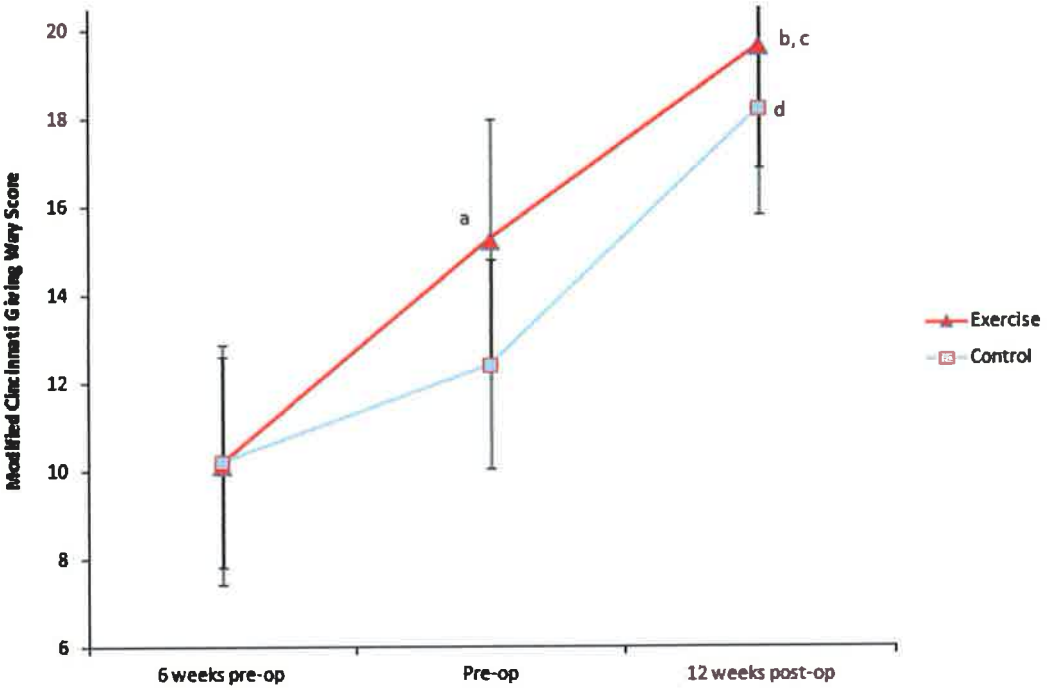


Figure 33. Swelling sub-scale of the Modified Cincinnati Knee Rating system score (N=11 and 9 for exercise and control groups respectively). Total scores were calculated at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op).* A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05.



[a] p= 0.34 v 6 wk pre-op
[b] p<0.001 v 6 wk pre-op
[c] p=0.005 v pre-op
[d] p=0.001 v 6 wk pre-op & pre-op

Figure 34. Giving way sub-scale of the Modified Cincinnati Knee Rating system score (N=11 and 9 for exercise and control groups respectively). Total scores were calculated at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op*). A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05.

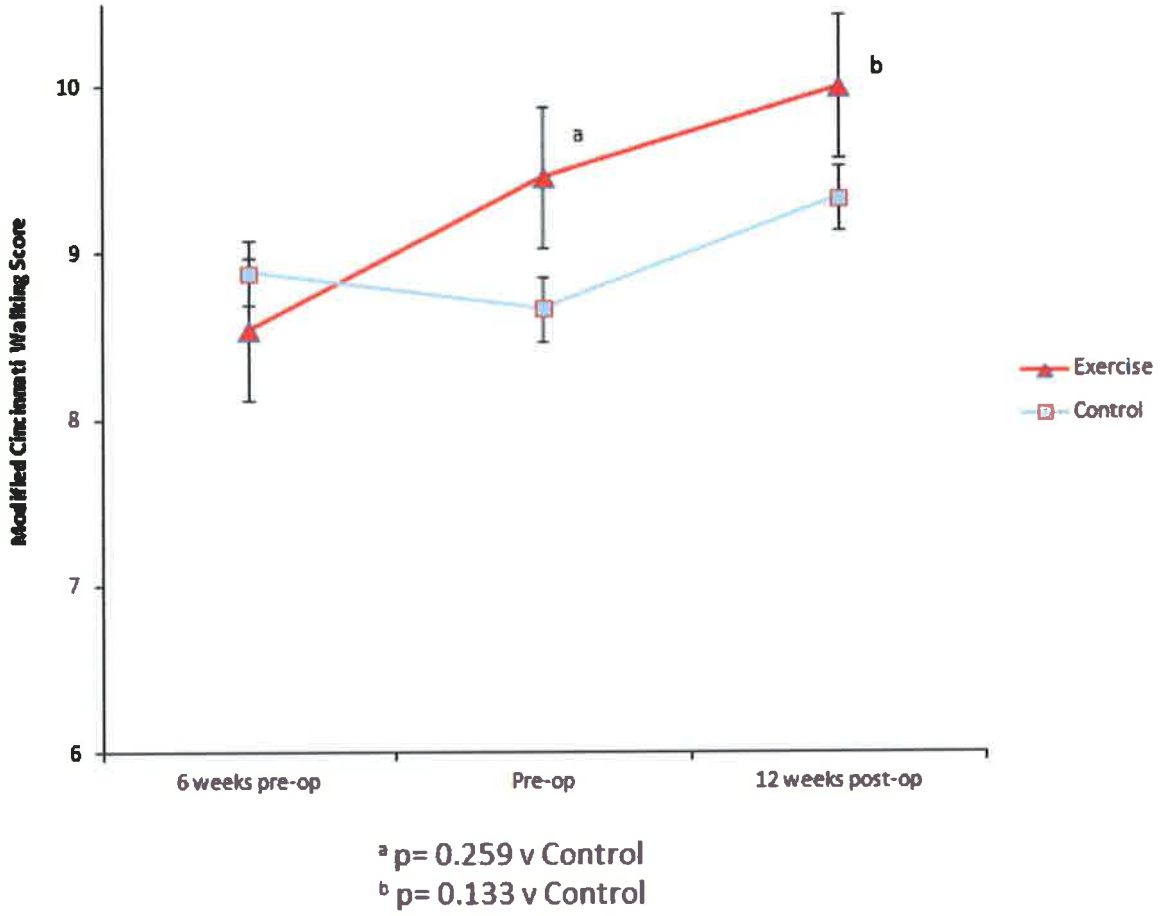


Figure 35. Walking sub-scale of the Modified Cincinnati Knee Rating system score (N=11 and 9 for exercise and control groups respectively). Total scores were calculated at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op*). A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05.

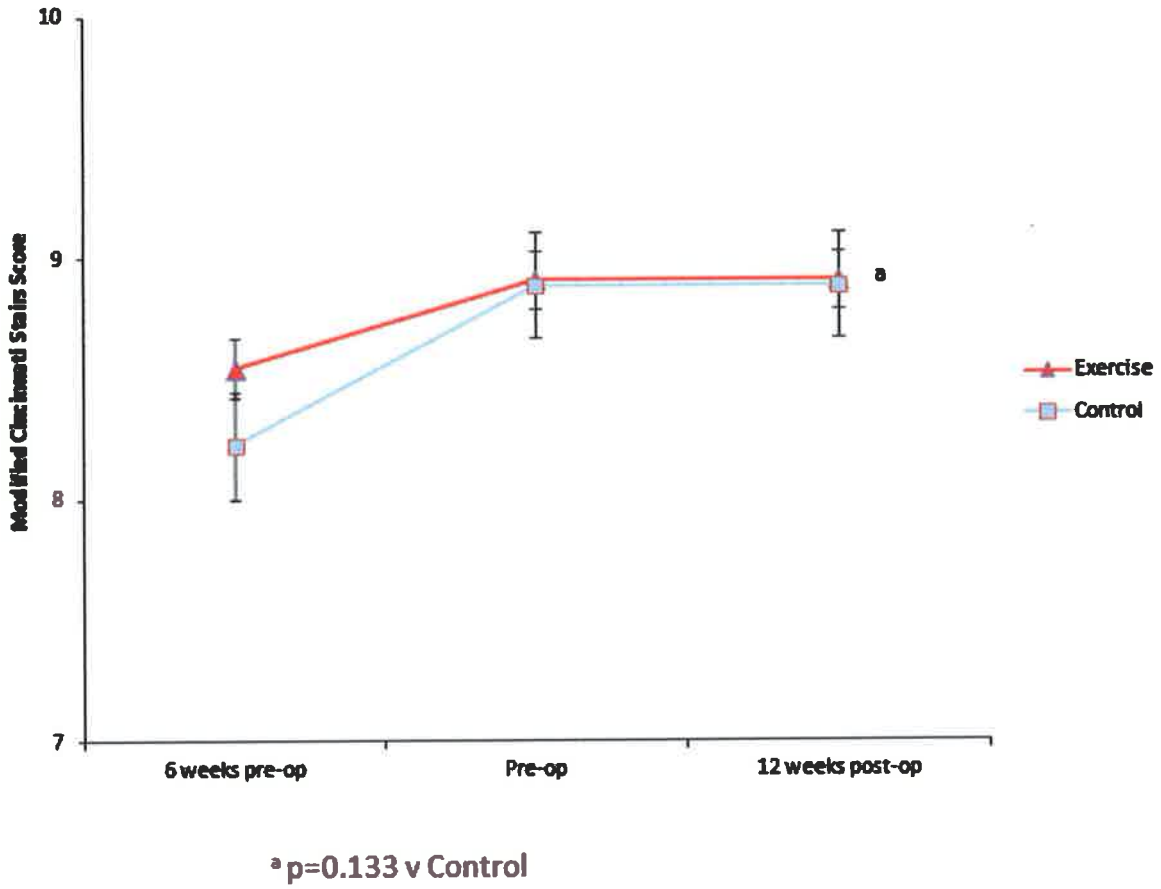


Figure 36. Stairs sub-scale of the Modified Cincinnati Knee Rating system score (N=11 and 9 for exercise and control groups respectively). Total scores were calculated at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op*). A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05.

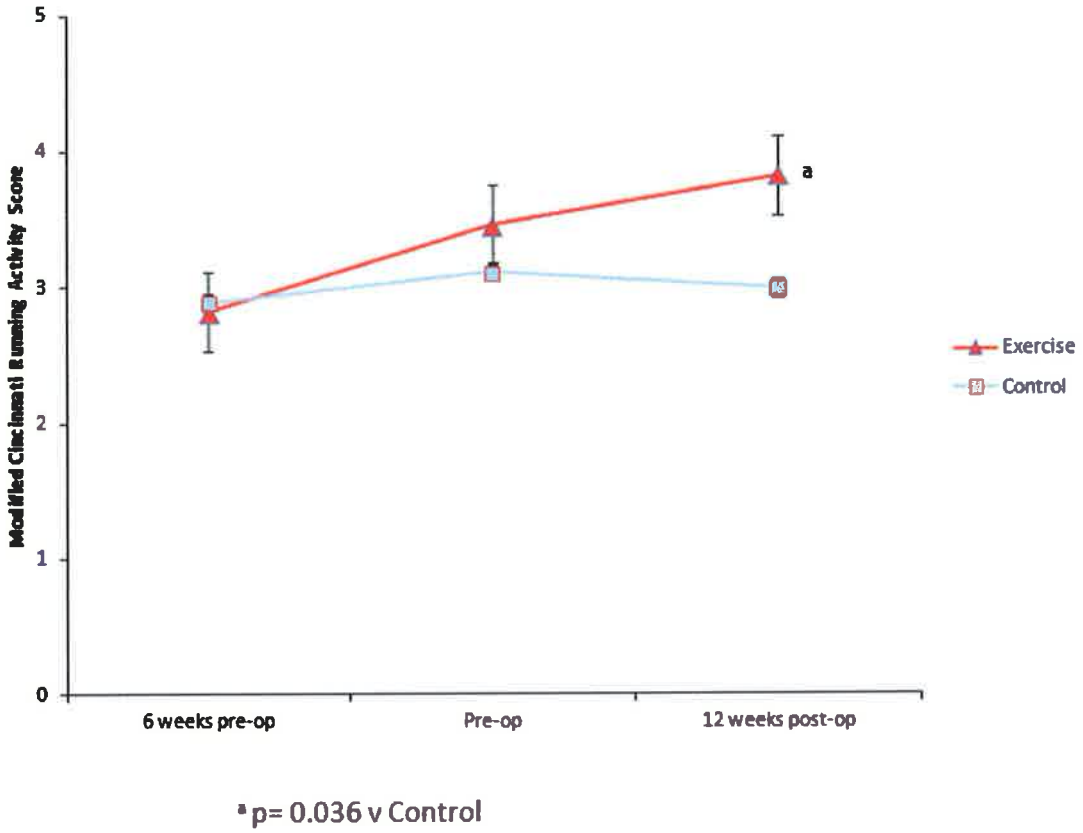
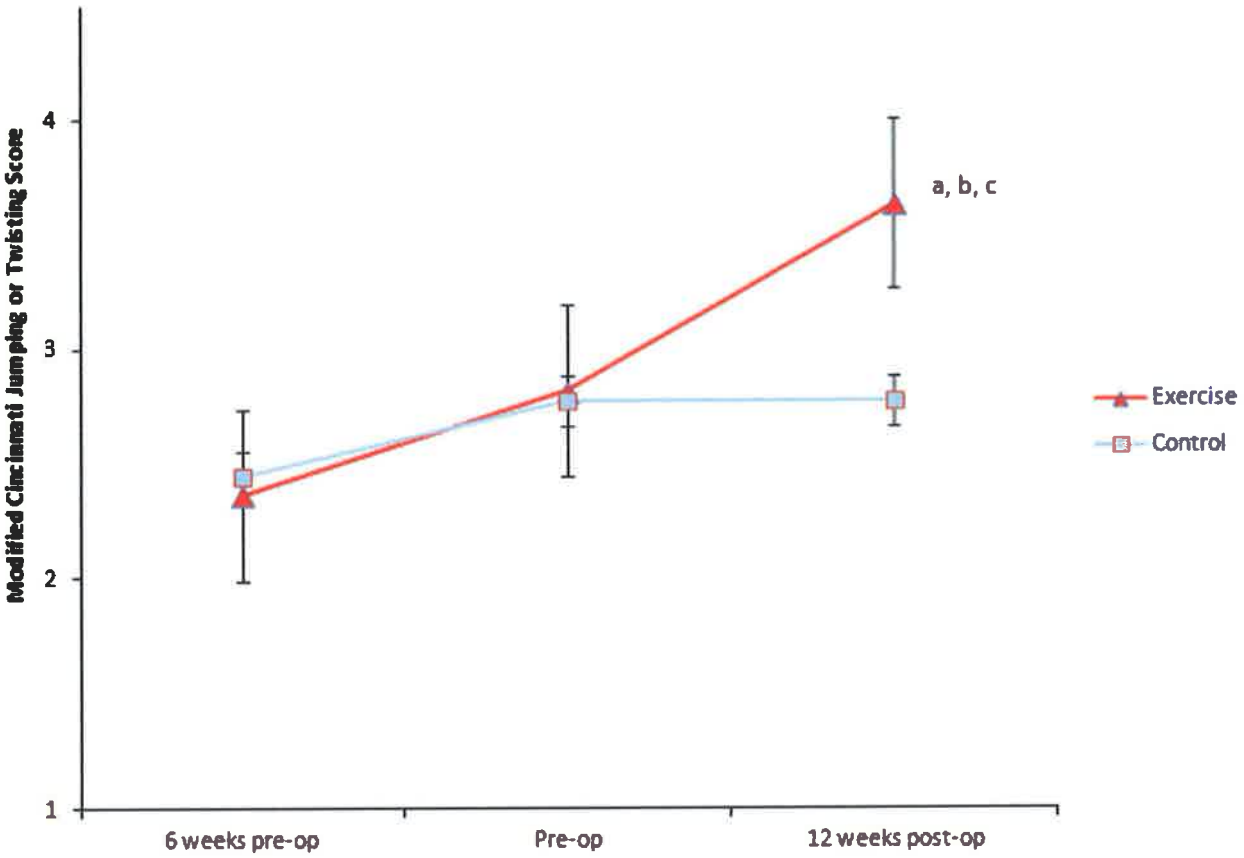


Figure 37. Running sub-scale of the Modified Cincinnati Knee Rating system score (N=11 and 9 for exercise and control groups respectively). Total scores were calculated at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op*). A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05.

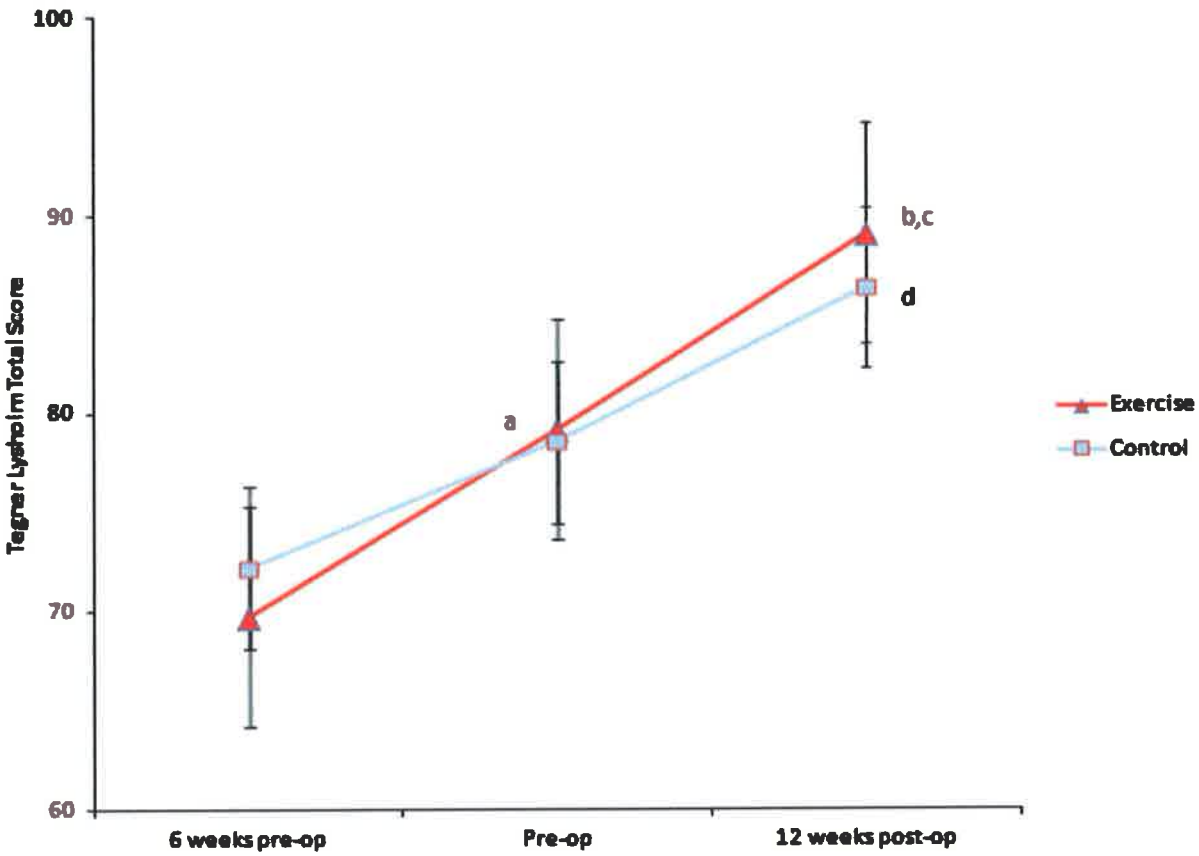


[a] p= 0.03 v 6 wk pre-op
[b] p= 0.01 v pre-op
[c] p= 0.01 v Control

Figure 38. Jumping or twisting sub-scale of the Modified Cincinnati Knee Rating system score (N=11 and 9 for exercise and control groups respectively). Total scores were calculated at three time points; 6-week preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op*). A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05.

Tegner-Lysholm Knee Score

The Tegner Lynsholm total scores are shown in Figure 39. There were similar improved trends for both groups at all time points compared to baseline. However, this was statistically significant in the exercise group only (p=0.006). This was also observed at 12-week postoperatively with statistically significant increment when compared to both baseline and preoperative time points (p=<0.001, p=0.014). However, there were no differences between the exercise and control group at all time points. Similar trends were observed for sub-scores of the Tegner Lynsholm Knee score including pain (Figure 41) and instability (Figure 42), albeit with different statistically significant p-values. There were no changes for the support sub-score (Figure 40). The breakdowns of the scores are illustrated in Table 5.



[a] p= 0.006 v 6 wk Exercise pre-op
[b] p= 0.014 v pre-op
[c] p<0.001 v 6 wk pre-op
[d] p=0.009 v 6 wk pre-op

Figure 39: Total Tegner-Lysholm Knee Score (N=11 and 9 for exercise and control groups respectively). Total scores were calculated at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op).* A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05.

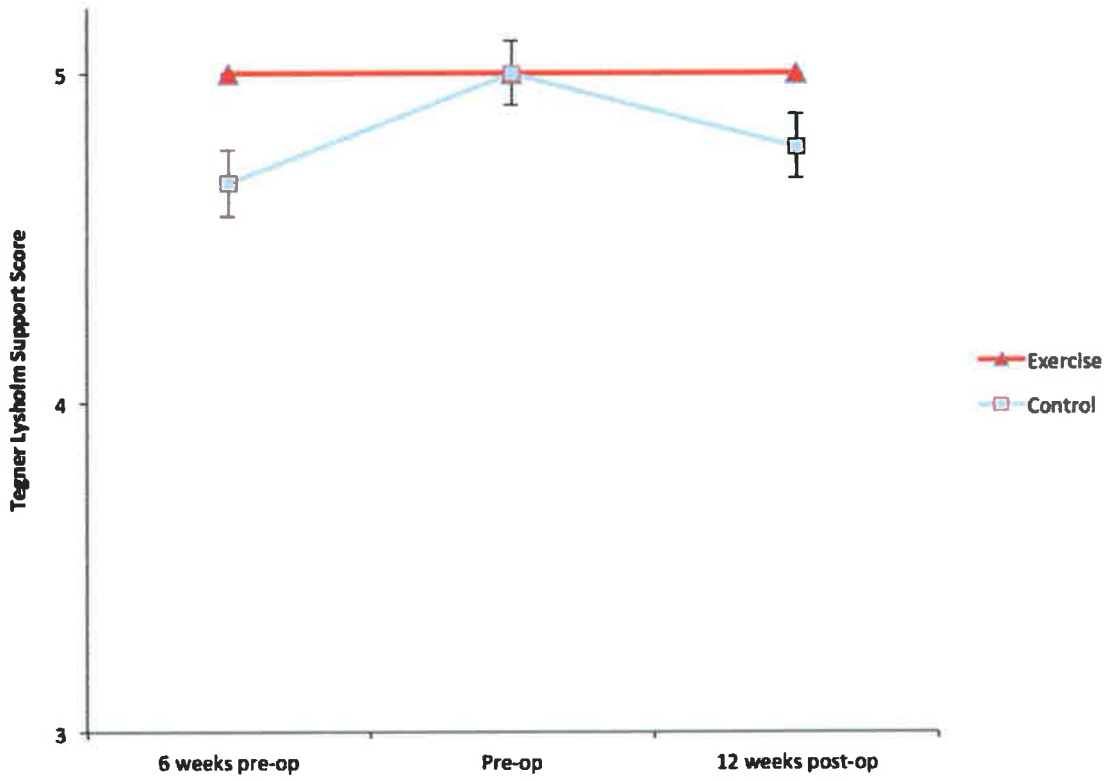
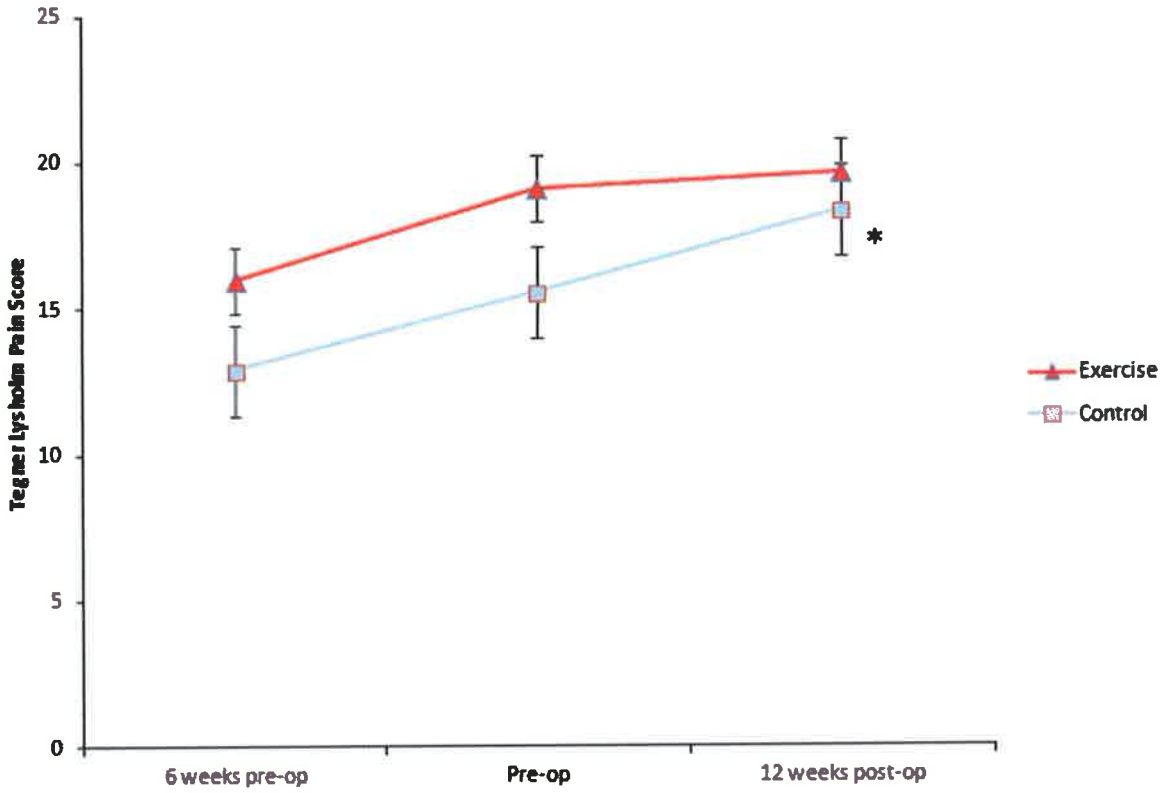
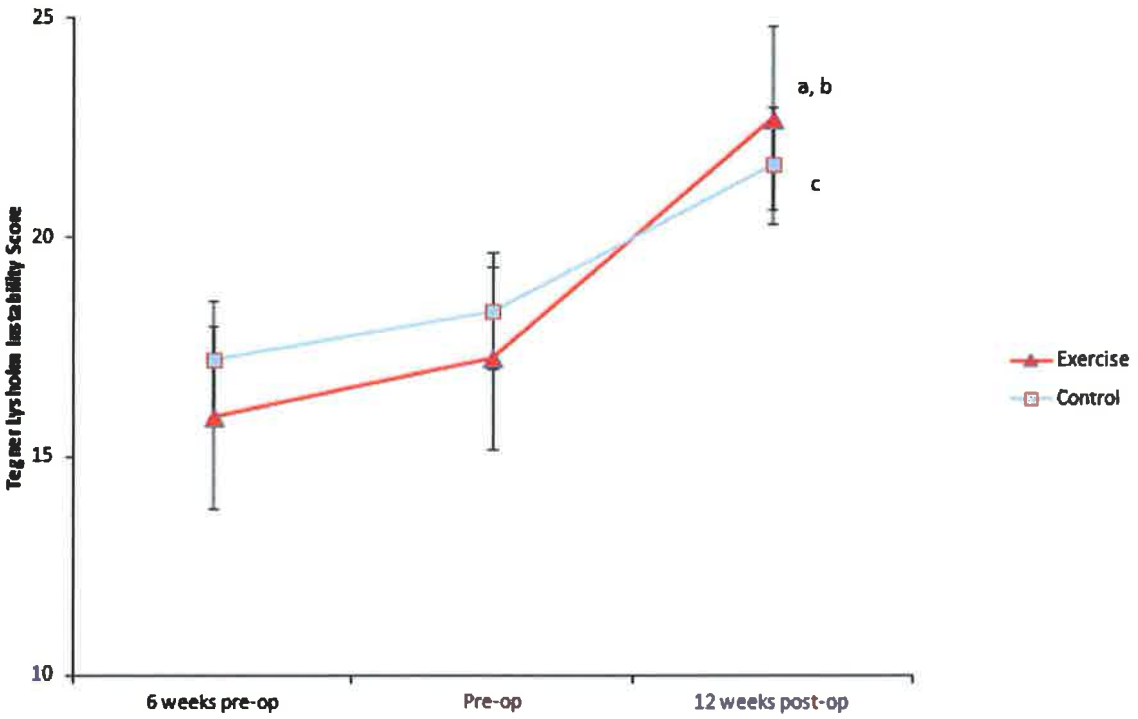


Figure 40: Support sub-scale of the Tegner-Lysholm Knee Score (N=11 and 9 for exercise and control groups respectively). Total scores were calculated at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op).* A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05.



*p= 0.016 v 6wk pre-op

Figure 41: Pain sub-scale of the Tegner-Lysholm Knee Score (N=11 and 9 for exercise and control groups respectively). Total scores were calculated at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op*). A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05.



[a] p< 0.001 v 6 wk pre-op
[b] p= 0.001 v pre-op
[c] p= 0.11 v pre-op

Figure 42: Instability sub-scale of the Tegner-Lysholm Knee Score (N=11 and 9 for exercise and control groups respectively). Total scores were calculated at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op).* A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05.

Table 5: Mean value of disease-specific questionnaires ± SD (N=11 and 9 in exercise and control groups respectively)

		Mean ± SD					
		Exercise			Control		
		6 wk pre-op	Pre-op	12 wk post-op	6 wk pre-op	Pre-op	12 wk post-op
Modified Cincinnati Knee Rating System							
	Total Score (max =100)	62.64 ± 13.29	76.55 ± 9.98	85.27 ± 4.77	65.78 ± 13.73	69.78 ± 10.23	77.56 ± 5.64
	Pain (max = 20)	10.91 ± 4.42	15.64 ± 2.16	16.73 ± 3.93	13.33 ±2.00	13.78 ± 2.91	14.67 ± 3.46
	Swelling (max= 10)	7.82 ± 1.40	8.91 ± 1.38	9.09 ± 1.04	8.00 ± 1.73	8.00 ± 1.73	8.22 ±1.20
	Giving Way (max = 20)	10.18 ±4.85	15.27 ± 4.32	19.64 ± 1.21	10.22 ± 6.67	12.44 ± 4.67	18.33 ± 2.91
	Overall Activity (max =20)	11.27 ± 3.00	12.00 ± 2.83	13.09 ± 2.59	12.00 ± 3.46	12.00 ± 2.60	12.44 ± 3.13
	Walking (max = 10)	8.55 ± 1.57	9.45 ± 1.29	10.00 ± 0	8.89 ± 1.76	8.67 ± 1.73	9.33 ± 1.41
	Stairs (max=10)	8.55 ± 0.93	8.91 ± 1.04	8.91 ± 1.04	8.22 ± 2.11	8.89 ± 1.45	8.89 ± 1.05

	Running (max= 5)	2.82 ± 1.08	3.45 ± 0.69	3.82 ± 0.41	2.89 ± 1.45	3.45 ± 0.69	3.00 ± 1.12
	Jumping or Twisting (max=5)	2.36 ± 0.92	2.82 ± 0.75	3.64 ± 0.15	2.44 ± 1.01	2.78 ± 0.83	2.78 ± 0.833
Tegner Lysholm Knee Scoring Scale							
	Total Score (max = 100)	69.73 ± 10.24	79.18 ± 10.43	89.09 ± 8.64	72.22 ± 17.98	78.56 ± 12.84	86.33 ± 7.94
	Limp (max= 5)	3.73 ± 1.01	4.45 ± 0.93	4.09 ± 1.04	3.56 ± 1.67	3.89 ± 1.05	4.09 ± 1.04
	Support (max= 5)	5.00 ± 0	5.00 ± 0	5.00 ± 0	4.67 ±1.00	5.00 ± 0	4.78 ± 0.67
	Pain (max=25)	16.00 ± 5.66	19.09 ± 4.37	19.64 ± 4.95	12.89 ± 6.99	15.56 ± 3.91	18.33 ± 4.33
	Instability (max= 25)	15.91 ± 3.75	17.27 ± 4.10	22.73 ± 3.43	17.22 ± 4.41	18.33 ± 2.50	21.67 ± 3.54
	Locking (max= 15)	12.09 ± 4.18	13.27 ± 3.13	15.00 ± 0	12.89 ± 3.37	13.33 ± 2.50	15.00 ± 0
	Swelling (max=10)	6.55 ±2.84	7.82 ± 2.09	8.55 ± 2.02	8.22 ± 2.11	9.11 ± 1.76	7.33 ± 2.83
	Stairs (max= 10)	7.09 ± 3.15	8.91 ± 1.87	8.91 ± 1.87	7.78 ± 2.91	10.00± 0	9.56 ± 1.33
	Squat	3.82 ±	4.36 ±	4.36 ±	9.56 ±	4.00 ±	4.44 ±

	(max=5)	0.98	1.21	0.51	1.33	0.87	0.53
Tegner Activity Level Score	Before injury	1.29 ± 1.01			3.91 ± 1.14		
	After injury	3.91 ± 1.14			4.44 ± 1.88		

3. 6 Genetic Testing

To normalise and measure the relative expression of mRNA, a qRT-PCR of the housekeeping gene GAPDH was measured in all samples to confirm that there were no differences in expression for both exercise and control groups at all time points. The PCR efficiency for GAPDH was measured to be similar to other PCR primers used in this study to accurately assess relative expression. The MHC I, IIa and IIx, IGF-1, MAFBx and MuRF-1 crossing point (Cq) values were normalised relative to GAPDH Cq values of the respective samples. All samples were run in triplicate.

IGF-1, MuRF-1 and MABFx Gene Expression

The IGF-1 mRNA was significantly increased in the exercise group compared to the control group preoperatively (p=0.028) (Figure 43). It was also increased when compared to

baseline within the same exercise group (p=0028). However, this was not sustained with a significant decrease back to baseline levels at 12-week postoperative time point (p=0.012).

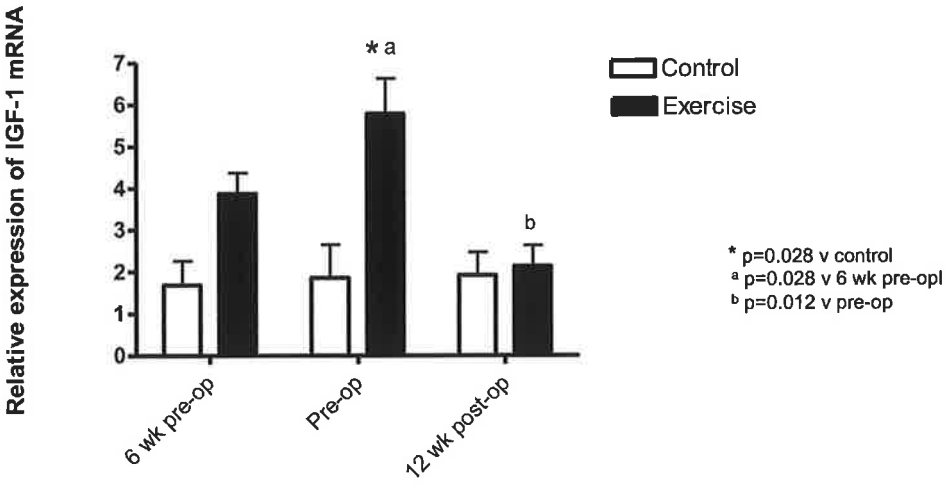


Figure 43. Relative expression of IGF-1 mRNA on qRT-PCR (N=11 and 9 for exercise and control groups respectively). Relative expression was quantified against GAPDH at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op).* A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05.

Conversely, MuRF-1 mRNA expression was significantly decreased preoperatively compared to baseline (p=0.05) (Figure 44). This was a transient effect as the MuRF-1 mRNA expression was increased 12-week postoperatively comparable to baseline values. When compared to preoperative levels, the increment was statistically significant (p=0.03). There were no significant differences between the exercise and control group at all time points. For MAFbx mRNA, there were no significant differences between both exercise and control groups and within the two groups at all time points (Figure 45).

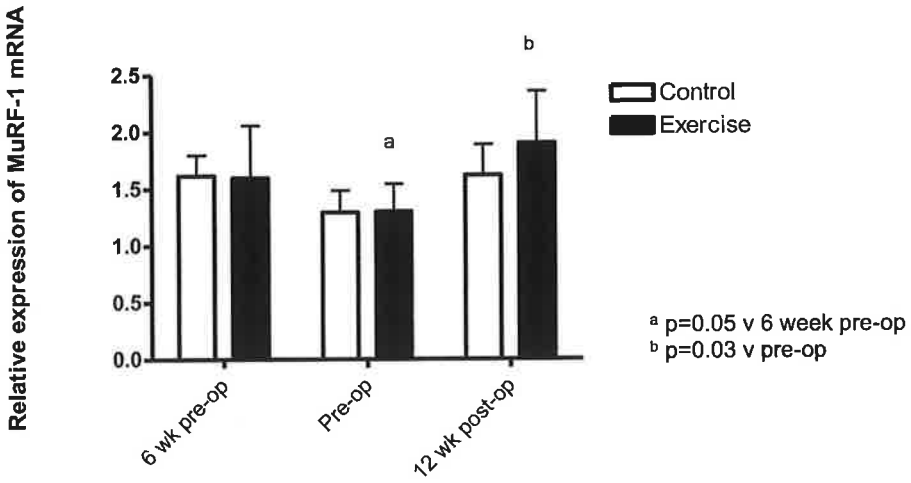


Figure 44. Relative expression of MuRF-1 mRNA on qRT-PCR (N=11 and 9 for exercise and control groups respectively). Relative expression was quantified against GAPDH at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12- week postoperatively (*12 weeks post-op).* A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05.

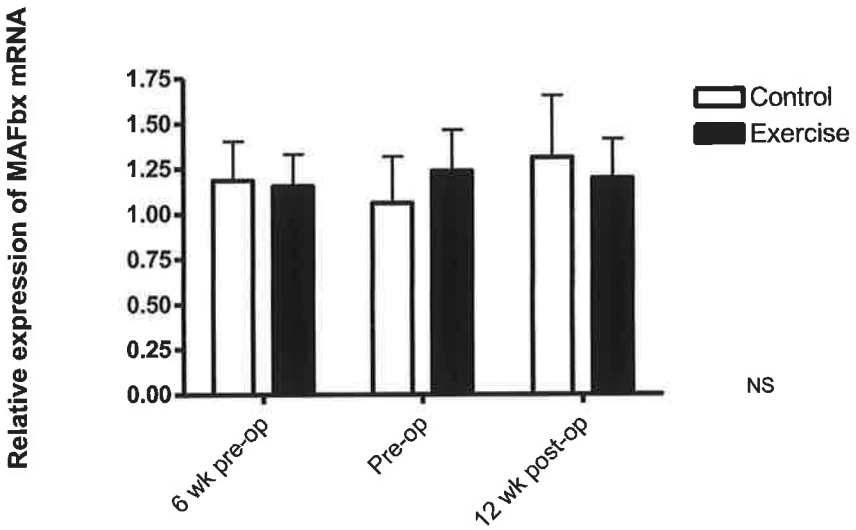


Figure 45. Relative expression of MAFbx mRNA on qRT-PCR (N=11 and 9 for exercise and control groups respectively). Relative expression was quantified against GAPDH at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op).* A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05.

MHC I, IIa and IIx mRNA Expression

For MHC I mRNA, there were no significant differences between both exercise and control groups and within the two groups at all time points (Figure 46). For the MHC IIa mRNA, there was a statistically significant increase in expression in the exercise group compared to control group preoperatively ($p=0.028$) (Figure 47). These levels were also significantly different when compared to baseline in the exercise group ($p=0.03$). However, this increment was not sustained with levels similar to baseline and significantly decreased values compared to levels preoperatively ($p=0.03$).

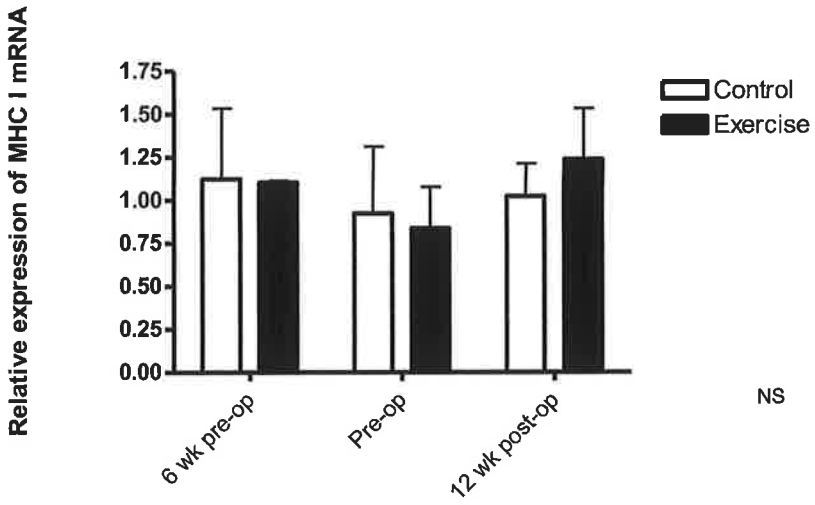


Figure 46. Relative expression of MHC I mRNA on qRT-PCR (N=11 and 9 for exercise and control groups respectively). Relative expression was quantified against GAPDH at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op).* A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05.

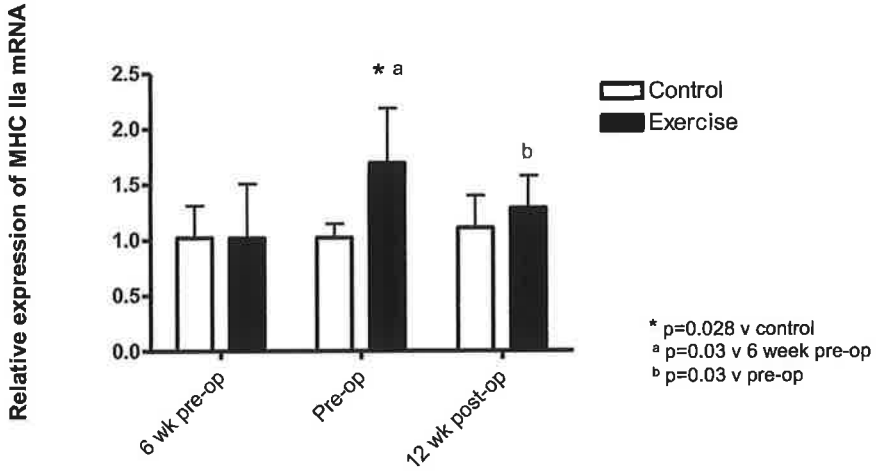


Figure 47. Relative expression of MHC IIa mRNA on qRT-PCR (N=11 and 9 for exercise and control groups respectively). Relative expression was quantified against GAPDH at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-week postoperatively (*12 weeks post-op).* A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05.

Conversely for MHC IIx mRNA, there was a significant decrease in expression preoperatively in the exercise group compared to baseline (p=0.05) (Figure 48). However, this was not sustained at 12-week postoperatively. There were no differences in MHC IIx

expression between the exercise and control groups and also between the time points within the control group.

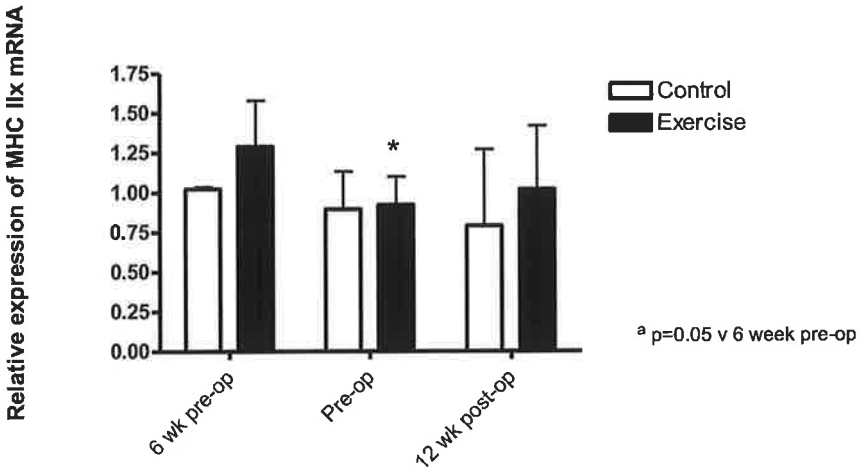
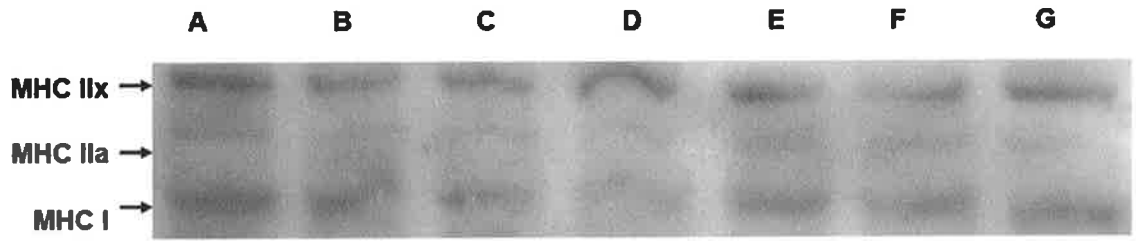


Figure 48. Relative expression of MHC IIx mRNA on qRT-PCR (N=11 and 9 for exercise and control groups respectively). Relative expression was quantified against GAPDH at three time points; 6 weeks preoperatively (*6 weeks pre-op*) equivalent to pre-exercise, preoperatively (*pre-op*) equivalent to post-exercise and 12-weeks postoperatively (*12 weeks post-op).* A three-way ANOVA test was used to calculate differences between the groups with a significant p-value cut-off of 0.05.

3.7 Protein testing

An example of the triplicate protein bands from the SDS-Page western blotting used for intensity analysis of MHC isoforms is shown in Figure 49. The percentages of expression of MHC isoforms were calculated and normalised based on the expression of B-actin from respective samples. Samples were pooled and run in triplicate.

There were no significant changes in MHC I isoform expression between both exercise and control groups and at all time points within the two groups (Figure 50). Similar to mRNA expression, there was a significant increase of MHC IIa expression preoperatively compared to the control group ($p=0.05$) (Figure 51). However, a reciprocal decrement in MHC IIx isoform expression was not observed (Figure 52).



A - Positive control from rabbit myosin heavy chain
B - Control 6 wk pre-op
C - Exercise 6 wk pre-op
D - Control pre-op
E - Exercise pre-op
F - Control 12 wk post-op
G - Exercise 12 wk post-op

Figure 49: Western blot analysis (SDS-PAGE) showing MHC isoforms. Total cell lysate from 25 mg of muscle biopsies were lysed and pooled for analysis. Rabbit myosin heavy chain was used as positive control.

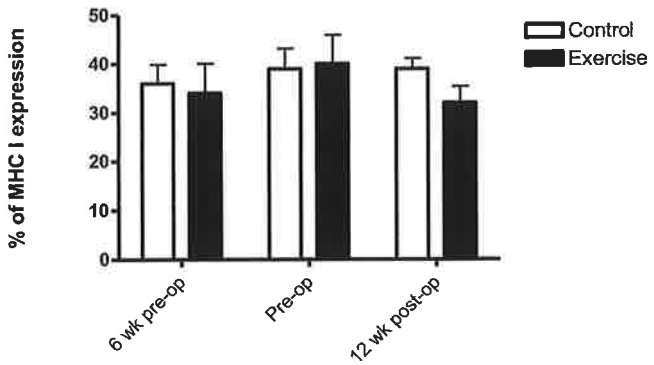


Figure 50. Relative densitometry of MHC I isoform protein in percentage (N=11 and 9 for exercise and control groups respectively). Percentage of expression was normalised to B-actin. Total cell lysate from 25 mg of muscle biopsies were lysed and pooled for analysis in triplicates.

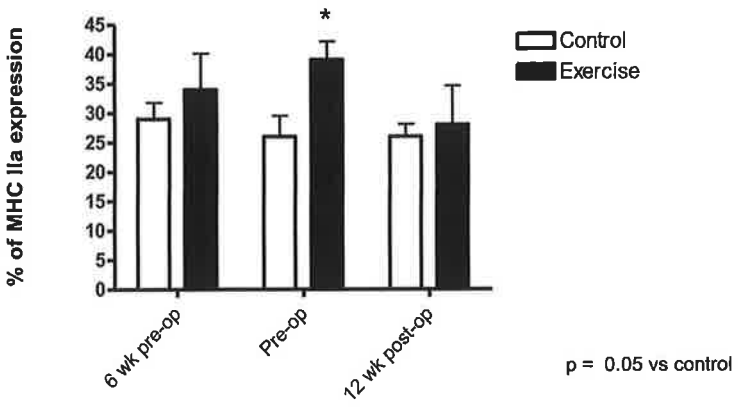


Figure 51. Relative densitometry of MHC IIa isoform protein in percentage (N=11 and 9 for exercise and control groups respectively). Percentage of expression was normalised to B-actin. Total cell lysate from 25 mg of muscle biopsies were lysed and pooled for analysis in triplicates.

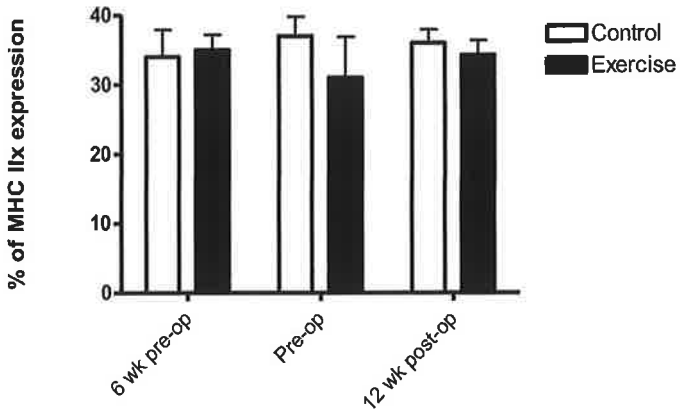


Figure 52. Relative densitometry of MHC IIx isoform protein in percentage (N=11 and 9 for exercise and control groups respectively). Percentage of expression was normalised to B-actin. Total cell lysate from 25 mg of muscle biopsies were lysed and pooled for analysis in triplicates.

3.8 Histopathology in Haematoxylin and Eosin stained muscle cross sections

5 samples from each treatment group were assessed for histological characterisation of atrophy in the vastus lateralis. Histology sections in both groups showed normal morphology in the majority of myofibres revealing polygonal shape with peripheral nuclei, intact sarcolemma, non-fragmented sarcoplasm and homogenous fibre size distribution

(Figure 53A). Small, angulated muscle fibres and fragmented sarcoplasm were more prominent at 6-wk postoperatively in both control and exercise groups indicating muscle atrophy without inflammation or fibrosis. (Figure 53C,F). Interestingly, in the exercise group, there was an improvement in the muscle fibre size and shape preoperatively (Figure 53E) compared to baseline (Figure 53D). This pattern was observed in 4 out of 5 patients in the exercise group.

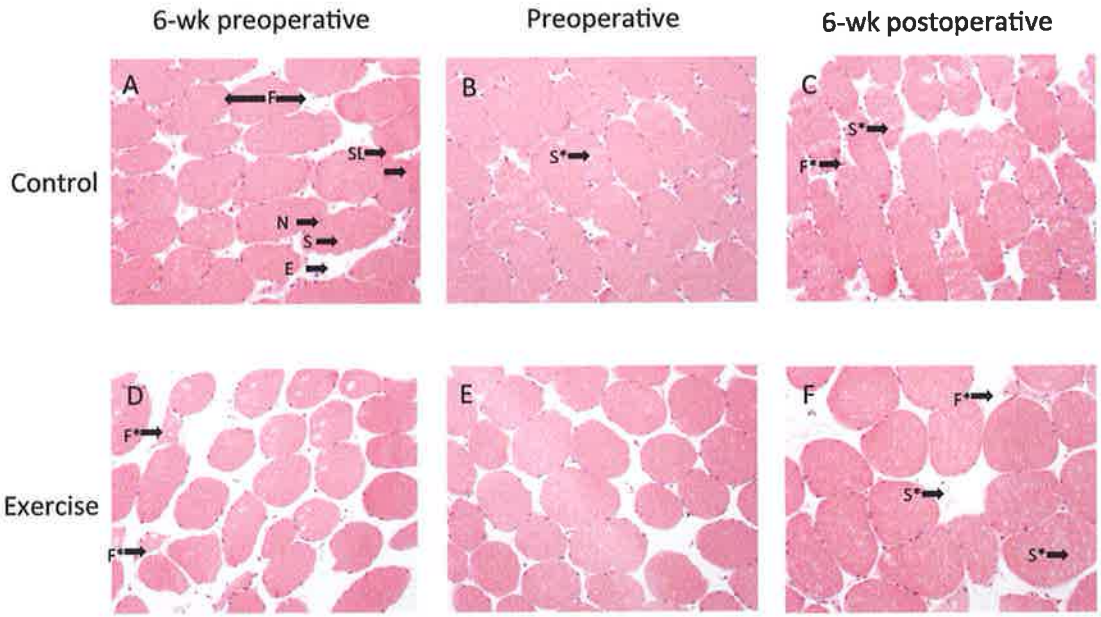


Figure 53. A representative cross-sectional histology (H & E stain, 200x) of the vastus lateralis in a patient from the control group and the exercise group at 6-wk preoperative, preoperative and 6-wk postoperative time points. Normal histological characteristics; F-fibre, N-nuclei, S-sarcoplasma, SP –sarcolemma and E-endomysium. Atrophic histological characteristics; F*- small and/or angulated fibre (degenerated fibre), S* - fragemented sarcoplasm

3.9 Correlations between patient demographics and study outcomes

To predict patient baseline demographics on the four primary outcomes, Pearson correlation coefficient (r) analysis was applied for several patient demographics including age, BMI and Tegner activity scores before injury. There were no correlations between pre-injury activity scores with quadriceps peak torque, quadriceps CSA, single leg hop test or Total Modified Cincinnati scores.

However, there was a strong negative correlation between age and functional test as measured by the single leg hop test 12-week postoperatively (r=-0.5416, p=0.0136) (Figure 54C). Though there was a strong correlation between age and quadriceps CSA (r=0.412), this was not statistically significant (p=0.07) (Figure 54B). There were no strong correlations between age and quadriceps peak torque or total Cincinnati scores (Figure 54 A,D).

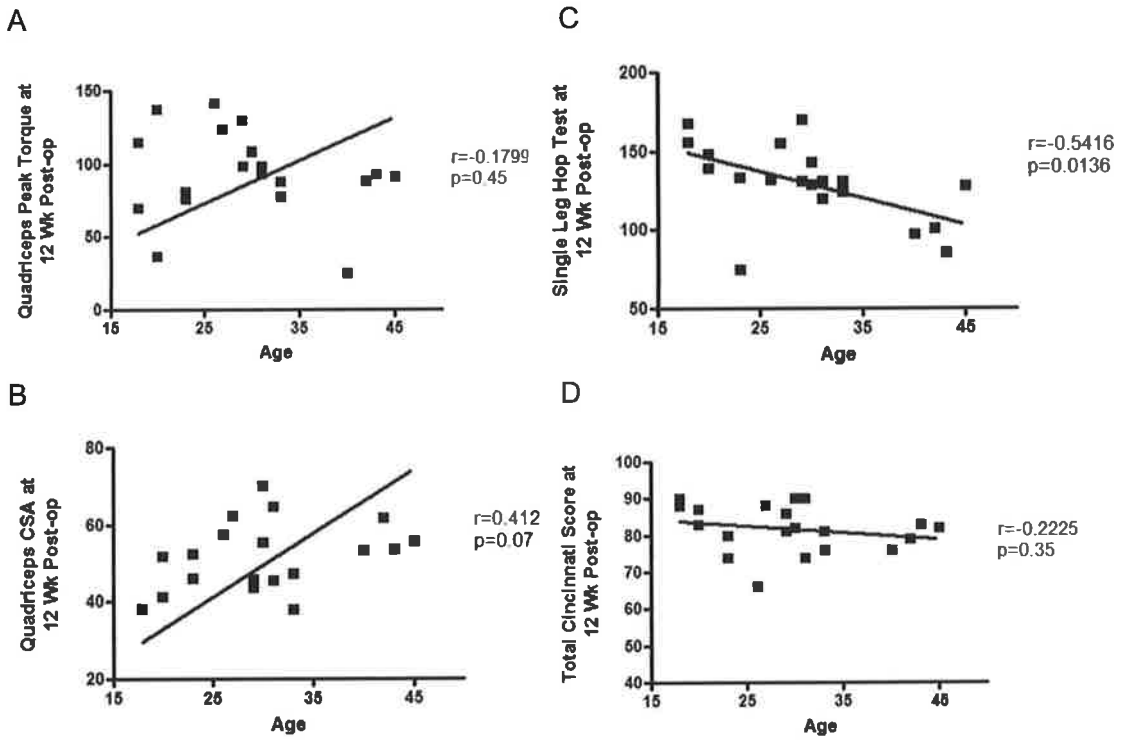


Figure 54. Pearson correlations between age and quadriceps peak torque (A), quadriceps CSA (B), single leg hop test (C) and total Cincinnati scores (D) at 12-week postoperatively (N=20). Pearson correlation coefficient r and p-value [95%CI] included in the analysis (N=11 and 9 for exercise and control groups respectively).

Meanwhile, there was a strong positive correlation between BMI and quadriceps CSA at 12-week postoperative time point (r=0.8599, p<0.0001) (Figure 55B). There was also a strong negative correlation between BMI and Total Modified Cincinnati scores (r=-0.4438, p=0.005) (Figure 55D). Weak positive and negative correlations were observed for quadriceps peak torque (r=0.3666, p=0.11) and single leg hop (r=-0.2218, 0=0.35) respectively however these were not statistically significant (Figure 55A,C).

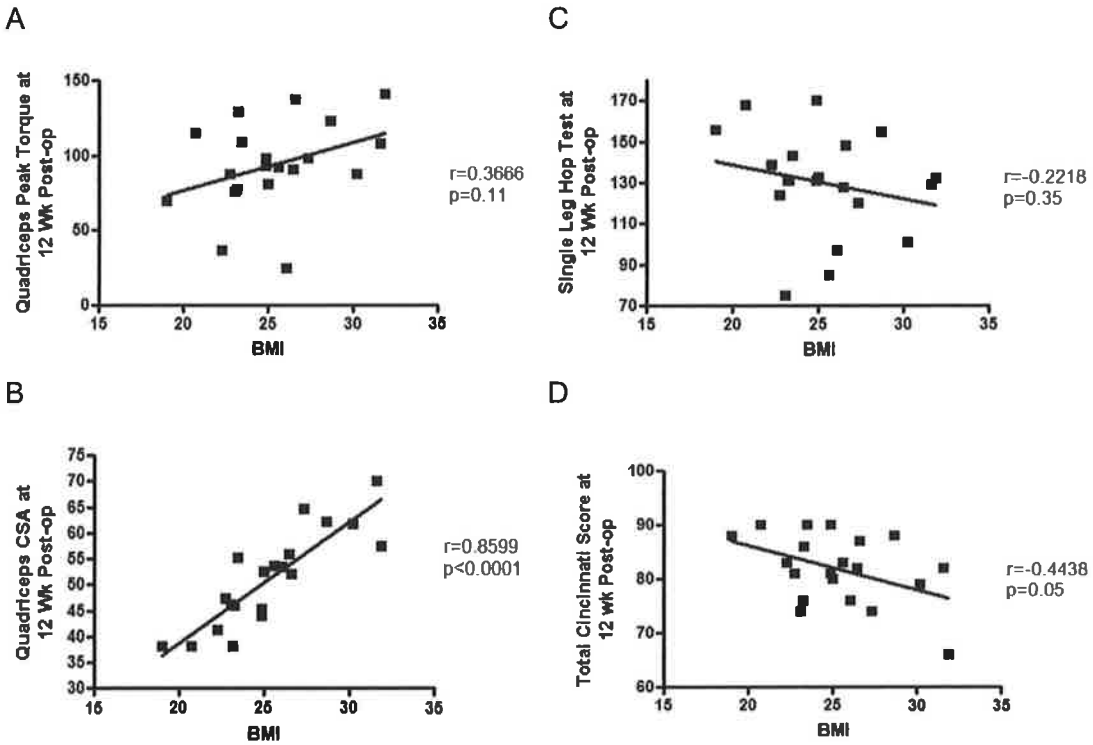


Figure 55. Pearson correlations between BMI and quadriceps peak torque (A), quadriceps CSA (B), single leg hop test (C) and total Cincinnati scores (D) at 12-week postoperatively (N=20). Pearson correlation coefficient r and p-value [95%CI] included in the analysis (N=11 and 9 for exercise and control groups respectively).

For correlations between different outcomes, only moderate to strong correlations (r=>0.4 or <-0.4) with p-values < 0.05 are shown in Table 6. There was a positive correlation between the single hop test at 12-week postoperative time point and the total Cincinnati scores (r=0.492, p=0.028). Meanwhile preoperatively, the single hop test positively correlates with quadriceps peak torque (r=0.457, p=0.043). As expected, there was a strong

association between baseline total Modified Cincinnati scores and Tegner-Lysholm scores (r=0.467, p=0.034).

The quadriceps peak torque correlates with quadriceps CSA consistently but strong correlations were observed for baseline (r=0.495, p=0.027) and preoperative time points (r=0.477, p=0.033). There was also a positive correlation in between quadriceps peak torque at baseline compared to CSA at 12-week postoperatively (r=0.495, p=0.027). When all the peak torque values were measured against their respective CSA, a strong correlation was observed (r=0.5396, p<0.0001) (Figure 56).

Table 6: Correlations (N=11 and 9 for exercise and control groups respectively)

Paired variables	Pearson Correlation coefficient	Sig. (2-tailed)
Single hop test 12wk post-op + Cincinnati score 12wk post-op	0.492	0.028
Single hop test pre-op + Quadriceps peak torque pre-op	0.457	0.043
Cincinnati 6wk pre-op + Tegner-Lysholm 6wk pre-op	0.467	0.034

Cincinnati 12wk post-op + Hamstring MRI CSA 12wk post-op	-0.599	0.005
Tegner-Lysholm 6wk pre-op + Hamstring torque pre-op	0.449	0.047
Quadriceps peak torque 6wk pre-op + Quadriceps MRI 6wk pre-op	0.495	0.027
Quadriceps peak torque 6wk pre-op + Quadriceps MRI 12wk post-op	0.530	0.016
Quadriceps peak torque 6wk pre-op + Hamstring MRI 6wk pre-op	0.566	0.009
Quadriceps peak torque pre-op + Quadriceps MRI pre-op	0.477	0.033

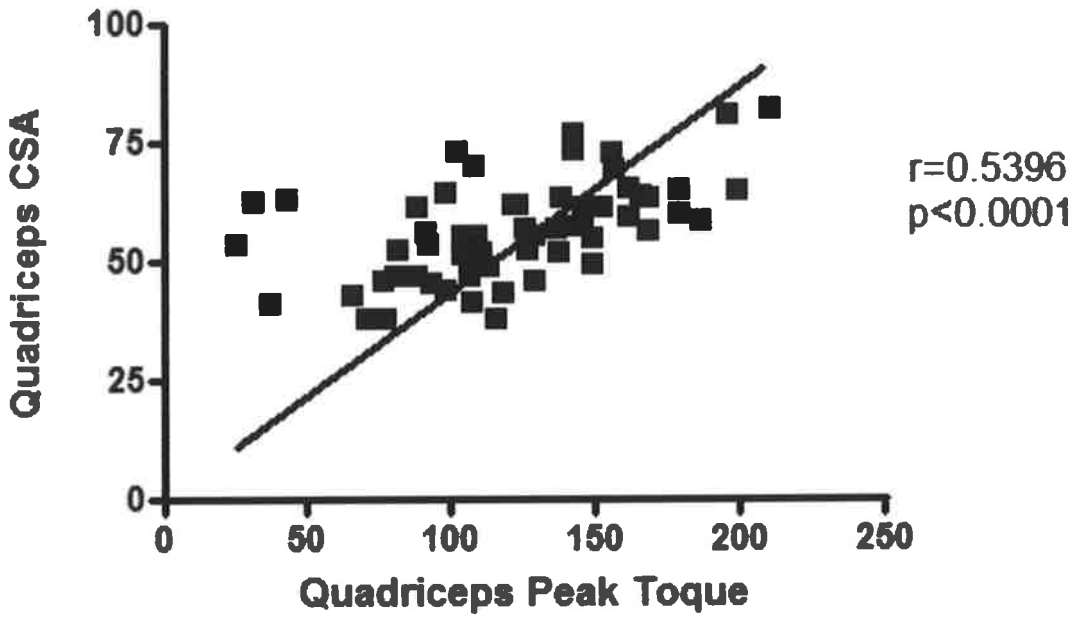


Figure 56. Pearson correlations between quadriceps peak torque with quadriceps CSA for 20 patients at all three time points (N=60). Pearson correlation coefficient r and p-value [95%CI] included in the analysis (N=11 and 9 for exercise and control groups respectively).

Chapter 4

Discussion

There is an increased prevalence of ACL injuries with emerging evidence of identifiable risk factors including gender, anatomical, biomechanical and hormonal predispositions. Preoperative quadriceps strength has been shown to be an important predictor of the functional outcome in the knee joint after ACLR. Enhancing the quadriceps strength and function of the knee preoperatively is a tempting intervention to improve the final outcomes of individuals undergoing ACLR. Currently, local and national guidelines in North America and Europe do not include preoperative rehabilitation due to lack of evidence [232].

Despite growing evidence on the effects of rehabilitation in non-operative and postoperative individuals with ACL injuries, there are currently only three studies published looking at the effects of prehabilitation in ACLR patients. These studies examined the effects of different aspects of physiotherapy. One study looked at the effects of a 5-week physical therapy including muscle strengthening which improved knee function and quadriceps peak torque [182]. Another study employed perturbation training prior to ACLR which improved symmetrical quadriceps strength and neuromuscular feedback, thereby enhancing dynamic stability during gait [183]. The controls in these two studies were paired to the same individuals using baseline measurements. The third study looked at the effectiveness of preoperative home-based physiotherapy and concluded that quadriceps strengthening

improve post-intervention preoperatively [233]. However, this study did not have a postoperative follow-up after ACLR. In addition, these studies tested functional and physical outcomes only. There is no published study examining the molecular effects of prehabilitation in individuals undergoing ACLR.

The main aim of this study was to investigate the effects of a 6-week preoperative resistance and proprioceptive training for patients awaiting ACLR in a single-surgeon, multicentre prospective randomised controlled trial. Besides testing macro effects using physical and functional tests, we also investigated the response of prehabilitation on quadriceps muscles at a cellular level. The primary aim of this physical therapy programme was to restore and optimise the muscle strength and neuromuscular response preoperatively which could be maintained post-ACLR. We recruited patients who had some degree of instability in their injured knee and were not able to return to their initial level of activity. Therefore, our study aimed to specifically examine the non-copers of ACL injury and the conclusion should not be extended to possible non-operative individuals [129].

Though there were 58 eligible individuals, the final number in our study was 22 with in each group to satisfy the sample size requirements for the two primary outcomes. The criteria for the power analysis of this study with quadriceps strengthening and the single leg hop test were based on standardised differences determined by preliminary measurements on the first 5 patients at the preoperative time point. Three of the initial participants did not complete the programme and therefore, their data were not used in the final analysis. Interestingly, the 11 patients that did complete the programme displayed more than 90% compliancy of the prescribed exercise regime. This is in line with a previously published

study that the level of motivation is high due to the well-described predictors of intentions in ACL injured patients in regards to rehabilitation [234].

The baseline characteristics of these patients were also similar in terms of age, height, weight, activities before and after injury and clinical indices based on the anterior drawer, Lachmann and pivot shift tests. However, despite adequate criteria for randomisation during recruitment, differences were observed for BMI although this was not statistically significant. Therefore, during analysis of function, isokinetic dynamometry and CSA of the injured and uninjured limbs, a common denominator (ie the injured limb at baseline) was used to normalise differences that were accountable to individual's body weight, height and activities over time. Although this is not a perfect model, the authors believe that this is the most adequate method in identifying the relative, as opposed to absolute differences that may be not be as discriminative when presented in graph format. However, the statistical analyses were performed with absolute values.

Of note, the limb symmetry index (LSI) is commonly used when assessing the progression of isokinetic muscle strength or functional testing. The LSI is a ratio of the injured limb over the contralateral non-injured side, with a value equal to or more than 90% to be taken as the normal value [133, 135, 136, 183]. We found this to be an unsatisfactory assessment for two reasons. The injured limb as a denominator ideally eliminates any biological difference between patients, however the non-injured limb could be affected from bilateral neuromuscular changes following an ACL injury and the prehabilitation programme itself [93]. As our evaluation takes into account 3 different time points, a baseline reference point was needed to assess relative differences. Our symmetry index, or

injury baseline index, is defined as the ratio between the value of the test of the limb being assessed at the current time over the injured limb value at baseline. Hence for the initial baseline assessment, all values of the injured limb started at '1'.

4.1 Functional Tests

In this study, the single leg hop test showed the most consistent beneficial effects of prehabilitation in ACLR individuals. Our data confirmed that preoperatively, after a 6-week exercise programme, there was an improvement with a 12% increase in the exercise group compared with a 6% increase in the control group which was statistically significant ($p=0.046$). At 12-week postoperatively, there was a decrease of 24% and 27% for the exercise and control groups respectively compared to their preoperative values. However, when compared to the baseline values, the exercise group fared better with only a 10% decrement whilst the control group suffered a 22% decrement in the single leg hop test. This effect was statistically significant ($p=0.001$).

The single leg hop test is a functional test considered by some investigators as the gold standard for assessing muscular strength and dynamic muscular coactivation [129]. Clinicians have used the single leg hop test to assess their patient's lower extremity muscle strength and ability to perform tasks that challenge knee stability. Previous single leg hop test studies have provided more information on the relationships with other physical impairments such as quadriceps weakness, passive joint laxity and knee joint proprioception deficits [235].

The limiting factors for the single leg hop test are pain and fear of re-injury. All patients were wearing the Don-Joy brace on the injured limb during all tests periods which functions primarily for patient confidence and, to a lesser degree, knee support. Only one control patient had complained of pain on their injured limb at the preoperative assessment due to a recent twisting injury with 5/10 pain score on the lateral aspect of the knee joint line. No patient experienced pain during the single leg hop test at the 12-week postoperative period; however they needed more assurance and relied heavily on verbal support together with the use of the brace.

Although the single leg hop test is believed to reflect lower extremity muscular strength, there are conflicting reports among studies investigating associations with quadriceps and hamstring strength measurement in both ACL deficient and reconstructed knees. The reported correlations between single leg hop test and measurements of muscle performance have been reported with varying consistencies [235]. As conclusive associations have not been established between knee function and muscle strength, ligament laxity and range of movement, many researchers in this field argued that the decision to return to sport activities in ACL deficient patients should be based on functional tests as opposed to static determinants and quadriceps peak torque [236]. The single leg hop has been shown to be successful in predicting short-term return to sport after specific non-operative management [237]. In addition, Barber et al, [238] reported that the single leg hop test is more reliable than the isokinetic strength test for assessing functional recovery after ACL reconstruction.

As shown in the result chapter, some of the effects of prehabilitation were not expected preoperatively, or even sustained postoperatively for other primary outcomes such as quadriceps peak torque, CSA and self-assessed questionnaires. However, in hindsight, the consistent positive results of the single leg hop test pre- and postoperatively shown in this study may in isolation support the positive effects of prehabilitation in ACLR.

The in-line lunge test is a simpler, quantitative assessment which may be used as a functional performance test of the ACL deficient and reconstructed subjects. In contrast to the single leg hop test, this test is less associated with pain, discomfort or perception of instability. Significant differences in the movement pattern performed suggest that this test could be used to discriminate functional performance [239]. It has been reported that ACLR subjects produced significantly less force during the initial step than the control, healthy group [240]. The impact index and force impulse measurements were significantly greater for the uninjured leg compared to the injured leg in ACLR. This suggests that the in-line lunge test is a promising tool for evaluation of function in ACLR.

In this study, compared to the single leg hop test, the in-line lunge test remained similar to baseline for the injured limb in the exercise group. In addition, there were no statistically significant differences among all 4 limbs between different time points. Though these results were unexpected in relation to the single leg hop test, a systematic bias has been observed in a previous study [241]. This bias involved a learning effect in the subjects involving the knee joint angular velocity during the braking phase of the lunge and the peak power generated by the knee extensors in the concentric phase of the movement.

In addition, the leading researchers of the in-line lunge test have published emerging evidence using computational modelling that the mechanical equilibrium of the knee joint in regards to a forward lunge movement may involve the posterior cruciate ligament as a more important stabilising player than the ACL [242]. Though the reliability of this test has been proven in previous studies examining functional performance in the ACL-deficient and ACLR patients, the unexpected data in this study may relate to a learner's effect and other unidentified biomechanical factors that may come to play. Also, to the author's knowledge, there is no study published looking at the direct correlation between the single leg hop test and in-line lunge test.

4.2 Isokinetic Dynamometry

Quadriceps weakness is one of the main dysfunctions following ACL injury. The preoperative quadriceps strength predicts the functional ability 1-year post-ACLR [180]. Isokinetic muscle strength using the peak torque value is the most frequently used muscle strength measurement for quadriceps in ACL injured subjects. Peak torque is the highest torque achieved during the test movement. Peak torque may give limited information about the muscle performance during range-of movement (ROM), however it is not an absolute measurement of maximal muscular tension. Rather, it represents a point in the test movement where length-tension factors and variations in level arm combine in an optimal fashion [243-246].

The isokinetic testing was performed using an anti-shear device limiting the movement arc from 20° - 110°. The conventional peak torque value during knee extension from 90-0° was not used due to safety reasons in post-ACLR subjects. The Johnson anti-shear device prevents excessive knee joint forces by having two points of contact in the proximal and distal tibia for adequate force redistribution and averting further damage to the knee. The restriction in the movement arc was also to prevent excessive strain in the ACL (or remnants of it) because ACL stress is greatest in the last 20 degrees of leg extension [215]. The velocity of the isokinetic test was set at 90°/sec as this appears to be the natural velocity of the knee. There are no absolute recommendations in testing speed, with most speeds used in previous studies at 60°/sec and 120°/sec. However, our institute testing speed is within the range of 0-180° which is recommended by the manufacturer [Cybex manual 2006].

From baseline, the isokinetic dynamometry demonstrated an increase of 20% in the average peak quadriceps torque in the exercise group compared to 10% in the control group preoperatively. This is a contrast to another study looking at resistance training in ACL-injured patient which only led to a 4% increase in peak torque after a 5-week rehabilitation programme [182]. However patients were supervised at least twice a week compared to our study which included four direct supervised sessions at home and the gym. Therefore we propose that continuous supervision during each session as well as increased home physiotherapy sessions per week, could potentially enhance isokinetic outcome.

At 12-week postoperatively, there was a decrease in the quadriceps peak torque by 19% versus 29% in the exercise and control group respectively compared to baseline.

Although this was not statistically significant when compared to the control group (p=0.31), this may be a clinically and scientifically relevant finding. Firstly, on a molecular level, this may reflect muscle hypertrophy preoperatively that was abled to inhibit to some extent the rapid decay of peak torque after an ACLR. Second, a delayed decrease of quadriceps strength deficit by 15% in the exercise compared to the control group should reflect a better outcome as this measurement is a strong predictor of function and return to normal activities. Studies have reported quadriceps strength deficit at 12 months post-ACLR, in which one particular study reporting a 10% deficit 7 years after reconstruction [247]. Natri et al, found the mean quadriceps strength to be 85% compared with the uninjured knee at 4 years postoperatively [248]. Therefore, a difference of 15% between the two groups favouring the exercise group in the injured limb 12-week postoperatively may be clinically meaningful.

Due to the study design, we observed interesting effects of prehabilitation on the uninjured limbs of the exercise group too. The uninjured limb illustrated a concurrent increase in average quadriceps peak torque similar to the injured limb but this was maintained above baseline value by more than 19% at 12-week postoperatively. There were no differences in the uninjured limb in the control group pre- and postoperatively. However, there are a few published studies examining early isokinetic testing postoperatively at 12 weeks which may reflect immediate term recovery pattern on muscle performance [249]. Our data provide further evidence that injured limbs in both the exercise and control group suffered a decreased in their peak torque values which were sustained 12-week postoperatively despite commencement of a postoperative accelerated rehabilitation programme. From this study, we conclude that prehabilitation appears to

benefit the exercise group preoperatively which may attenuate the rapid decay of muscle strength associated with the immediate postoperative factors such as immobilisation, pain and inflammation.

Peak torque angle-specific measurements could theoretically provide added information on interaction between specific moment arm and length of a muscle especially due to differences in ACL deficient and reconstructed knees. However, this was not performed due to additional sources of variability in which problematic inferential capacity to measure this test reliably has been questioned.

Interestingly, a moderate association between quadriceps strength and the single leg hop test has been reported especially in non-copers [250]. The trend between the single leg hop test and quadriceps peak torque in this study was similar. In fact, there were moderate correlations between these two measurements at all three time points but were not statistically significant albeit at the preoperative time point ($p=0.043$). Our data supported other studies that the ability to perform the single leg hop test is variably dependent on quadriceps strength.

The hamstring isokinetic peak torque showed an increase of 33% in the exercise group at the preoperative assessment compared to 22% in the control group. Although this effect was not maintained postoperatively, the hamstring strength remained 21% higher than baseline compared to 3% in the control group. The control group appeared to have a higher hamstring peak torque in the uninjured limb which could signify an abnormal or increased usage of their hamstrings disproportionately to the contralateral quadriceps as a

result of change in their gait pattern. Future studies may include the nerve firing potential in the quadriceps and hamstring with electromyography (EMG) to characterise further the neurophysiologic changes involved in both limbs revealing difference reflex contraction latencies in both muscle groups.

Hamstring strength deficits in ACLR have been reported consistently in the literature. Bizzini et al, reported 10.4+/-3.6% deficits in hamstring muscles strength after 11 months postoperatively [251]. Aune et al, reported an approximate 15% deficit in hamstring tendon grafts one year after surgery [252]. Other studies claimed that hamstring strength deficit decreases and only returns to normal after 24 months postoperatively [253]. Current rehabilitation protocols emphasise early and aggressive hamstring training following an ACLR on the basis that hamstring contraction can generate posterior tibial translation to reduce the strain on the maturing ACL substitute [254-256]. Although there were no statistically significant differences between the exercise and control group at any time point in this study, we conclude that the improved trend in hamstring strength preoperatively and the delayed attenuation in strength deficit postoperatively compared to baseline support current practice to enhance the hamstring strength in ACLR.

As mentioned, the prehabilitation programme focuses on quadriceps strengthening but also works on hamstring strengthening as the latter plays a vital role in the ACL-injured knee in increasing stability by reducing the degree of anterior tibial translation [257]. Proprioceptive training comprising closed kinetic chain exercises combined with progressive reduction in stability (wobble cushion, eyes opened, then closed, then closed with the head facing upwards) was included in this programme. Although not measured, this may enhance

reflex hamstring contraction latency to protect the knee joints in the preoperative phase and subsequently the graft in its healing period soon after ACL reconstruction. It would be interesting to study the effects of prehabilitation on specific measures such as speed and facility of hamstring contractions by measuring reflex hamstring contraction latency. Interestingly the 'giving way' and 'knee-instability' subsets in the total Cincinnati scores and Tegner-Lysholm scores respectively improved at post-exercise and postoperatively in the exercise group compared to the control group (although this did not reach statistically significant) which may reflect an improvement in proprioceptive deficit. Unfortunately, a device to measure this accurately still does not exist. From the current findings, we concluded that proprioception must receive the same importance as joint movement and muscle strength in prehabilitation programmes in ACLR.

4.3 Muscle Cross-Sectional Area (CSA)

The recovery and enhancement of quadriceps strength involve morphological alterations including muscle CSA. A larger fibre diameter leads to a greater number of cross-bridges and therefore, higher capacity to develop force [258]. When measuring the CSA in this study, only the outline of the muscle was taken to ensure minimal inclusion of connective tissue or fluid inclusion. Three repeated measurements were taken to reduce error and the average was calculated. Mitsiupolous et al, stated that CT and MRI were able to detect a 2% difference in either skeletal muscle or subcutaneous adipose tissue [228]. The MRI was taken 15 cm above the joint line and twenty-four 4mm axial cuts were available for assessment. The author chose the 12^{th} MRI slice as this appeared to have

almost all the individual muscles in every patient for analysis. Therefore the level of the measurement was 19.8cm from the joint line. All MRI were analysed by another independent consultant radiologist who was blinded to the subject's group as the confounding patient's details of the scan were removed prior to analysis.

Quadriceps muscle CSA

The quadriceps cross-sectional area (CSA) showed a significant increase of 7.6% for the injured limb in the exercise group preoperatively compared to baseline (p=0.001) whilst no change was seen in the control group. The exercise group then regressed to 12.3% below baseline value at 12-week postoperatively. However the control group showed a 2.5% decrease in CSA at preoperative assessment and this was unchanged postoperatively.

When specific muscles were analysed, the three vastus lateralis, medialis and intermedius CSA improved after the 6-week exercise programme in the range of 5-13%. The vastus medialis had the most appreciable improvement of 13% preoperatively which supports conventional clinical wisdom that the vastus medialis is the earliest of the heads of the quadriceps to atrophy and therefore showed the best improvement post-exercise therapy. However these effects were reversed with regression of vastus lateralis>vastus medialis>vastus intermerdius at 23%: 19%: 17% respectively at 12-week postoperatively. The 4th muscle rectus femoris was not included for analysis as this was not always available in the MRI cut of interest.

The vastus lateralis and intermedius are the largest of the quadriceps muscles, making them more vulnerable to the effects of neural disruption as seen in ACL injury. However, this may not always relate to altered morphological features due to other factors such as increased oxidative stress and depression of heat shock proteins with antioxidant properties shown previously in animal models [259]. Therefore, the authors can only infer that the disproportionate effects of ACL injury and prehabilitation on different muscles are related to other, non-established combinatorial factors.

Gerber et al, showed a significant increase in quadriceps CSA and strength with progressive eccentric training after ACLR [260]. Our study included both eccentric and concentric exercises. Interestingly, the pattern of CSA enlargement preoperatively which regressed postoperatively correlates with our functional and peak torque results. Fukunaga et al, has established that muscle volume calculated from multiple axial cuts correlated considerably with joint torque ($r= 0.92 – 0.94$) [89]. A single peak CSA can also be used however this correlated less with joint torque ($r= 0.71 – 0.88$). Our study supports published evidence that quadriceps peak torque correlates strongly with CSA ($r=0.5396$, $p<0.0001$).

However, the decrement in the single leg hop test and peak torque seen in the injured limb of the exercise group postoperatively was not as extensive as muscle CSA. Some researchers have argued that even though peak torque and occasionally functional test correlate with quadriceps CSA, as supported in this study, peak torque gains are almost exclusively attributed to neural changes relating to better motor unit efficacy as opposed to significant changes in hypertrophy leading to morphological alterations corresponding to increased muscle volume and size [261].

Previous studies have shown that quadriceps CSA was still reduced at 1 year postoperatively supporting the current data [262, 263]. We propose that the increased CSA observed in the exercise group could be related to hypertrophy, supporting the present findings for peak torque and single leg hop test. However, other neuromuscular tropism may come to play postoperatively, leading to a discrepancy in the results postoperatively when quadriceps CSA was compared to strength and function. Nevertheless, this present study supports previous evidence that quadriceps CSA strongly correlates with peak torque measurement supporting that muscle strength decrease or increase can only be partially explained by muscle hypertrophy or atrophy respectively, which may lead to macro changes such as CSA.

Hamstring muscle CSA

The CSA of three hamstring muscles were measured in this study. Although the semitendinosus and semimembranosus showed an increase in CSA compared to baseline, this was not sustained postoperatively. In addition, the biceps femoris showed no significant changes at all three time points. The individual hamstring muscles showed a downward trend with the rate of atrophy at 17% : 16% : 9% for biceps femoris : semitendinosis : semimembranosis respectively at 12-week postoperative compared to preoperative time points. This was an unexpected finding as we hypothesised a higher rate of hypertrophy for the exercise group due to hamstring strengthening and conversely, higher rate of atrophy in the control group. In addition, semitendinosus regeneration with the expected hypertrophy

for biceps femoris and semimembranosus muscles should only be relevant to ACLR with semitendinosus-gracilis grafts. In the recent years, possible consequences of hamstring muscle atrophy have been discussed in ALCR. Though it is well-established that functional deficit after ACL injury and ACLR are due to decrease in quadriceps activation and atrophy, other muscles including the hamstring often showed no changes [264]. Therefore, as hamstring CSA studies have received less attention especially in BPTB graft, the current study contributes by providing insight into the morphometric changes that occur with prehabilitation and subsequent ACLR. This data offers supporting evidence regarding the dominant role of the quadriceps muscles which correlate with strength. However, this cannot be generalised to ACLR with semitendonosus-gracilis graft which results in different mechanism of compensation in these muscles. In addition, as discussed, the effects of prehabilitation on the hamstring muscles in terms of proprioceptive as opposed to strength deficit should include examination of the reflex hamstring contraction latency which has been shown to correlate with functional instability [144].

4.4 Questionnaires

Self-reported questionnaires have become a common part of patient follow-up in ACLR. There are several validated disease-specific questionnaires concerning ACL injuries (KOOS, Mohtadi, IKDC, Tegner, Cincinnati) [220]. The Cincinnati Knee Rating System (or Modified Cincinnati), commonly used more in North America has multiple parts: symptoms, sports activity and activities of daily living scales, along with clinical parameters. Introduced in 1983, this was originally designed to assess ACL injuries but with an emphasis on patient's

symptoms and perception of their knee function. Tegner-Lysholm Knee score is a condition-specific outcome which was originally designed for assessment of ligament injuries of the knee, and has been used for a variety of knee conditions. It contains eight domains: limp, locking, pain, stair-climbing, use of supports, instability, swelling and squatting. For both scores, an overall score of 0 to 100 is divided into multiple sub-outcomes with a higher score depicting a favourable outcome. The psychometric properties of these two knee scoring systems have been vigorously established including reliability, validity and responsiveness [220].

Although self-assessment scoring systems are more subjective than dynamic functional tests, peak torques and CSA, these scoring are becoming more vital in the accurate evaluation of interventions for the knee. Over the past few decades, there has been a paradigm shift in the determinants of successful outcomes in both ACL injury and ACLR subjects, which emphasised physical examination and radiographic characteristic, to a more patient-centred assessment. There is no general consensus that recommends a single best knee scoring system.

Modified Cincinnati Knee Rating System

The Modified Cincinnati total scores improved in an upward trend for the 3 time points. The baseline mean score for the exercise group was 62.6, which was 3 points lower than the control group score. At pre- and postoperative time points, the scores for the exercise group significantly improved over time compared with the control group. The mean

12-week postoperative score was 85.3 in the exercise group versus 77.6 in the control group which was statistically significant (p= 0.004). The breakdown of the Modified Cincinnati scores in the exercise group depicted a superior improvement in pain, swelling, overall activity, walking, running, jumping and twisting scores compared to baseline. However the stairs scores were similar to the control group postoperatively. This finding supports the advantage of prehabilitation in ACLR based on this self-administered, patient-centred functional outcome instrument.

For the correlation analysis, the Cincinnati scores at 12-week postoperatively had a positive moderate correlation with the single leg hop test (r=0.492, p=0.028). This supports conventional wisdom that an objective functional knee test should correlate with self-assessment questionnaire that test function. Studies have reported correlation between muscular strength and the single leg hop test with Cincinnati scores [265].

Tegner-Lysholm Knee Score

The Tegner-Lysholm scores showed a similar improvement in both groups pre- and postoperatively compared to baseline. This was only statistically significant in the exercise group only. The breakdown of the questionnaire showed an improvement in pain and instability. Whilst an advantageous effect of the exercise group was not as appreciable as in the Cincinnati scores, the trend still supports prehabilitation in ACLR. Arguably, the Tegner-Lynsholm score is more appropriate for the ACL-deficient knee as opposed to ACLR subjects. The score was designed to validate disability; therefore, low-demand patients tend to

perform highly with this score as depicted in this study [266]. The differences in biomechanics and psychological factors between baseline, post-exercise and post-ACLR time points may have potential confounding factors that may affect these scores. A sham-control group would be ideal in discriminating the placebo effects on a self-reported scoring assessment which validates disability such as this outcome.

4.5 Molecular and histological adaptation of muscle hypertrophy and atrophy

IGF-1, MURF-1 and MAFbx mRNA

Exercise-induced muscle hypertrophy is observed mostly as a consequence of resistance training which leads to muscle fibre hypertrophy. Muscle fibre hypertrophy is generated by two mechanisms; the induction of satellite cell activation and recruitment and the increase of muscle protein synthesis in which IGF-1 has been implicated. Circulating IGF-1 is produced mainly by the liver, however it is also synthesised in stretch and overload-induced skeletal muscle. In skeletal muscle IGF-1 peptide expression increases muscular protein synthesis and stimulates differentiation of satellite cells [267, 268]. Overexpression or exogenous administration of IGF-1 in muscle has been shown to increase muscle mass [269]. Potential gene therapies have extended to include recombinant and transgenic or viral expression of IGF-1 to increase muscle mass in animal models [270,271]. Muscle levels of IGF-1 mRNA have been reported to be increased in response to compensatory overload

necessary for hypertrophic response [272]. IGF-1 mRNA has been repeatedly measured to determine skeletal muscle hypertrophy in other diseases such as pulmonary rehabilitation in chronic obstructive lung disease [273].

In our study, the vastus lateralis IGF-1 gene was increased by 3-fold in the exercise group at the preoperative time point as compared to the control group. There was a 30% increase when compared to baseline. However this decreased to a similar baseline value comparable to the control group at 12-week postoperatively. This current data supports previous published report that increased provision of IGF-1 within skeletal muscles can stimulate a hypertrophic response by which resistance exercise may counter atrophy mechanisms related to ACL injury. To date, there are no studies examining IGF-1 levels post ACLR. This study not only provides first evidence that IGF-1 levels regressed post ACLR but also suggests that factors other than IGF-1 changes may come to play in the 12-week recovery period post ACLR.

In a recent mouse study [274], the liver IGF-1-deficient gene knockout reduced the level of serum by 80% but still induced a muscle hypertrophy as a result of resistance training from local muscular IGF-I production. However, the reverse phenomenon in which reduced local IGF-1 production may be compensated by increased liver production. Also, the expression of IGF-1 within the individual muscles is correlated not only with hypertrophy but also with the muscle phenotypic adaptation that results from stretch and overload [275]. The authors propose that future studies looking at prehabilitation in ACLR should include serum IGF-1 levels as well. Although widely used as a marker for hypertrophy, due to the specificity issues, other emerging genes of interest that may be useful for assessment

include cyclin D1 and MyoD which regulate the expression of key muscle specific proteins during muscle development [276].

Skeletal muscle atrophy is characterized by a reduction in muscle mass maintenance caused by an imbalance of protein synthesis and degradation [277]. This results in a loss of protein content in the myofibres. The principle behind atrophy relates to an increase in intracellular proteolysis by the ubiquitin proteasome system. Two ubiquitin protein ligase, MAFbx and MuRF-1 occurs in different models of muscle atrophy such as denervation and immobilisation [278]. The mRNA levels of these two atrogins (or atrophic genes) have been validated in the studies of muscle atrophy as their increase precede the onset of muscle weight loss and are appropriate given the fact that quadriceps muscle atrophy is reported in ACL injuries and persist after reconstruction and rehabilitation [279]. A recent study showed an increase in the levels of MAFbx and MURF-1 after 48 hours of limb immobilisation [280]. More importantly, significant reductions in both genes were achieved within 24 hours of commencing rehabilitation [280].

The atrophy gene MuRF-1 gene expression was significantly decreased in the exercise group after intervention compared to baseline ($p=0.05$). At 12-week postoperatively, this effect was reversed with a 70% increase compared to baseline. Higher levels of inflammatory cytokines such as interleukin-6 and tumour necrosis factor-alpha related to swelling and inflammation post ACLR may increase the expression of MuRF-1 as previously observed [281]. Interestingly, the MAFbx level displayed no significant changes between the two groups and time points within the same group. Studies measuring MAFbx levels in muscle atrophy including specific focus on quadriceps muscle atrophy are published

[282] as MAFBx and MuRF-1 are overexpressed in numerous catabolic conditions. Accordingly, investigators were quick to use increased expression of either gene as a convenient marker of enhanced muscle protein breakdown. However, there are emerging reports observing no correlation between the expression of MAFbx and rates of protein breakdown [283]. Significant advances to elucidate the signalling pathways in muscle atrophy should shed light on more robust assays to measure muscle atrophy. Also, physiological measurements such as electrical impedence and T2-weighted MRI are emerging novel biomarkers of muscle atrophy and hypertrophy [284].

MHC Isoforms

Slow-and fast-twitch fibres have been shown to adapt differently in terms of metabolic, structural and contractile properties to physical training. We studied the changes in both mRNA and protein expression of human slow-twitch MHC I and fast-twitch MHC IIa because MHC isoforms can correlate with several isometric knee extensor performance variables including peak torque [285]. Exercise physiotherapy is capable of transforming 'fast' contracting muscles into 'slow' contracting muscles such that MHC isoform expression proceeds in the general direction from MHC IIx to MHC IIa to MHC I. This is similar to findings by Sharman et al, who demonstrated a 10% increase in MHC IIa and a similar decrease in MHC IIx [286]. Fast-twitch/slow-twitch fibre area and body weight adjusted to isometric force were found to be positively correlated [287]. MHC IIx has been reported to negatively correlate with peak force measures. To the author's knowledge,

there has been no study published examining MHC isoforms in the ACL deficient or ACLR knees.

There were no significant changes in the MHC-I relative gene expression in both groups. However the MHC IIa gene was elevated in the exercise group when compared to the control group preoperatively and within the same group at baseline. Conversely the expression of MHC IIx was decreased preoperatively compared to baseline. Similar findings were seen at protein level. Therefore, this study supports current evidence that MHC isoforms after exercise training shifts from MHC IIx to MHC IIa [288]. However, this adaptation was not maintained postoperatively. 'Detraining' from ACLR may increase the expression of fast MHC isoform although both groups underwent accelerated post-ACLR rehabilitation programme [289]. In addition, a study has shown that MHC isoforms did not differ after 6-week bed rest concluding that muscle unloading in humans do not always induce important changes in fibre type or MHC composition *in-vivo* [290]. It is argued that skeletal muscle adaptations would not depend on a single 'master switch' but also a number of broad genetic programmes which may differ postoperatively. The mRNA expression of genes encoding mitochondrial proteins and transcriptional regulators does not seem to be fibre type specific [291].

Histology of the Vastus Lateralis

Several studies have observed muscle hypertrophy using overload as an experimental model in animals and humans demonstrating a proportionate increase in

muscle fibre size and myonuclear number [292, 293]. On the other hand, atrophy was shown to be accompanied by a decrease in the number of myonuclei [294] corresponding to a reduction in muscle fibre size. In this study, there were minimal abnormalities observed histologically in the vastus lateralis of the injured limbs in both control and exercise groups. Some but not all characteristics of atrophy were observed such as the presence of variable, smaller and angulated fibres and fragmented sarcoplasm in the pre-exercise and postoperative time points. However, normal myofibres were present in the majority of the slides examined. More noticeable signs of atrophy such as central nuclei and non-intact sarcolemma were not observed. Interestingly, there was no evidence of large fibres in the post-exercise slides to suggest hypertrophy. Furthermore, the histological features of hypertrophy are best distinguished with the presence of Type 1 fibre. Eccentric exercise has been shown to elicit remodelling of myofibrillar structure with major effects such as muscle protein leakage and myofibrillar organization pathway however these were not observed [295]. It is important to note that biopsies of the uninjured limbs were not obtained for comparison and individual fibre sizes were not quantified. In addition, longitudinal cuts and staining with myofibrillar ATPase for fibre types assessment were not performed. Although there are studies examining the histology of the ligamentalisation process in ACL deficient, exercised and reconstructed knees, there are no histological studies examining the quadriceps muscle. Recent studies have employed MRI to evaluate overall muscle size as a morphometric measurement [68, 296]. Additionally, mRNA and protein expression involved in the hypertrophic and atrophic signalling pathway are more quantifiable and reproducible than histological examination (297). As 5 patients from each group were selected randomly for histological examination, statistical and correlative analyses were not performed.

4.6 General discussion

Our exercise programme meets the current recommendations for progression models of resistance training programme for healthy individuals by the American College of Sports Medicine [129]. The 2 gym sessions per week were selected, as this is the minimum number of training required for adequate muscular strengthening but this programme also further included 2 home sessions. After the prehabilitation programme protocol was developed, 5 healthy controls were used to assess the ease, time spent and adequacy of the exercises. The programme could be completed within one hour with adequate satisfaction. All muscle groups of the lower limb were included with emphasis on the quadriceps and hamstring muscles. The initial baseline weight during the gym sessions was calculated from their maximal 1-repetition maximum (RM) effort, to be able to perform 3 sets of 12 repetitions. The gym session weights were increased at a rate of 10-15% per week. As the injured limb can be considerably weaker, the weight increase for the injured limb would be minimally higher than the non-injured limb to ensure patient were abled to lift approximate symmetrical weights by the end of the 6-week exercise programme. Patients performed one set of exercise on one limb and alternated to the other side prior to moving on to the next station after a rest period of approximately 2-3 minutes. This allowed for a rest period of approximately 45 seconds – 1 minute between each limb within the same exercise. For the last gym session prior to ACLR, the previous session's weights were used to increase muscular endurance [298-301]. We interspersed the 2 gym sessions on alternate days from the 2 supervised home sessions. The supervision during home sessions was to ensure standardisation among the exercise group, where the subjects underwent the same routine as in the gym but with the use of a Theraband ™ instead of weights. As expected, we

deduced that the combination of dedicated gym and home sessions to be easier for the patients in terms of time off from work and family commitments or travelling to gym sessions. We therefore propose, that future studies and recommendations on prehabilitation in ACLR patients should employ both dedicated gym and home sessions.

Our correlation analysis revealed that certain patient characteristics might predict a better outcome for ACLR prehabilitation at 12-week postoperatively. Age was strongly and negatively correlated with the single leg hop test. Therefore, younger patients were associated with an improved single leg hop test compared to older patients. A positive but statistical insignificant correlation was seen with quadriceps CSA however this did not accord with peak torque. Intuitively, BMI was found to be very strongly and positively correlated with quadriceps CSA at 12-week postoperatively. Meanwhile, a low BMI was associated with improved total Cincinnati scores at the same time point. Currently, there is no consensus on what constitutes a satisfactory outcome post ACLR. Therefore, these baseline demographics may or may not be predictive of subjects likely to gain the most benefit of prehabilitation. The authors cannot propose that these isolated correlation findings reflect direct causation of improved outcome without influence of a third variable or a coincidence. Larger studies specifically to define baseline and patient demographics as predictors for a better outcome from prehabilitation are required to answer this question.

There was a general improvement in almost all the macro outcomes post-exercise therapy compared to baseline. For outcomes such as the single leg hop test, quadriceps and hamstring peak torque and self-assessed questionnaires, there was an advantageous effect for the exercise group at 12-week postoperatively even though these differences were not

all statistically significant. However, statistically insignificant does not always mean the effect is absent, small or clinically irrelevant. Although the power of the study was met, the small sample sizes means that we cannot conclude that for some of these results, the null hypothesis is true. For example, the decrease in average quadriceps peak torque was 19% in the exercise group compared to 31% in the control group at 12-week postoperatively. This was not statistically significant but a reported 10% residual deficit of quadriceps peak torque 7 years after reconstruction as reported in one study makes this current finding clinically relevant [302].

Another interesting aspect of this study was that the 12-week postoperative time point comprised of both groups having undergone standard physiotherapy care. This standard therapy within three months postoperatively did not commence weight training with open chain exercises or treadmill running. Hence, we could propose that any maintenance of muscle power should hypothetically be due to the increase observed in the preoperative period. The control group was still allowed to undergo their normal weekly routine of exercise either at home or in the gym preoperatively.

Due to the study design, multiple comparisons could then be made within different time points, treatment groups and limbs. For the latter, the uninjured limb should not be mistaken as equivalent to having normal knee function. This is further discussed in the next chapter.

Future efforts in this area must include a consensus on what constitutes a satisfactory outcome post ACLR especially in determining return to sport activities that does

not always correlate with peak torque, joint laxity and muscle CSA. For example, isokinetic strength test alone, although predictive cannot appropriately evaluate the patient's ability to return to sports. We also confirmed in our correlation analysis that peak torque is not consistently associated with functional performance. The authors are confident that although some of the outcomes examined in this study regressed back to baseline postoperatively, a long-term follow-up may still reveal a better outcome due to the phenomenon of 'muscle memory' and the theory that previously strong muscle can easily be retrained. Bruusgaard et al, proved that elevated number of nuclei was maintained even after months of denervation resulting in severe atrophy [303].

In this study, we looked at multiple effects including dynamic function, self-reported function, strength, morphology and molecular changes of a 6-week prehabilitation programme with heavy resistance strength training and neuromuscular exercises in ACLR. This study supports prehabilitation for patients awaiting ACLR however further studies are required before such recommendation can be introduced into ACLR guidelines.

Chapter 5

Limitations

Although the sample size was powered to measure two of the primary outcomes i.e. peak torque and the single leg hop test, the main limitation of the study was due to its small sample size of 22. Despite 58 eligible individuals, the major factor for the sample size is the inability of patients to participate due to work and time constraints. Due to the nature of the pathology of ACL injury, the subjects eligible for this study are mainly young, active individuals who are either working or studying full-time.

The length of follow-up period in this study was limited to 12-week postoperatively. As studies have shown quadriceps strength and subjective outcomes were impaired up to 7 and 9 years of follow-up respectively [302], a longer follow-up time at least up to one year would be useful. An interesting outcome includes time to return to sports or normal activities.

Control subjects received standard preoperative care, which included written instructions to perform simple exercises. Subjects were also allowed to do their self-routine exercise. Several studies have incorporated sham exercises into their protocols to ensure that improvements in the exercise group were not due to the extra attention including direct supervision and advise given in regards to pain and symptoms relating to their pathology.

All patients were operated by a single surgeon who uses BPTB graft. Hamstring graft reconstruction were not included in the study as the duration of postoperative rehabilitation is longer and the use of the isokinetic dynamometry would require a different time assessment later than 12-week postoperatively. Therefore, the current findings cannot be generalised to ACLR using hamstring graft reconstruction. Importantly, this study examined the effects of prehabilitation in 'non-copers' subjected to subsequent ACLR. The improved outcomes observed post-exercise should not be generalised to potential non-operative candidates.

Muscle sample processing did include a mounted sample for sectioning and examination under light microscopy. Although cross-section cuts were obtained, high quality longitudinal sections were unavailable. Histological analysis looking at changes in the ratio of fibre typed muscle would provide further support on microscopic outcomes. Muscle biopsy of the contralateral uninjured limb would have also been beneficial. A vital assumption with muscle biopsies is that the sample obtained is representative of the entire quadriceps muscle. The individual muscle MRI CSA has shown a stronger correlation with the vastus medialis muscle between groups especially in the preoperative period. However it would be interesting to investigate hypertrophic and atrophic pathways in each individual muscle.

The ideal criterion for standard control would be to have pre-injury data in the same subjects, but this is not practical as these individuals would not be considered for ALCR in the first place. Therefore studies examining ACLR are faced with making assumptions that involve the injured and uninjured limb, in which the latter does not always represent the

normal pre-injury status having subjected to compensatory adaptations from the contralateral leg. This argument can be extended to using pretest and posttest for comparison as employed in this study i.e. baseline versus post-exercise versus post-ACLR.

Female subjects were not recruited in this study due to the additional precautions required to schedule the muscle biopsy during a specific time in the menstrual cycle. We hypothesised that the aromatisation pathway relating to the oestrogen:testosterone ratio would affect muscle physiology. Specific rehabilitation studies looking at female subjects with ACLR are currently emerging [304].

A cost-benefit analysis was not performed in the exercise intervention group. From this study we cannot state that a prehabilitation programme is economically viable. We performed supervised home sessions to ensure exercise adherence in this study therefore the current findings must be interpreted with caution if generalized to a practical prehabilitation programme that included unsupervised sessions at home.

Finally, the patients included in this study were relatively young, active individuals who might have had higher motivation for exercise than other subgroups of patients with ACL injury. A prehabilitation programme that requires 6-week commitment of both gym and home based exercise limits the potential of this intervention to highly motivated population. The current findings depended on high compliancy with low dropout rates from the proposed exercise programme.

Chapter 6

Conclusion

There is an increased prevalence of ACL injuries with evidence of functional and strength impairment at medium to long-term follow-up. Enhancing the quadriceps strength and function of the knee preoperatively may improve the final outcomes of subjects undergoing ACLR. There are currently three studies published looking at the effects of prehabilitation in ACLR subjects. Some of these studies do not include a non-treated control group, postoperative assessment or molecular effects of prehabilitation. This current study examined muscular strength, dynamic function, muscle CSA, self-reported assessment and molecular changes of prehabilitation in a randomised controlled manner.

Our study has shown that significant increase in quadriceps strength can be achieved with a preoperative, prehabilitation programme. Factors that impact on this gain include a relative high intensity exercise regime interspersed with home exercise and direct supervision of the exercise and progressive resistance training. This finding correlates with quadriceps CSA preoperatively implying that an increase in strength may be variably due to muscular hypertrophy, with supporting evidence from the 3-fold increase in IGF-1 and a reciprocal decrease of MuRF-1 mRNA expression. At 12-week postoperatively, the isokinetic dynamometry showed an attenuated regression in quadriceps strength at 19% from baseline value, compared to 29% in the control group. Although these benefits were not reproducible in the hamstring muscles, we proposed that the reflex hamstring contraction latency is a better measurement to measure the proprioceptive effects on the hamstring in improving functional stability in ACL injured and reconstructed knees.

There were also improvement in the Modified Cincinnati Knee Rating System and the Tegner-Lysholm Knee functional scores. The reported pain levels during tasks and the functional pain scores also improved. The 'giving way' and 'knee instability' also improved pre- and postoperatively, which could be related to an enhancement in the proprioceptive deficit involved in the ACL injured knees apart from the mechanical stability provided with ACLR. The functional single leg hop test improved significantly compared to baseline and the control group. Postoperatively, the exercise group only suffered a 10% decrement of the single leg hop test compared to 22% in the control group. These results support the benefits associated with a preoperative exercise programme in ACL injured patients.

Although the general consensus of what constitutes the most optimal outcome for ACLR is inconclusive, we used multiple valid outcomes such as isokinetic dynamometry, functional tests, self-reported questionnaires and MRI quadriceps CSA in ACL injury. These tests have shown high levels of test-retest reliability and repeatability.

Long-term follow-up is needed to examine the phenomenon of 'muscle memory' on the effects of prehabilitation in ACLR. Future studies should attempt to examine more patients undergoing both types of ACL reconstruction including hamstring graft. The proprioceptive effects on hamstring activity and overall knee stability should also be examined. Studies with female subjects may help predict gender-specific outcomes. The cost-effectiveness of the prehabilitation programme could also be studied with specific attention to supervised and home physiotherapy programmes. Neuromuscular stimulation devices which can be used together or as a substitute to exercise may improve outcome and

be more cost-effective. The complex pathway of muscle hypertrophy and atrophy should be explored to find more robust markers for muscular adaptation to exercise training. Emerging physiological measurements such electromyogram, electrical impedence and T2-weighted MRI may be more helpful and less invasive in elucidating the changes in muscular activation and/or adaptation to post-exercise therapy.

We conclude that a 6-week progressive prehabilitation programme for subjects undergoing ACLR is safe and led to improved knee function, quadriceps strength and CSA, and self-reported assessment pre- and postoperatively. A 6-week period of preoperative exercise in ACL injury should be considered in the surgical management of ACL injury however further studies are required to definitely include this intervention in standard guidelines.

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Appendix A

Patient Information Sheet

and Consent form

The effect of Pre-conditioning on the outcome of Anterior Cruciate Ligament Reconstruction

This Informed Consent Form has two parts:

- **Information Sheet (to share information about the study with you)**
- **Certificate of Consent (for signatures if you agree to participate)**

PART I: Information Sheet

Introduction

This project is under the guidance of Professor John O'Byrne (Professor of Orthopaedics at the Royal College of Surgeons) in Cappagh National Orthopaedic Hospital, Mr. Ray Moran (Consultant Orthopaedic Surgeon in Sports Surgery Clinic) and Professor Niall Moyna (Professor of Health and Human Performance) in Dublin City University. The research concerns the effect that "pre-conditioning" or exercise

prior to your anterior cruciate ligament reconstruction will have on the success of the operation.

You are in no way obliged to partake in the study and if you do not wish to take part, it will in no way delay or affect your scheduled operation.

If at any stage there is anything that you do not understand, please stop and ask me to explain or alternatively you can ask me at the end.

Purpose

It is well known by clinicians that one of the main factors influencing the success of an anterior cruciate ligament reconstruction is the strength of the quadriceps muscles – that is those muscles at the front of your leg. By taking a little sample of your muscle tissue or biopsy, we are planning to look for genes that may cause weakness in these muscles. In addition, by undertaking an exercise programme before your operation, we are looking to see if this affects the activity of any of these genes. The ultimate outcome will be whether or not it will improve your recovery from the operation.

Participant selection

You are being asked to participate as you are currently awaiting an anterior cruciate ligament reconstruction and satisfy the age requirements of the study.

Voluntary Participation

Your decision to participate in this study is entirely voluntary. It is your choice whether to participate or not. If you choose not to consent, all the services you receive will continue and nothing will change. You may also choose to change your mind later and stop participating, even if you agreed earlier, and your operation will

not be in jeopardy. Permission will also have been sought from the Consultant looking after you before asking you to participate.

Procedures and Protocol

The main intervention involved will be 3 muscle biopsies: one 6 weeks before your operation, the other just before/during it and the final biopsy 3 months after the operation. The biopsies are small samples of muscle that are taken through a tiny incision – smaller than your finger nail in length. This can easily be done under a local anaesthetic and you do not need to be put to sleep for it.

In addition, you will be assessed both clinically and with an MRI scan of your leg at these times and again 3 months following your operation. Note that an MRI scan does not involve any ionizing radiation that could potentially be harmful to you. Part of the clinical assessment will include a test of muscle strength on a specially designed machine known as an Isokinetic machine.

Half of the patients in the study will be asked to partake in an exercise programme to improve their muscle strength. These will be adapted to your own abilities and will not involve severe discomfort. Some students from Dublin City University will be available to help you perform these.

In order to see if there is any benefit in boosting your leg muscle strength preoperatively, we need to make comparisons. Patients taking part in the study will be randomly placed into one of two groups. One group will partake in the exercise programme, the second will continue on as normal until their operation. Patient who are randomly selected into the exercise group will require to undergo 6 weeks prehabilitation programme (which includes 2 gym session in the Sports Surgery

Clinic and 2 home sessions per week).After the operation, both groups will receive full Physiotherapy in the normal manner.

The muscle biopsies obtained during the study will be used only for the research outlined and will be destroyed once the research is complete. The muscle biopsies will be retained for genetic testing which will be conducted in Dublin City University.

Duration

Part of the design of the study is to minimize the inconvenience to you, the patient. Thus it is hoped that all the necessary data can be collected and the first sample of muscle taken when you are attending the hospital for your routine preoperative workup. The second sample and assessments will be done whilst you are an inpatient for your operation. The final data collection will be around 3 months after your operation and would involve you attending the hospital for an hour or two.

Side Effects

With regard to the muscle biopsy, the side effects would be very minor and would include the risk of a wound infection; some bruising; some discomfort after the local anaesthetic wears off and very rarely patients have reported a small temporary area of numbness.

Benefits

The potential benefits to you are a faster post-operative recovery and a better outcome from your anterior cruciate ligament reconstruction in the long term. In addition, you will receive much more incentive to do well from the actual assessments that we make, for example if we can prove that you have made a significant increase in muscle strength or can return to sports at an earlier period.

The potential benefits for the community would be that this may evolve into a programme that is undertaken by all patients before their operation in order to improve the outcome. Also, we may identify genes that will one day be the target of a different type of therapy.

Incentives

This study will not involve any monetary incentive to you. However, you will incur no costs for any tests performed and every effort will be made to minimize the inconvenience.

Confidentiality

The information that we collect from this research project will be kept confidential. Information about you that will be collected from the research will be put away and no-one but the researchers will be able to see it.

Sharing of the results

The knowledge that we get from this study can be shared with you before it is made widely available to the public. Confidential information will not be shared. Afterwards, we will publish the results and present our findings at conferences in order that other interested people may learn from our research.

Right to Refuse or Withdraw

You do not have to agree to take part in this research if you do not wish to do so and refusing to participate will not affect your treatment in any way. You may stop

participating in the research at any time that you wish without losing any of your rights as a patient here.

Retention of muscle biopsies

As you have read from the above section, the retention of muscle biopsies will be necessary for the testing of the genes involved in growth and shrinking of muscles. These samples will be securely stored in the laboratory in Dublin City University and will be destroyed if you decide to stop participating in this research or when the research is completed.

Who to Contact

If you have any questions you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact myself at the following address;

Chief Researcher: **Shahril Shaarani**

c/o Sports Surgery Clinic

Santry, Dublin 9

Email: sshaarani@rcsi.ie

This proposal has been reviewed and ethical approval granted by the Health Research Board whose task it is to make sure that the research participants are protected from harm.

PART II: Certificate of Consent

I have been invited to participate in a study on Muscle Strength prior to my anterior cruciate ligament reconstruction. I understand that this will involve a total of 3 muscle biopsies (taking of muscle samples), several clinical assessments and scans, and possibly an exercise programme before my operation. I have been informed of the minimal risks involved.

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate in this study and understand that I have the right to withdraw from the study at any time without in any way affecting my medical care.

Print Name of Participant: _____

Signature of Participant: _____ **Date:**

/ / 2011

I have accurately read or witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print Name of Researcher: _____

Signature of Researcher: _____ **Date:**

/ / 2011

A copy of this Informed Consent Form has been provided to the participant _____(initialed by researcher)

Appendix B

Programme for ACL Prehabilitation

Gym based

Warm up	Bike	10 mins	
Group 1	Leg Press	12reps	
	Leg curl	12 reps	3 sets
	Leg extension	12 reps	
	(leg press machine- range of motion to -20 degrees)		
Group 2	1/2 Lunge	12 reps	
	45° squat	12 reps	3 sets
	Calf raise	12 reps	
Group 3	Hip abduction	12 reps	
	Hip adduction	12 reps	3 sets
	Hip Flexion (machine)	12 reps	
Group 4	Balance Progamme	*see below*	
Stretching	*see below*		

Home based

Warm up	Walk	5-10 mins	
Group 1	Leg press (theraband)	12 reps	
	Leg Curl (theraband)	12 reps	3 sets
	Knee Ext (theraband)	12 reps	
	(range of motion limited to -20 degrees)		
Group 2	1/2 Lunge	12 reps	
	45^0 squat (swiss ball)	12 reps	3 sets
	Calf raise	12 reps	
Group 3	Hip abduction (theraband)	12 reps	
	Hip adduction (theraband)	12 reps	3 sets
	Hip Flexion (theraband)	12 reps	
Group 4	Balance Programme	*see below*	
Stretching	*see below*		

Balance Programme

This is a progressive programme to allow you to improve your Strength and Proprioception. All exercises should be completed on both legs for counts of ***30 SECONDS.*** *You may only progress to the next stage when you can complete the previous stage* ***3 TIMES IN A ROW WITHOUT YOUR OPPOSITE LEG TOUCHING THE GROUND.***

Phase 1 - standing on one leg with your eyes **OPEN**. Hands on hips and count the amount of times the opposite leg needs to touch the ground for you to maintain balance. Balancing leg must remain flat on the ground at all times - no hopping allowed.

Phase 2 - standing on one leg with your eyes **CLOSED**. Hands on hips and count the amount of times the opposite leg needs to touch the ground for you to maintain balance. Balancing leg must remain flat on the ground at all times - no hopping allowed.

Phase 3- standing on wobble cushion with both legs

Stretching Programme

Flexibility - 3 sets of 30 seconds

All stretches should be held for a minimum of 30 seconds and longer if time permits – there is no limit on how long you can hold a stretch.

Hamstrings – lie flat on your back bringing one leg up, with hands behind the knee to assist, as far as you can while leaving the leg not being stretched **FLAT** on the ground.

Quadriceps – stand with one hand on a wall/chair for balance. Bring the heel of the leg you wish to stretch to the glutes and hold in position with same hand.

If unable, patient should hold trouser/sock to reduce ROM

QUADRICEPS STRETCH

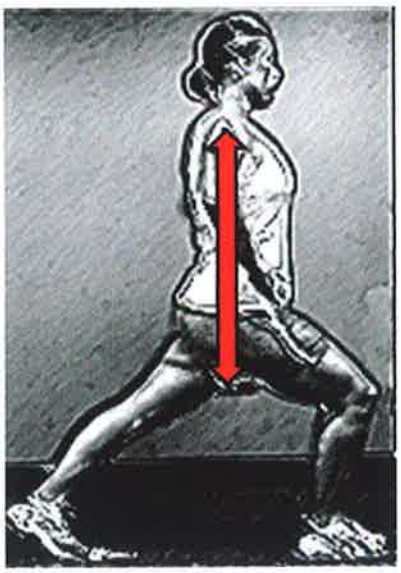
Upper calf – stand with both feet facing the same direction about 1 meter apart and face the wall. Lean into the wall with your hands and lock out the back knee. You should feel a stretch in the upper part of the calf on the back leg. It is important that both heels remain on the ground at all times. To increase the stretch get the heel on the back leg flat on the ground. Ensure the foot of the back leg is facing straight forward.

Standing calf stretch

Strength and Control

Half- Lunge

- Have the feet slightly narrower than shoulder width apart with the core muscles engaged and the lower back stabilized.
- Inhale as you step out with one foot, keeping both feet pointing straight ahead and get your knee right out over your toes; keeping your body upright as you step
- Exhale while pushing yourself back into a standing position and repeat with alternative leg.

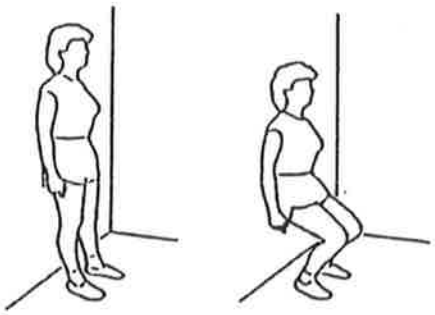


Wall Squat

- Push your back against the wall in a standing position
- Feet must be shoulder width apart while keeping the back and shoulders

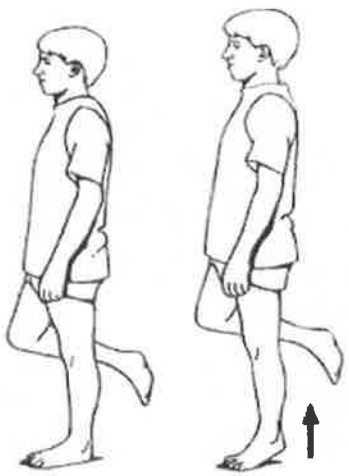
straight.

- Engage the core muscles and perform a squat to 45 degrees of knee flexion.
- Make sure the knees don't come out over the toes.
- Breathe in on the way down and exhale as you are coming up.



Calf Raises

- Do this exercise on flat surface.
- Stand on one foot with the other leg bent.
- Raise up on the ball of foot.
- Slowly lower the leg back down to the starting position to the count of 3
- Repeat 12 times and then swap legs.



Modified Cincinnati Knee Rating System

Section 1 - Pain Intensity
No pain, normal knee, performs 100%
Occasional pain with strenuous sports or heavy work, knee not entirely normal, some limitations but minor and tolerable
Occasional pain with light recreational sports or moderate work activities, running or, heavy labour, strenuous sports
Pain, usually brought on by sports, light recreational activities or moderate work. Occasionally occurs with walking, standing or light work
Pain is a significant problem with simple activity such as walking, relieved by rest, unable to do sports
Pain present all the time. Not relieved by rest

Section 2 -Swelling
No swelling
Occasional swelling with strenuous sports or heavy work. Some limitations but minor and tolerable
Occasional swelling with light recreational sports or moderate work ativities. Frequently brought on by vigorous activities running, heavy labour, and strenuous sport
Swelling limits sports and moderate work. Occurs infrequently with simple walking activities or light work (approx 3 times year)
Swelling brought on by simple walking activities and light work. Relieved by rest
Severe problem all the time, with simple walking activities

Section 3 - Giving Way
No giving way
Occasional giving way with strenuous sports or heavy work. Can participate in all sports but some guarding or limitations present
Occasional giving way with light sports or moderate work. Able to compensate but limits vigorous activities, sports, or heavy work not able to cut or twist suddenly, are conveniently positioned (e.g., on a table)
Giving way limits sports and moderate work, occurs infrequently with walking or light work (approx 3 times per year)
Giving way with simple walking activities and light work. Occurs once per month, requires guarding
Severe problem with simple walking activities, cannot turn or twist while walking without giving way

Section 4 - Overall activity level
No limitation, normal knee, able to do everything including strenuous sports or heavy labour
Perform sports including vigorous activities but at lower performance level: involves guarding or some limits to heavy labour
Light recreational activities possible with rare symptoms, more strenuous activities cause problems.Active but in different sports, limited to moderate work
No sports or recreational activities possible. Walking with rare symptoms; limited to light work
Walking, ADL cause moderate symptoms, frequent limitations
Walking, ADL cause severe problems, persistent symptoms

Section 5 - Walking
Walking unlimited
Slight/mild problem
Moderate problem: smooth surface possible up to approx 800m
Severe problem, only 2-3 blocks possible
Severe problem; requires stick or crutches

Section 6 - Stairs
Normal, unlimited
Slight/mild problem
Moderate problems only 10-15 steps possible
Severe problem; requires bannister support
Severe problem on 1-5 steps possible

Section 7 - Running activity
Normal, unlimited; fully competitive, strenuous
Slight mild problem, run half speed
Moderate problem 2-4 km
Severe problem only 1-2 blocks possible
Severe problem only a few steps

Section 8 - Jumping or Twisting
Normal, unlimited, fully competitive, strenuous
Slight to mild problem; some guarding but port possible
Moderate problem; gave up strenuous sports, recreational sports possible
Severe problem; affects all sports; must constantly guard
Severe problem; only light activity possible (golf, swimming)

Tegner-Lysholm Knee Scoring

Section 1 -Limp

- ○ None
- ○ Slight or periodical
- ○ Severe and constant

Section 3 - Pain

- ○ None
- ○ Inconstant and slight during severe exertion
- ○ Marked during severe exertion
- ○ Marked on or after walking more than 2 km
- ○ Marked on or after walking less than 2 km
- ○ Constant

Section 5 -Locking

- ○ No locking and no catching sensations
- ○ Catching sensation but no locking
- ○ LockingOccasionally
- ○ Frequently
- ○ Locked joint on examination

Section 7 - Stair-climbing

- ○ No problems
- ○ Slightly impaired
- ○ One step at a time
- ○ Impossible

Section 2 -Support

- ○ None
- ○ Stick or crutch
- ○ Weight-bearing impossible

Section 4 - Instability

- ○ Never giving way
- ○ Rarely during athletics or other severe exertion
- ○ Frequently during athletics or other severe exertion (or incapable of participation)
- ○ Occasionally in daily activities
- ○ Often in daily activities
- ○ Every step

Section 6 - Swelling

- ○ None
- ○ On severe exertion
- ○ On ordinary exertion
- ○ Constant

Section 8 - Squatting

- ○ No problems
- ○ Slightly impaired
- ○ Not beyond 90°
- ○ Impossible

Tegner Activity Level Scale

Please indicate in the spaces below the HIGHEST level of activity that you participated in BEFORE YOUR INJURY and the highest level you are able to participate in CURRENTLY.

BEFORE INJURY: Level _____ CURRENT: Level _____

Level 10	Competitive sports- soccer, football, rugby (national elite)
Level 9	Competitive sports- soccer, football, rugby (lower divisions), ice hockey, wrestling, gymnastics, basketball
Level 8	Competitive sports- racquetball or bandy, squash or badminton, track and field athletics (jumping, etc.), down-hill skiing
Level 7	Competitive sports- tennis, running, motorcars speedway, handball Recreational sports- soccer, football, rugby, bandy, ice hockey, basketball, squash, racquetball, running
Level 6	Recreational sports- tennis and badminton, handball, racquetball, down-hill skiing, jogging at least 5 times per week
Level 5	Work- heavy labor (construction, etc.) Competitive sports- cycling, cross-country skiing, Recreational sports- jogging on uneven ground at least twice weekly
Level 4	Work- moderately heavy labor (e.g. truck driving, etc.)
Level 3	Work- light labor (nursing, etc.)
Level 2	Work- light labor Walking on uneven ground possible, but impossible to back pack or hike
Level 1	Work- sedentary (secretarial, etc.)
Level 0	Sick leave or disability pension because of knee problems

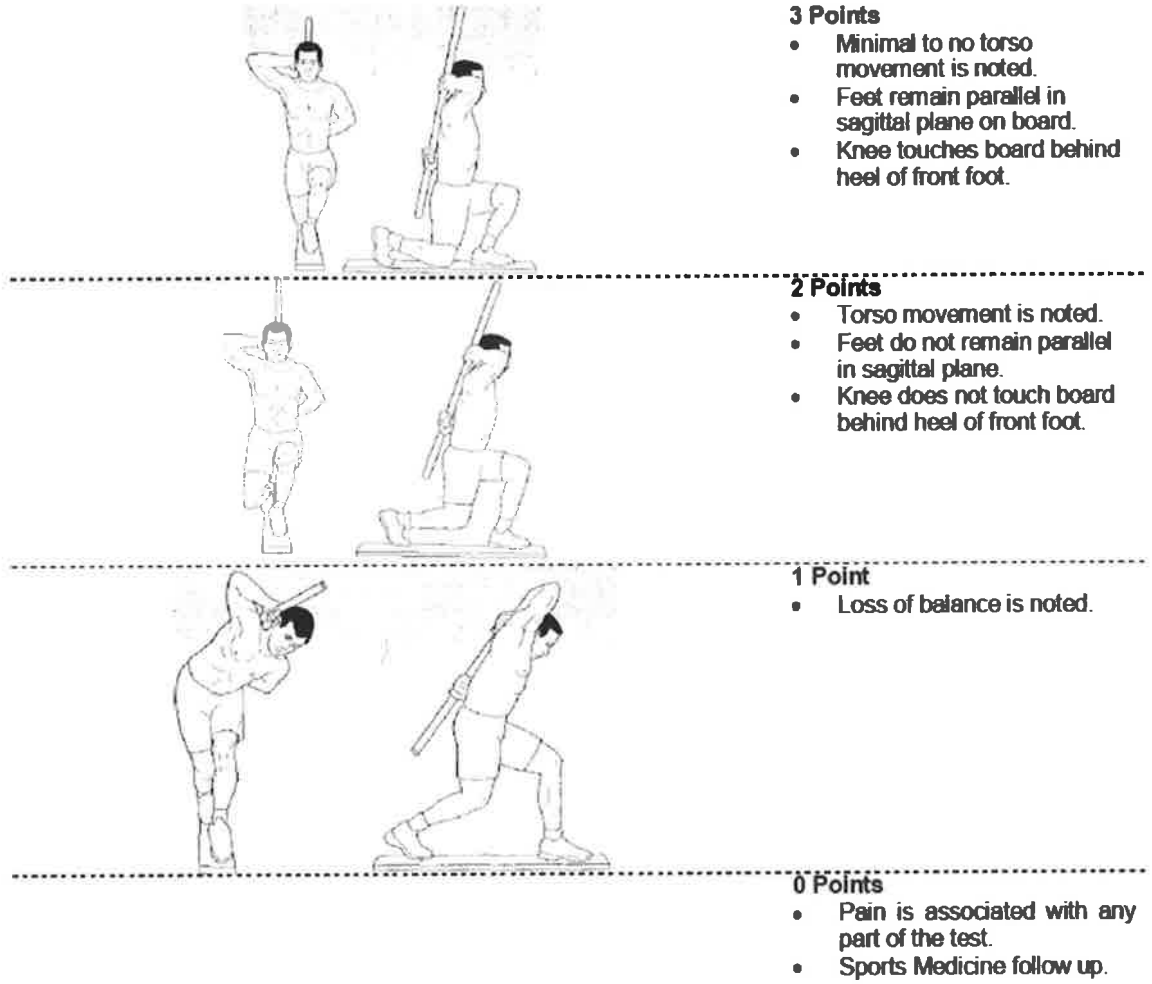
Appendix D Physical performance test

Single leg hop test

Adapted from *J Orthop Sports Phys Ther 2011;41(6):388. doi:10.2519/jospt.2011.0504*

Patients commenced the test with their non-injured leg. They initially were given a trial jump, followed by three further jumps on the same leg. The best single leg hop value was recorded.

In-line Lunge test



Adapted from *N Am J Sports Phys Ther*, vol 1(2), pp. 62-72, 2006.

The tester measures the individual's tibial length with a tape measure. The athlete then places one foot on the end of the 2" x 6" board. The athlete places the dowel behind their back touching the head, thoracic spine, and sacrum. The hand ipsi-lateral to the back foot should be the hand grasping the top of the dowel; the contra-lateral hand grasps the bottom. The tester then measures the tibial length from the end of the individual's toes and a mark is made on the board. The athlete is then asked to take a step and place their heel on the mark. The athlete then lowers their back knee enough to touch the board behind the

front foot. The feet should be on the same line and pointing straight throughout the movement. The lunge is performed up to three times bilaterally in a slow controlled fashion. If one repetition is completed successfully then a three is given.

Appendix E

CAPPAGH
NATIONAL
ORTHOPAEDIC
HOSPITAL

FOUNDED 1908
THE SISTERS OF CHARITY

FINGLAS, DUBLIN 11, IRELAND.
TEL:8140 400 FAX:8140 327

Dr. Sharil Shaarani
23 Chancery Court,
Bride Street
Dublin 8.

1st December 2009

Our Ref: JOB.10.2009.28
Re: Cappagh National Orthopaedic **Hospital Research Ethics Committee**

Dear Dr. Shaarani,

The Research Ethics Committee of Cappagh National Orthopaedic Hospital reviewed your renewed application. It was the decision of the committee to fully approve your application of research "The effects of prehabilitation on the outcome of anterior cuciate ligament (ACL) reconstruction"

The Research Ethics Committee requests that the committee be formally notified of the date of finalisation of the study and provided with a formal report as to the outcome and success of this trial.

Should you require any further information please do not hesitate to contact me.

Regards.

Yours sincerely,

Gordon Dunne
General Service Manager

SSC
SPORTS SURGERY CLINIC
DUBLIN

Sports Surgery Clinic
Santry Demesne
Dublin 9

Tel: +353 1 5262000
Fax: +353 1 5262011
Web: www.sportssurgeryclinic.com

January 2010

Mr Sharil Shaarani
23 Chancery Court
Bride Street
Dublin 8

Re: Application to carry out research in SSC

Dear Mr Shaarani

Further to your application to carry out research in SSc as detailed in your letter together with the relevant documentation submitted, I am pleased to inform you that the Research & Ethics committee have approved your application.

Yours sincerely

Professor Kevin Mulhall
SSC Research & Ethics Committee

Appendix F Raw Data

Label	Age	ACLSide	DominantLeg	Height	Weight	BMI	Lachmann	Pivot	AnteriorDrawer	SingleHopACLT1	SingleHopACLT2
P1	18	Left	Right	174	63	20.8	Positive	Positive	Postive	170	188
P2	29	Right	Right	200	93	23.3	Positive	Positive	Postive	144	156
P3	29	Right	Right	176	77	24.9	Positive	Positive	Postive	182	200
P4	27	Right	Right	174	87	28.7	Positive	Positive	Postive	183	190
P5	23	Right	Left	180	81	25	Positive	Positive	Postive	176	204
P6	33	Right	Right	176	72	23.2	Positive	Positive	Postive	123	181
P8	20	Left	Right	183	89	26.6	Positive	Positive	Postive	162	176
P9	45	Left	Left	179	85	26.5	Positive	Positive	Postive	157	174
P11	18	Left	Right	185	65	19	Positive	Positive	Postive	176	197
P12	30	Right	Right	182	78	23.5	Positive	Positive	Postive	156	188
P13	31	Right	Right	177	78	24.9	Positive	Positive	Postive	144	160
C1	20	Right	Right	164	60	22.3	Positive	Positive	Postive	183	185
C2	26	Left	Right	193	119	31.9	Positive	Positive	Postive	155	155
C3	23	Left	Left	180	75	23.1	Positive	Positive	Postive	168	213
P7	31	Right	Right	176	85	27.4	Positive	Positive	Postive	133	168
C5	42	Right	Right	181	99	30.2	Positive	Positive	Postive	100	95
C6	33	Left	Right	179	73	22.8	Positive	Positive	Postive	156	154
C7	43	Right	Right	181	84	25.6	Positive	Positive	Postive	110	116
C8	40	Right	Right	176	81	26.1	Positive	Positive	Postive	105	108
C9	30	Right	Right	178	100	31.6	Positive	Positive	Postive	181	210

SingleHopACLT3	SingleHopContraT1	SingleHopContraT2	SingleHopContraT3	InLineLungeACLT1	InLineLungeACLT2	InLineLungeACLT3	InLineLungeContraT1
168	185	211	203	2	2	2	3
131	218	225	215	2	2	2	3
170	182	190	185	3	2	3	3
155	196	191	181	2	2	3	3
133	196	207	205	3	3	3	3
131	188	207	183	2	3	2	2
148	183	180	222	3	3	3	3
128	189	188	178	3	3	3	3
156	180	209	223	3	3	3	3
143	210	215	185	3	3	3	3
131	168	191	179	3	3	3	3
139	214	208	205	3	3	3	3
132	189	179	170	2	2	3	3
75	240	244	217	3	3	3	3
120	186	209	192	2	3	3	3
101	175	187	181	3	2	2	3
124	174	172	212	3	3	3	3
85	118	124	117	2	2	3	2
97	127	125	123	2	3	3	2
129	170	174	180	3	3	3	3

InLineLungeContraT2	InLineLungeContraT3	IsoQuadsTorqueACLT1set2	IsoQuadsTorqueACLT2set2	IsoQuadsTorqueACLT3set2	IsoQuadsTorqueContraT1set2
3	2	118	113	115	126
3	2	80	106	129	142
3	3	145	161	98	163
3	2	165	156	123	164
3	3	121	168	81	155
3	3	107	149	77	149
3	3	152	199	137	151
3	3	161	155	91	175
3	3	65	108	70	85
3	3	145	179	109	167
3	3	126	168	93	138
3	3	104	110	37	117
3	3	179	186	141	221
3	3	104	149	76	165
3	3	142	142	98	155
3	2	102	138	88	155
3	3	129	136	88	123
2	3	122	125	92	136
3	3	31	43	25	108
3	3	195	210	108	233

IsoQuadsTorqueContraT2set2	IsoQuadsTorqueContraT3set2	IsoHamsTorqueACLT1set2	IsoHamsTorqueACLT2set2	IsoHamsTorqueACLT3set2
142	126	66	83	68
225	236	73	119	119
175	160	94	85	95
159	190	83	76	71
174	152	103	152	115
161	191	61	91	113
214	137	85	142	127
157	170	94	123	107
121	114	43	80	77
193	179	96	119	86
183	129	84	111	93
127	130	66	85	56
188	225	108	119	132
153	151	68	96	73
152	144	92	98	83
195	167	68	111	81
142	126	83	88	87
139	125	75	79	74
115	104	24	35	24
243	247	94	119	94

IsoHamsTorqueContraT1set2	IsoHamsTorqueContraT2set2	IsoHamsTorqueContraT3set2	QuadsMRIT1	QuadsMRIT2	QuadsMRIT3	HamsMRIT1	HamsMRIT2
76	87	92	43.54	49.04	38.12	28.97	30.97
106	160	152	47.12	47.28	45.97	35.04	33.92
88	100	94	59.34	59.52	43.89	59.18	56.91
97	114	113	64.25	69.35	62.22	55.87	58.19
136	146	137	62.18	63.65	52.51	61.3	53.15
80	108	127	41.73	49.49	38.04	48.73	45.94
106	129	127	61.62	65.3	51.99	45.31	50.73
102	115	119	65.77	72.88	55.85	50.55	55.01
50	95	74	43.1	50.52	38.12	33.27	33.55
107	146	115	61.72	65.2	55.34	49.92	46.55
91	126	103	52.37	56.6	45.43	36.28	37.45
61	77	87	51.57	52.18	41.24	47.31	47
125	115	155	60.04	58.76	57.44	65.3	66.74
95	102	102	55.35	55.21	46.25	44.04	47.68
113	121	126	77.14	73.57	64.67	68.74	65
99	107	113	73.31	63.38	61.65	57.42	53.93
89	87	107	55.38	57.26	47.28	36.16	36.07
69	75	74	62.23	57.32	53.71	43.98	43.04
60	64	72	62.5	63.17	53.43	47.74	48.21
104	111	136	81.19	82.02	70.07	76.42	77.87

HamsMRIT3	QuadsMRIT1Contra	QuadsMRIT2Contra	QuadsMRIT3Contra	HamsMRIT1Contra	HamsMRIT2Contra	HamsMRIT3Contra	VLmriT1	VLmriT2
29.78	46	50.33	50.93	34.23	33.29	31.87	11.69	12.69
30.93	66.18	63	68.06	35.43	34.16	30.04	14.09	10.59
50.06	65.12	55.76	54.85	57.92	60.3	53.24	11.86	12.24
52.41	69.36	69.45	68.71	56.29	50.31	52.75	15.83	16.66
59.95	55.28	59.96	59.96	63.64	63.09	63.74	11.86	11.65
49.48	62.3	55.01	56.62	55.77	49.6	44.21	8.23	8.49
44.45	64.02	68.3	65.17	60.92	52.96	51.7	14.39	15.85
45.84	66.15	68.23	66.17	50.96	51.68	40.21	15.38	20.92
34.17	47.66	51	51.63	36.88	40.02	35.34	9.51	10.51
44.64	58.67	68.88	62.55	51.73	47.44	50.43	13.94	14.26
33.38	56.98	59.49	60.91	36.55	34.82	35.38	11.32	11.93
44.29	53.94	53.62	52.79	44.91	45.78	43.03	9.69	10.76
69.78	74.49	70.81	76.65	68.18	68.51	69.85	11.03	8.59
44.79	69.07	67.51	67.2	51.44	52.75	51.03	10.24	13.06
64.99	76.25	75.52	76.93	68.77	58.88	67	19.38	15.3
53.31	76.21	71.66	73.24	60.18	57.49	60.09	13.26	12.69
35.44	59.93	60.78	61.03	39.76	40.56	37.32	13.88	13.03
45.12	60.77	58.12	59.4	43.44	41.17	42.55	12.87	10.51
45.94	54.4	52.04	54.67	38.74	39.34	37.78	14.82	15.74
77.21	86.26	87.59	86.76	71.53	71.97	71.97	18	18.45

VLmriT3	VMmriT1	VMmriT2	VMmriT3	VImriT1	VImriT2	VImriT3	VLmriT1Contra	VLmriT2Contra	VLmriT3Contra	VMmriT1Contra
9.82	18.23	18.9	16.04	13.52	16.12	14.65	11.3	11.85	12.08	21.22
9.53	19.69	20.17	19.01	12.28	13.12	13.14	16.29	12.95	13.4	28.54
9.29	21.02	25.58	16.3	17.06	17.05	14.34	13.94	12.99	13.8	19.09
13.48	21.3	23.9	21.83	18.35	18.67	17.46	18.14	18.08	17.95	21.58
9.33	21.53	22.48	14.22	19.85	21.15	22.34	12.68	12.04	12.71	24.14
7.38	17.36	22.05	18	14.21	15.18	11.25	12.34	8.44	7.84	25
11.61	19.16	22.03	17.74	20.2	22.24	11.6	14.63	15.87	15.57	24.62
13.83	25.43	25.36	22.54	20.62	22.56	17.34	14.59	15.46	15.32	26.81
8.76	15.4	19.06	13.27	14.85	15.96	13.87	9.84	11.08	10.21	17.61
10.21	27.06	27.7	26.09	16.5	17.16	12.46	12.91	15.13	14.16	25.83
9.02	20.16	22.71	17.33	15.41	18.13	16.04	11.14	11.95	11.68	22.92
8.32	16.58	15.02	14.56	20.16	21.13	14.77	11.7	11.38	9.12	16.27
10.62	27.1	29.82	26.26	16.28	15.77	16.44	13.44	10.41	13.46	34.43
9.13	25.39	25.02	19.85	25.39	16.37	13.91	14.62	17.37	13.86	31.48
15.67	23.64	25.78	22.65	24.08	23.48	19.81	17.62	18.81	20.3	27.93
11.74	27.71	26.46	23.65	21.1	17.5	19.26	19.64	16.41	17.5	28.06
12.23	24.91	25.25	22.23	14.51	16.49	11.69	13.03	13.69	16.28	26.76
10.23	26.86	24.27	22.86	17.57	15.01	13.63	13.93	12.48	12.31	24.55
12.09	22.65	23.84	20.67	19.5	18.88	15.95	13.62	12.73	14.02	21.5
18.9	34.31	36.39	30.45	28.06	27.34	23.13	23.12	25.77	25.45	34.96

VMmriT2Contra	VMmriT3Contra	VImriT1Contra	VImriT2Contra	VImriT3Contra	SamriT1	SamriT2	SamriT3	GrmriT1	GrmriT2	GrmriT3
21.55	22.22	14.47	15.26	15.94	3.14	2.72	2.61	2.34	2.24	2.01
28.01	33.45	16.87	16.52	13.16	3.52	3.62	2.73	1.33	1.37	1.12
23.58	22.07	18.46	14.31	14.18	2.6	2.62	2.04	3.06	3.09	3.17
21.82	22.08	19.66	17.72	19.38	3.3	3.25	3.15	3.67	3.4	3.02
20.26	16.59	17.77	19.93	22.16	4	5.33	5.48	3.56	3.59	3.45
24.85	25.72	21.04	18.25	18.16	2.42	1.87	2.4	3.1	2.75	2.87
26.99	26.22	20.89	24.13	20.76	2.75	3.08	2.84	2.6	3.11	2.66
28.46	28.13	19.1	20.8	20.42	2.92	3.28	2.96	2.22	2.73	2.14
20.01	18.88	16.87	16.8	15.94	2.75	2.6	2.79	2.83	2.86	2.62
31.43	27.06	16.23	18.94	17.79	3.81	3.9	3.65	2.26	3.06	2.08
26.01	24.92	17.38	18.95	14.87	3.53	3.75	3.47	2.33	2.19	2.27
15.95	16.59	19.29	20.8	17.42	2.6	3.04	2.62	3.28	3.55	3.08
35.43	35.37	20.13	20.44	21.31	4.7	5.4	4.24	3.14	2.53	3.12
30.85	30.02	17.29	17.69	17.62	3.43	3.72	3.52	2.43	2.78	2.76
26.63	29.36	23.74	21.2	18.47	3.62	3.65	3.32	5.33	4.78	4.82
29.05	29.22	20.39	18.84	20.35	4.03	3.67	3.65	2.28	2.29	2.36
25.82	28.79	17.23	17.31	13.34	3.3	3.3	3.14	2.12	2.3	2.12
23.36	23.78	16.03	14.92	13.6	3.13	3.45	3.1	2.89	2.53	2.72
21.27	23.87	11.69	10.53	12.04	3.29	3.17	3.12	3.84	4.12	4.23
36.52	32.91	25.76	28.85	22.69	3.58	3.92	3.31	4.96	5.31	4.85

SamriT1Contra	SamriT2Contra	SamriT3Contra	GrmriT1Contra	GrmriT2Contra	GrmriT3Contra	BFmriT1	BFmriT2	BFmriT3	STmriT1	STmriT2
3.53	2.89	2.71	3.39	3.25	2.74	14.98	15	14.98	5.15	5.18
3.52	3.59	3.77	1.34	1.08	1	11.66	11.16	10.64	2.75	3.87
2.86	2.71	2.75	2.82	2.87	2.9	17.5	20.15	17.19	4.91	4.88
3.3	3.25	3.15	3.67	3.4	3.02	24.55	22.49	18.53	5.65	6.9
3.68	4.04	5.37	2.84	3.03	3.08	21.33	24.65	22.83	9.93	9.42
2.52	2.42	2.37	1.09	2.35	2.16	19.38	15.81	17.75	7.14	7.07
3.71	3.35	3.1	2.94	3.17	2.81	19.22	22.59	22.8	6.4	8.26
2.93	3.28	2.96	2.23	2.74	2.14	23.79	23.36	20.08	5.96	10.4
2.45	2.49	2.63	2.62	3.08	2.58	13.79	13.46	12.98	5.05	4.95
5.46	4.61	5.34	2.01	2.43	2.25	24.94	22.56	22.52	3.61	3.19
3.08	3.17	2.96	1.63	1.57	1.69	14.24	14.94	14.64	5.97	5.87
3.79	3.64	2.89	2.6	2.8	2.71	19.69	19.23	17.47	5.41	6.05
4.57	4.49	4.52	3.32	3	3.33	23.05	23.77	26.91	5.86	5.13
3.35	3.05	4.27	2.33	2.29	2.36	19.28	19.31	18.74	3.53	3.31
3.07	3.2	3.45	4.49	3.92	3.52	32.66	29.94	28.62	18.43	12.88
3.84	3.85	4.16	2.83	2.73	2.72	22.35	23.06	22.71	7.72	7.74
3.06	3.26	3.19	2.9	2.95	2.79	18.08	16.94	17.85	3.25	4.48
2.88	2.76	2.95	2.41	2.29	2.27	18.23	17.56	17.73	5.97	5.23
3.27	3.34	3.48	3.09	2.88	3.14	20.37	21.12	20.37	6.78	7.58
3.58	3.62	3.77	4.96	4.96	4.65	32.77	30.87	31.81	12.45	12.97

STmriT3	SMmriT1	SMmriT2	SMmriT3	BFmriT1Contra	BFmriT2Contra	BFmriT3Contra	STmriT1Contra	STmriT2Contra	STmriT3Contra	SMmriT1Contra
4.51	9.21	9.15	8.89	19.61	16.85	18.19	4.69	4	4.13	11.29
2.73	11.23	11.91	9.9	10.83	11.55	13.15	4.17	3.28	2.68	12.23
5.21	15.3	17.04	13.8	18.74	17.84	18.08	5.11	4.04	4.94	16.61
4.53	13.26	13.95	10.94	18.96	16.25	16.27	8.17	7.25	6.1	13.97
10.64	14.97	16.58	18.94	22.64	20.64	21.24	8.87	9.54	18.3	17.87
7.02	11.96	11.43	11.12	20.26	18.87	16.71	7.03	9.7	6.94	15.16
6.31	14.29	12.66	13.41	37.39	24.14	23.23	6.12	6.86	5.94	17.61
5.61	12.99	13.97	12.28	24.35	25.73	19.02	4.35	6.95	3.96	14.81
4.32	8.81	10.34	9.41	14.51	13.96	11.31	6.26	6.16	5.45	11.15
4.13	18.3	17.93	17.76	25.73	24.66	25.76	3.03	3.47	3.81	16.69
5.35	10.68	12.01	8.9	15.96	15.23	14.23	6.23	6.05	3.82	10.06
6.55	14.49	13.45	12.91	17.81	19.24	17.47	6.06	6.05	6.55	12.34
6.18	18.23	21.58	18.87	26.11	23.77	26.91	6.38	5.46	6.38	21.51
3.06	13.91	14.16	14.15	23.24	21.04	22.63	3.85	3.99	3.69	14.38
13.62	9.55	13.26	13.69	30.32	25.68	29.52	15	11.13	12.28	14.74
7.75	14.8	15.36	15.98	22.45	23.06	24	8.38	7.27	7.86	15.96
3.19	10.81	13.73	11.82	16.68	17.19	16.81	4.6	5.92	4.42	13.5
5.51	16.23	17.82	17.72	18.71	13.96	11.31	4.95	6.16	5.45	15.46
6.91	12.06	13.94	12.06	14.53	24.66	25.76	6.37	3.47	3.81	11.12
12.54	18.15	20.78	18.69	26.52	15.23	14.23	13.82	6.05	3.82	23.32

SMmriT2Contra	SMmriT3Contra
10.26	9.9
11.25	10.75
18.88	16.54
14.19	15.34
18.31	16.97
12.39	11.23
17.19	16.65
15.41	13.57
13.11	10.45
16.25	16.03
9.93	8.86
13.45	12.91
23.68	21.52
13.88	15.35
13.84	14.72
16.07	18.62
13.02	14.26
14.95	16.33
12.33	11.78
22.89	24.91

Group	Label	Age	ACLSide	DominantLeg	WeeksTillSurgery	Height	Weight	BMI	Lachmann	Pivot	AnteriorDrawer
2	C10	32	1	1	5	172	85	28.7	1	1	1
2	C11	25	1	1	5	168	75	26.6	1	1	1

SingleHopACLT1	SingleHopACLT2	SingleHopACLT3	SingleHopContraT1	SingleHopContraT2	SingleHopContraT3
150	162	122	165	170	168
169	155	131	183	190	189

IsoQuadsTorqueACLT1 set2	IsoQuadsTorqueACLT2 set2	IsoQuadsTorqueACLT3 set2	IsoQuadsTorqueContra T1set2	IsoQuadsTorqueContra T2set2	IsoQuadsTorque ContraT3set2
160	172	132	170	179	157
111	115	97	121	131	100